Muscle contraction increases palmitate esterification and oxidation and triacylglycerol oxidation

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Dyck, D. J., and A. Bonen. Muscle contraction increases palmitate esterification and oxidation and triacylglycerol oxidation. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E888–E896, 1998.—We examined the oxidation and esterification of palmitate and the hydrolysis and oxidation of intramuscular lipids in isolated soleus muscles at rest and during tetanic contractions (2–40 tetani/min). Muscles were pulsed with [14C]palmitate to prelabel all intramuscular lipid pools. Muscles remained at rest or were then stimulated to contract at 2, 8, 20, or 40 tetani/min (30 min) in the presence of [3H]palmitate. Palmitate oxidation was increased 412% at 2 tetani/min (P < 0.05) and 880% at 8 tetani/min (P < 0.05). During contraction there was an absolute increase in esterification of palmitate to triacylglycerol in proportion with the increasing rate of palmitate oxidation. Intramuscular lipid oxidation provided ~77% of the total muscle energy compared with ~3% provided by exogenous palmitate under all conditions, with carbohydrate sources (glycogen and glucose) providing ~20% of the total energy. Thus, during muscle contraction, the oxidation rates of both exogenous and intramuscular lipids are increased in proportion to each other, while concomitantly palmitate is esterified in proportion to its oxidation.

Fatty acids (FA), derived from the plasma or hydrolysis of intramuscular triacylglycerol(s) (TG), represent a major oxidizable fuel for muscular work, especially at rest and during low and moderate exercise intensities (25, 26). During prolonged exercise, plasma FA concentrations become elevated as the respiratory exchange ratio (RER) decreases, indicating a shift toward greater lipid utilization (2, 5, 6, 8). This is also supported by early studies using radiolabeled FA, in which circulating FA provide more of the energy as exercise duration is increased (12, 13). However, when the exercise intensity is greater [i.e., >80% maximum O2 consumption (VO2max)], the rate of FA oxidation by muscle is diminished, due in part to a decrease in FA release from adipose tissue (25). However, reductions in the circulating FA do not entirely account for the reduced rate of FA oxidation during intense aerobic exercise (25).

It has been difficult to determine the contribution of intramuscular TG to energy provision during exercise or in isolated contracting muscles. Some studies have provided direct evidence for a decrease in intramuscular TG during exercise (17) or during electrically stimulated muscle contraction in situ (3, 9, 29) or in vitro (16). Others, however, have not observed a decrease in intramuscular TG during exercise (32) or during muscle contraction (4, 21). These discrepancies may be related to the unusually high variability associated with the measurement of intramuscular TG, both in animals (29) and humans (32). Another approach has been to assume that the difference between whole body lipid metabolism (indirect calorimetry) and 13C FA oxidation (13CO2 production) reflects the contribution of intramuscular TG to energy provision (25).

Few studies have examined lipid utilization by isolated muscles. It appears that some of the FA taken up by contracting muscles are not only oxidized but simultaneously esterified. For example, a recent study from this laboratory (10) showed that a bolus injection of [14C]palmitate rapidly increased (<5 min) incorporation of the radiolabel into the phospholipid (PL) and TG pools in tetanically contracting rat hindlimb muscles, with incorporation rates being greater in the slow-twitch than the fast-twitch muscles. In other studies, FA were simultaneously esterified and oxidized during intermittent twitch contraction in the isolated rat flexor digitorum brevis, and concomitantly, intramuscular TG were also utilized (16). However, the relationship, if any, between the oxidation rates of exogenously provided FA and intramuscular TG is not known, and the quantity of exogenously provided FA that may be esterified has not been determined in contracting muscle.

We have recently developed a pulse-chase technique for measuring the hydrolysis and oxidation of intramuscular lipid pools while simultaneously assessing esterification and oxidation of exogenously provided FA in isolated skeletal muscles at rest (6). With this procedure, we have now determined 1) the rate of lipid metabolism in contracting soleus muscle over a wide range of contraction rates and 2) the effects of exogenous FA availability on intramuscular hydrolysis and oxidation during contraction. We hypothesized that, with increasing rates of muscle contraction, 1) lipid oxidation rates would increase, 2) FA esterification would increase, 3) there would be a greater reliance on intramuscular TG, and 4) in the absence of FA, dependence on intramuscular TG oxidation would increase further.

