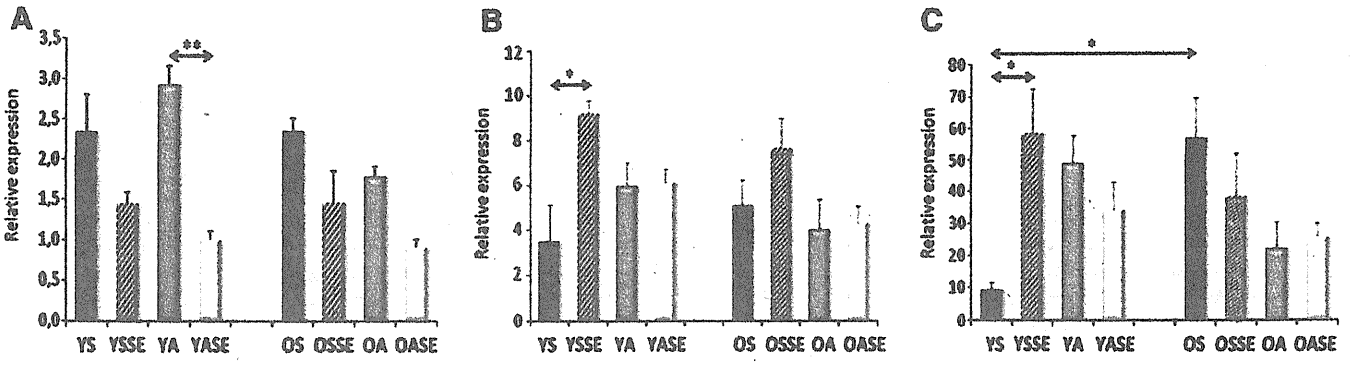

Fig. 3. Effect of SEB on expression of (A) Ku70, (B) Cu,Zn-superoxide dismutase (SOD), and (C) Mn-SOD. RNA was isolated from muscle biopsies excised before and 24 h after SEB. Quantitative RT-PCR was carried out as described under Materials and methods. YS, young sedentary; YSSE, young sedentary after a single bout of exercise; YA, young active; YASE, young active after a single bout of exercise; OS, old sedentary; OSSE, old sedentary after a single bout of exercise; OA, old active; and OASE, old active after a single bout of exercise. Values are means ± SE for six subjects per group. $^{*}p<0.05$, $^{**}p<0.01$.

preexercise levels in physically active individuals, both OA and YA, its level in DNA remained high in sedentary young and old subjects after a 24-h recovery period (Fig. 1A). For example, 8-oxoG levels were approximately four times higher in untrained older (Fig. 1A) compared to younger individuals without SEB (Fig. 1A). Importantly, there was no change in genomic 8-oxoG levels in muscle biopsies of OA individuals after SEB (Fig. 1A).

The subphysiological level of genomic 8-oxoG in physically active subjects suggested an efficient repair of DNA. We observed that OGG1 levels did not significantly change in younger subjects, but they increased in the older subjects in response to SEB (Fig. 1B). In contrast, Ac-OGG1 levels were significantly increased in younger individuals, whereas in the older subjects no significant change was observed in response to SEB. Ac-OGG1 level was approximately threefold higher in active compared to older, sedentary individuals (Figs. 1E and C). SEB did not change Ac-APE1 (Fig. 2A), which was similar to APE1 levels (data not shown), suggesting that neither Ac-APE1 nor APE1 is limiting in the repair of 8-oxoG.

