Differential attenuation of AMPK activation during acute exercise following exercise training or AICAR treatment

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1Department of Physiology, The University of Melbourne, Melbourne, Victoria, Australia; 2Department of Physiology, Khon Kaen University, Khon Kaen, Thailand; and 3St. Vincent’s Institute, Fitzroy, Victoria, and Commonwealth Scientific and Industrial Research Organisation Molecular Health Technologies, Parkville, Victoria, Australia

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McConell GK, Manimmanakorn A, Lee-Young RS, Kemp BE, Linden KC, and Wadley GD. Differential attenuation of AMPK activation during acute exercise following exercise training or AICAR treatment. J Appl Physiol 105: 1422–1427, 2008. First published August 14, 2008; doi:10.1152/japplphysiol.01371.2007.—Short-term exercise training in humans attenuates AMP-activated protein kinase (AMPK) activation during subsequent exercise conducted at the same absolute workload. Short-term 5-aminoimidazole-4-carboxamide-ribonucleoside (AICAR) administration in rats mimics exercise training on skeletal muscle in terms of increasing insulin sensitivity, mitochondrial enzymes, and GLUT4 content, but it is not known whether these adaptations are accompanied by reduced AMPK activation during subsequent exercise. We compared the effect of 10 days of treadmill training (60 min/day) with 10 days of AICAR administration (0.5 mg/g body weight ip) on subsequent AMPK activation during 45 min of treadmill exercise in male Sprague-Dawley rats. Compared with no exercised control rats, acute exercise significantly (P < 0.05) increased AMPKα Thr172 phosphorylation (p-AMPKα; 1.6-fold) and ACCβ Ser218 phosphorylation (p-ACCβ; 4.9-fold) in the soleus and p-ACCβ 2.2-fold in the extensor digitorum longus. Ten days of exercise training abolished the increase in soleus p-AMPKα and attenuated the increase in p-ACCβ (nonsignificant 2-fold increase). Ten days of AICAR administration also attenuated the exercise-induced increases in AMPK signaling in the soleus although not as effectively as 10 days of exercise training (nonsignificant 1.3-fold increase in p-AMPKα; significant 3-fold increase in p-ACCβ). The increase in skeletal muscle 2-deoxyglucose uptake during exercise was greater after either 10 days of exercise training or AICAR administration. In conclusion, 10 days of AICAR administration substantially mimics the effect of 10 days training on attenuating skeletal muscle AMPK activation in response to subsequent exercise.

glucose uptake; glycogen skeletal muscle; acetyl-CoA carboxylase

AMP-activated protein kinase (AMPK) is a serine/threonine protein kinase that is involved in maintaining cellular ATP levels by inhibiting ATP-consuming pathways and activating ATP-producing pathways (12). AMPK is activated by metabolic stress that includes muscle contraction, hypoxia, mitochondrial uncoupling, and glucose deprivation, and it is also activated by the pharmacological agents 5-aminoimidazole-4-carboxamide-ribonucleoside (AICAR), metformin, glitazones, and the thienopyridone A769662 (9, 12, 13).

Increases in skeletal muscle glucose uptake and fat oxidation occur in response to both acute exercise and acute AICAR administration (3, 14, 22). Similarly, skeletal muscle GLUT4 mRNA and protein levels, hexokinase protein expression, glycogen levels, mitochondrial enzymes, and insulin sensitivity are all increased in response to both exercise training and chronic AICAR administration in rats (11, 15–17, 28, 35). The physiological effects of AICAR appear to be dependent on AMPK activation in skeletal muscle (18, 23); however, suppression or ablation of AMPK activation only partially attenuates increases in glucose uptake during contraction, indicating that AMPK is not essential for the response (18, 23). Furthermore, increases in mitochondrial enzymes and hexokinase expression in response to acute and chronic exercise are essentially normal in mice lacking AMPK, even though AICAR induced increases are abolished in these mice (17, 19). Thus, whereas AMPK is essential for responses to AICAR treatment, it is not essential for glucose uptake during contraction.

We have shown that skeletal muscle AMPK activity is increased ∼10-fold during prolonged exercise in untrained humans, yet this increase in AMPK activity during exercise is essentially abolished following 10 days of exercise training (20). This raises the question as to whether AMPK signaling alone during exercise training is itself sufficient to attenuate the extent of AMPK activation during subsequent exercise bouts. To investigate this, we tested the extent to which daily AICAR treatment could mimic exercise training in reducing the AMPK responsiveness to an exercise challenge. AICAR administration at rest naturally differs to exercise because there is no muscle contraction and there are not the large increases in blood flow or oxygen consumption that occur during exercise/contraction. However, both result in alterations in muscle that signal that there is an energy deficit. Exercise/contraction increases free AMP and reduces creatine phosphate content (20, 32, 33), whereas AICAR is phosphorylated to 5′-aminoimidazole-4-carboxamide-ribonucleotide with no apparent changes in skeletal muscle AMP, ATP, ADP, or creatine phosphate (15, 32).

