Effects of chronic AICAR treatment on fiber composition, enzyme activity, UCP3, and PGC-1 in rat muscles

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Effects of chronic AICAR treatment on fiber composition, enzyme activity, UCP3, and PGC-1 in rat muscles. J Appl Physiol 95: 960–968, 2003. First published May 30, 2003; 10.1152/japplphysiol.00349.2003.—This study was designed to determine the histological and metabolic effects of the administration of 5′-AMP-activated protein kinase (AMPK) activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) for 14 successive days. AICAR treatment caused a significant decrease in the percentage of type IIB fibers and the concomitant increase in the percentage of type II fibers in extensor digitorum longus (EDL) muscle. The capillary density and the capillary-to-fiber ratio were not altered by AICAR. AICAR treatment increased the uncoupling protein 3 (UCP3) level in EDL and the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1) protein level in the soleus and EDL muscles, whereas the myogenin level was not altered by AICAR. These results seem to imply that the chronic activation of AMPK alters such muscle histochemical and metabolic characteristics.

5′-AMP-activated protein kinase; antioxidant capacity; mitochondrial enzymes; muscle fiber type composition; peroxisome proliferator-activated receptor-γ coactivator-1α; 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; uncoupling protein 3

ENDURANCE EXERCISE TRAINING modifies various physiological characteristics of the skeletal muscle, including the muscle fiber composition (16), capillary network (25), metabolic capacity (4), and antioxidant systems (37). Despite the fact that a number of signaling pathways inducing such adaptations have been analyzed for several years (9, 30, 51, 54), the mechanisms of such adaptations remain to be fully elucidated.

Recently, the activation of 5′-AMP-activated protein kinase (AMPK) by the injection of 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) or β-guanidinopropionic acid feeding showed an increase in fatty acid oxidation (31), glucose uptake (31), mitochondrial biogenesis (2, 58), hexokinase (HK) activity (20, 53), mitochondrial enzyme activities (53), glucose transporter 4 (GLUT-4) protein (20), and uncoupling protein 3 (UCP3) (57). AMPK was activated by muscle contraction such as exercise (14, 52) and electrical stimulation (22) by depression of ATP-to-AMP ratio. It is thus hypothesized that such skeletal muscle adaptations to endurance exercise training occur at least in part through the AMPK pathway.

Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1) is a transcriptional coactivator that interacts with several nuclear transcriptional factors (40). PGC-1 promoted GLUT-4 expression (32), mitochondrial biogenesis (30, 56), and fiber type transformation (30) in skeletal muscle cells. PGC-1 mRNA expression was enhanced by AICAR (46). Acute endurance exercise increased skeletal muscle PGC-1 mRNA (1, 36, 46) and protein (1) levels. Endurance exercise training also increased skeletal muscle PGC-1 mRNA (36). On the basis of these data, it is hypothesized that some skeletal muscle adaptations due to endurance exercise training such as an increase in the oxidative enzyme activities and fiber type transformation are induced by the activation of AMPK and the consequent increase in the PGC-1 expression. However, a study to determine whether such skeletal muscle adaptations are related to the chronic activation of AMPK and increased PGC-1 protein content has yet to be conducted. Therefore, one purpose of the present study was to determine whether the chronic injection of AICAR influences the PGC-1 protein content, muscle histochemical characteristics including the fiber composition and capillary density, and metabolic enzyme activities in rat slow- and fast-twitch skeletal muscles.

Endurance exercise training enhances skeletal muscle antioxidant enzyme activities (37). In addition, acute exercise and AICAR increase skeletal muscle UCP3 (57), which is a possible regulator of free radical production (49). These reports raise the possibility that the activation of AMPK also enhances antioxidant systems. However, very few studies concerning the relationship between AMPK and antioxidant systems have so far been conducted (43, 57). Another purpose of the present study was to test the hypothesis that the chronic activation of AMPK by AICAR increases the skeletal muscle UCP3 content and antioxidant enzyme activities.

