Regulation of contraction-induced FA uptake and oxidation by AMPK and ERK1/2 is intensity dependent in rodent muscle

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Raney, Marcella A., and Lorraine P. Turcotte. Regulation of contraction-induced FA uptake and oxidation by AMPK and ERK1/2 is intensity dependent in rodent muscle. Am J Physiol Endocrinol Metab 291: E1220–E1227, 2006. First published July 11, 2006; doi:10.1152/ajpendo.00155.2006.—Muscle contraction activates AMP-activated protein kinase (AMPK) and extracellular signal-regulated kinase (ERK1/2), two signaling molecules involved in the regulation of muscle metabolism. The purpose of this study was to determine whether activation of AMPK and/or ERK1/2 contributes to the regulation of muscle fatty acid (FA) uptake and oxidation in contracting muscle. Rat hindquarters were perfused during rest (R) or electrical stimulation (E) of increasing intensity by manipulating train duration (E1 = 25 ms, E2 = 50 ms, E3 = 100 ms, E4 = 200 ms). For matched FA delivery, FA uptake was significantly greater than R during E1, E2, and E3 (7.8 ± 0.7 vs. 14.4 ± 0.3, 16.9 ± 0.8, 15.2 ± 0.5 nmol·min⁻¹·g⁻¹, respectively, P < 0.05), but not during E4 (8.3 ± 0.3 nmol·min⁻¹·g⁻¹, P > 0.05). FA oxidation was significantly greater than R during E1 and E2 (1.5 ± 0.1 vs. 2.3 ± 0.2, 2.5 ± 0.2 nmol·min⁻¹·g⁻¹, P < 0.05) before returning to resting levels for E3 and E4 (1.8 ± 0.1 and 1.5 ± 0.2 nmol·min⁻¹·g⁻¹, P > 0.05). A positive correlation was found between FA uptake and ERK1/2 phosphorylation from R to E2 (R² = 0.55, P < 0.05) and between FA oxidation and ERK1/2 phosphorylation from R to E2 (R² = 0.76, P < 0.05), correlations that were not maintained when the data for E4 and E3 and E4, respectively, were included in the analysis (R² = 0.04 and R² = 0.03, P > 0.05). A positive correlation was also found between FA uptake and FA oxidation and AMPK activity for all exercise intensities (R² = 0.57, R² = 0.65 respectively, P < 0.05). These results, in combination with previous data from our laboratory, suggest that ERK1/2 and AMPK are the predominant signaling molecules regulating FA uptake and oxidation during low- to moderate-intensity muscle contraction and during moderate- to high-intensity muscle contraction, respectively.

Fatty acids (FA) are an important energy source for skeletal muscle at rest and, at a given FA concentration, FA utilization in muscle has been shown to be severalfold higher during muscle contraction than at rest (7, 10, 23, 29). In incubated muscle, FA oxidation during muscle contraction has been shown to correlate positively with the stimulation frequency up to 40 tetani/min (7, 10). Similarly, in healthy subjects performing bicycle exercise, the rate of muscle FA oxidation is higher during low- and moderate-intensity muscle contraction than at rest (23, 26, 31, 34). The increase in FA utilization during whole body exercise is associated with an increase in plasma FA concentration and, hence, FA delivery to the working muscle (18, 23, 25, 42), leading some to hypothesize that the increase in FA delivery is the main factor regulating the increase in FA utilization during muscle contraction (4, 8, 21). However, studies performed in our laboratory and others’ have provided additional evidence that FA uptake and oxidation increase during acute exercise independently of FA delivery (2, 29, 30, 33), suggesting that intracellular mechanisms must exist in the regulation of contraction-induced FA metabolism.

Data show that multiple intracellular signaling cascades are activated by muscle contraction. Specifically, it is known that muscle contraction of increasing intensity is associated with an increase in AMP-activated protein kinase (AMPK) activity (20, 41) as well as extracellular signal-regulated kinase-1/2 (ERK1/2) phosphorylation (24, 36). Both AMPK activity and ERK1/2 phosphorylation have been implicated in the regulation of various aspects of lipid metabolism including regulation of contraction-induced acetyl-CoA carboxylase (ACC) phosphorylation and subsequent long-chain FA oxidation (19, 38) as well as regulation of contraction-induced activation of muscle hormone-sensitive lipase (6), respectively. Therefore, it is reasonable to hypothesize that ERK1/2 and AMPK may also participate individually in the regulation of contraction-induced FA uptake and oxidation in skeletal muscle.

