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Short-term adenosine monophosphate–activated protein kinase activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside treatment increases the sirtuin 1 protein expression in skeletal muscle

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Abstract

Adenosine monophosphate–activated protein kinase (AMPK) has been proposed to stimulate mitochondrial biogenesis and fat and glucose metabolism in skeletal muscle. Nicotinamide adenine dinucleotide–dependent histone deacetylase sirtuin 1 (SIRT1) is also thought to play a pivotal role for such metabolic adaptations. The purpose of the present study was to examine the effect of AMPK activation with the administration of AMPK activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) to rats on skeletal muscle SIRT1 protein expression as well as peroxisome proliferator activated receptor γ coactivator–1α (PGC-1α) and glucose transporter 4 (GLUT4) protein expression and hexokinase activity. The AICAR promoted the phosphorylation of AMPK α-subunit (Thr^{172}) and acetyl–coenzyme A carboxylase (Ser^{79}) without any change of total AMPK α-subunit or acetyl–coenzyme A carboxylase protein levels in both the slow-twitch soleus and fast-twitch extensor digitorum longus (EDL) muscles. The SIRT1 protein expression increased at 24 hours after administration of AICAR in the EDL muscle but not in the soleus muscle. The PGC-1α protein expression increased in both the soleus and EDL muscles and GLUT4 did in the EDL muscle at 24 hours after an administration of AICAR. The hexokinase activity increased at 18 and 24 hours in the soleus and at 12, 18, and 24 hours in the EDL after an AICAR treatment. These results suggest that short-term AICAR treatment to rats promotes skeletal muscle AMPK phosphorylation and then coincidently increases the SIRT1 protein expression. In addition, such treatment also enhances the PGC-1α and GLUT4 protein contents and hexokinase activity in skeletal muscle.

1. Introduction

Silence information regulator 2 (Sir2) proteins are the nicotinamide adenine dinucleotide–dependent acetylases that regulate longevity in *Caenorhabditis elegans* [1] and *Saccharomyces cerevisiae* [2] in response to caloric restriction. In mammals, the Sir2 ortholog, sirtuin 1 (SIRT1)/Sir2α plays an important role in various biological processes via functionally interacting and deacetylating several proteins [3]. SIRT1 controls both energy homeostasis and metabolic adaptations [4]. The activation of SIRT1 with its activator resveratrol improved the glucose tolerance and survival in mice fed high-fat diet [5,6]. SIRT1 can promote mitochondrial biogenesis and fatty acid oxidation in skeletal muscle cells via deacetylation and functionally activating the peroxisome proliferator activated receptor γ coactivator–1α (PGC-1α) [7-9]. This metabolic role of SIRT1 is associated with 5′-adenosine monophosphate–activated protein kinase (AMPK), which is also a key regulator of energy metabolism [4].

5′-Adenosine monophosphate–activated protein kinase is a heterotrimer consisting of 3 subunits: α, β, and γ [10]. Two isoforms exist for both the α-subunit (α1 and α2) and β-subunit (β1 and β2) and 3 for the γ-subunit (γ1, γ2, and γ3). The α-subunit contains the catalytic domain. The β-subunit mediates the assembly of the heterotrimeric AMPK complex [11] and glycogen binding [12]. The γ-subunit binds the AMP and following phosphorylation of threonine

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172 in the α-subunit and kinase activation [13]. The AMPK functions as an energy sensor and is activated when the cellular AMP to adenosine triphosphate ratio is increased [10]. The phosphorylation of threonine 172 in α-subunit strongly correlates with the AMPK activity [14]. The AMPK phosphorylation is mainly regulated by an upstream kinase LKB1 in skeletal muscle [15]. Skeletal muscle AMPK is activated by exercise [16], adipocytokines including leptin [17] and adiponectin [18], and antidiabetic drug metformin [19,20]. The activation of AMPK by its activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) stimulates both glucose uptake and fatty acid oxidation in skeletal muscle cells [21] and increases insulin-stimulated glucose uptake, insulin signaling such as phosphatidylinositol 3-kinase and protein kinase B activities, glucose transporter 4 (GLUT4) protein expression, hexokinase activity, and mitochondrial oxidative enzyme activities in skeletal muscle [22-24]. The activation of AMPK by AICAR also increases the PGC-1α expression in skeletal muscle [25], which controls mitochondrial biogenesis and glucose metabolism [25,26]. The AMPK is indirectly phosphorylated by SIRT1 through LKB1 deacetylation [27]. In addition, AMPK promotes SIRT1 activation by enhancing the transcription and activity of nicotinamide phosphoribosyltransferase [28].

The skeletal muscle SIRT1 protein expression [29] and activity [30] have been observed to increase with endurance exercise in rat skeletal muscle. Endurance exercise has a great impact on the skeletal muscle metabolic characteristics, including mitochondrial biogenesis and GLUT4 expression [31], while also activating AMPK [16]. The activation of AMPK with AICAR also induces such metabolic adaptations in skeletal muscle [23,24], thus suggesting that the activation of AMPK mediates the effect of endurance exercise training on metabolic characteristics. It is hypothesized that AMPK regulates SIRT1 expression. The purpose of the present study was to investigate whether the activation of AMPK with short-term AICAR treatment to rats induced the expression of SIRT1 protein as well as the expression of PGC-1α and GLUT4 protein and also the hexokinase activity in slow- and fast-twitch skeletal muscles.