In response to SEB, the expression of p300/CBP increased approximately fivefold in the younger subjects, but unexpectedly, it significantly decreased in older subjects (Fig. 3A). If indeed p300/CBP is the acetyltransferase in muscle, these results are in line with the levels of Ac-OGG1 (Figs. 1C and E). In physically active subjects SEB did not significantly alter p300/CBP levels (Fig. 2B). Expression of the deacetylase SIRT1 showed a significant increase only in younger sedentary subjects in response to SEB (Fig. 2C). The expression of SIRT3, which has no deacetylase activity, was the highest in muscle biopsies of active, younger subjects (Fig. 2D), and its expression did change upon SEB (Fig. 2D). SIRT6 expression (Fig. 2E), along with Ku70 (Fig. 3A), decreased in both young and old muscles after SEB. Together these data suggest that a physically active lifestyle induces an adaptive response by generating mild oxidative stress and prevents the age-associated increase in genomic 8-oxoG levels possibly due to the age-independent increase in OGG1's acetylation.

Discussion

Age-related and physical exercise-associated changes in DNA damage levels in skeletal muscle of experimental animals have been reported previously [13,14,48]. This study analyzed levels of 8-oxoG in DNA and the abundance of rate-limiting BER enzymes in human muscle biopsies before and after a single exercise bout. We also examined expression of acetyltransferases and deacetylases linked to DNA repair pathways and antioxidant genes that could reflect on cellular redox conditions. We show that the genomic 8-oxoG level is lastingly elevated in sedentary young and old subjects, but it returned rapidly to preexercise levels in physically active individuals independent of age upon a single exercise bout. The 8-oxoG level in DNA inversely correlated with the abundance of Ac-OGG1, but not with total OGG1, APE1, or Ac-APE1. Importantly, our data also demonstrate a physical activity-dependent increase in the acetylated forms of OGG1 in human skeletal muscle. Accordingly, it is possible that an exercise-induced acetylation pathway would enhance OGG1 activity, not only in muscles, but in other tissues, and thereby exercise may decrease the incidence of various pathological conditions, such as inflammation, that have been linked to carcinogenesis, cardiovascular diseases, strokes, or Alzheimer disease.

8-oxoG is arguably one of the important forms of DNA base damage induced by ROS, and it has been proposed to play a role in the aging process and is also linked to age-associated diseases [1–3,5]. This hypothesis is consistent with the severalfold increase in 8-oxoG (and possibly of other oxidized bases) content in nuclear and mtDNA from aged tissues [1–3,5]. A single bout of exercise has been shown to cause mild oxidative stress [32,49,50], and thus we applied a SEB and determined cellular oxidative states, changes in 8-oxoG levels, and abundance of selected repair enzymes. Because of a limited amount of muscle biopsies, we used quantitative fluorescence analysis [36,38,41] to assess 8-oxoG levels, as the quantity of DNA isolated did not allow us to use HPLC with electrochemical detection [7,8], which would provide a better estimates. By using a highly specific, anti-8-oxodG-specific antibody, we observed significantly higher levels of genomic 8-oxoG in human skeletal muscle of sedentary, older individuals compared to the levels in younger subjects, in line with previous observations [13,14,43,44]. In response to SEB-induced ROS, 8-oxoG levels increased further and were not repaired, even after a 24-h period, in sedentary individuals, independent of age. In contrast, 8-oxoG levels returned to preexercise levels in physically active individuals, a finding that may mean regular physical activity could prevent accumulation and/or increase repair efficacy of 8-oxoG and possibly other bases in DNA human skeletal muscle.

The observed increase in 8-oxoG levels in sedentary individuals points to a possible age-dependent decrease in levels of OGG1. In contrast, our data show a significantly increased OGG1 level in elderly subjects and, interestingly, SEB furthered its level. Unexpectedly, the 8-oxoG level was also enhanced. These paradoxical observations suggested to us that OGG1 may have a low DNA glycosylase/AP lyase activity or that BER activities are significantly lower in aged human muscle. Indeed, a recent publication documents decreased overall BER activities in both the nuclei and the mitochondrial extracts from skeletal muscles, compared to those from liver or kidneys of the same mice [51]. Although decreased overall BER activity could be a possibility, our data also imply that a lack of or delayed repair of 8-oxoG could be linked to a deficiency in posttranslationally modified OGG1 in aged muscles. Indeed, OGG1's glycosylase/AP-lyase activity is