Therefore, the aim of this study was to determine, in rats, the extent of skeletal muscle AMPK activation during exercise following either 10 days of AICAR administration or 10 days of exercise training. We hypothesized that 10 days of AICAR administration would be insufficient to attenuate skeletal muscle AMPK activation during subsequent exercise.

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METHODS

Animals

This study was approved by the Animal Experimentation Ethics Committee of The University of Melbourne. Male Sprague-Dawley rats (244 ± 3 g) were housed under controlled temperature (22–23°C) and lighting (12:12-h light-dark cycle) and were given free access to water and a standard chow diet. Animals were randomly divided into five groups: control (Con), acute AICAR, acute exercise (acute Ex), 10-day AICAR-treated (10 days AICAR), and 10-day training (10 days Training) groups (n = 5–8 per group). These animals were involved in the experiments where AMPK activation was determined. Four days before the start of the experiment, all rats underwent 15-min periods of running on a treadmill (25 m/min, 8% grade) on 2 separate days to familiarize the rats to treadmill running.

Experimental Procedures

To confirm that AMPK was activated in response to a single injection of AICAR, five rats were subcutaneously injected with a single dose of AICAR (0.5 mg/g body wt; divided among 3 sites: upper, middle, and lower back). Sixty minutes after the AICAR administration rats received an anesthetic overdose of xylazine-HCl (30 mg/kg) and ketamine-HCl (240 mg/kg) followed by cervical dislocation. The 10-day AICAR-treated group was injected with AICAR (0.5 mg/g body wt) and the other 10-day groups (Con and 10 days Training) were injected with an equal volume of saline every morning (between 9:00 and 10:00 AM) for 10 days. The 10-day training group performed treadmill running for 60 min (speed 25 m/min at a 8% grade) each day for 10 days. Body weight and food intake were measured daily.

Twenty-four hours following the treatment, all rats (except the acute AICAR group) received an intraperitoneal injection of 2-deoxy-D-[3H]glucose (2-DG; 50 Ci). Con rats then rested for 45 min on the treadmill, whereas the other rats were subjected to 45 min of treadmill running (25 m/min at an 8% grade). All rats were then immediately killed as described above. Soleus and extensor digitorum longus (EDL) muscles were rapidly removed and immediately frozen in liquid nitrogen for later determination of AMPKα Thr172 phosphorylation and ACCβ Ser218 phosphorylation, muscle glycogen, and 2-DG uptake into muscle. After conducting the main study two further groups were utilized for determination of muscle glycogen content at rest after 10 days of AICAR administration and at rest after 10 days of exercise training (n = 8 per group).

Analytical Techniques

AMPK signaling. Frozen muscle (10 μl buffer/mg muscle) was homogenized in freshly prepared ice-cold buffer [50 mM Tris-HCl at pH 7.5 containing 1 mM EDTA, 10% vol/vol glycerol, 1% vol/vol Triton X-100, 50 mM NaF, 5 mM Na3P2O7, 1 mM DTT, 1 mM PMSF, and 5 μl/ml protease inhibitor cocktail (P8340, Sigma)]. Tissue lysates were incubated on ice for 20 min and then spun at 16,000 g for 20 min at 4°C. Protein concentration was determined using a bichinchoninic acid protein assay (Pierce, Rockford, IL) with BSA as the standard.

For AMPKα Thr172 and ACCβ Ser218 phosphorylation, 90 μg of total protein was subjected to SDS-PAGE and binding was detected by immunoblotting with either phospho-ACCβ Ser218 polyclonal antibody (Upstate Biotechnology, New York, NY) or an AMPKα pan antibody that recognizes both AMPKα1 and AMPKα2 (Cell Signaling Technology, Hartsfordshire, UK). Binding was detected with IRDye 800-conjugated anti-rabbit IgG (Rockland, Gilbertsville, PA) secondary antibody. Direct fluorescence was detected and quantified using the Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NB). Membranes were then stripped [2% SDS (wt/vol) in 25 mM glycine, pH 2.0] and reprobed with IRDye 800-labeled strepta-vidin (Rockland, Gilbertsville, PA) and anti-phospho-AMPK Thr172 antibody (Upstate Biotechnology, New York, NY). Phosphorylation was expressed relative to protein abundance.