2. Materials and methods

2.1. Animals

Male Wistar rats that were 4 weeks of age and with a body weight of 70 to 90 g (Kyudo, Tosu, Saga, Japan) were used for the current study. All rats were handled daily for at least 5 days before beginning their experiment regimen. All rats were housed in a temperature- (22°C ± 2°C) and humidity- (60% ± 5%) controlled room with a 12-hour light (7:00 AM-7:00 PM) and 12-hour dark (7:00 PM-7:00 AM) cycle. Food and water were provided ad libitum. All experimental procedures were strictly conducted in accordance with the Nakamura Gakuen University Guidelines for the Care and Use of Laboratory Animals and were approved by the University Animal Experiment Committee.

2.2. AMPK and acetyl–coenzyme A carboxylase phosphorylation study

The rats were randomly assigned to pre (n = 12) and AICAR treatment (n = 36) groups. The rats of AICAR treatment group were then given a subcutaneous ingestion of AICAR (Toronto Research Chemicals, North York, Ontario, Canada; 1 mg/g body weight). The rats were anesthetized with pentobarbital sodium (60 mg/kg body weight IP), and the slow-twitch soleus and fast-twitch extensor digitorum longus (EDL) muscles were rapidly dissected out at 1 (n = 12), 2 (n = 12), and 4 (n = 12) hours after the AICAR treatment. The rats of the pre group were also anesthetized, and the soleus and EDL muscles were dissected out. The muscles were frozen in liquid nitrogen and stored at −80°C until determinations of phosphorylated and total AMPKα and acetyl–coenzyme A carboxylase (ACC) protein expression were performed.

A lysis buffer was used to inhibit phosphatases and determine the phosphorylated AMPK and ACC protein levels as well as total AMPKα and ACC (50 mmol/L HEPES, 0.1% Triton X-100, 4 mmol/L EGTA, 10 mmol/L EDTA, 15 mmol/L $Na_4P_2O_7$, 100 mmol/L β-glycerophosphate, 25 mmol/L NaF, 5 mmol/L Na_3VO_4, and 1 tablet per 50 mL Complete Protease Inhibitor Cocktail Tablets [Roche Diagnostics, Tokyo, Japan], pH 7.4). The muscle specimens were homogenized in ice-cold lysis buffer (1:10 wt/vol) with a Polytron-type homogenizer operating at maximum speed for 30 seconds. The homogenate was centrifuged at 15 000*g* (4°C) for 25 minutes. The protein concentration of the supernatant was then determined by use of a protein determination kit (Bio-Rad, Richmond, CA). The muscle protein homogenate was solubilized in sample loading buffer (50 mmol/L Tris-HCl, pH 6.8, 2% sodium dodecyl sulfate (SDS), 10% glycerol, 5% β-mercaptoethanol, and 0.005% bromophenol blue).

2.3. SIRT1, PGC-1α, and GLUT4 proteins and hexokinase activity study

The rats were randomly assigned to pre (n = 12), AICAR treatment (n = 48), and saline treatment (n = 12) groups. The rats of AICAR treatment group were then given a subcutaneous ingestion of AICAR (1 mg/g body weight). The rats were anesthetized with pentobarbital sodium (60 mg/kg body weight IP); and then the soleus and EDL muscles were rapidly dissected out at 6 (n = 12), 12 (n = 12), 18 (n = 12), and 24 (n = 12) hours after the AICAR treatment. The rats of pre group were also anesthetized, and the muscles were dissected out. In the rats of saline treatment group, a comparable volume of saline was administered subcutaneously. The rats were anesthetized, and the muscles were dissected out at 24 hours after the saline injection. The

muscles were frozen in liquid nitrogen and stored at −80°C until analyses were performed.

The frozen samples were homogenized with homogenizer in ice-cold homogenizing buffer (1:10 wt/vol) (25 mmol/L HEPES, 250 mmol/L sucrose, 2 mmol/L EDTA, 0.1% Triton X-100, and 1 tablet per 50 mL Complete Protease Inhibitor Cocktail Tablets [Roche Diagnostics], pH 7.4). The homogenate was centrifuged at 15000*g* (4°C) for 25 minutes. The protein concentration of the supernatant was determined by the use of a protein determination kit (Bio-Rad). The muscle homogenate was used for Western blotting to determine the SIRT1, PGC-1α, and GLUT4 protein contents and hexokinase activity. For Western blotting, the muscle protein homogenate was solubilized in sample loading buffer as described above.

2.4. Gel electrophoresis and Western blotting

The proteins (20 μg) of these homogenates were separated by SDS polyacrylamide gel electrophoresis using 5% (phospho- and total ACC), 7.5% (SIRT1 and PGC-1α), and 10% (GLUT4 and phospho- and total AMPKα) resolving gels. The proteins separated by SDS polyacrylamide gel electrophoresis were then electrophoretically transferred onto the polyvinylidene difluoride membrane. The membrane was incubated with a blocking buffer of casein solution (SP-5020; Vector Laboratories, Burlingame, CA) for 1 hour at room temperature. The membrane was reacted with affinity-purified rabbit polyclonal antibody to phospho-AMPKα (Thr^{172}; 1:500 dilution, #2532, Cell Signaling, Beverly, MA), total AMPKα (1:1000 dilution, #2531S, Cell Signaling), phospho-ACC (Ser^{79}; 1:500 dilution, #3661, Cell Signaling), total ACC (1:500 dilution, #3662, Cell Signaling), Sir2 (1:1000 dilution, #07-131, Upstate Biotechnology, Lake Placid, NY), PGC-1α (1:500 dilution, AB3242, Chemicon International, Temecula, CA), or GLUT4 (1:8000 dilution, AB1346, Chemicon International) overnight at 4°C and then was incubated with biotinylated anti-rabbit/mouse immunoglobulin G (1:1000 dilution, BA-1400, Vector