modulated via acetylation, phosphorylation, and redox [23,25]. For example, OGG1 is acetylated on lysines 338 and 341 and has an approximately 10-fold increase in its 8-oxoG excision activity compared to unacetylated OGG1 [23]. To explore this possibility we show that approximately one-fifth of OGG1 is in an acetylated form in younger individuals and, importantly, Ac-OGG1 was nearly undetectable in the sedentary elderly. This observation is a feasible possibility, as 8-oxoG level in DNA was inversely correlated with levels of Ac-OGG1 in muscles of young and old individuals.

Repair of 8-oxoG is initiated by OGG1 during the BER pathway, followed by APE1-mediated cleavage of the DNA strand at the abasic site. After removal of this 3′-blocking group, the single-nucleotide gap is filled in by a DNA polymerase, and DNA ligase seals the nick to restore DNA integrity [17]. It has also been shown that OGG1 remains tightly bound to its AP product after base excision, and APE1 prevents its reassociation with its product, thus enhancing OGG1 turnover [45]. Accordingly, APE1 is considered to be rate-limiting in the BER of 8-oxoG [17,39]. However, neither APE1 nor Ac-APE1 showed significant changes with aging and/or physical activity. Therefore, it may be proposed that the Ac-OGG1 is limiting in the repair of 8-oxoG lesions in human skeletal muscle during BER processes. As modification by phosphorylation substantially alters the incision activity of only OGG1 [24], our earlier observations of an exercise-induced increase in OGG1 activity in skeletal muscles of human and experimental animals [14,43] may be attributed to Ac-OGG1.

Acetylation levels of OGG1 and APE1 are dependent on the level/activity of the acetyltransferase p300/CBP [23,25] and possibly on a deacetylase(s) such as some of the sirtuins [52]. Results from our studies show that p300/CBP's expression was increased in young individuals by SEB, independent of whether they were sedentary or active. However, we were not able show such consistency in the elderly. SIRT1, a NAD-dependent histone deacetylase [53], has been shown to interact with p300/CBP to regulate its acetyltransferase activity [52]. SIRT1 levels increased in both young and elderly muscles in response to exercise. These observations are in line with the general role of SIRT1 in the DNA damage response and maintenance of genomic integrity, as it promotes proper chromatin structure and DNA damage repair foci formation for repair of DNA base lesions [27,28]; however, the patterns of change in SIRT1 expression in young vs old or sedentary vs physically active suggest an inverse correlation between SIRT1 and the level of Ac-OGG1.

Among sirtuins, only SIRT3 expression correlates with the life span of humans [54]. Interestingly, SIRT3 expression was increased with physical fitness level only in young subjects in this study. SIRT3 has two isoforms with different molecular masses (44 and 28 kDa), which are localized in mitochondria and nucleus, respectively [55]. The translocation of SIRT3 from the nucleus to the mitochondria has been shown to be induced by oxidative stress [55]. SIRT3 is also a modulator of apoptosis [56]. Recent findings also indicate that SIRT3 is a downstream target of PGC-1α and one of the regulators of mitochondrial ROS production [57].

Exercise has been shown to cause mild oxidative stress [32,49,50,58]. Although the 8-oxoG level is a documented measure of such an oxidative insult [14], MDA levels and expression of superoxide dismutase(s) were used to evaluate further SEB-induced oxidative stress. An increase in MDA levels in plasma correlated with genomic 8-oxoG level in both young and old subjects in response to SEB. Interestingly, only the expression of Cu,Zn-SOD showed age-independent and exercise-associated changes, and Mn-SOD expression was increased only in the younger sedentary group. Based on these observations, it appears that Cu,Zn-SOD expression is a better measure of an adaptive response to ROS than that of mitochondrial Mn-SOD. These data also imply a decline in adaptive response with age at the level of Mn-SOD. These observations are in line with those showing that the adaptive capability of an organism to withstand oxidative stress challenge(s) is markedly decreased as a function of age [59,60]. Based on our data, however, we propose that adaptive responses to ROS are not age dependent, but decided by the physical status of an individual.