Indeed, a link between AMPK activation and translocation of the FA transporter FAT/CD36 to the plasma membrane and subsequent FA uptake has been observed in rat cardiac myocytes (14), suggesting that, at least in cardiac muscle, AMPK signaling is involved in the regulation of contraction-induced FA uptake. However, in skeletal muscle perfused with the pharmacological agent 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranside (AICAR), a significant increase in AMPK activity was associated with a significant increase in FA uptake at rest but no change in FA uptake during low-intensity muscle contraction (19). Although these results suggest that AMPK activation may be involved in the regulation of muscle FA uptake at rest, it is not clear whether AMPK activation plays a critical role in the regulation of the contraction-induced increase in FA uptake during low-intensity muscle contraction. The possible role AMPK activation plays in the regulation of contraction-induced FA uptake at other contraction intensities is unknown. A previous study performed in our laboratory has also demonstrated that inhibition of contraction-induced ERK1/2 phosphorylation with the MAP/ERK kinase-1/2 (MEK1/2) inhibitor PD-98059 results in complete inhibition of
contraction-induced FA uptake during moderate-intensity muscle contraction (30), suggesting that ERK1/2 plays a crucial role in the regulation of contraction-induced FA uptake at this contraction intensity. In that same study, it was demonstrated that ERK1/2 inhibition had no effect on total or AMPK contraction intensity. In that same study, it was demonstrated that ERK1/2 plays a crucial role in the regulation of contraction-induced FA uptake, it must lie upstream of ERK1/2 (30). However, the possible interaction between AMPK and ERK1/2 activity in the regulation of contraction-induced FA uptake during contraction of other intensities has yet to be explored.

The role of these two signaling molecules in the regulation of contraction-induced FA oxidation is similarly uncertain. Although AMPK is widely known for its phosphorylation of ACC and subsequent increase in FA oxidation during muscle contraction (16, 39), our laboratory has shown that low-intensity muscle contraction results in a significant increase in FA oxidation despite no change in AMPK activity (19). These results suggest that AMPK-independent mechanisms may also exist in the regulation of contraction-induced FA oxidation. The potential involvement of ERK1/2 activation in the regulation of contraction-induced FA oxidation has not been studied.

Therefore, the purpose of this study was to more clearly define the role of AMPK and ERK1/2 in the regulation of contraction-induced FA uptake and oxidation in skeletal muscle. To verify the involvement or lack of involvement of either signaling molecule in the regulation of contraction-induced FA metabolism, we chose to manipulate contraction intensity to determine whether a linear relationship exists between FA uptake or oxidation and the activity of each kinase. On the basis of published data, we expected that a positive correlation would be found between FA kinetics and ERK1/2 phosphorylation and not necessarily between FA kinetics and AMPK activity.

**MATERIALS AND METHODS**

**Animal preparation.** Male Wistar rats (~250–330 g) were housed in pairs and kept on a 12:12-h light-dark cycle. They received standard rat chow and water ad libitum. Rats were randomly assigned to either the rest group (R; n = 10) or to one of four electrical-stimulation groups representing increasing contraction intensity via manipulation of the train duration stimulation parameter: 25 ms (E1; n = 9), 50 ms (E2; n = 10), 100 ms (E3; n = 8), 200 ms (E4; n = 9) (13). Ethical approval for the present study was obtained from the Institutional Animal Care and Use Committee at the University of Southern California.

**Hindquarter perfusion.** On the day of the experiment, rats were anesthetized with an intraperitoneal injection of a ketace-thalozidine mixture (80 and 12 mg/kg body wt, respectively). Rats were then prepared surgically for hindquarter perfusion as previously described (32). Before the perfusion catheters were inserted, heparin (150 IU) was administered into the inferior vena cava. The rats were killed with an intracardial injection of pentobarbital sodium (0.4 mg/g body wt), and arterial and venous catheters were inserted immediately into the descending aorta and vena cava, respectively. The preparation was placed in a perfusion apparatus where the left iliac vessels were tied off and a clamp was fixed tightly around the proximal part of the leg to prevent bleeding (32).