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MUSCLE CHARACTERISTICS WITH AICAR TREATMENT

METHODS

Animals. Five-week-old male Wistar rats with a body weight of ~140 g were used for this experiment. All rats were handled daily for at least 6 days before beginning the treatment regimen. All rats were housed two to three per cage (42 × 25 × 20 cm deep) in a temperature- (22 ± 2°C) and humidity-controlled (60 ± 5%) room with a 12-h light (0700 to 1900) and 12-h dark (1900 to 0700) cycle. Food and water were provided ad libitum. All experimental procedures were approved by the University Committee for the Use of Animals and were in strict accordance with the American Physiological Society Guiding Principles in the Care and Use of Animals. The rats were divided into control (n = 9) or AICAR (n = 7) groups. The rats of the control and AICAR groups were given daily subcutaneous injections of saline vehicle and AICAR (Toronto Research Chemicals, North York, ON, Canada) (1 mg/g body wt) in saline, respectively. This dose of AICAR certainly enhanced the skeletal muscle AMPK activity at 60 and 120 min after injection (20, 42). Such procedures were performed between 0800 and 1000 for 14 successive days. The nonfasted rats were anesthetized ~24 h after the last injection, with pentobarbital sodium (60 mg/kg body wt ip). The soleus and extensor digitorum longus (EDL) muscles of both legs were rapidly dissected. The muscles of the right leg were used for an enzyme assay and those of the left leg were used for histochemistry and Western blotting. The abdominal fat pads (perirenal, epididymal, and mesenteric) were also excised and weighed.

Muscle histochemistry. Skeletal muscle fibers were roughly categorized as type I, IIA, and IIB fibers (6). An analysis of single muscle fibers demonstrated that the histochemically defined type I, IIA, and IIB fibers expressed myosin heavy chains 1, 2a, and 2b, respectively (47). Furthermore, an additional myosin heavy chain 2x, which is also called 2d, has been identified (29, 47). In addition, the type IIC fibers that coexpress type 1 and type 2a myosin heavy chain proteins were also observed (42, 47). In this study, the muscle fibers were categorized as type I, IIC, IIA, IIX, and IIB fibers. The rank order of maximum contraction velocity in rat skeletal muscle fibers was I < IIA < IIX < IIB (15). Muscle transverse sections (7 μm) were cut from each muscle by using a cryostat maintained at −20°C, and the sections were then mounted on a cover glass. Myosin adenosine triphosphatase (ATPase) was determined by using the previously described procedures (44). In brief, consecutive serial sections were processed by using three different pretreatments, preincubation at pH 4.3, 4.6, and 10.4. The muscle fibers were identified as type I, IIC, IIA, IIX, and IIB fibers on the basis of the myosin ATPase staining intensity. Each section was photographed by use of an Axioskop 2 plus microscope (Carl Zeiss, Hallbergmoos, Germany) mounted with an AxioCam HRm CCD camera (Carl Zeiss), and then each fiber was identified and counted by use of a hand counter. Next, the muscle fiber composition was determined by evaluating all countable fibers in both muscles. A remaining transverse section was stained to determine the succinate dehydrogenase activity (33).

To visualize the capillaries, another cross-section (7 μm) was also cut. The section was fixed with 100 mM phosphate buffer containing 4% formaldehyde for 4 min at room temperature and then myosin ATPase (preincubation at pH 10.5) was demonstrated as described previously (45). The stained sections were photographed, and then the artifact-free three 0.147-mm² areas in each section were analyzed to determine the capillary density (capillaries/mm²), capillary-to-fiber ratio (capillaries/fibers), and fiber density (fibers/mm²).

Enzyme assay. The frozen muscle samples were homogenized 1:20 (wt/vol) in 175 mM KCl, 10 mM GSH, 2 mM EDTA, and 0.1% Triton X-100, pH 7.4. Enzyme activities were measured spectrophotometrally. All enzymatic assays were carried out at 30°C by using saturating concentrations of substrates and cofactors as determined in preliminary analyses. Citrate synthase (CS; tricarboxylic acid cycle) and carnitine palmitoyltransferase (CPT; transport of fatty acids to mitochondria) activities were measured at 412 nm to detect the transfer of sulphydryl groups to 5,5’-dithiobis(2-nitrobenzoic acid) (DTNB). HK (mobilization of blood glucose), pyruvate kinase (PK; glycolysis), lactate dehydrogenase (LDH; anaerobic glycolysis), malate dehydrogenase (MDH; tricarboxylic acid cycle), β-hydroxyacyl CoA dehydrogenase (HAD; β-oxidation of fatty acids), glutathione peroxidase (GPX; antioxidant system), and glutathione reductase (GR; antioxidant system) activities were measured at 340 nm by following the production or disappearance of NADH or NADPH.

