AMPK activation attenuates S6K1, 4E-BP1, and eEF2 signaling responses to high-frequency electrically stimulated skeletal muscle contractions

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Thomson DM, Fick CA, Gordon SE. AMPK activation attenuates S6K1, 4E-BP1, and eEF2 signaling responses to high-frequency electrically stimulated skeletal muscle contractions. J Appl Physiol 104: 625–632, 2008. First published January 10, 2008; doi:10.1152/japplphysiol.00915.2007.—Regulation of protein translation through Akt and the downstream mammalian target of rapamycin (mTOR) pathway is an important component of the cellular response to hypertrophic stimuli. It has been proposed that 5’-AMP-activated protein kinase (AMPK) activation during muscle contraction may limit the hypertrophic response to resistance-type exercise by inhibiting translational signaling. However, experimental manipulation of AMPK activity during such a stimulus has not been attempted. Therefore, we investigated whether AMPK activation can attenuate the downstream signaling response of the Akt/mTOR pathway to electrically stimulated lengthening muscle contractions. Extensor digitorum longus muscles (n = 8/group) were subjected to a 22-min bout of lengthening contractions by high-frequency sciatic nerve electrical stimulation (STIM) in young adult (8 mo) Fischer 344 × Brown Norway male rats. Forty minutes before electrical stimulation, rats were subcutaneously injected with saline or 5-aminimidazole-4-carboxamide-1–4-ribofuranoside (AICAR; 1 mg/g body wt), an AMPK activator. Stimulated and contralateral resting muscles were removed at 0, 20, and 40 min post-STIM, and AMPK, acetyl CoA carboxylase (ACC), Akt, eukaryotic initiation factor 4E-binding protein (4E-BP1), 70-kDa ribosomal protein S6 kinase (S6K1), and eukaryotic elongation factor 2 (eEF2) phosphorylations were assessed by Western blot. AICAR treatment increased (P ≤ 0.05) post-STIM AMPK (Thr172) and ACC phosphorylation (Ser21/22), inhibited post-STIM S6K1 (Thr389) and 4E-BP1 (ser 106/240) phosphorylation and elevated post-STIM eEF2 phosphorylation (Thr56). These findings suggest that translational signaling downstream of Akt/mTOR can be inhibited after lengthening contractions when preceded by AMPK activation and that energetic stress may be antagonistic to the hypertrophic translational signaling response to loaded muscle contractions.

high-frequency electrical stimulation; resistance exercise; AMPK-activated protein kinase; mammalian target of rapamycin; protein synthesis

SIGNALING through the mammalian target of rapamycin (mTOR) is crucial in the hypertrophic response of skeletal muscle to overload (4, 5, 41) likely due to its stimulating effect on the rate of protein translation and synthesis (20, 46). mTOR is activated downstream of Akt, and it in turn promotes enhanced translation through its targets eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) and 70-kDa ribosomal protein S6 kinase (S6K1). Another potentially important signaling protein in the control of translation is eukaryotic elongation factor 2 (eEF2), which is indirectly regulated by mTOR through S6K1 (53). Elevated translational signaling through this pathway occurs quickly after skeletal muscle overload in rats (2, 7, 33, 38) and humans (11, 13, 14). It has been postulated that the rapid response of the mTOR pathway immediately after overload may represent an important priming event in the elevation of protein synthesis hours later in the recovery period (7). Indeed, inhibition of mTOR just before resistance exercise prevents the normal increase in protein synthesis 16 h post-exercise (28), suggesting that this acute mTOR activity is indeed important in the hypertrophic response.

While phosphocreatine stores help to maintain a relatively constant skeletal muscle ATP concentration during exercise, ADP and AMP levels can become greatly elevated, and this increased AMP/ATP ratio activates 5’-AMP-activated protein kinase (AMPK) (18). AMPK is thus an energy-sensing protein that, when activated, promotes processes that act to further replenish ATP while concurrently inhibiting pathways that consume ATP (18). In line with this role, AMPK phosphorylates and activates TSC2 (25), which, in turn, inhibits mTOR and S6K1 activity (24). Furthermore, treatment with the AMPK activator 5-aminimidazole-4-carboxamide-1–ribofuranoside (AICAR) inhibits mTOR-mediated signaling and protein synthesis in resting skeletal muscle (6), and protein translation in cultured myotubes (54). AICAR activates AMPK as it is converted to the AMP analog ZMP, thus mimicking an increase in intracellular AMP without disturbing the actual energy status of the cell (12). We (49, 50) and others (2, 14) have noted an inverse association between AMPK phosphorylation and activation of the mTOR signaling pathway in response to skeletal muscle overload. As such, it has been suggested that activation of AMPK after resisted muscle contraction may be inhibitory to translational signaling through mTOR (14). However, direct experimental manipulation of AMPK activity after such a contraction bout has not been performed to confirm this. Accordingly, in this study, we tested the hypothesis that AMPK activation via AICAR treatment would result in reduced activation of signaling pathways that promote translation as assessed by the phosphorylations of Akt, S6K1, 4E-BP1, and eEF2 in the extensor digitorum longus (EDL) after a single bout of high-frequency electrical stimulation (HFES) of the sciatic nerve.