The initial perfusate (250 ml) consisted of Krebs-Henseleit solution, 5% bovine serum albumin (Bovuminar, pH 7; Serologicals, Norcross, GA), 550 µM albumin-bound palmitate, 8 µCi of albumin-bound [1-14C]palmitate, and 6 mM glucose. Insulin was not included so as to isolate contraction effects on muscle metabolism. The perfusate (37°C) was continuously gassed with a mixture of 95% O2-5% CO2, which yielded arterial pH values of 7.4–7.5 and arterial Po2 and PCO2 values that were typically 500–530 and 28–46 mmHg, respectively. Mean perfusion pressures were not affected by muscle contraction and averaged 85.5 ± 8.6, 81.9 ± 11.9, 93.5 ± 9.1, 81.2 ± 6.2, and 101.4 ± 10.9 mmHg in the R, E1, E2, E3, and E4 groups, respectively (P > 0.05).

The first 25 ml of perfusate that passed through the right hindquarter were discarded, whereupon the hindquarter was equilibrated for 20 min. Animals were then perfused for an additional 20 min at a perfusate flow of 15 ml/min (average for all groups: 0.86 ± 0.01 ml/min g dry muscle−1 g−1 perfused muscle). In the electrically stimulated animals, the right leg was immobilized at the tibiofemoral ligament, and a hook electrode was placed around the sciatic nerve and connected to an S48 Grass stimulator (Grass Telefactor, West Warwick, RI). The resting length of the gastrocnemius-soleus-plantaris muscle group was adjusted to obtain maximum active tension upon stimulation. As done previously to show that glucose transport is intensity dependent (13), we manipulated train duration to increase contraction intensity. Thus, stimulation was delivered every 2 s, and lasting 2, 5, 10, 20, and 200 ms, respectively. During the 20-min muscle stimulation, the tension developed by the gastrocnemius-soleus-plantaris muscle group was recorded with a modular chart recorder (Cole Parmer, Vernon Hills, IL). The decrease in tension development over the stimulation period was used as an indicator of performance.

After equilibration, arterial and venous perfusate samples were taken at 5, 10, 15, and 20 min for analysis of [1-14C]FA and 14CO2 radioactivities, as well as FA, glucose, and lactate concentrations. Arterial and venous perfusate samples for determinations of Pco2, Po2, and pH were taken at 10 and 20 min. At the end of the 20-min experimental period, the gastrocnemius-soleus-plantaris muscle group of the right leg was freeze-clamped in situ with aluminum clamps precooled in liquid N2, taken out, and stored for later analysis.

**Blood sample analyses.** A YSI-1500 was used to analyze both plasma glucose and lactate concentrations (Yellow Springs Instrument, Yellow Springs, OH). Plasma FA concentrations were determined spectrophotometrically using the WAKO NEFA-C test (Biochemical Diagnostics, Edgewood, NY). Plasma [1-14C]FA and 14CO2 radioactivities were determined in duplicate as previously described in detail (29, 31, 32). Pco2, Po2, and pH were measured with an ABL-5 analyzer (Radiometer America, Westlake, OH).

**Muscle sample analyses.** To quantify dual phosphorylation of ERK1/2, muscle samples (~90 mg) were powdered under liquid N2, homogenized with 1 ml of ice-cold RIPA buffer, and prepared for Western blot analysis as previously described (14). For AMPK activity determinations, powdered muscle (~400 mg) was homogenized in AMPK buffer (210 mM sucrose, 1 mM EDTA, 5 mM sodium pyrophosphate, 50 mM NaF, 1 mM DTT, 2 mM PMSF, and 50 mM HEPES, pH 7.4) and centrifuged for 45 s at 15,000 g. The supernatant was used to determine total AMPK activity, and isoform-specific AMPK activity was determined in immunoprecipitates from 200 µg of supernatant protein after overnight incubation at 4°C with ~1.5 µg of affinity-purified isoform-specific goat IgG against either AMPKα1 or AMPKα2 in 20 µl of protein A/G-agarose beads (Santa Cruz Biotechnology). [32P]ATP incorporation into SAMS peptide was used to measure total, AMPKα1, and AMPKα2 activity from the respective preparations (39). ACC phosphorylation was determined via Western blot analysis (28) using anti-phospho-ACC (Ser79) (1:5,000; Upstate Cell Signaling, Charlotteville, VA), according to the manufacturer’s instructions, using equal quantities of protein from the supernatant of the total AMPK activity preparations.