For the HK (EC 2.7.1.1) assay, 100 mM Tris-HCl, 0.4 mM NADP, 5 mM MgCl₂, 750 μM glucose–6-phosphate dehydrogenase (EC 1.1.1.49), 1 mM ATP, pH 7.0, were used.

For the PK (EC 2.7.1.40) assay, 50 mM Tris-HCl, 0.1 mM KCl, 10 mM MgCl₂, 0.28 mM NADH, 1.5 mM ADP, 6 U/ml LDH, and 5 mM phosphoenolpyruvate, pH 7.6, were used.

For the LDH (EC 1.1.1.27) assay, 50 mM Tris-HCl, 0.28 mM NADH, and 2.4 mM pyruvic acid, pH 7.6, were used.

For the MDH (EC 1.1.1.37) assay, 50 mM Tris-HCl, 0.28 mM NADH, and 0.5 mM oxalacetate, pH 7.6, were used.

For the CS (EC 4.1.3.7) assay, 100 mM Tris-HCl, 0.1 mM DTNB, 0.3 mM acetyl-CoA, 3.33 mM K₂HPO₄, and 0.5 mM oxalacetate, pH 8.0, were used.

For the CPT (EC 2.3.1.21) assay, 75 mM Tris-HCl, 0.2 mM DTNB, 1.5 mM EDTA, 2 mM L-carnitine, 0.05 mM palmityl-CoA, pH 8.0, were used.

For the HAD (EC 1.1.1.35) assay, 100 mM Tris-HCl, 0.28 mM NADH, 5 mM EDTA, and 0.1 mM acetooxyl-CoA, pH 6.9, were used.

For the GPX (EC 1.11.1.9) assay, 100 mM Tris-HCl, 0.5 mM EDTA, 2 mM GSH, 0.2 mM NADPH, 1 U/ml GR, and 0.07 mM t-butyl hydroperoxide, pH 8.0, were used.

For the GR (EC 1.6.4.2) assay, 50 mM phosphate buffer, 1 mM EDTA, 1 mM GSSG, 0.2 mM NADPH, and 0.1% BSA, pH 7.6, were used.

Primary antibodies. Affinity-purified rabbit polyclonal antibody to UCP3 (AB3046, Chemicon International, Temecula, CA), PGC-1 (H-300, Santa Cruz Biotechnology, Santa Cruz, CA), and myogenin (M-225, Santa Cruz Biotechnology) were used in this study.

Gel electrophoresis and Western blotting. The tissue specimens from each muscle were homogenized (1:10) in 50 mM Tris-HCl pH 7.4, 5 mM EDTA, 10 μg/ml PMSF, 0.5 μg/ml leupeptin, 0.2 μg/ml aprotinin, 0.1% Triton X-100, 0.2% NP-40, 0.05% mercaptoethanol, and 1 mM Na₃VO₄ for 30 s. The homogenate was centrifuged at 15,000 g (4°C) for 25 min. The supernatant was removed, and its protein concentration was determined by use of a protein determination kit (Protein Assay II, 500-0006, Bio-Rad, Richmond, CA). Sodium dodecyl sulfate-polyacrylamide gel (12.5% for UCP3 and myogenin and 7.5% for PGC-1) electrophoresis (SDS-PAGE) was performed. The proteins separated by SDS-PAGE were transferred onto the polyvinylidene difluoride membrane electrophoretically. The membrane was incubated with a blocking buffer of casein solution (SP-5020, Vector Laboratories, Burlingame, CA) for 30 min. The membrane was reacted with the primary antibodies for 1 h and then incubated with biotinylated anti-rabbit IgG (1:800 dilution, Vector Laboratories) for
was considered to be significant.

**RESULTS**

**Body composition.** The body mass and abdominal fat content of the rats in each group are shown in Fig. 1. No statistically significant differences in the body mass were observed between the control and AICAR groups \((P = 0.599)\) (Fig. 1A). The abdominal fat content of AICAR \(3.99 \pm 0.20 \text{ g}\) was 26% lower than the control \(5.39 \pm 0.20 \text{ g}\) \((P = 0.0002)\) (Fig. 1B). Although the rats were fed ad libitum and food consumption was not measured, it is speculated that the observed difference was at least partially due to the chronic activation of AMPK by AICAR rather than any difference in food intake because a previous study using pair-fed rats \((53)\) also indicated a lower fat pad weight in chronic AICAR-injected rats than saline-injected rats.