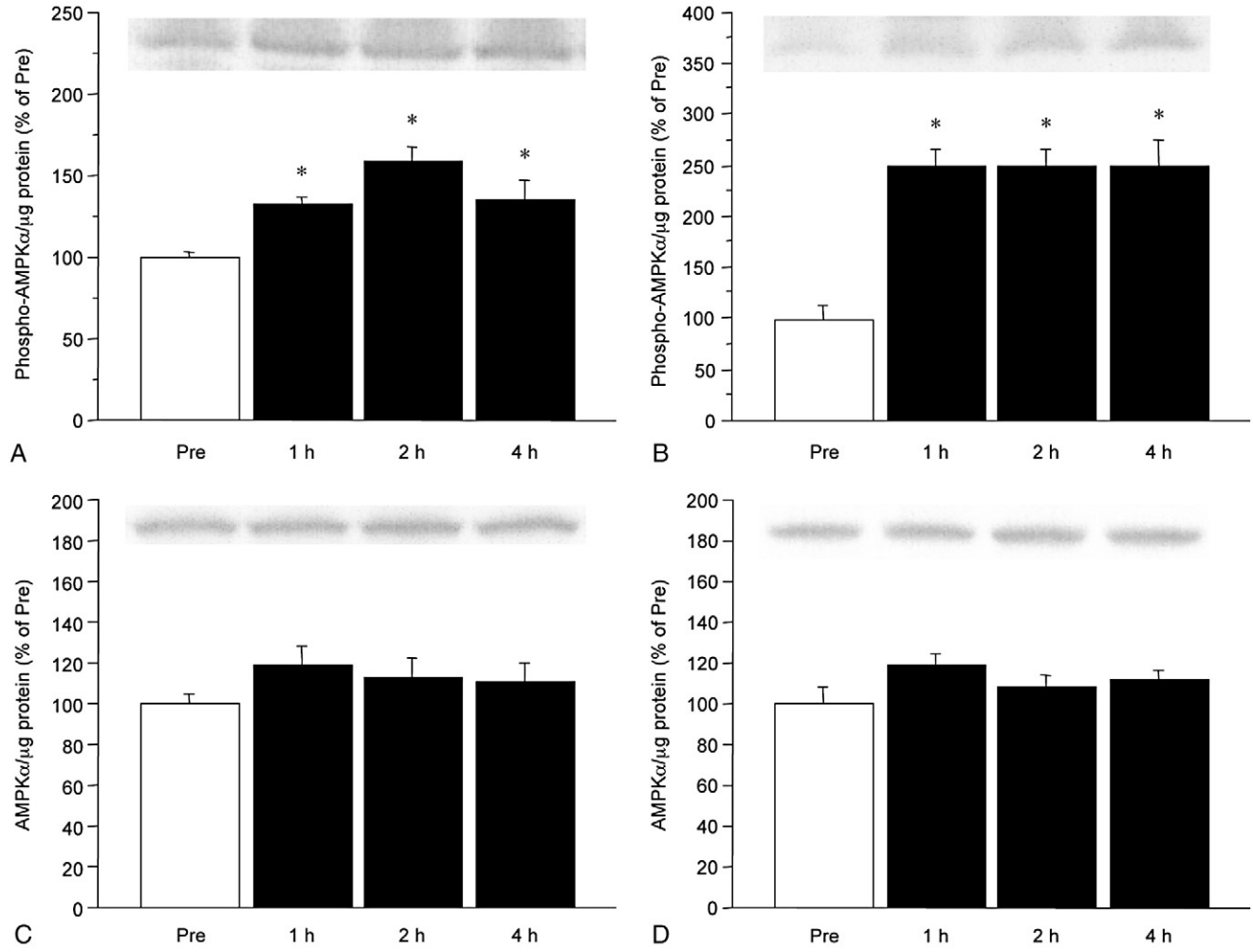

Fig. 1. Phospho- and total AMPKα protein expression in the soleus and EDL muscles before and 1, 2, and 4 hours after AICAR treatment. A and B, Phospho-AMPKα in soleus and EDL muscles, respectively. C and D, Total AMPKα in soleus and EDL muscles, respectively. Values are the means ± SE; n = 12 muscles per group. *$P < .05$ vs pre.

Laboratories) for 30 minutes. The band on the membrane was visualized by avidin and biotinylated horseradish peroxidase macromolecular complex technique (PK-6100, Vector Laboratories). The band densities were determined using the Image 1.62 software package (National Institute of Health, Bethesda, MD).

2.5. Hexokinase activity

The hexokinase activity was measured spectrophotometrically. The enzymatic assay was carried out at 30°C using saturating concentrations of substrates and cofactors as determined in preliminary analyses. The hexokinase activity was measured at 340 nm by following the production of reduced form of beta-nicotinamide adenine dinucleotide phosphate (NADPH) for 3 minutes. The extinction coefficient for NADPH, which is a reference of the hexokinase activity, was 6.22. For the hexokinase assay, 100 mmol/L Tris-HCl, 0.4 mmol/L beta-nicotinamide adenine dinucleotide phosphate (NADP), 5 mmol/L $MgCl_2$, 700 U/mL glucose-6-phosphate dehydrogenase, 1 mmol/L glucose (omitted for the measurement of nonspecific activity), and 5 mmol/L adenosine triphosphate (omitted for the measurement of nonspecific activity), pH 7.0, were used.