**Calculations and statistics.** Palmitate delivery, fractional and total palmitate uptake, and percent and total palmitate oxidation were
calculated as previously described in detail (29, 32). Both percent and total palmitate oxidation values were corrected for label fixation by using previously calculated acetate correction factors (32). The arterial specific activity for palmitate did not vary over time and was not significantly different between groups, averaging 40.3 ± 2.2, 43.8 ± 1.3, 42.4 ± 1.0, 45.8 ± 1.8, and 48.9 ± 3.4 μCi/mmol in R, E1, E2, E3, and E4 groups, respectively (P > 0.05). Oxygen and glucose uptake as well as lactate release were calculated as previously described (32) and are expressed per gram of perfused muscle, which was previously determined to be 5.6% of body weight for unilateral hindquarter perfusion (33). At rest and during muscle contraction, time effects for glucose, lactate, and FA concentrations and kinetic data were analyzed using a one-way ANOVA with repeated measures. Because there was no significant difference in values measured after 10, 15, and 20 min of perfusion, average values were used for each animal in subsequent analyses. The effects of contraction intensity on the same variables as well as on muscle ERK1/2 and ACC phosphorylation and AMPK activities were analyzed using a one-way ANOVA (Statistica v. 5.0; StatSoft, Tulsa, OK). Sheffe’s test for post hoc multiple comparisons was performed when appropriate. The existence of association(s) between enzyme activity and FA metabolism and trends with changes in contraction intensity were initially examined with a running interval smooth analysis, which determines the trend of data independently of any preestablished statistical line equations (37). The plot of the running interval smooth analysis uncovered curvature within the data and confirmed that this was not due to randomness. The curvilinear pattern then allowed us to limit our correlational analysis to specific data points. The square of Pearson’s product moment coefficient was used to determine the significance of correlation between FA uptake and oxidation and ERK1/2 phosphorylation as well as FA uptake and oxidation and ERK1/2 phosphorylation. A significance level of 0.05 was chosen for all statistical methods.

RESULTS

Muscle performance and O2 uptake. Oxygen uptake did not vary over time in any of the groups (P > 0.05). Muscle contraction increased oxygen uptake in an intensity-dependent manner (P < 0.05; Table 1). The initial amount of tension developed by the contracting muscles demonstrated intensity dependence (P < 0.05; Table 1). Muscle tension development decreased markedly during the first 10–15 min of electrical stimulation and was followed by a more gradual decrease. After 10 min of electrical stimulation, muscle tension development had decreased to 60–67% of initial tension development in all groups. No statistical difference in percentage of initial tension was found between contraction groups at 5, 10, 15, or 20 min of stimulation (P > 0.05; Fig. 1).

Palmitate metabolism. Perfusion palmitate concentration and palmitate delivery did not change during the 20-min experimental period for any group (P > 0.05) and, as dictated by the protocol, were not significantly different between groups (P > 0.05; Table 1). Fractional (fractional units) and total FA uptake (mmol·min⁻¹·g⁻¹; Fig. 2B) were significantly greater than R during E1, E2, and E3 (0.018 ± 0.001 vs. 0.031 ± 0.002, 0.036 ± 0.005, and 0.031 ± 0.003 and 7.8 ± 0.7 vs. 14.4 ± 0.3, 16.9 ± 0.8, and 15.2 ± 0.5, respectively, P < 0.05), but not during the high-intensity protocol E4 (0.021 ± 0.002 and 8.3 ± 0.3, P > 0.05). Muscle contraction did not affect the percentage of palmitate oxidized (P > 0.05; Fig. 3A). However, total palmitate oxidation (Fig. 3B) was significantly greater than R during E1 and E2 (1.5 ± 0.1 vs. 2.3 ± 0.2 and 2.5 ± 0.2 mmol·min⁻¹·g⁻¹, P < 0.05) before returning to resting levels for E4 (1.5 ± 0.2 mmol·min⁻¹·g⁻¹, P > 0.05).