METHODS

Animals

Female Sprague-Dawley rats (140–200 g) were used for all experiments. Animals were housed in a controlled environment on a 12:12-h light-dark cycle and fed Purina rat chow ad libitum. All procedures were approved by the animal care committee at the University of Waterloo. Animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (6 mg/100 g body mass) before all experimental procedures.

Preparation of Soleus Muscle Strips

Longitudinal strips of soleus were carefully prepared without damaging its junction to the tendon. Tendons were tied, and the strip was cut at the tendons without damage to the actual muscle and placed in a 4-ml glass incubation reservoir...
10 mM glucose, 1.0 mM palmitate, and 2 µCi of [1-14C]palmitate (Amersham Life Science, Oakville, ON, Canada) were immediately added to the incubation reservoir. The incubation medium was continuously gassed with 95% O2-5% CO2, and temperature was maintained at 30°C by circulating distilled water from a bath through an outer water jacket of the incubation reservoir. Muscles were “pulsed” with [1-14C]palmitate (ICN, Mississauga, ON, Canada) for 30 min to prelabel all endogenous lipid pools.

After the pulse incubation buffer was drained, muscles were washed for 15 min with incubation medium containing no radiolabeled palmitate. During this process, [1-14C]palmitate trapped in the interstitium was removed, with little or no net change in the amount of [14C] label already incorporated in the endogenous lipid pools (6). After the wash, some of the muscles were removed and processed for lipid extraction to determine the amount of [14C] label incorporated into the intramuscular lipid pools. The remaining muscles were incubated with [9,10-3H]palmitate (ICN) for an additional 30 min while the contraction studies were performed.

Preexperimental Labeling of Intramuscular Lipids

Three milliliters of warmed (30°C), pregassed (95% O2-5% CO2, pH = 7.4) Krebs-Henseleit buffer containing 4% fatty acid-free BSA (Boehringer Mannheim, Laval, QC, Canada), 10 mM glucose, 1.0 mM palmitate, and 2 µCi of [9,10-3H]palmitate to monitor the palmitate oxidation as well as its incorporation into the intramuscular lipids. Simultaneously, the degradation of the intramuscular lipid pools was monitored by measuring the changes of the prelabeled [14C] lipids. A 0.5 cm layer of heavy mineral oil was placed on top of the incubation medium to prevent escape of 14CO2 from the labeled substrate. For these purposes, 1.0 ml of incubation buffer was added to 5.0 ml of 2:1 chloroform-methanol and mixed thoroughly for 20 min. Two milliliters of 2 M KCl-2 M HCl were added, and the solution was mixed for an additional 20 min and centrifuged for 5 min at 3,000 g. One milliliter of the aqueous phase was removed for liquid scintillation counting.

Lipid oxidation. Simultaneous determination of palmitate oxidation and intramuscular lipid oxidation during contraction were determined as previously outlined (6), with minor modifications. 14CO2 produced during the experiments was determined by transferring a 0.5-ml aliquot of incubation buffer to a sealed glass vial and acidifying the buffer with 1.0 ml of 1 M H2SO4. Liberated 14CO2 was captured by a suspended center well containing benzethonium hydroxide. Center wells were placed in scintillation vials and counted using standard liquid scintillation techniques. Oxidation of [9,10-3H]palmitate was determined by separation of 3H2O from the labeled substrate. For these purposes, 1.0 ml of incubation buffer was added to 5.0 ml of 2:1 chloroform-methanol and mixed thoroughly for 20 min. Two milliliters of 2 M KCl-2 M HCl were added, and the solution was mixed for an additional 20 min and centrifuged for 5 min at 3,000 g. One milliliter of the aqueous phase was removed for liquid scintillation counting.