METHODS

Animals. The East Carolina University Animal Care and Use Committee approved all procedures before this investigation. Young adult (8 mo old) Fischer 344 × Brown Norway F1 hybrid male rats were subjected to a high-frequency electrically stimulated skeletal muscle contractions.

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were clarified by centrifugation at 9,500 g.

AICAR administration and electrical stimulation. Rats (n = 8/group) were weighed and given a subcutaneous injection of either AICAR (AIC; 1 mg/g body wt; 75 mg AICAR/ml saline) or an equivalent volume of saline. Rats were then anesthetized using vaporized isoflurane (2–4%) and nitrous oxide in supplemental oxygen sufficient to achieve surgical anesthetic depth. The sciatic nerve of the left hindlimb was isolated just proximal to the point of trifurcation where stainless steel electrodes were attached. Using HFS (100 Hz; Grass Model S48 Stimulator, Quincy, MA), 10 sets of six maximal contractions (3-s duration with 10 s rest between contractions) of the hindlimb musculature were elicited shortly (within 2–3 min) after electrode placement, with 1-min rest periods between sets, for a total contraction bout of ~22 min. The contratralateral (right) hindlimb was not subjected to the operation or stimulation, and its EDL was analyzed as an internal control. Timing of the contraction bout was such that it ended 1 h after the AICAR/saline injection when AMPK is known to be greatly activated by AICAR (21). This stimulation protocol results in an initial maximal eccentric contraction of the dorsiflexor muscles (EDL and tibialis anterior (TA)) due to the greater overall force production of the simultaneously contracting plantar flexors (gastrocnemius, plantaris, and soleus muscles). This is followed by a period of maximal isometric contraction in a lengthened position at full plantar flexion. This protocol acutely activates S6K1 in the EDL and TA muscles and generates significant hypertrophy of these muscles (but not of the plantaris and soleus), when performed on a chronic basis (4). Isoflurane anesthesia was maintained continually up until 10 min before the designated muscle harvest time point (0, 20, or 40 min postexercise) at which time isoflurane/nitrous oxide administration was stopped and an intraperitoneal injection of a ketamine (1.25 mg/100 g body wt) and xylazine (1.25 mg/100 g body wt) mixture was given for terminal anesthesia. Tissue was collected from living animals.

Collection of muscles and homogenization. At the designated time, the EDL muscles were quickly removed from both hindlimbs in randomized order, cleaned of extraneous tissue, and frozen (within 10 s after removal from the living animal) between stainless steel plates chilled to the temperature of liquid nitrogen. To control for differences at the time of death that might be due to the time of day, animals were killed at time points evenly distributed across groups throughout the final 6 h of the light cycle.