**Histochemical analyses.** In the EDL muscle, from the myosin ATPase stained sections (Fig. 2A, a and b), the intermediated stained type IIB fibers appear to decrease in the AICAR group. In addition, in the succinate dehydrogenase-stained sections (Fig. 2A, c and d), the intensely stained oxidative fibers seem to increase in the AICAR group. As shown in Fig. 2B, the percentage of type IIB fibers in AICAR was significantly lower than in control \((26.9 \pm 2.7 \text{ and } 36.1 \pm 2.4\%\), respectively, \(P = 0.024)\), and the percentage of IIX fibers in AICAR was significantly higher than in control \((49.0 \pm 2.6 \text{ and } 40.9 \pm 2.1\%\), respectively, \(P = 0.030)\). In the soleus muscle, as shown in Fig. 2B, no significant differences were observed in fiber composition of either fiber type. No type IIX or IIB fibers were observed in the soleus muscle.

The capillary density, capillary-to-fiber ratio, and fiber density (an index of fiber size) are indicated in Table 1. No significant differences were observed regarding the capillary network between the groups.

**Enzyme activities.** Table 2 indicates the muscle enzyme activities. HK, PK, CS, MDH, and HAD activities in the AICAR group was significantly greater than control \((P < 0.05)\) in both the soleus and EDL muscles. On the other hand, the LDH, CPT, GPX, and GR activities in AICAR were not significantly different from control in either the soleus or EDL muscles.

**Western blotting.** Figure 3A shows representative Western blot detections of UCP3 in the EDL muscle of rats from both groups. Compared with control, UCP3 was markedly increased in the EDL muscle of the AICAR group. The densitometric data (Fig. 3B) indicated that UCP3 protein level in AICAR was significantly higher than in control \((1.96 \pm 0.28 \text{ and } 1.00 \pm 0.19\), respectively, \(P = 0.010)\). In the soleus muscle, UCP3 was undetected.

Figure 4A shows representative Western blot detections of PGC-1 in the soleus and EDL muscles of the rats from both groups. PGC-1 appeared to markedly increase in both the soleus and EDL muscles of the AICAR group. The densitometric data (Fig. 4B) indicated that the PGC-1 protein level of the soleus and EDL muscles in AICAR were significantly higher than control \((2.14 \pm 0.32 \text{ and } 1.00 \pm 0.08, P = 0.003 \text{ in soleus, and } 1.87 \pm 0.39 \text{ and } 1.00 \pm 0.07, P = 0.025 \text{ in EDL, respectively})\). We also determined the myogenin protein level because it was a possible regulator of oxidative enzyme activities \((21)\). In Fig. 5, the myogenin content was shown to demonstrate no change after chronic AICAR treatment in the soleus muscle \((1.00 \pm 0.14 \text{ in control and } 1.26 \pm 0.23 \text{ in AICAR, } P = 0.33)\). In the EDL muscle, no myogenin was detected.

**DISCUSSION**

In this study, we demonstrated that chronic AICAR treatment for 2 wk decreased the percentage of type IIB fibers and concomitantly increased the percentage of type IIX fibers, which had a slower shortening ve-

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**Fig. 1.** Body mass (A) and abdominal fat content (B) of rats. AICAR, 5-aminomimidazole-4-carboxamide-1-β-D-ribofuranoside. Values are means ± SE; \(n = 7–9 \text{ rats per group}. \*P < 0.001 \text{ vs. control.} \)**
locity (5) and a more oxidative capacity (38) than IIB fibers, in fast-twitch EDL muscle. It is well recognized that exercise training and chronic electrical stimulation induced skeletal muscle fiber type transformation (35). The chronic low-frequency stimulation for 28 days in rat EDL muscle showed a marked decrease in the percentage of type IIB fibers and an increase in the percentage of type IIX fibers (11). Voluntary running exercise over 45 days in rats resulted in a reduced percentage of type IIB fibers and an increased percentage of type IIA/IIX fibers in the EDL muscle (27). These reports suggested that IIB → IIX conversion occurred in the early phase of chronic muscle contraction. On the basis of these data, AICAR treatment appears to mimic the effect of chronic electrical stimulation and endurance exercise training on muscle fiber type trans-