Graphical representation of apparatus used for stimulating soleus muscles. Muscle is tied at each tendon, adjusted to resting length, and set to 0.1 g of tension. Muscle is attached to a force transducer that is connected to a computer, thereby permitting monitoring of force production. Stimulating electrodes are located on walls of incubation vessel. Double-walled glass vial permits preparation to be surrounded by warmed water jacket. Gas (95% O2-5% CO2) is bubbled into the buffer, and mineral oil prevents escape of CO2.
Calculations and Statistics

The quantity of palmitate esterified and oxidized was calculated from the specific activity of labeled palmitate in the incubation medium (i.e., radiolabeled palmitate in dpm/total palmitate in nmol). Hydrolysis of intramuscular lipids was calculated as the net loss of preloaded 14C label from PL, monoacylglycerol (MG), and diacylglycerol (DG) pools. Because in a previous study the 14C label tracked only a small fraction of the TG pool in resting muscles (6), it was important to determine in contracting muscles the relationship between the changes in the radiolabeled TG pool and the enzymatically measured TG depots. Therefore, in separate experiments with enzymatic techniques (32), we compared the loss of TG with the loss of the 14C label from this pool. This demonstrated that during muscle contraction the 14C label tracked 51–68% of the TG pool, depending on the contraction condition employed, and, hence, this relationship was used to calculate the total amount of TG hydrolyzed in contracting muscles.

Results were analyzed using analysis of variance (ANOVA) procedures, and a Fisher’s protected least significant difference post hoc test was used to test significant differences revealed by the ANOVA. Significance was accepted at P < 0.05. All data are reported as means ± SE.

RESULTS

Muscle Tension

Force production generated by contracting SOL strips was recorded at 10-min intervals (Table 1). Initial force production was similar at all contraction rates (68.6 ± 8.1 g/g wet wt). No muscle fatigue was observed when the contractile rate was <20 tetani/min. At 20 tetani/min, force production decreased by 33% in the first 10 min (P < 0.05) and thereafter remained constant (Table 1).

Exogenous Palmitate Utilization

Palmitate uptake. With the onset of muscle contraction, the total amount of palmitate taken up (i.e., sum of palmitate esterified and oxidized) increased markedly (Fig. 2). At low stimulation rates (2 tetani/min), palmitate uptake into the muscle was doubled compared with the uptake at rest (P < 0.05). At stimulation rates of 10 tetani/min a further increase was observed (P < 0.05), with no further increase thereafter at stimulation rates of 20 and 40 tetani/min (P > 0.05) (Fig. 2).

Table 1. Force production during 30 min of muscle contraction

<table>
<thead>
<tr>
<th>Contraction Rate, tetani/min</th>
<th>Time, min</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>97.3 ± 3.5</td>
<td>101.9 ± 5.3</td>
<td>105.4 ± 4.9</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>91.4 ± 3.7</td>
<td>112.2 ± 11.5</td>
<td>105.6 ± 8.6</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>98.9 ± 5.5</td>
<td>99.7 ± 8.5</td>
<td>97.9 ± 7.3</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>67.3 ± 8.9</td>
<td>67.5 ± 10.7</td>
<td>69.8 ± 11.9</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE for 5–9 animals/group, expressed as percentage of initial tension (100%). Initial force production in all groups was 68 ± 8.1 g/g wet wt. Significantly different from: *P < 0.05; **P < 0.01.

Table 2. Palmitate esterification into PL, MG, and DG pools during 30 min of contraction

<table>
<thead>
<tr>
<th>Lipid Pool</th>
<th>Contraction Rate, tetani/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL</td>
<td>0 (Rest) 2 8 20 40</td>
</tr>
<tr>
<td>MG</td>
<td>3.1 ± 0.6 3.2 ± 0.8 3.0 ± 0.3 4.1 ± 0.5 3.8 ± 0.7</td>
</tr>
<tr>
<td>DG</td>
<td>26.1 ± 1.2 23.3 ± 4 24.3 ± 1.6 24.2 ± 2.4 28.7 ± 3.9</td>
</tr>
</tbody>
</table>

Values are means ± SE of 5–9 animals/group, expressed in nmol/g wet wt. PL, phospholipids; MG, monoacylglycerol; DG, diacylglycerol.