Muscles were ground-glass homogenized on ice in 20 volumes of homogenization buffer [50 mM HEPES (pH 7.4), 0.1% Triton X-100, 4 mM EGTA, 10 mM EDTA, 15 mM Na3PO4·12H2O, 100 mM β-glycerophosphate, 25 mM NaF, 50 μg/ml leupeptin, 50 μg/ml pepstatin, 33 μg/ml aprotenin, and 5 mM NaN3·V03·]. The homogenates were clarified by centrifugation at 9,500 g for 10 min. Protein concentration was assessed in triplicate using a modified Lowry procedure (DC Protein Assay, Bio-Rad, Hercules, CA). SDS-PAGE, Western blotting, and immunodetection. Clarified muscle homogenates were solubilized in sample loading buffer (50 mM Tris·HCl, pH 6.8, 10% glycerol, 2% SDS, 2% β-mercaptoethanol, 0.1% bromophenol blue) at a concentration of 2 mg/ml and boiled for 5 min. Samples were loaded on sodium dodecyl sulfate-polyacrylamide gels [7.5% for phospho-S6K1 and phospho-eEF2; 4–15% for phospho-AMPK and phospho-acetyl CoA carboxylase (phospho-ACC); 15% for phospho-Akt and 4E-BP1]; then proteins were separated by electrophoresis. Western blotting was for 1 h at 4°C onto a PVDF membrane (Millipore, Bedford MA) at 100 V in transfer buffer (25 mM Tris base, 192 mM glycine, 20% methanol). After transfer, membranes were stained with Ponceau S to verify transfer and equal protein loading among lanes. For immunodetection, membranes were blocked for 1 h at room temperature in blocking buffer [5% nonfat dry milk in TBS-T (20 mM Tris-base, 150 mM NaCl, 0.1% Tween-20), pH 7.6], serially washed in TBS-T at room temperature, then probed for specific signaling proteins using antibodies for the detection of phospho-AMPK (Thr172), phospho-ACC (Ser79 on the α-isozyme and the analogous Ser21 residue on the β-isozyme), phospho-Akt (Ser473), phospho-S6K1 (Thr389), phospho-S6K1 (Thr421/Ser424), 4E-BP1, and phospho-eEF2 (Thr56). All antibodies were from Cell Signaling Technology (Beverly, MA), except for phospho-S6K1 (Thr389), which was from Santa Cruz Biotechnology, (Santa Cruz, CA). Membranes were incubated overnight at 4°C in primary antibody buffer [5.0% BSA in TBS-T, pH 7.6, primary antibody diluted 1:1,000, except for phospho-eEF2, which was diluted 1:2,000, and phospho-S6K1 (Thr389), which was diluted 1:500]. The next morning, the membranes were serially washed in TBS-T, incubated with horseshadish peroxidase (HRP)-conjugated secondary antibody (Amersham Pharmacia Biotech, Piscataway, NJ) in blocking buffer for 1 h, and again serially washed in TBS-T. The HRP activity was detected using enhanced chemiluminescence reagent (Femtoglow HRP Substrate Plus; Michigan Diagnostics, Troy, MI) and exposure to autoradiographic film (Classic Blue Sensitive, Midwest Scientific, St. Louis, MO). Relative antigen concentration was calculated by quantification of the integrated optical density (IOD) of the appropriate band(s) using Gel Pro Analyzer software (Media Cybernetics, Silver Spring, MD). As 4E-BP1 becomes phosphorylated at various residues, its migration during electrophoresis becomes progressively impeded such that the protein is separated into multiple distinct bands on immunodetection. We observed as many as four total bands for 4E-BP1, and phosphorylation status was calculated as the percentage of the total protein signal that was encompassed in the two slower-migrating, hyperphosphorylated bands. Phosphorylation of AMPK, ACC, Akt, and eEF2 was expressed normalized to the average value for saline-treated control muscles. Phosphorylation of S6K1 at both Thr389 and Thr421/Ser424 was expressed in arbitrary IOD units because the chemiluminescent signal for these antibodies was generally not detectable in the saline-treated control muscles.

Statistical analysis. Data were analyzed using Statistica data analysis software, version 6 (StatSoft, Tulsa, OK). Differences between groups were analyzed by factorial ANOVA for the resting and contralateral contracted muscles. When necessary, square root data transformations were utilized to satisfy the equality of variance assumption for ANOVAs. Fisher’s least significant difference post hoc analyses were used when appropriate. Differences were considered significant at P < 0.05. Data are expressed as means ± SE.

RESULTS

AMPK and ACC phosphorylation (Figs. 1 and 2). HFES had no effect on AMPK phosphorylation in muscles from salinetermined rats at any time point, but ACC phosphorylation was elevated by HFES at the immediately post-STIM (IP) time point in saline-treated rats. AICAR substantially increased AMPK and ACC phosphorylation, as expected, and this effect persisted throughout the measured time course. Interestingly, HFES in AICAR-treated rats led to an additional increase in AMPK phosphorylation, but not ACC phosphorylation, at all three time points. Data are presented relative to the salinetreated control muscle at each time point.

Akt phosphorylation (Fig. 3). HFES led to increased Akt phosphorylation immediately after STIM in both salinetreated AICAR-treated muscles (Fig. 3A), but the increase was greater with the AICAR (~410% increase) than the saline (~130% increase) treatment. Under both treatments, Akt phosphoryla-
tion returned to control levels by 20 min post-STIM (Fig. 3, B and C). At 20 min post-STIM, a main effect was observed, with Akt phosphorylation being ~44% lower in AICAR- vs. saline-treated muscles (Fig. 3B), but AICAR-treated control (unstimulated) muscles were not different from saline-treated counterparts at the IP or 40-min post-STIM time points. No differences were observed between groups at 40 min post-STIM (Fig. 3C).