In conclusion, this investigation offers insight into interactions between aging processes, exercise, and regulation of the repair of oxidized DNA base lesions in human skeletal muscle. We show for the first time that (1) acetylated forms of OGG1 and APE1 are present in human tissues, but (2) only Ac-OGG1 seems to be rate limiting in the BER processes of 8-oxoG, and (3) repair of 8-oxoG seems to be independent of age, but (4) is dependent on the physical state of muscles. Our data also imply that regular exercise induces an adaptive response that involves an improved, more efficient antioxidant and DNA repair machinery.

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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.
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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

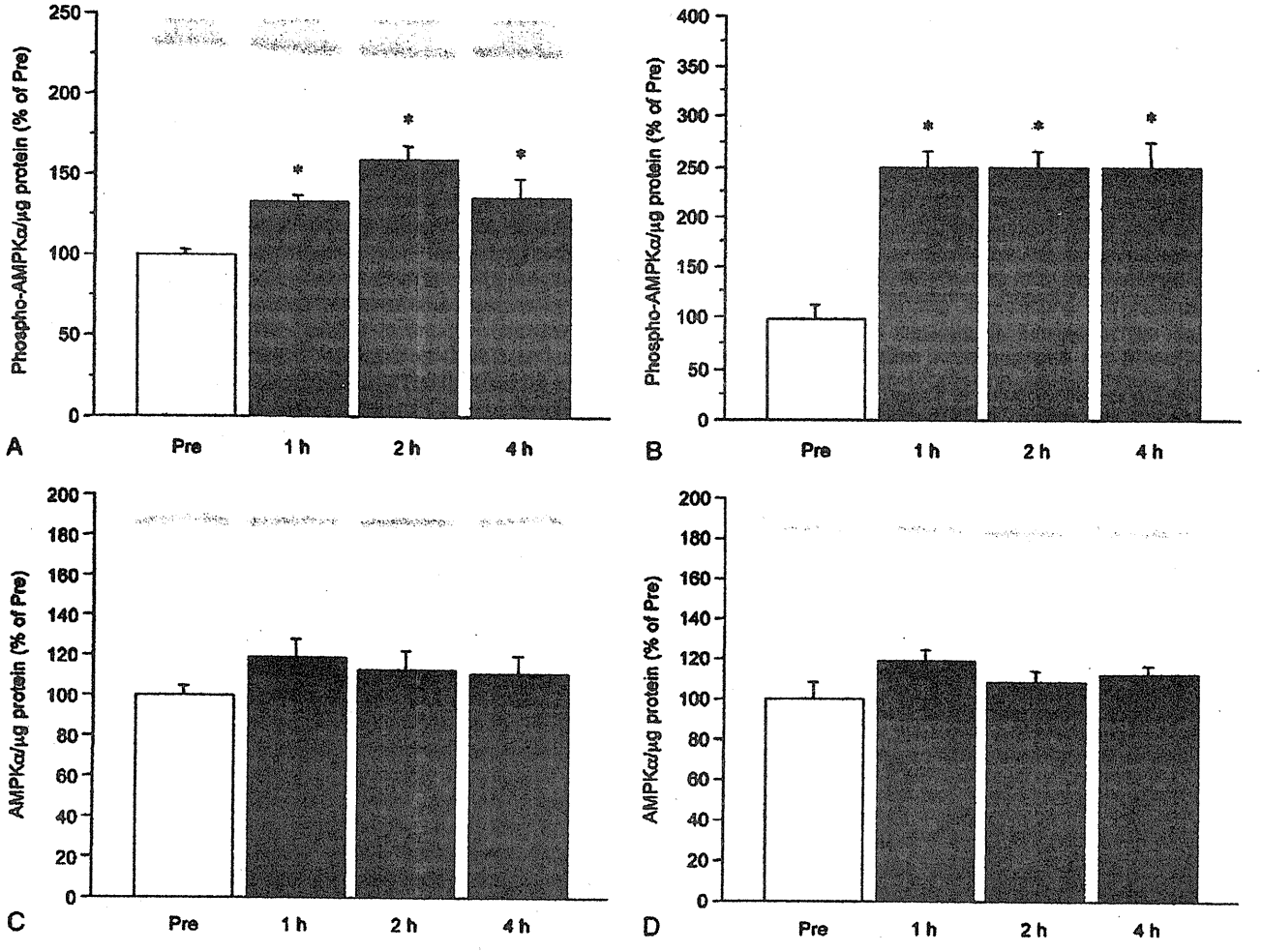

Fig. 1. Phospho- and total AMPKα protein expression in the soleus and EDL muscles before and 1, 2, and 4 hours after AICAR treatment. A and B, Phospho-AMPKα in soleus and EDL muscles, respectively. C and D, Total AMPKα in soleus and EDL muscles, respectively. Values are the means ± SE; n = 12 muscles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