Fig. 2. Palmitate incorporation into isolated soleus muscles at rest and during muscle contraction. Total palmitate was determined by summing all palmitate deposited into intramuscular depots and palmitate that was oxidized. Values are means ± SE; n = 5–9 animals/group.
Palmitate esterification relative to palmitate oxidation. The relative (%) quantity of palmitate esterified to TG decreased by ~40% in the transition from rest to the lowest contraction rate used, 2 tetani/min (Fig. 4A). With further increases in muscle contraction, the relative quantity (%) of palmitate that was esterified remained remarkably constant (~50%; Fig. 4A). Concomitantly, the proportion of palmitate oxidized increased in a reciprocal manner (Fig. 4A). Such comparisons, however, are somewhat deceptive, because the total uptake of palmitate was increased during muscle contraction (Fig. 2). Therefore, when we compared the absolute rates of palmitate oxidized with the palmitate that was esterified to TG, a highly linear relationship was observed during the various stimulation rates (2–40 tetani/min) (Fig. 4B).

Intramuscular Lipids

During the pulse phase of our studies the intramuscular lipids were prelabeled with $^{14}$C. Therefore, we were able to determine, during 30 min of muscle contraction (2–40 tetani/min), the changes in the $^{14}$C label in the PL, DG, and MG pools (i.e., dpm·g$^{-1}$·min$^{-1}$). No changes were observed in the PL and MG pools during contraction (data not shown), whereas $^{14}$C decrements in the DG pool were doubled when the contraction rate was ≥8 tetani/min (P < 0.05, data not shown).

During contraction, intramuscular TG hydrolysis was increased, whether this was monitored by changes in TG depots (Table 3) or by measurement of the changes in the radiolabeled TG pool (P < 0.05). The relative changes in $^{14}$C label content and TG concentration are shown in Fig. 5. The rate of hydrolysis increased with increasing muscle work rates up to a rate of 8 tetani/min (Fig. 6). At this point TG hydrolysis was maximal, because at the higher contraction rates no further decrease in intramuscular TG was observed (P > 0.05, Fig. 6).

At the lowest stimulation rate (2 tetani/min), oxidation of endogenous lipids was increased 88% above rest (P < 0.05). At a contraction rate of 8 tetani/min the increase was +152%, whereas at the highest contraction rates (20 and 40 tetani/min), intramuscular lipid oxidation was increased +220% above the oxidation rate at rest. Differences between the rates of TG hydrolysis and $^{14}$CO$_2$ production reflect the contributions of the other labile lipid pools.

Comparison of Palmitate Oxidation with Intramuscular Lipid Oxidation and TG Hydrolysis

Because muscle contraction increased the oxidation of exogenous palmitate and intramuscular lipids at the same time (Fig. 3), we compared the relationship...
between them. There was a linear relationship between the amount of palmitate oxidized and the quantity of intramuscular lipids that were concomitantly oxidized (Fig. 7). The intramuscular lipids provided most of the total lipids metabolized (>90%) during muscle contraction (Fig. 7).

Carbohydrate Metabolism

Glucose oxidation rates were sharply increased by muscle contraction, increasing 117% at 2 tetani/min and 700% at 8 tetani/min over the oxidation rates observed at rest. At higher rates of contraction, glucose oxidation rates were not increased further (Table 3). Concomitantly, muscle glycogen content decreased during contraction. At 8 tetani/min, the decrease in glycogen (~10%) was not significant, but at 20 tetani/min there was a ~30% decrease in glycogen content (P < 0.05). No further decrease was observed during contractions at 40 tetani/min (Table 3).