Table 1. Muscle capillary density, capillary-to-fiber ratio, and fiber density

<table>
<thead>
<tr>
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<th>Soleus</th>
<th>EDL</th>
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<tbody>
<tr>
<td>Capillary density, capillaries/mm²</td>
<td>Control 1,047 ± 40</td>
<td>AICAR 1,185 ± 54</td>
</tr>
<tr>
<td>Capillary-to-fiber ratio, capillaries/fibers</td>
<td>2.32 ± 0.07</td>
<td>2.41 ± 0.10</td>
</tr>
<tr>
<td>Fiber density, fibers/mm²</td>
<td>453 ± 21</td>
<td>498 ± 35</td>
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Values are means ± SE; n = 6–9 muscles. EDL, extensor digitorum longus; AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside.
formation. The reason why AICAR treatment alters the muscle fiber composition is unclear at present. One possible mechanism for this is that increased PGC-1 protein by AICAR alters the fiber type-related gene expression. PGC-1 coactivated transcriptional factor myocyte enhancer factor-2 (30, 32) and skeletal muscle fiber type transformation (30). Myocyte enhancer factor-2 activated the expression of slow or oxidative muscle genes (55). The induction of PGC-1 has been proposed to play an important role in coordinating the activation of various genes linking to the skeletal muscle fiber phenotype.

It should be noted that the fiber-type transformation from type II to type I fibers was not observed in the present study. Although any evidences of the transformation from type II to type I fibers with AICAR treatment have not yet been observed, our results did not completely negate the potential role of AMPK for such transformation. In the early phase of high-intensity endurance exercise training (26) and chronic electrical

Table 2. Effect of chronic AICAR treatment on enzyme activities

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Soleus Control</th>
<th>EDL Control</th>
<th>AICAR</th>
<th>AICAR</th>
</tr>
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<tbody>
<tr>
<td>HK</td>
<td>2.29 ± 0.15</td>
<td>3.01 ± 0.17*</td>
<td>2.32 ± 0.09</td>
<td>4.82 ± 0.20*</td>
</tr>
<tr>
<td>PK</td>
<td>25.9 ± 1.1</td>
<td>31.0 ± 1.4*</td>
<td>166.3 ± 5.7</td>
<td>216.6 ± 8.9*</td>
</tr>
<tr>
<td>LDH</td>
<td>166 ± 8</td>
<td>187 ± 9</td>
<td>623 ± 20</td>
<td>568 ± 26</td>
</tr>
<tr>
<td>CS</td>
<td>13.9 ± 0.7</td>
<td>16.7 ± 0.8*</td>
<td>10.8 ± 0.4</td>
<td>12.5 ± 0.2*</td>
</tr>
<tr>
<td>MDH</td>
<td>735 ± 32</td>
<td>874 ± 37*</td>
<td>614 ± 26</td>
<td>746 ± 46*</td>
</tr>
<tr>
<td>CPT</td>
<td>0.115 ± 0.007</td>
<td>0.118 ± 0.009</td>
<td>0.073 ± 0.003</td>
<td>0.076 ± 0.004</td>
</tr>
<tr>
<td>HAD</td>
<td>36.2 ± 1.4</td>
<td>46.2 ± 1.5*</td>
<td>14.2 ± 0.5</td>
<td>17.1 ± 0.6*</td>
</tr>
<tr>
<td>GPX</td>
<td>184 ± 2</td>
<td>189 ± 5</td>
<td>134 ± 2</td>
<td>135 ± 2</td>
</tr>
<tr>
<td>GR</td>
<td>1.91 ± 0.05</td>
<td>1.91 ± 0.06</td>
<td>1.02 ± 0.02</td>
<td>0.99 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE (in μmol·g⁻¹·min⁻¹); n = 7–9 muscles. HK, hexokinase; PK, pyruvate kinase; LDH, lactate dehydrogenase; CS, citrate synthase; MDH, malate dehydrogenase; CPT, carnitine palmitoyl transferase; HAD, β-hydroxyacyl CoA dehydrogenase; GPX, glutathione peroxidase; GR, glutathione reductase. *P < 0.05 vs. control.

Fig. 3. Western blot analysis of uncoupling protein 3 (UCP3) in the EDL muscle. A: representative blots made by use of an antibody against UCP3 showing increases in UCP3 by AICAR treatment. B: average values for the UCP3 in EDL muscle. Values are means ± SE; n = 7–9 muscles per group. *P < 0.05 vs. control.