Substrate exchange across the hindquarter. Arterial perfusate glucose concentration did not change during the 20-min experimental period for any group (P > 0.05). Average glucose concentration was also not significantly different between groups (R: 6.6 ± 0.7, E1: 6.4 ± 0.2, E2: 6.4 ± 0.3, E3: 6.6 ± 0.2, and E4: 6.9 ± 0.2 mmol/l, P > 0.05). Glucose uptake did not change over time in any of the groups (P > 0.05) and demonstrated a gradual intensity-dependent increase (R: 5.4 ± 1.4 vs. E1: 11.8 ± 1.6, E2: 12.95 ± 1.0, E3: 13.0 ± 2.2, and E4: 19.2 ± 0.5 mmol·h⁻¹·g⁻¹, P < 0.05; Fig. 2A). Initial arterial perfusate lactate concentration was not significantly different between groups (P > 0.05; Table 1). At the conclusion of the perfusion, arterial lactate concentration was not significantly different than initial concentration in R (P > 0.05) and increased 1.7- to 2.4-fold in contraction groups (P < 0.05).

Table 1. Perfusion characteristics of rat hindquarters perfused at rest or during electrical stimulation of increasing train duration

<table>
<thead>
<tr>
<th></th>
<th>R (n = 10)</th>
<th>E1 (n = 9)</th>
<th>E2 (n = 10)</th>
<th>E3 (n = 8)</th>
<th>E4 (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA concentration, μmol/l</td>
<td>560.5 ± 27.8</td>
<td>534.8 ± 23.6</td>
<td>543.3 ± 24.9</td>
<td>527.7 ± 21.3</td>
<td>529.8 ± 34.4</td>
</tr>
<tr>
<td>FA delivery, mmol/min/g</td>
<td>483.2 ± 38.4</td>
<td>462.0 ± 30.8</td>
<td>450.8 ± 32.5</td>
<td>488.6 ± 29.3</td>
<td>472.1 ± 35.3</td>
</tr>
<tr>
<td>Initial lactate concentration, mmol/l</td>
<td>0.51 ± 0.10</td>
<td>0.51 ± 0.04</td>
<td>0.56 ± 0.06</td>
<td>0.51 ± 0.03</td>
<td>0.53 ± 0.05</td>
</tr>
<tr>
<td>Final lactate concentration, mmol/l</td>
<td>0.55 ± 0.09</td>
<td>1.40 ± 0.09#</td>
<td>1.50 ± 0.12#</td>
<td>1.52 ± 0.09#</td>
<td>1.84 ± 0.09#</td>
</tr>
<tr>
<td>Lactate release, μmol/h/g</td>
<td>15.5 ± 1.1</td>
<td>23.6 ± 2.1#</td>
<td>26.2 ± 1.9#</td>
<td>29.4 ± 2.8#</td>
<td>33.7 ± 1.8#</td>
</tr>
<tr>
<td>Oxygen uptake, μmol/h/g</td>
<td>12.4 ± 1.3</td>
<td>17.4 ± 1.6#</td>
<td>19.11 ± 1.4#</td>
<td>27.2 ± 1.3#</td>
<td>28.2 ± 1.9#</td>
</tr>
<tr>
<td>Initial force production, g</td>
<td>N/A</td>
<td>357.8 ± 29.4*</td>
<td>621.3 ± 44.8*</td>
<td>752.1 ± 32.0*</td>
<td>1010.7 ± 96.6*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. R: rest; E1: 25 ms; E2: 50 ms; E3: 100 ms; E4: 200 ms. *P < 0.05 vs. all other groups; #P < 0.05 vs. R; SP < 0.05 vs. E4.
Lactate release did not vary significantly over time in any of the groups (P > 0.05) and demonstrated a gradual intensity-dependent increase (P < 0.05; Table 1).