ATP Provision During Muscle Contraction

Because we are able to measure lipid utilization and carbohydrate utilization in our studies, it was possible to estimate the energy provided for muscle contraction from exogenously provided glucose and palmitate as well as from the intramuscular glycogen and lipid depots. The majority of the fuel during muscle stimulation at 8, 20, and 40 tetani/min was provided by intramuscular lipids (74–80%), considerably smaller contributions were derived from palmitate (2.4–3.2%) and glucose (4.5–6.1%), and glycogen provided 10–20% of the total energy for the contracting muscles (Fig. 8).

DISCUSSION

These are the first studies to examine the simultaneous metabolism of exogenous FA (palmitate) and intramuscular lipids in contracting skeletal muscle. It should be recognized that these studies were performed in isolated muscles in the absence of the normal hormonal and neurological milieu. However, by using an isolated muscle preparation, we were able to take advantage of the extremely sensitive pulse-chase labeling procedures (6) to investigate lipid metabolism in a contracting oxidative muscle (soleus). With these procedures we were able to measure changes of as little as ~2–5 nmol/g wet wt in small soleus muscle strips. This enabled us to track with great precision the changes in intramuscular lipid TG stores. Additionally, with the pulse-chase approach it is possible to simultaneously determine the esterification and hydrolysis of the intra-

Table 3. Triacylglycerol and glycogen utilization and glucose oxidation in isolated contracting soleus muscles

<table>
<thead>
<tr>
<th>Substrate</th>
<th>0 (Rest)</th>
<th>2</th>
<th>8</th>
<th>20</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triacylglycerol concentration, µmol/g dry wt</td>
<td>17.7 ± 4.2</td>
<td>ND</td>
<td>10.2 ± 2.2  a</td>
<td>8.8 ± 1.5 a</td>
<td>10.5 ± 1.2 a</td>
</tr>
<tr>
<td>Glycogen concentration, µmol/g dry wt</td>
<td>102.6 ± 3.5</td>
<td>ND</td>
<td>91.8 ± 6.9</td>
<td>70.0 ± 5.2 c</td>
<td>78.9 ± 5.1 a</td>
</tr>
<tr>
<td>Glucose oxidation, nmol/g wet wt</td>
<td>212.0 ± 343</td>
<td>460.4 ± 140.3 b</td>
<td>1468.3 ± 168.7 b</td>
<td>1511.1 ± 111.3 b</td>
<td>1508.0 ± 301.8 b</td>
</tr>
</tbody>
</table>

Values are means ± SE of 5 animals/group. ND, not determined. Significantly different from: a rest; b 2 tetani/min; c 8 tetani/min.
muscular lipids, either at rest (6) or during a metabolic challenge such as muscle contraction (present study). A number of novel observations were made in the present study. First, the rates of both palmitate oxidation and esterification are increased with muscle contraction, and the absolute increase in these two processes is correlated. Second, the incorporation of exogenous palmitate into PL and the smaller MG and DG pools is unaffected by muscle contraction. Third, the oxidation of intramuscular lipids is increased with muscle contraction, providing ~75% of the total metabolism in the contracting soleus muscle.

Muscle Viability During Contraction

In our previous study (6), the viability of incubating quiescent muscles at 30°C was verified by the maintenance of ATP, phosphocreatine, and glycogen contents over a 3-h period. Intracellular phosphagens were not measured in the present study. Adequate oxygenation and muscle viability were assessed by the ability to maintain force production over the duration of contractions, as has previously been done (24). No significant decrease in force production was observed at contractile rates of 2, 8, or 20 tetani/min, indicating that the muscles were viable in these experiments. At 40 tetani/min, force production was decreased by 33% of initial value by 10 min and thereafter remained constant. The fact that the decrease in force production occurred only during the first 10 min of stimulation suggests that early fatigue of the small percentage of type IIA fibers [31%; (6)] may have been responsible for the decline in force, as opposed to inadequate oxygenation of the muscle fibers. It is also likely that because of the limited glycolytic potential of the soleus, metabolic provision of ATP could not be further increased beyond a contraction rate of 20 tetani/min, resulting in a compensatory decrease in force production. Indeed, this possibility is supported by the observation that both glucose oxidation and glycogen utilization were not significantly increased beyond 20 tetani/min (Table 3 and Fig. 8).