Glycogen analysis. Frozen muscle samples were extracted in 250 μl of 2 M HCl at 95°C for 2 h and then neutralized with 750 μl of 0.667 M NaOH. The extract was then analyzed for glucosyl units using an enzymatic, fluorometric assay as previously described (25).

2-DG uptake. Muscle 2-DG uptake was assessed on portions of soleus and EDL muscles in an identical fashion to that described by Campbell and Febbraio (6). Briefly, the muscle was weighed then digested using NaOH and HCl. One portion of the digest was then deproteinized using Ba(OH)2 and ZnSO4, and the other portion was deproteinized using perchloric acid and then spun. Each respective supernatant was then added to water and scintillant and the radioactivity of both treatments was measured using a β-counter. The first treatment yields unphosphorylated 2-DG, and the second treatment measures total 2-DG; therefore, the difference gives phosphorylated 2-DG (6).

Statistics

Results were analyzed by the SPSS statistical package using two-factor repeated-measures ANOVA (between factor: treatment; within factor: time) for food intake and body weight. All other results were analyzed using a one-factor ANOVA. If the ANOVA revealed a significant interaction, specific differences between mean values were located using the Fisher’s least significance difference test. The level of significance was set at P < 0.05.

RESULTS

Body Weight and Food Intake

During the 10 day treatment period, all groups increased body weight (body weight: Con increased by 95.1 ± 3.5 g, acute Ex increased by 87.1 ± 6.0 g, 10 days AICAR increased by 65.5 ± 3.2 g, and 10 days Training increased by 70.1 ± 4.8 g). However, the increase in body weight was significantly lower for the 10 days AICAR group compared with the other three groups (P < 0.05). There was no difference in the increase in body weights between Con, acute Ex, and 10 days Training. The 10 days Training group appeared to have a smaller increase in body weight than Con, but this was not significant (P = 0.19). Food intake (food intake) in 10 days AICAR was significantly (P < 0.05) lower than Con and 10 days Training. Ten days Training food intake was significantly (P < 0.05) lower than Con (food intake: Con 29.2 ± 0.4 g/day, Acute Ex 28.2 ± 1.1 g/day, 10 days AICAR 23.3 ± 0.5 g/day and 10 days Training 25.6 ± 0.9 g/day). There was no difference in food intake between Con and Acute Ex.

AMPK Signaling

Soleus. Acute AICAR administration had no significant affect on AMPKα Thr172 phosphorylation but significantly increased ACCβ Ser218 phosphorylation (Fig. 1, A and B). Acute exercise significantly (P < 0.05) increased AMPKα Thr172 phosphorylation and ACCβ Ser218 phosphorylation (compared with Con) (Fig. 1, A and B). Ten days of exercise training fully suppressed the exercise-induced increases in AMPKα Thr172 and attenuated the exercise-induced increases in ACCβ Ser218 phosphorylation (not significantly different to Con) (Fig. 1, A and B). AICAR administration attenuated the exercise-induced increases in AMPKα Thr172 phosphorylation and ACCβ Ser218 phosphorylation during subsequent exercise.
but not to the same extent as training (compared with Con) (Fig. 1B).

**EDL.** Acute AICAR administration increased AMPKα Thr\(^{172}\) phosphorylation (AMPKα pThr\(^{172}\); A) and ACCβ Ser\(^{218}\) phosphorylation (ACCβ pSer\(^{218}\); B) in soleus muscle. Control, muscle dissected without any exercise or 5-aminoimidazole-4-carboxamide-ribonucleoside (AICAR) treatment; Acute AICAR, muscle dissected after 45 min of AICAR treatment; Acute Ex, muscle dissected after 45 min of treadmill exercise; Ex after 10 d AICAR, muscle dissected after 45 min of treadmill running that was preceded by 10 days of AICAR treatment; Ex after 10 d training, muscle dissected after 45 min of treadmill running that was preceded by 10 days of treadmill exercise training; Streptavidin IR800, IRDye 800-labeled streptavidin. *P < 0.05 vs. Control. **P < 0.05 vs. Ex after 10 d training. +P < 0.05 vs. Ex after 10 d AICAR.

**Muscle Glycogen Content**

**Soleus.** Muscle glycogen levels significantly decreased following acute AICAR, acute exercise, and exercise after 10 days of AICAR treatment (P < 0.05 vs. Con; Fig. 3A). Ten days of exercise training significantly increased resting muscle glycogen levels compared with Con (Con vs. No exercise after 10 days training; Fig. 3A), and this may be why muscle glycogen content at the end of exercise was not significantly decreased compared with Con (Con vs. Exercise after 10 days training; Fig. 3A). Ten days of AICAR treatment tended to increase resting muscle glycogen levels; however, this was not statistically significant (Con vs. No exercise after 10 days AICAR; Fig. 3A).