2.6. Statistical analysis

All data are expressed as the means ± SE. To estimate the time course of the protein expressions and hexokinase activity with AICAR treatment, we used the 1-way analysis of variance. Dunnett post hoc test was conducted if the analysis of variance indicated a significant difference. The unpaired *t* test was used to compare the saline and AICAR groups. A value of $P < .05$ was considered to be significant.

3. Results

3.1. AMPK and ACC protein phosphorylation

Fig. 1 shows the change in the phosphorylated and total AMPKα protein expression after an AICAR treatment. In the

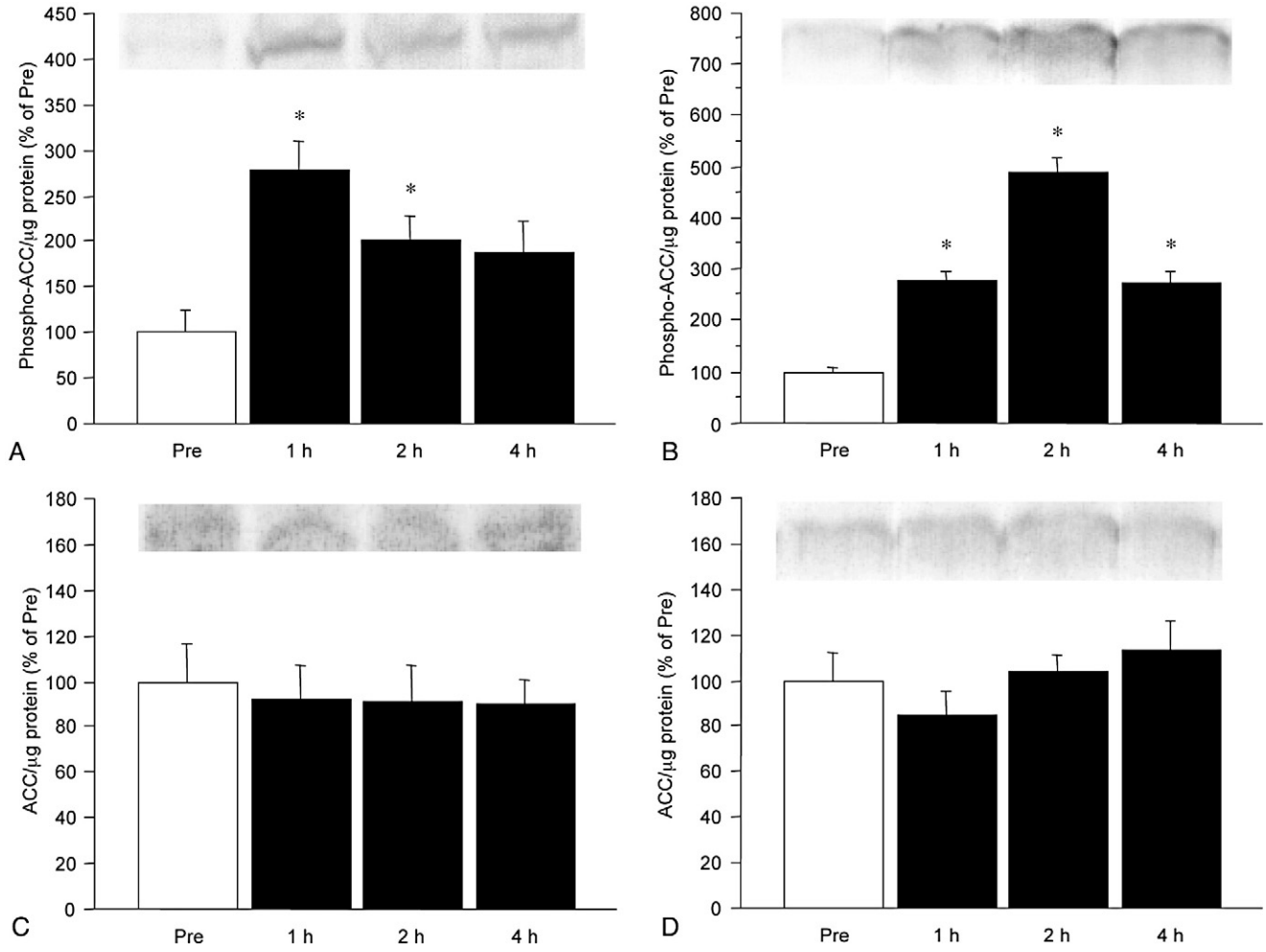

Fig. 2. Phospho- and total ACC protein expression in soleus and EDL muscles before and 1, 2, and 4 hours after AICAR treatment. A and B, Phospho-ACC in soleus and EDL muscles, respectively. C and D, Total ACC in soleus and EDL muscles, respectively. Values are the means ± SE; n = 12 muscles per group. *$P <$ .05 vs pre.