Oxidation and Esterification of Exogenous Palmitate

In the present study, ~25% of the palmitate incorporated into resting soleus was oxidized. In the transition from rest to contraction, a progressively greater percentage of the palmitate was directed toward oxidation, with 48% of the palmitate incorporated being oxidized at the lowest contraction rate (2 tetani/min) and ~63% thereafter (Fig. 3). Concomitantly, a lower percentage of palmitate was esterified with the onset of contraction, but this remained remarkably constant (~30%) during contractions ranging from 8 to 40 tetani/min. Thus, with increasing energy demand, a greater absolute amount of palmitate is incorporated into the contracting muscle, resulting in greater absolute rates of oxidation and esterification into TG. However, a greater percentage of the FA that are taken up is shunted toward oxidation for energy production, and a lower percentage is directed toward storage. This implies either the transport of FA into the mitochondria or that the enzymes involved in FA oxidation are more sensitive to changes in intracellular factors associated with increased energy demand than are the enzymes.
LIPID METABOLISM IN CONTRACTING SOLEUS MUSCLE

Hydrolysis and Oxidation of Intramuscular Lipids

Estimates of intramuscular lipid hydrolysis and oxidation during contraction are scarce. In exercising humans, intramuscular TG oxidation has been estimated from the difference between total body lipid oxidation (indirect calorimetry) and exogenous FA oxidation by muscle (rate of disappearance of labeled plasma FA) (25). However, this is an indirect measurement of intramuscular TG metabolism, and this estimate also makes the assumption that all FA taken up by the contracting muscle are oxidized. This assumption is challenged by the results of the present study, in which only ~65% of incorporated FA are oxidized during contraction, with the remainder directed toward storage. Our observation that a considerable proportion of the FA taken up by the contracting muscle is esterified agrees with other studies, in humans, in which 52–68% of oleate taken up by muscle was oxidized during low- and high-intensity exercise (28), and with studies of contracting rat hindlimb muscles (10) and in the isolated rat flexor digitorum brevis (16). Thus use of the RER obtained with indirect calorimetry to calculate the oxidation of circulating FA will overestimate the contributions of this energy source to muscle metabolism.

The present study is, to our knowledge, the first to directly measure the hydrolysis of intramuscular TG and the oxidation of intramuscular lipid oxidation. TG hydrolysis increased markedly during contraction rates up to 8 tetani/min and was greatest at 20–40 tetani/min. Net hydrolysis of intramuscular TG has been documented during exercise (7, 17, 30) when circulating levels of norepinephrine and epinephrine are elevated. We have recently shown that epinephrine increases TG hydrolysis and oxidation in isolated soleus muscle (23). However, endogenous TG utilization has also been shown to occur in isolated contracting muscles (15, 27), when no catecholamines are present. This finding is extended in the present study, in which there was a contraction rate-dependent hydrolysis of intramuscular TG in the absence of catecholamines. This suggests that activation of hormone-sensitive lipase during contraction may be mediated by factors other than cAMP (1), such as changes in cellular energy status or increases in cytosolic Ca²⁺. The role of cytoplasmic Ca²⁺ in the activation of hormone-sensitive lipase has been previously suggested (9) but remains to be clarified. In other studies in our laboratory, we have been able to detect, with Western blotting, hormone-sensitive lipase in muscle and adipose tissue by use of the same antibody (23), suggesting that the same hormone-sensitive lipase isoform is present in both tissues. Intramuscular lipid oxidation was also increased with contraction. The production of ¹⁴CO₂ mirrored the hydrolysis of the DG and TG stores. As noted in results, we were unable to quantify the absolute rate of DG hydrolysis, but in other studies it has been noted that the DG concentrations are only ~5% of TG deposits (J. Gorski, personal communication). The MG depots and intracellular FFA concentrations are presumably much smaller, indicating that most of the ¹⁴CO₂ from intramuscular lipids is derived from intramuscular TG. It should be pointed out that there is the possibility of recycling, or reincorporation of a small amount of the ¹⁴C tracer that was released from the TG pool or between various endogenous lipid pools. Therefore, estimates of hydrolysis based on loss of ¹⁴C label may be slight underestimates. Nevertheless, our results indicate that, at rest and over the entire contractile range, virtually all of the [¹⁴C]palmitate released from intramuscular lipids was oxidized (Fig. 6). Interestingly, there was a linear relationship between the oxidation of exogenous palmitate and the oxidation of intramuscular lipids (Fig. 7). Clearly, the intramuscular lipids provided most of the aerobic energy (~75% of total energy, Fig. 8) over the range of muscle contraction used.