Fig. 4. Western blot analysis of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) in the soleus and EDL muscles. A: representative blots made by using an antibody against PGC-1α showing increases in PGC-1α in both the soleus and EDL muscles by AICAR treatment. B: average values for the PGC-1α in soleus and EDL muscles. Values are means ± SE; n = 7–9 muscles per group. *P < 0.05 vs. control.

Fig. 5. Western blot analysis of myogenin in the soleus muscle. A: representative blots made by using an antibody against myogenin. B: average values for the myogenin in soleus muscle. Values are means ± SE; n = 7–9 muscles per group.
stimulation (11), the fiber-type transformation within subtypes of type II fibers was only seen. However, in the later phase, alternation from type II to type I fibers may possibly occur (11, 16, 26). Therefore, it might be possible that AICAR treatment for over 14 days induces the transformation from type II to type I fibers. Further research is needed to examine whether AICAR treatment affects all fiber types.

Acute and chronic AICAR treatment increased the skeletal muscle glucose uptake (8, 18, 28, 31) and GLUT-4 protein content (20, 23), respectively. Like previous studies (20, 53), we demonstrated that the AICAR treatment increased the skeletal muscle HK activity, which is a possible determinant of the glucose uptake in skeletal muscle during exercise and hyperinsulinemic conditions (17). Interestingly, the present study also demonstrated that the glycolytic PK activities of the soleus and EDL muscles were also increased by AICAR. Collectively, these findings provide evidence that the activation of AMPK by AICAR totally enhances the glucose metabolism. We demonstrated that chronic AICAR treatment increased the tricarboxylic acid cycle and β-oxidation enzyme activities in skeletal muscle. Furthermore, a previous study demonstrated that acute AICAR treatment enhanced fat oxidation through the inhibition of the acetyl-CoA carboxylase activity and the consequent reduction in the malonyl-CoA content, which inhibits the transport of fatty acids to the mitochondria, in skeletal muscle (31). Collectively, such evidence suggests that the activation of AMPK may totally enhance the capacity of energy utilization.

It is noteworthy that the HK activity of EDL was increased 108% by 14 successive days of AICAR treatment, whereas all other enzyme activities affected by AICAR increased ~15–31%. Winder et al. (53) demonstrated that the 4 wk of AICAR treatment increased GLUT-4 protein content in fast-twitch quadriceps muscle but not in slow-twitch soleus muscle. In addition, Buhl and coworkers (7, 8) indicated that chronic AICAR treatment enhanced GLUT-4 protein expression, insulin-stimulated glucose uptake, and GLUT-4 translocation in primarily fast-twitch glycolytic muscles. Collectively, these findings provide evidence that chronic AICAR treatment improves the capacity of skeletal muscle glucose uptake especially in fast-twitch glycolytic muscles.

Because PGC-1 and myogenin controlled the mitochondrial biogenesis and oxidative enzyme activities (21, 30, 56), we herein examined their protein content in skeletal muscles. As shown in the results, the PGC-1 protein contents in the rat soleus and EDL muscles increased after chronic AICAR treatment. In addition, a previous study indicated that acute AICAR treatment increased the PGC-1 mRNA expression in rat epitrochlearis muscle (46). Therefore, the activation of AMPK with AICAR should enhance the PGC-1 expression. PGC-1 increased the mRNA expression and transcriptional activity of nuclear respiratory factors 1 and 2 (1, 56), which were transcriptional factors related to mitochondrial function (40). Although the transcriptional activities of nuclear respiratory factors were not determined in this study, on the basis of these data we speculate that the increase in the muscle mitochondrial oxidative enzyme activities with chronic AICAR treatment is at least partially induced by the interaction of PGC-1 and nuclear respiratory factors. On the other hand, myogenin protein was not altered in the soleus muscle by AICAR or undetected in the EDL muscle. Therefore, an upregulation of myogenin by exercise (19, 50) is probably not related to the AMPK pathway.

Both the present study (treatment for 2 wk) and a previous study (treatment for 4 wk) (53) demonstrated that chronic AICAR treatment increased the mitochondrial tricarboxylic acid cycle enzyme activities. In addition, the HAD activity, which catalyzes mitochondrial β-oxidation of fatty acids, was also increased by AICAR in the present study. On the other hand, Winder et al. (53) failed to demonstrate such an increase. They also showed that treatment with AICAR for 2 wk increased the CS activity in the rat red quadriceps muscle, whereas 4 wk of such treatment did not. These results raise the possibility that the increase in the mitochondrial oxidative enzyme activities by AICAR occurs in the early phase but downregulation occurs if the treatment period is extended. To resolve the effect of the time course on the AICAR-treated muscle oxidative enzyme activities, further studies are thus called for.