_S6K1 phosphorylation (Figs. 4 and 5)._ Phosphorylation of S6K1 at Thr389 (Fig. 4) was generally not detectable in unstimulated control muscles. STIM led to a great increase in S6K1 phosphorylation in saline-treated muscles at all time points measured. In AICAR-treated rats, the S6K1 phosphorylation response to STIM was dramatically suppressed, being significantly less than saline-treated STIM muscles at all three time points, and only statistically increased with HFES at 20 min post-STIM (Fig. 4B).

S6K1 phosphorylation at Ser421/Thr424 (Fig. 5) was generally undetectable in unstimulated control muscles but increased robustly at all time points after STIM in both saline- and AICAR-treated muscles. Phosphorylation at this site in the stimulated muscles was much less affected by AICAR than was S6K1 Thr389 phosphorylation. S6K1 phosphorylation at Ser421/Thr424 was only modestly suppressed by AICAR treatment at 20 (36% lower) and 40 (19% lower) min but was not suppressed by AICAR immediately post-STIM.

_4E-BP1 phosphorylation (Fig. 6)._ STIM increased 4E-BP1 phosphorylation at all three time points. 4E-BP1 phosphorylation was significantly lower after STIM in AICAR- vs. saline-treated muscles at 20 and 40 min post-STIM. 4E-BP1 phosphorylation in AICAR vs. saline-treated control (unstimulated) muscles was only different at 40 min post-STIM.

_eEF2 phosphorylation (Fig. 7)._ eEF2 phosphorylation at Thr56, which is inhibitory on its activity, was decreased at 20 and 40 min post-STIM in saline- and AICAR-treated muscles. However, AICAR treatment led to higher eEF2 phosphorylation in AICAR- vs. saline-stimulated muscles at those same two time points. AICAR also elevated eEF2 phosphorylation in control (unstimulated) muscles at all three time points.

**DISCUSSION**

Signaling through mTOR to its downstream targets S6K1 and 4E-BP1 is a crucial component of the response of skeletal muscle to hypertrophic stimuli (4, 5). In resting tissue, pharmacological activation of AMPK was previously shown to...
decrease Akt, S6K1, and 4E-BP1 phosphorylation (6), and increase eEF2 phosphorylation (23), which taken together are indicative of suppressed growth signaling and protein translation. We and others (2, 14, 49, 50) have speculated that AMPK activity associated with muscle contraction might negatively regulate translational signaling through this pathway, based on the association of suppressed growth signaling downstream of Akt/mTOR with elevated AMPK signaling. However, until now, AMPK activity during and after contractile activity has not been directly manipulated to test this hypothesis. Thus the purpose of this study was to determine if AMPK activation during and after an acute bout of high-frequency electrically stimulated contractions (STIM) can suppress growth signaling downstream of Akt/mTOR with elevated AMPK signaling. However, until now, AMPK activity during and after contractile activity has not been directly manipulated to test this hypothesis. Thus the purpose of this study was to determine if AMPK activation during and after an acute bout of high-frequency electrically stimulated contractions (STIM) can suppress growth signaling downstream of Akt/mTOR. We found that AICAR activation of AMPK did suppress phosphorylation of S6K1 and 4E-BP1, and elevated phosphorylation of eEF2, consistent with an inhibition of protein translation. This effect appears to be mediated by factors downstream of Akt, since Akt phosphorylation immediately after contraction was actually enhanced by AMPK activation. Our data are consistent with previous data showing that AMPK inhibits mTOR by activating TSC2, which is a signaling intermediate between Akt and mTOR (25).