Enzyme activities. Both total (Fig. 4A) and AMPKα2 activity (Fig. 4B) increased from R to E2 (total AMPK: R: 759.7 ± 20.5 vs. E1: 1,271.3 ± 66.1 and E2: 1,325.2 ± 191.3; AMPKα2: R: 30.2 ± 0.6 vs. E1: 33.9 ± 0.3 and E2: 37.2 ± 1.1 pmol·min⁻¹·g⁻¹, respectively, P < 0.05) and then gradually decreased back to resting levels (total AMPK: E4: 751.27 ± 67.7; AMPKα2: E4: 25.7 ± 1.6 pmol·min⁻¹·g⁻¹, respectively, P > 0.05). ACC phosphorylation (Fig. 4C) paralleled these directional changes in total and AMPKα2 activity, increasing from R to E2 before gradually decreasing as intensity was increased further (R: 100, E1: 118.6 ± 4.1, E2: 134.3 ± 7.6, E3: 114.4 ± 1.5, E4: 111.4 ± 2.9 relative density units, P < 0.05). AMPKα1 activity (Fig. 4D) was not significantly different from R to E1, E2, or E3 (R: 23.0 ± 0.4, E1: 21.8 ± 0.7, E2: 21.1 ± 1.9, and E3: 21.6 ± 2.6 pmol·min⁻¹·g⁻¹, P > 0.05), but it was significantly increased during the high-intensity stimulation protocol (E4: 29.4 ± 1.3 pmol·min⁻¹·g⁻¹, P < 0.05). ERK1/2 protein expression was not different between groups (P > 0.05; Fig. 5A), whereas ERK1/2 phosphorylation gradually increased with exercise intensity (R: 100 vs. E1: 105.6 ± 4.3, E2: 105.8 ± 5.7, E3: 112.2 ± 3.4, and E4: 120.8 ± 2.1 rel. density units) and was significantly greater than R for E3 and E4 (P < 0.05; Fig. 5B).

Correlational analysis. A positive correlation was found between FA uptake and total and AMPKα2 (Fig. 6A) activity for all exercise intensities (R² = 0.57 and 0.71 respectively, P < 0.05). A similar relationship was found between FA oxidation and total and AMPKα2 (Fig. 6B) activity (R² = 0.65 and 0.66, respectively, P < 0.05). When FA uptake and oxidation were each plotted against ERK1/2 phosphorylation, a running interval smooth analysis indicated a curvilinear association peaking with E3 and E2 data, respectively. Due to this trend with changes in contraction intensity, no significant correlation was found between FA uptake and ERK1/2 phosphorylation (R² = 0.04, P > 0.05) or between FA oxidation and ERK1/2 phosphorylation (R² = 0.03, P > 0.05) for all exercise intensities. However, as accepted by methods used to deal with curvature in running interval smooth analysis (37), elimination of data resulted in significant correlations with ERK1/2 phosphorylation. Indeed, FA uptake shared a significant positive correlation with ERK1/2 phosphorylation (Fig. 7A) from R to E3 (R² = 0.55, P < 0.05). Similarly, FA oxidation shared a significant positive correlation with ERK1/2 phosphorylation (Fig. 7B) from R to E2 (R² = 0.76, P < 0.05).

DISCUSSION

It is well accepted that FA utilization during whole body exercise is intensity dependent. However, the cellular signaling mechanisms regulating FA metabolism during muscle contraction of increasing intensity are still unclear. Our results provide novel information regarding these signaling mechanisms and suggest that both AMPK and ERK1/2 may play a significant...
role in the regulation of FA metabolism during muscle contraction. Combined with data from previous studies performed in our laboratory (19, 30), the results from this study suggest that FA uptake and oxidation are regulated in part by ERK1/2 signaling from low- to moderate-intensity muscle contraction when rates of FA uptake and oxidation are maximized. The results also suggest that FA uptake and oxidation are regulated by AMPK signaling from moderate- to high-intensity muscle contraction when these rates return to basal levels.