Although the HAD activity was enhanced by AICAR treatment, this study and the previous study (53) demonstrated that CPT activity, which may be the rate-limiting step in fatty acid uptake and oxidation by mitochondria, was not changed by chronic AICAR treatment. Because both HAD and CPT are the enzymes of mitochondrial fatty acid metabolism, our results suggested that CPT was not regulated by the AMPK pathway. The upregulation of CPT in response to endurance exercise training (3, 48) has thus been proposed to depend on the other signaling pathways. It should be noted that such results did not exactly negate the effect of AICAR treatment on fatty acid uptake by mitochondria. Merrill et al. (31) demonstrated that acute AICAR perfusion to the hindlimb immediately inactivated acetyl-CoA carboxylase and decreased malonyl-CoA, an inhibitor of CPT, and then the fatty acid oxidation was increased. In the present study, the muscles were dissected ~24 h after the last AICAR injection. It is possible that such an acute effect is not maintained at least 24 h after injection.

In this study, we demonstrated that chronic AICAR treatment increased the UCP3 protein content in EDL muscle. UCPs inhibited the production of reactive oxygen species in the mitochondria (12, 34, 49). Exercise and hypoxia, which are considered to produce reactive oxygen species, immediately enhanced the skeletal muscle UCP3 protein level as well as AICAR (57). In addition, exercise (14, 52), hypoxia (13), and hydrogen peroxide (10) all activated AMPK. As a result, the increased muscle UCP3 protein by the AMPK pathway
thus appears to inhibit the reactive oxygen species production in fast-twitch muscle. On the other hand, this study indicated that the antioxidant enzyme activities, including GPX and GR, were not altered after chronic AICAR treatment in either the soleus or EDL muscles. The increase in the antioxidant enzyme activities by exercise training (37) is thus suggested to be independent of the AMPK pathway. As a result, our original hypothesis was proven to be incorrect. To resolve the underlying process for the increased antioxidant enzyme activities in response to exercise, further experimental studies are called for.

In the EDL muscle, both the UCP3 levels and mitochondrial enzyme activities except for CPT increased after chronic AICAR treatment. These results were consistent with previous reports describing that the UCP3 appeared to increase as a component of the exercise-induced increase in skeletal muscle mitochondria (24). These results seem plausible because the PGC-1 protein, which regulated both mitochondrial biogenesis (30, 56) and expression of UCPs (56), increased with chronic AICAR treatment. In other words, it is speculated that the activation of AMPK increased the PGC-1 protein level, and it consequently increased both mitochondrial enzyme activities and UCP3.

Both this study and a previous one (41) demonstrated that UCP3 protein was not detectable in the control rat slow-twitch soleus muscle. In addition, the rank order of the UCP3 protein content in human skeletal muscle fibers is IIX > IIA > I (39). These results suggest that UCP3 protein preferentially accumulates in fast-twitch muscle fibers. The factors causing a difference in the UCP3 protein content between slow- and fast-twitch muscles remain to be elucidated. One possibility might be the difference in the recruitment of muscles. Because the slow-twitch soleus is an antiglidy and postural muscle, it would be recruited to a much greater extent than EDL muscle at least in the sedentary condition. The muscle UCP3 protein content in endurance-trained subjects was lower than in untrained subjects (39), thus suggesting that an increase in the muscle activity results in a decrease in the UCP3 expression. As a result, the UCP3 protein of the soleus muscle dramatically decreased until reaching an undetectable level.

In summary, we herein demonstrated that chronic AICAR treatment for 2 wk decreased the percentage of type IIB fibers and increased the percentage of type IIX fibers in EDL muscle. In addition, such treatment also increased the glycolytic and oxidative enzyme activities in the soleus and EDL muscles and the UCP3 protein content in the EDL muscle of rats. It is speculated that at least several such adaptations with AICAR treatment may be due to an increased PGC-1 protein content. On the other hand, the capillary network, antioxidant enzyme activities, and myogenin protein content may be independent of the AMPK pathway.

REFERENCES