We did not observe an increase in AMPK phosphorylation after STIM in saline-treated muscles. This finding is in agreement with Atherton et al. (2), who found that in vitro isometric contractions also failed to increase AMPK phosphorylation. AMPK in skeletal muscle is phosphorylated primarily by LKB1 (in complex with STRAD and MO25) (19, 43, 51, 57), although other kinases such as calcium-calmodulin-dependent protein kinase kinase (CaMKK) may also play a role (27). AMPK activation occurs during metabolic stress as ATP is broken down to AMP (55). As the AMP/ATP ratio increases, AMPK is activated first by covalent means, likely through protection from dephosphorylation (44, 47). In the phosphorylated state, AMPK is also further activated allosterically by AMP (47). Consequently, phosphorylation of AMPK alone is not fully indicative of true in vivo AMPK activity. Phosphorylation of ACC is thus widely used as an index of true in vivo AMPK activity (35, 36). Under saline conditions in our study, ACC phosphorylation was significantly higher (3-fold) in STIM vs. control muscles immediately after contractions and still greater than twofold higher (nonsignificant) 20 min post-STIM. Thus a subtle increase in actual AMPK activity likely occurred with normal stimulation in the present investigation, which would be in agreement with work in humans showing that ATP and PCr levels are diminished after intense, multiple-set resistance exercise (48). Accordingly, AMPK activity is increased for at least 1 h following resistance exercise in humans, although AMPK phosphorylation was not reported in that study (14).
The fact that AMPK has been shown to be activated by resistance exercise in human skeletal muscle (14) for a longer postexercise period than that seen in our investigation may be due to work-rest differences between contractions. The contraction protocol in the present study allowed 10 s of rest between contractions, while the rest periods between individual contractions in humans are shorter, which probably lead to less recovery of energy status, thereby increasing AMPK activation. Regardless, even the increase observed in human studies after resistance exercise is small (75%) relative to the increase (150% and higher) shown after various cycling exercise protocols of sufficient intensity (10, 15, 31, 52, 56, 58). This is likely due to the intermittent nature of resistance exercise, which allows for at least a partial recovery of energy balance between contractions and may thus present less of a stimulus for AMPK activation. An alternate possibility involves differences in Akt, which we (in this report) and others (2, 33) have shown to be activated immediately after HFES. Since Akt can inhibit AMPK activity (17), it may be that elevated Akt activity during and after resistance exercise limits AMPK activation by energy stress or other factors. This may be important in the hypertrophic response to exercise since AMPK has been shown to limit protein synthesis and growth in skeletal muscle and other tissues. In contrast to resistance exercise, aerobic exercise such as running may not activate Akt (30), perhaps allowing a full activation of AMPK.

Like others (2, 4, 34, 37, 38), we observed a rapid increase in S6K1 and 4E-BP1 phosphorylation and a decrease in eEF2 phosphorylation with STIM, which has also been observed in humans within 1–2 h after resistance exercise (14). This reinforces the concept that mTOR is an important component in the cellular response to muscle overload. It has been postulated that the acute increase in mTOR-mediated signaling in the hour immediately after resistance-type exercise is an important priming step in the stimulation of protein synthesis that is typically observed 6–48 h later (6). Indeed, treatment with rapamycin (a specific mTOR inhibitor) before weightlifting prevented the increase in protein synthesis that normally occurs 16 h postexercise (28). Here, we have shown that AICAR activation of AMPK also suppresses the growth signaling response downstream of mTOR (indicated by decreased S6K1 and 4E-BP1 phosphorylation and increased eEF2 phosphorylation) after STIM. We did not measure phosphorylation of mTOR itself in the present investigation. Although phosphorylation of Ser2448 has been used as an indicator of mTOR activity, the usefulness of this measure is in doubt because mutation of Ser2448 to alanine has no effect on mTOR activity toward S6K1 or 4E-BP1 (45). Thus we examined the phos-
phosphorylation of S6K1 and 4E-BP1, as they are accepted markers of mTOR activity (20, 26, 46).

The phosphorylation response of S6K1 at Thr^{389} was strongly inhibited by AICAR in this investigation, while phosphorylation at Ser^{211}/Thr^{224} was only modestly suppressed. Out of the various phosphoacceptor sites on S6K1, Thr^{389} is most reflective of in vivo activity (26) and is also the primary mTOR-dependent phosphorylation site (39). When activated, S6K1 promotes protein translation and cell growth. These effects have been traditionally attributed to phosphorylation and activation of ribosomal protein S6 (rpS6) by S6K1, although the relative importance of rpS6 phosphorylation has recently been called into question (20, 42). Other targets of S6K1 that are likely important in the control of protein translation are eukaryotic initiation factor 4B (20) and eEF2 kinase (eEF2k) (53). 4E-BP1 is also phosphorylated by mTOR at several sites (9), and this, along with phosphorylation by other proteins, promotes the dissociation of 4E-BP1 from eukaryotic initiation factor 4E (eIF4E), which in turn frees eIF4E so that it may complex with eukaryotic initiation factors 4G and 4A, forming together what is termed eukaryotic initiation factor 4F (eIF4F). Formation of this complex is crucial in the recruitment of the 40S ribosome to the vast majority of mRNAs. Thus phosphorylation of 4E-BP1 is a major mechanism in promoting the initiation of protein translation. It should be noted that regulation of protein translation and synthesis can occur via other signaling pathways independent of mTOR (1, 8); however, taken together, the S6K1 and 4E-BP1 data in the present study suggest that mTOR activation by STIM was greatly blunted by AMPK activation.