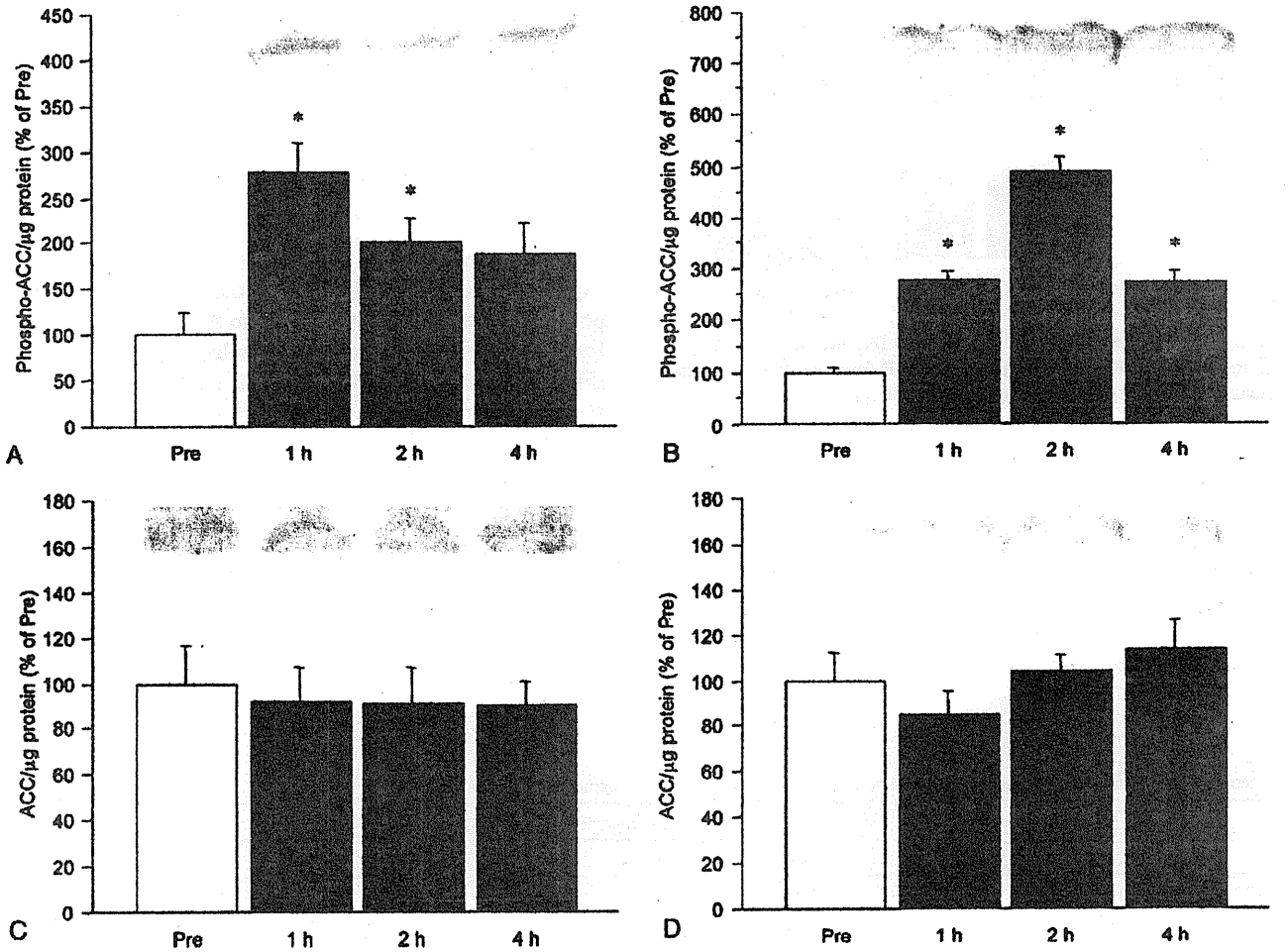

Fig. 2. Phospho- and total ACC protein expression in soleus and EDL muscles before and 1, 2, and 4 hours after AICAR treatment. A and B, Phospho-ACC in soleus and EDL muscles, respectively. C and D, Total ACC in soleus and EDL