As shown repeatedly during whole body exercise, the increase in FA utilization during muscle contraction is associated with an increase in plasma FA concentration (18, 23, 25, 42), and it has been suggested that the accompanying increase in FA delivery to the working muscle is directly responsible for the measured increase in FA uptake and oxidation (4, 8, 21). Thus we designed our protocol to eliminate increases in contraction-induced FA delivery (see Table 1) so that conclusions about the role of AMPK and ERK1/2 signaling mechanisms on the observed changes in FA metabolism with muscle contraction of increasing intensity could be made. We are therefore confident that intracellular mechanisms were responsible for the regulation of contraction-induced FA metabolism in the current study.

Our results show that both AMPK activity (total and AMPKα2) and ERK1/2 phosphorylation correlate positively with FA uptake and oxidation from rest to moderate-intensity muscle contraction. In other words, as contraction intensity...
was increased from rest to moderate, total and AMPKα2 activities, ERK1/2 phosphorylation, and FA uptake and oxidation increased. As contraction intensity was increased from moderate to high, the rate of FA uptake and oxidation decreased, and this was matched by a decrease in total and AMPKα2 activity. In contrast to total and AMPKα2 activity, ERK1/2 phosphorylation continued to increase with an increase in contraction intensity. Alone, these results would suggest that, although both AMPK and ERK1/2 signaling might be involved in the regulation of FA metabolism during low- to moderate-intensity muscle contraction, AMPK rather than ERK1/2 signaling appears to be involved at higher contraction intensities. However, we have shown that, during moderate-intensity muscle contraction, ERK1/2 inhibition prevents the contraction-induced increase in FA uptake without affecting AMPK activity and that, during low-intensity muscle contraction, AMPK activation is not necessary to measure an increase in FA uptake or oxidation (19, 30). Thus, in combination with these previous findings, the present data suggest that, although both ERK1/2 and AMPK activation may be involved in the regulation of FA uptake and oxidation during low- to moderate-intensity muscle contraction, it is likely that ERK1/2 activation plays a more critical role than AMPK. Furthermore, our results extend our previous findings and suggest that, beyond moderate-intensity muscle contraction, AMPK signaling may become more important than ERK1/2 as a mechanism of regulation for FA metabolism.

The use of ERK1/2 phosphorylation as a means to estimate ERK1/2 activation in vivo has been questioned (22) because it has been shown that, although ERK1/2 phosphorylation correlates positively with exercise intensity in human muscle, ERK1/2 activity does not and is maximized during low-intensity exercise protocols (22). However, we are confident that our conclusions regarding the role of ERK1/2 signaling in the regulation of FA metabolism during muscle contraction of increasing intensity would not have changed had ERK1/2 activity been measured. This is because with our experimental conditions ERK1/2 activity would most certainly have shown an increase from rest to low- to moderate-intensity muscle contraction, and this would have been associated with a positive correlation between ERK1/2 activity and FA uptake and oxidation during low- to moderate-intensity muscle contraction. Thus our conclusions about the importance of ERK1/2 signaling in the regulation of FA uptake during these contraction intensities would have been the same.

In previous studies where exercise intensity was modulated by changes in treadmill speed or stimulation frequency, con-

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**Fig. 6.** Correlation analysis between AMPKα2 activity and FA uptake (A) and AMPKα2 activity and FA oxidation (B) in rat hindquarters perfused at rest and during electrical stimulation of increasing train duration. R (n = 10, ●), E1 (n = 9, ○), E2 (n = 10, ●), E3 (n = 8, □), E4 (n = 9, ▲). In A, dashed and straight lines represent the correlation between FA uptake and AMPKα2 activity, respectively ($R^2 = 0.71, y = 0.7239x - 11.8$, and $R^2 = 0.66, y = 0.096x - 1.1$, respectively, $P < 0.05$).