Activity of eEF2 is suppressed by phosphorylation at Thr^{56} by eEF2 kinase (eEF2k) (53). While S6K1 activates eEF2 by phosphorylating and inactivating eEF2k (53), AICAR-induced AMPK activation in cardiomyocytes phosphorylates (deactivates) eEF2 via direct phosphorylation of eEF2k by AMPK itself (at a stimulatory eEF2k site that is different from the inhibitory site phosphorylated by S6K1) (22, 23). Ours is the first report showing that AMPK activation also increases eEF2 phosphorylation (thus likely reducing its activity) in skeletal muscle, and we also show that AICAR prevents a full decrease in eEF2 phosphorylation in response to resisted contractions. This effect might have been due to either a direct stimulatory effect of AMPK on eEF2k, and/or AMPK’s suppression of mTOR-S6K1 signaling (since S6K1 phosphorylation and presumed activity was also suppressed by AICAR).

Contrary to our hypothesis, AICAR did not attenuate the phosphorylation of Akt immediately after muscle contraction, but instead enhanced the response. As previously noted, the lack of inhibition of Akt by AICAR is not entirely surprising since the inhibitory effect of AMPK on mTOR appears to be integrated downstream of Akt (25). AICAR suppresses Akt phosphorylation in resting muscle (6), and we did observe such a decrease (although slight) 20 min after the contraction bout. This effect may be due to increased insulin signaling instead of a direct effect of AMPK, since AICAR has been shown to decrease serum insulin concentrations (40). It is not known why AICAR had the opposite effect immediately after contractions, but one possibility may be that the activation of S6K1 after contractions normally attenuates Akt phosphorylation and activity, since S6K1 inhibits the IRS1/2 upstream of Akt through negative feedback (29). The lack of S6K1 activation (as suggested by the lack of an increase in Thr^{389} phosphorylation) immediately after contraction in the AICAR-treated muscles may have thus allowed the enhanced Akt phosphorylation that we observed.

In conclusion, we have shown that AICAR activation of AMPK suppresses the normal elevation in 4E-BP1 and S6K1 phosphorylation and attenuates the depression of eEF2 phosphorylation after acute electrically stimulated lengthening contractions in vivo. A robust activation of AMPK after muscle contraction, such as occurs with endurance exercise (10, 15, 31, 52, 56, 58), may serve to attenuate the translational signaling response to the contractions, thereby limiting the energetically costly process of protein synthesis at a time when the cell is trying to restore ATP levels. This may contribute to the lack of muscle hypertrophy and strength gain generally observed with endurance training (3, 32). On the other hand, after resisted contractions, the limited activation of AMPK as observed here may allow a robust increase in translational signaling and a greater hypertrophic response than seen with endurance exercise. This idea is supported by our results since activation of AMPK with AICAR before and during resisted muscle contractions greatly attenuated translational signaling. Our results suggest that the degree to which AMPK is activated after resistance exercise may play an important role in regulating the hypertrophic translational signaling response to that

Fig. 7. Eukaryotic elongation factor 2 (eEF2) phosphorylation (p-eEF2) at Thr^{56} in the extensor digitorum longus muscle from saline or AICAR-treated rats immediately after (A), 20 min after (B), and 40 min after (C) unilateral high-frequency electrical stimulation of the sciatic nerve. Data are presented as means ± SE relative to the saline-treated control muscle at each time point. *Significant difference (P < 0.05) in stimulated vs. corresponding control muscles. †Significant difference (P < 0.05) in AICAR-treated vs. corresponding saline-treated muscles.
exercise bout. As AMPK is activated robustly by aerobic exercise, this has important implications in the design of optimal exercise programs. Furthermore, as ingestion of an amino acid/carbohydrate mixture has been shown to decrease AMPK phosphorylation while enhancing protein synthesis and anabolic signaling (16), AMPK may also serve as an important mechanistic link between the nutrient intake and protein synthesis with training. Last, since the most commonly prescribed diabetes drugs (thiazolidinediones and biguanides) activate AMPK, our findings are also of importance in the treatment of diabetes and insulin resistance in frail populations where the treatment of sarcopenia with strength training may also be desirable.

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REFERENCES