**EDL.** Only acute exercise significantly (P < 0.05) decreased muscle glycogen content compared with Con (Fig. 3B). Muscle glycogen content after acute exercise was also lower than in the acute AICAR, Exercise after 10 days AICAR, and no exercise after 10 days AICAR groups. Ten days of exercise training prevented a significant decrease in muscle glycogen content.

Fig. 1. AMP-activated protein kinase (AMPKα) Thr\(^{172}\) phosphorylation (AMPKα pThr\(^{172}\); A) and ACCβ Ser\(^{218}\) phosphorylation (ACCβ pSer\(^{218}\); B) in soleus muscle. Control, muscle dissected without any exercise or 5-aminoimidazole-4-carboxamide-ribonucleoside (AICAR) treatment; Acute AICAR, muscle dissected after 45 min of AICAR treatment; Acute Ex, muscle dissected after 45 min of treadmill exercise; Ex after 10 d AICAR, muscle dissected after 45 min of treadmill running that was preceded by 10 days of AICAR treatment; Ex after 10 d training, muscle dissected after 45 min of treadmill running that was preceded by 10 days of treadmill exercise training; Streptavidin IR800, IRDye 800-labeled streptavidin. *P < 0.05 vs. Control. **P < 0.05 vs. Ex after 10 d training. +P < 0.05 vs. Ex after 10 d AICAR.

Fig. 2. AMPKα Thr\(^{172}\) phosphorylation (A) and ACCβ Ser\(^{218}\) phosphorylation (B) in EDL muscle. Groups as indicated in Fig. 1. *P < 0.05 vs. Control.
compared with Con, despite 10 days of exercise training not increasing resting muscle glycogen content (Fig. 3B).

**Glucose Uptake**

Although glucose uptake tended to increase during an acute exercise bout, this was not significant (Fig. 4). Glucose uptake increased significantly during exercise after 10 days of AICAR treatment and 10 days of exercise training.

**DISCUSSION**

Acute exercise activated AMPK signaling in the rat soleus muscle and 10 days of exercise training greatly suppressed this exercise-induced increase in AMPK signaling (AMPKα Thr<sup>172</sup> phosphorylation and ACCβ Ser<sup>218</sup> phosphorylation). These findings in the rat are consistent with our laboratory’s previous findings in humans (20). Although 10 days of AICAR administration substantially attenuated the exercise-induced activation of AMPK signaling during exercise in the soleus, ACCβ Ser<sup>218</sup> phosphorylation was not reduced to the same extent as in the trained rats. Thus 10 days of exercise training is more effective in attenuating the extent of activation of AMPK during exercise than 10 days of AICAR administration.

As was the case in our laboratory’s previous human study (20), we found that 10 days of exercise training in rats greatly attenuated the extent of skeletal muscle AMPK activation during exercise (Fig. 1). Soleus muscle AMPKα Thr<sup>172</sup> phosphorylation and ACCβ Ser<sup>218</sup> phosphorylation increased during treadmill running, but after 10 days of exercise training there was no significant increase in these during subsequent exercise challenge (Fig. 1). Less activation of AMPK during exercise after short-term exercise training would be expected due to better matching of ATP production to ATP use and therefore attenuated increases in free AMP during contraction/exercise after exercise training (8, 20). Surprisingly, Durante et al. (11) found that although red quadriceps AMPK was activated less during treadmill exercise after 7 wk of exercise training in rats, soleus AMPK activation was not reduced during exercise after training. Compared with the present study, the exercise training intensity and duration used by Durante et al. (11) was greater and the experimental exercise bouts were shorter but more intense, and these differences may have accounted for the different results in the two studies.