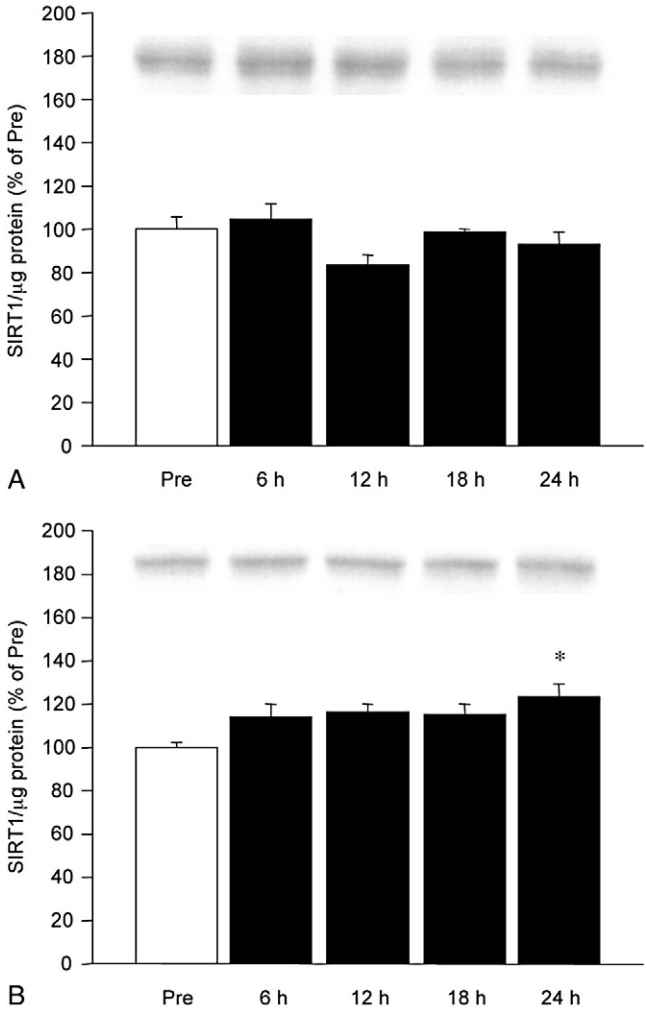

Fig. 3. SIRT1 protein expression in the soleus (A) and EDL (B) muscles before and 6, 12, 18, and 24 hours after AICAR treatment. Values are the means ± SE; n = 12 muscles per group. *$P < .05$ vs pre.

soleus muscle, the phosphorylated AMPKα protein increased at 1, 2, and 4 hours after the AICAR injection from the preinjection period (Fig. 1A; +32%, +59%, and +36%, respectively, from pre; $P < .05$). In the EDL muscle, the phosphorylated AMPKα protein also increased at 1, 2, and 4 hours after the AICAR injection from the preinjection period (Fig. 1B; +150%, +151%, and +150%, respectively, from pre; $P < .05$). Total AMPKα protein expression did not change in the soleus or EDL muscles (Fig. 1C, D).

The effect of AICAR was further examined on the phosphorylation of ACC, a downstream target of AMPK controlling the entry of fatty acids into mitochondrial matrix in skeletal muscle [21]. Fig. 2 shows the change in the phosphorylated and total ACC protein expression after an AICAR treatment. In the soleus muscle, the phosphorylated ACC protein increased at 1 and 2 hours after the AICAR injection from the preinjection period (Fig. 2A; +178% and +101%, respectively, from pre; $P < .05$). In the EDL muscle, the phosphorylated ACC protein also increased at 1, 2, and 4 hours after the AICAR injection from the preinjection period (Fig. 2B; +178%, +392%, and +173%, respectively, from pre; $P < .05$). Total ACC protein expression did not change in the soleus or EDL muscles (Fig. 2C, D).

3.2. SIRT1 protein expression

Fig. 3 shows the change in the SIRT1 protein expression after an AICAR administration. In the soleus muscle, no changes were observed after the treatment (Fig. 3A). In the EDL muscle, the SIRT1 protein increased (+24%) at 24 hours after the treatment from the pretreatment period (Fig. 3B, $P < .05$). In addition, the SIRT1 protein expression in the EDL muscle at 24 hours after the AICAR treatment was significantly higher than that in the saline treatment (Table 1, $P < .05$).

3.3. PGC-1α protein expression

Fig. 4 shows the change of the PGC-1α protein expression after an AICAR administration. The PGC-1α protein increased at 24 hours after an AICAR administration from the pretrial period in both the soleus (Fig. 4A) and EDL (Fig. 4B) muscles (+21% and +26%, respectively, from pre; $P < .05$). In addition, the PGC-1α protein expression in both the soleus and EDL muscles at 24 hours after the AICAR treatment was significantly higher than that in the saline treatment (Table 1, $P < .05$).

3.4. GLUT4 protein expression

Fig. 5 shows the change in the GLUT4 protein expression after an AICAR administration. In the soleus muscle, no changes were observed after the treatment (Fig. 5A). In the

Table 1
Skeletal muscle protein expression and hexokinase activity 24 hours after either saline or AICAR administration

	Soleus muscle		EDL muscle	
	Saline	AICAR	Saline	AICAR
SIRT1 (% of saline)	100.0 ± 1.8	104.1 ± 2.5	100.0 ± 6.2	117.6 ± 2.1*
PGC-1α (% of saline)	100.0 ± 6.0	116.3 ± 3.4*	100.0 ± 6.7	122.0 ± 8.1*
GLUT4 (% of saline)	100.0 ± 4.1	102.5 ± 5.9	100.0 ± 6.9	137.0 ± 5.8*
Hexokinase activity (μmol L^{-1} g^{-1} min^{-1})	2.02 ± 0.07	2.33 ± 0.07*	2.49 ± 0.07	3.45 ± 0.11*

Data are expressed as the mean ± SE; n = 12 muscles per group.
* $P < .05$ vs saline-treated group.