**Fig. 7.** Correlation analysis between ERK1/2 phosphorylation and FA uptake (A) and ERK1/2 phosphorylation and FA oxidation (B) in rat hindquarters perfused at rest and during electrical stimulation of increasing train duration. R (n = 10, ●), E1 (n = 9, ○), E2 (n = 10, ●), E3 (n = 8, □), E4 (n = 9, ▲). In A, dashed line represents the correlation between FA uptake and ERK1/2 phosphorylation for R-E3 data ($R^2 = 0.55, y = 0.359x - 25.4, P < 0.05$). E4 data were excluded from correlation analysis ($R^2 = 0.04, P > 0.05$). In B, straight line represents the correlation between FA oxidation and ERK1/2 phosphorylation for R-E2 data ($R^2 = 0.76, y = 0.068x - 5.06, P < 0.05$). E3 and E4 data were excluded from correlation analysis ($R^2 = 0.03, P > 0.05$).
traction intensity was shown to correlate positively with AMPK activity and muscle glycogen utilization in human and rodent muscle (3, 12, 13, 20). Given that lactate release can be used as an index of glycogen utilization in contracting muscle (44), and that lactate release was significantly increased with contraction intensity in the current study, it might be expected from these previous data that AMPK activity should have increased linearly with an increase in contraction intensity. We are confident, however, that our measurements of AMPK activity were accurate, since the intensity-dependent decrease in AMPK activity as exercise intensity was increased from moderate to high was accompanied by a concomitant decrease in the phosphorylation of ACC, the downstream substrate most often used to indirectly measure AMPK activation in vivo (17). Interestingly, a close inspection of the data presented in the alternative studies referenced above reveals that AMPK activity did not increase significantly when contraction intensity was raised from moderate to high, suggesting that the muscle contraction protocols used in these studies were not equivalent to the high-intensity 200-ms train duration protocol used in our study. In addition, Atherton et al. (1) have shown that isolated rodent muscles subjected to high-frequency electrical stimulation resulted in no change in AMPK phosphorylation. Combined with the results from our study, these data suggest that, in perfused muscle, AMPK activity may plateau during moderate- to high-intensity muscle contraction before returning to basal levels as contraction intensity continues to increase.

Other studies have shown that low initial muscle glycogen content is associated with greater AMPK activity during exercise (5, 40). However, because all animals were studied during the same postabsorptive phase with equal arterial perfusate glucose levels, we are confident that initial muscle glycogen content was similar in all groups of animals and that the differences found in AMPK activity between groups were a function of contraction intensity and not differences in precontraction glycogen content between groups. Thus, without differences in preglycogen levels, the decrease in AMPK activity during high-intensity muscle contraction may be a result of dephosphorylation of the kinase by additional cellular factors that may include accumulation of long-chain acyl-CoA esters. Indeed, exercise has been shown to increase the intracellular concentration of long-chain acyl-CoA esters leading to a decrease in the phosphorylation of AMPK in a dose-dependent manner (27, 35). Recently, it has also been hypothesized that the release of calcium from the sarcoplasmic reticulum during muscle contraction may lead to the activation of signaling molecules involved in the regulation of substrate utilization (6, 43). Therefore, the increase in calcium release and force production (Table 1) with contraction of increasing intensity may be associated with a gradual activation of calcium-dependent signaling mechanisms that could be involved in the regulation of contraction-induced FA metabolism. In fact, it has been shown that the upstream kinase of calcium/calmodulin-dependent protein kinase has the ability to phosphorylate and activate AMPK independently of AMP levels (9, 11). It is therefore possible that a combination of mechanically and metabolically dependent signaling mechanisms regulate FA metabolism during muscle contraction of various intensities. Additional studies will need to be performed to delineate the impact of these factors on the regulation of contraction-induced FA metabolism.

In summary, this study has confirmed in the perfused hind-quarter model that FA utilization increases from low- to moderate-intensity muscle contraction and decreases as intensity is increased further. This study also provides evidence for the potential involvement of AMPK and ERK1/2 in the regulation of contraction-induced FA metabolism. Our results indicate that ERK1/2 and AMPK signaling may play significant roles in the regulation of FA uptake and oxidation during low- to moderate-intensity muscle contraction and during moderate- to high-intensity muscle contraction, respectively. The relative importance of ERK1/2 and/or AMPK activation and the identity of alternative signaling molecules, whether dependent or independent of AMPK and ERK1/2 in the regulation of contraction-induced FA metabolism, cannot be determined from this study. However, our study has provided valuable information that will help direct future research in the quest to delineate the signaling mechanisms that regulate FA metabolism during contraction of various intensities.

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