In the present study, only one daily dose of AICAR was used for the chronic AICAR treatment (0.5 mg/g body wt ip). It is therefore possible that a higher daily dose of AICAR (e.g., 1 mg/g) could be as effective as exercise training in attenuating that activation of AMPK during exercise. However, we think that this is unlikely because in the soleus the acute AICAR administration increased ACCβ Ser<sup>218</sup> phosphorylation similarly to the acute exercise bout (Fig. 1B). In addition, muscle glycogen breakdown was similar in response to acute exercise and acute AICAR administration (Fig. 3A).
It will be important to now examine the mechanisms whereby 10 days of AICAR treatment attenuates the increase in AMPK activation during exercise. It is possible that there may have been an attenuation of the increase in skeletal muscle free AMP during exercise following 10 days of AICAR treatment. Previously, our laboratory found that short-term exercise training attenuates the increase in skeletal muscle free AMP in response to subsequent exercise in human biopsy samples (20). Unfortunately, it was not possible to assess this in the present study because the time to dissect the muscle makes such measures unreliable. It is also possible that the higher preexercise muscle glycogen content in the soleus after 10 days of exercise training may have contributed to the attenuated activation of AMPK during exercise after training. Skeletal muscle AMPK activity increases less during contractions in perfused rat soleus muscles (10) and during exercise in humans (36) when begun with high muscle glycogen levels compared with low muscle glycogen levels. However, although AMPK binds to glycogen, addition of glycogen to purified rat liver AMPK does not directly alter AMPK activity (29) Furthermore, our laboratory has shown that the short-term training effect on the extent of AMPK activation persists independently of preexercise muscle glycogen content in humans (20). Finally, it has been shown that the enzyme that dephosphorylates AMPK, protein phosphatase 2C (PP2C), is responsible for the decrease in skeletal muscle AMPK activity due to TNF-α (31). It is possible that exercise training and AICAR treatment increase PP2C activity and/or expression and that this results in lower levels of AMPK phosphorylation during exercise due to greater AMPK dephosphorylation. However, to date this concept has not been examined.

Unlike exercise, which predominately activates AMPK in slow-twitch muscles (30), AICAR activates AMPK to a greater extent in fast-twitch muscle (1, 2, 18). Indeed, we found that acute AICAR administration greatly increased AMPKα Thr172 phosphorylation in the EDL muscle (Fig. 2), but acute AICAR administration had no effect on AMPKα Thr172 phosphorylation in the soleus (Fig. 1). However, acute AICAR administration increased ACCβ Ser218 phosphorylation substantially in both the soleus and the EDL (Figs. 1 and 2). This could reflect ACCβ Ser218 phosphorylation being a more sensitive indicator of AMPK signaling in vivo than AMPKα Thr172 phosphorylation (7, 24, 34).

Chronic AICAR treatment significantly increases skeletal muscle glycogen content in the gastrocnemius/plantaris and in red and white quadriceps but not in the soleus muscle (15, 35). In the present study, 10 days of AICAR treatment tended to increase muscle glycogen content in the soleus and EDL, but these increases were not significant. It seems, therefore, that some muscles respond differently to others in terms of effects of AICAR on muscle glycogen content.

Unlike findings in humans in whom short-term exercise training attenuates the increase in glucose uptake during exercise (20, 21, 26), we found that skeletal muscle glucose uptake was actually greater in both soleus and EDL during subsequent exercise after 10 days of training (Fig. 4). It appears that this may be a species difference because several other studies have also demonstrated higher skeletal muscle glucose uptake during contraction/exercise in trained than untrained rats. Ploug et al. (27) found in a hindlimb perfusion study that exercise-trained Wistar rats had significantly higher soleus- and EDL-stimulated glucose uptake than untrained rats. Very similar results were also obtained in a hindlimb perfusion study using obese Zucker rats (5). In addition, even though skeletal muscle glucose uptake is reduced during easy treadmill exercise in trained rats compared with untrained rats, it is greater during more intense treadmill exercise in trained than untrained rats (3). The running in the present study was difficult for the rats, and based on previous research examining similar running speeds and grade would have been ~75% maximal O₂ uptake (4). We also found that skeletal muscle glucose uptake during exercise was greater after 10 days of AICAR treatment (Fig. 4). This novel finding suggests that the exercise training like effects of chronic AICAR treatment also extends to glucose uptake during exercise.

Both chronic AICAR treatment and exercise training increased the extent of skeletal muscle glucose uptake in response to the subsequent exercise challenge (Fig. 4). Because AMPK activation was attenuated by these treatments (Fig. 1), it suggests that AMPK is not important for skeletal muscle glucose uptake in response to exercise after training. This is not altogether a surprising finding, however, because dissociations between skeletal muscle AMPK activation and glucose uptake during exercise/contraction have been observed in both humans (20) and mice (18). It will be important to determine what factors are contributing to the augmentation in glucose uptake during exercise after 10 days of training or AICAR administration. It will also be important to examine whether 10 days of AICAR administration increases exercise performance.

In conclusion, 10 days of AICAR administration substantially mimics exercise training of skeletal muscle by attenuating the increases in AMPK activation in response to a subsequent exercise challenge.

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GRANTS

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