EDL muscle, the GLUT4 protein increased (+38%) at 24 hours after the treatment from the pretreatment period (Fig. 5B, $P < .05$). In addition, the GLUT4 protein expression in the EDL muscle at 24 hours after the AICAR treatment was significantly higher than that in the saline treatment (Table 1, $P < .05$).

3.5. Hexokinase activity

Fig. 6 shows the change in the hexokinase activity after an AICAR administration. In the soleus muscle, the hexokinase activity increased at 18 and 24 hours after an AICAR administration from the pretrial period (Fig. 6A; +12% and +12%, respectively, from pre; $P < .05$). In the EDL muscle, the activity increased at 12, 18, and 24 hours after an AICAR administration from the pretrial period (Fig. 6B; +24%, +36%, and +30%, respectively, from pre; $P < .05$). In addition, the hexokinase activity in both the soleus and EDL muscles at 24 hours after the AICAR treatment was significantly higher than that in the saline treatment (Table 1, $P < .05$).

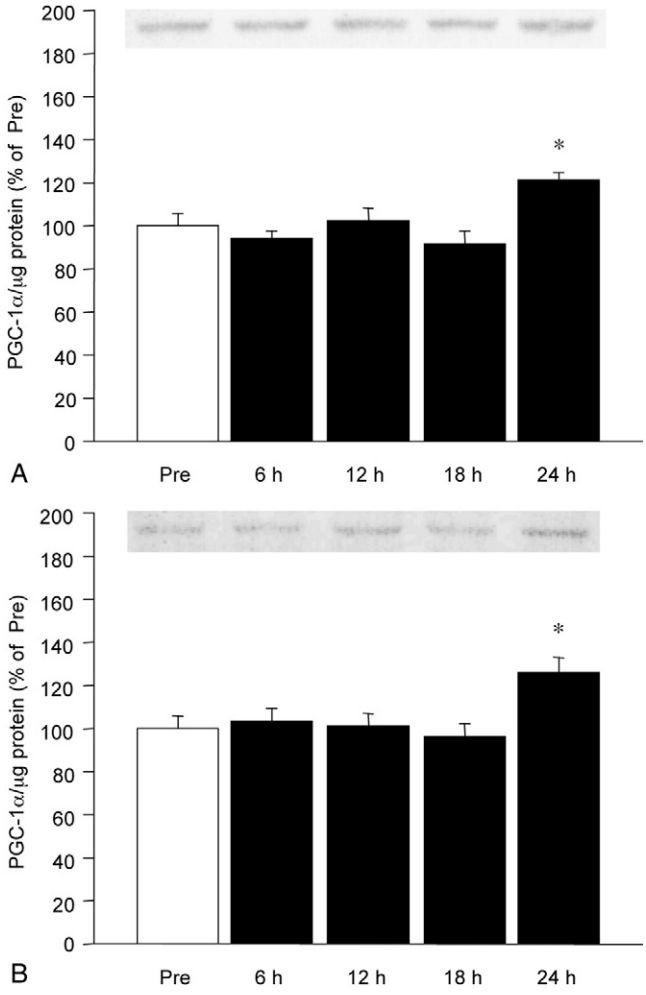

Fig. 4. PGC-1α protein expression in the soleus (A) and EDL (B) muscles before and 6, 12, 18, and 24 hours after AICAR treatment. Values are the means ± SE; n = 12 muscles per group. *$P < .05$ vs pre.

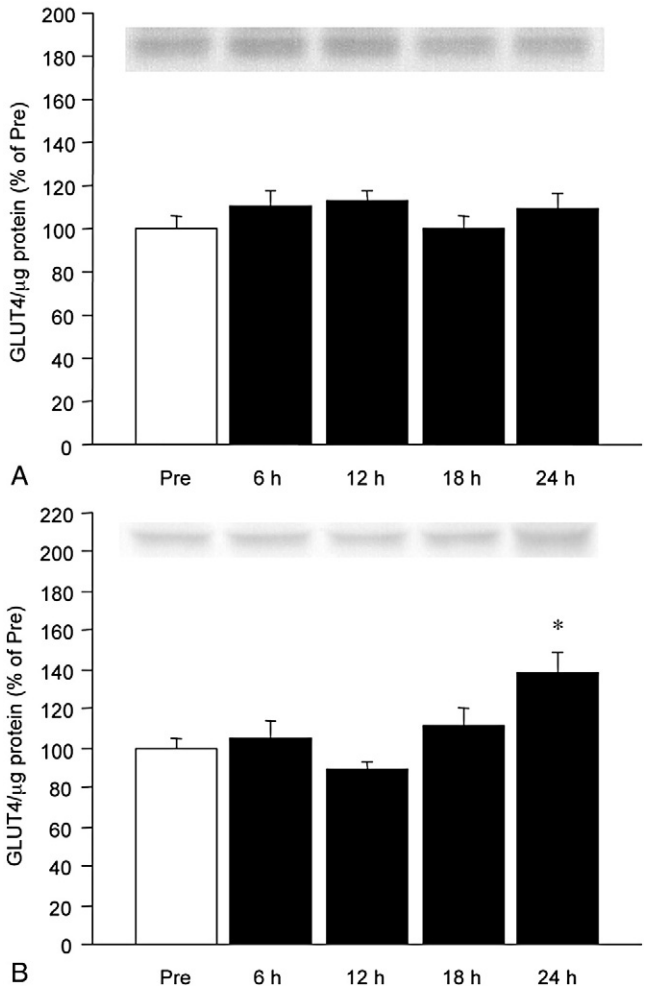

Fig. 5. GLUT4 protein expression in the soleus (A) and EDL (B) muscles before and 6, 12, 18, and 24 hours after AICAR treatment. Values are the means ± SE; n = 12 muscles per group. *$P < .05$ vs pre.