Our estimates of exogenous palmitate contributions to lipid metabolism in contracting muscle appear to be much lower than those generally believed. However, the relative contributions of exogenous lipids and intramuscular lipids to energy provision in the contracting muscle have been hampered by the extraordinary variability in the determinations of the intramuscular TG concentrations. The contribution of intramuscular TG to energy production in exercising humans varies considerably. Romijn et al. (25) estimated the intramuscular lipid utilization as the difference between whole body lipid metabolism and ¹³C-FA oxidation. Intramuscular TG oxidation was greatest at 65% VO₂max and contributed ~35% of the total energy provision, which was similar to the contributions from endogenous FA. However, it was acknowledged by the authors that the energy contributions from the endogenous sources represented minimal estimates, whereas those from exogenous fuels represented maximal estimations, because it was assumed that all FA incorporated into exercising muscles were oxidized. This assumption is in sharp disagreement with this and other studies, which have demonstrated that a significant FA fraction taken up into the muscle cell is esterified (Figs. 3 and 4B, and Refs. 10 and 16). Thus, in the studies of Romijn et al., the oxidation of exogenous FA was likely overestimated, because the investigators were unable to determine the fraction of FA that was esterified. This would bring their results more in line with studies that have demonstrated that endogenous FA contribute less to the energy provision during exercise in humans [i.e., 8% (18) to 19% (19)] than endogenous lipids (35% (18)]. In the trained state, the reliance on endogenous FA is apparently further reduced (17, 20). Animal studies...
have also indicated that endogenous lipids provide the majority of the muscle energy during aerobic exercise (–62% (29)) or tetanic muscle contraction (–90% (16)). Thus our findings that intramuscular TG deposits rather than circulating FA provide most of the lipid-derived energy for muscle contraction are consistent with a number of studies in humans (18, 19) and animals (16, 29). It may be that use of an incubated muscle preparation in which FA enter the muscle by diffusing through the interstitial space rather than being delivered to the muscle via the circulation, gives a somewhat lower estimate of the contribution of exogenous FA utilization. Nevertheless, it does appear from a number of studies that intramuscular lipids provide the bulk of the lipids that are oxidized during tetanic muscle contraction in a highly oxidative muscle such as the soleus.

Although the present study has examined only the contribution of exogenous FA and intramuscular lipids to energy provision in contracting oxidative muscle, it should be recognized that other sources of lipid, such as circulating TG chylomicrons and very low density lipoproteins, may also be important during muscle contraction. The contribution of plasma-derived TG is debatable, with estimates being that they provide only 5% to the energy provision during exercise (22). Although evidence for the utilization of 14C-labeled chylomicrons has also been documented (31), the actual amount of chylomicron-derived TG that were oxidized could not be quantified. Much of the discrepancy is likely due to the variability in measuring small arteriovenous differences in plasma TG concentrations.

In summary, a pulse-chase technique was employed to directly measure exogenous palmitate oxidation and esterification into intramuscular lipids, as well as the hydrolysis and oxidation of intramuscular lipids during different rates of tetanic contraction. Oxidation of palmitate and its incorporation into TG were increased with contraction, reaching maximal rates at a contraction rate of ~20 tetani/min. Hydrolysis and oxidation of intramuscular TG pools were also augmented with contraction, also plateauing at ~20 tetani/min. Quantitatively, intramuscular TG provided most of the substrate for muscle metabolism during contraction, increasing greatly from rest with the onset of contractions.

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