4. Discussion

The current study demonstrated that the activation of AMPK with AMPK activator AICAR treatment in vivo increases the SIRT1 protein expression in the rat EDL muscle. The AMPK phosphorylation level in human hepatoma cell line HepG2 is associated with the SIRT1 protein level [32]. Incubation of HepG2 cells in a high-glucose medium (25 mmol/L) decreases the phosphorylation of AMPK and its downstream target ACC with parallel decline of SIRT1 protein level in comparison to that in low-glucose medium (5 mmol/L). In contrast, incubation of HepG2 cells with pyruvate (0.1 or 1 mmol/L) increases the phosphorylation of AMPK and ACC and SIRT1 protein content. These results suggest that AMPK controls SIRT1 protein content.

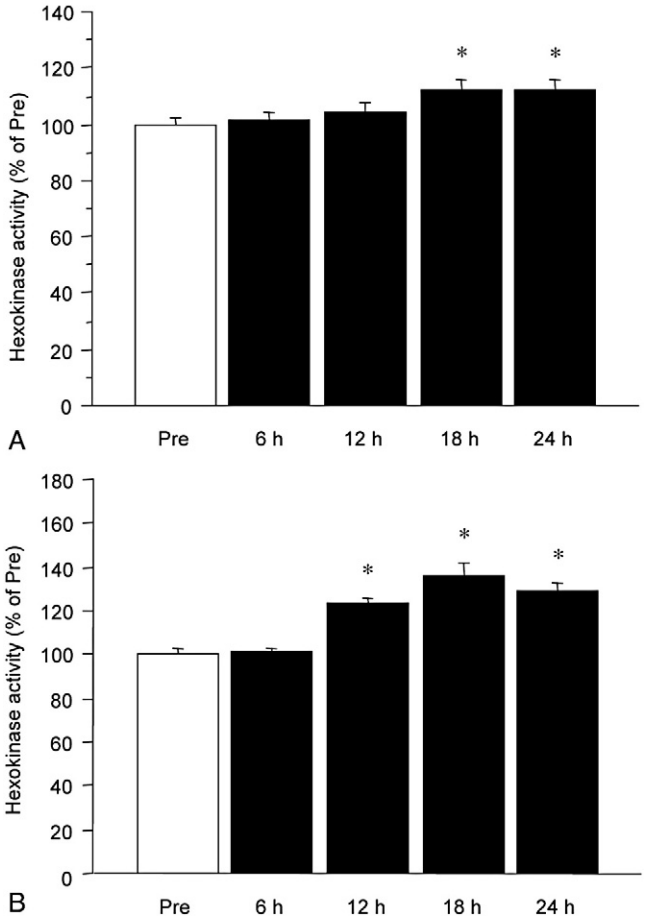

Fig. 6. Hexokinase activity in the soleus (A) and EDL (B) muscles before and 6, 12, 18, and 24 hours after AICAR treatment. Values are the means ± SE; n = 12 muscles per group. *$P < .05$ vs pre.

The effects of AICAR treatment to animals seem similar to those of endurance exercise training with regard to glucose uptake, mitochondrial fatty acid oxidation, and mitochondrial and GLUT4 biogenesis in skeletal muscle [10]. The endurance exercise increased the skeletal muscle SIRT1 protein expression [29]. Consequently, the results regarding SIRT1 in the current study further suggest that the AICAR treatment mimics the benefits of endurance exercise. In skeletal muscle cells, SIRT1 plays an important role in metabolic adaptations including mitochondrial biogenesis, fatty acid oxidation, and glucose homeostasis through deacetylation of PGC-1α [7-9]. Collectively, these observations raise the possibility that the AMPK-SIRT1-PGC-1α pathway may, in part, contribute to the metabolic adaptations with endurance exercise training in skeletal muscle.

However, AMPK may not be the only way to regulate the SIRT1 expression with exercise. The ablation of the AMPK activity experiments using AMPK dominant negative or AMPKα2 knockout mice models demonstrates that AMPK is not always essential for the regulation of downstream targets including ACC, fatty acid oxidation, mitochondrial biogenesis, or the glucose metabolism [33-35], thus suggesting that the redundant signaling pathways cooperate with AMPK in many kinds of adaptations and that signaling other than AMPK may compensate for such metabolic characteristics in the AMPK ablation state. To elucidate the mechanisms, other than AMPK, which regulate the SIRT1 expression with exercise, further experiments using AMPK ablation animal models subjected to various types of exercise are thus called for.

The mechanisms underlying the increase of SIRT1 protein content with AICAR treatment are unclear at present. One potential mechanism for this phenomenon is that nitric oxide synthase (NOS) mediates the SIRT1 expression after an AICAR treatment. The AMPK-induced skeletal and cardiac muscle glucose uptake depends on NOS [36]. In addition, AMPK seems to enhance the NOS activity and phosphorylation of endothelial NOS at Ser^{1177} [36,37]. The level of expression and phosphorylation of endothelial NOS is associated with SIRT1 expression in endothelial cells [38,39]. Furthermore, long-term treatment of NOS inhibitor N^G-nitro-L-arginine-methyl ester decreases the skeletal muscle SIRT1 protein content (M Suwa and S Kumagai, unpublished observation). Overall, it is likely that increasing SIRT1 protein expression with AICAR treatment is mediated by NOS. However, other studies have demonstrated that NOS inhibition does not affect the AICAR- or contraction-induced glucose uptake in rat skeletal muscle [40,41]. Further studies are necessary to clarify the mechanisms in the increase of skeletal muscle SIRT1 dependent on NOS after AMPK activation.

In the current study, the SIRT1 protein expression in the EDL muscle increased with AICAR treatment but not in the soleus. In addition, other characteristics examined in this study indicate inconsistent results between EDL and soleus muscles. The GLUT4 protein expression significantly increased with AICAR in the EDL muscle but not in the soleus muscle. In the hexokinase activity, AICAR treatment also seems more effective to the EDL than soleus muscle. The increase of AMPK phosphorylation level with AICAR in the EDL (~+150% from pre) seems greater than that in soleus (+32%-59% from pre) as well as ACC phosphorylation level (EDL, +173%-391%; soleus, +89%-179%; from pre), raising the possibility that such difference in the effect of AICAR against the AMPK phosphorylation partially causes the different results between soleus and EDL muscles. Another potential cause for such differences in regard to AICAR treatment is the difference in the AMPK subunit isoform distribution between muscle fiber types. The soleus muscle possesses dominantly slow-twitch type I fibers (type I, 84%; type IIA, 7%; type IIX, 9%; type IIB, 0%), whereas EDL muscle possesses dominantly fast-twitch type II fibers (type I, 4%; type IIA, 20%; type IIX, 38%; type IIB, 38%) in rats [42]. In rodents, the γ3-subunit of AMPK is dominantly expressed in the fast-twitch muscle in comparison to the slow-twitch muscle [43]. The γ3-containing AMPK complexes contain only α2- and β2-subunits [43], thus suggesting that α2/β2/γ3 heterotrimer preferentially expressed in the

fast-twitch muscle. Because α2- and β3-subunits play an important role for metabolic and contractile properties in skeletal muscle [44-46], it is likely that the different effects between soleus and EDL muscles on AMPK activation observed in this study are, at least in part, attributable to such differences in the subunit expression pattern between muscle fiber types.

The current study demonstrated that short-term AICAR treatment to rats promotes the skeletal muscle SIRT1 protein expression. On the other hand, a previous study has shown that long-term AICAR treatment to rats for 5 successive days decreases (white gastrocnemius and red and white tibialis anterior muscles) or fails to change (heart and red gastrocnemius muscles) the SIRT1 protein expression [47]. In addition, AICAR treatment for 14 successive days does not alter the SIRT1 protein expression in the rat red and white gastrocnemius muscles (M Suwa and S Kumagai, unpublished observation). These observations suggest that the effect of AICAR treatment on SIRT1 protein expression may thus differ depending on the treatment period. The SIRT1 transcription is regulated by the transcriptional factors E2F transcriptional factor 1 and hypermethylated in cancer 1 [48]. SIRT1 binds to these transcriptional factors, and the complexes repress its transcription [49,50]. This negative feedback loop in SIRT1 regulation might be at least partially associated with the inconsistent results observed among the different treatment period.

Although several previous studies have demonstrated that long-term AICAR treatment enhances the PGC-1α and GLUT4 protein expression and hexokinase activity in the skeletal muscles of rodents in vivo [23,24], the present study is the first to demonstrate that short-term administration of AICAR to rats also promotes them. These results suggest that only a single AICAR treatment is sufficient to promote such phenotypes. Previous studies have demonstrated that short-term endurance exercise augments the PGC-1α and GLUT4 expression and the hexokinase activity and expression [51-53]. These short-term exercise–induced changes may be at least partially associated with AMPK.

Several observations may explain the mechanisms in such changes with AICAR treatment. The PGC-1α and hexokinase II genes have a cyclic AMP–response element, and their transcription is thought to be controlled by the transcriptional factor cyclic AMP–response element binding protein [54-56]. The GLUT4 transcription is regulated by the transcriptional factors myocyte enhancer factor 2 and GLUT4 enhancer factor [57,58]. All these transcriptional factors are phosphorylated and/or transcriptionally activated by AMPK [55,59]. Presumably, such mechanisms are the possible causes for the increase in PGC-1α and GLUT4 expression and hexokinase activity with short-term AICAR treatment.

SIRT1 is associated with insulin sensitivity [7], insulin [60] and adiponectin [61] secretion, mitochondrial biogenesis, fatty acid oxidation [9], protection of neurodegenerative disorders, [62], and longevity [7]. The current study contributes to the understanding of the role of AMPK in the regulation of SIRT1 protein expression and further supports the strategies aimed to activate AMPK as a means of improving the outcome of chronic diseases.

In summary, these results show that short-term AMPK activator AICAR treatment to rats enhances the skeletal muscle AMPK and ACC phosphorylation and then coincidently increases the SIRT1 protein expression. The PGC-1α and GLUT4 protein expression and hexokinase activity also increases with AICAR treatment. Some of these changes preferentially occur in fast-twitch EDL muscles. Therefore, the observations in this study may provide new insights into the mechanisms of SIRT1 regulation and thereby help in both the prevention of and therapy for some chronic diseases including insulin resistance, type 2 diabetes mellitus, metabolic syndrome, and neurodegenerative disorders.

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