muscles, respectively. Values are the means ± SE; n = 12 muscles per group.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

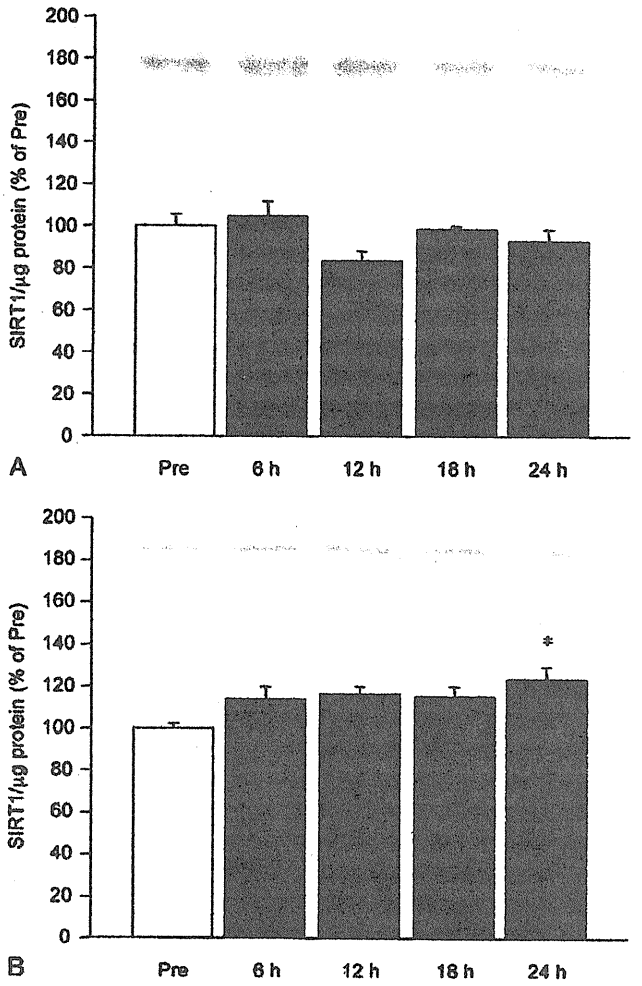

Fig. 3. SIRT1 protein expression in the soleus (A) and EDL (B) muscles before and 6, 12, 18, and 24 hours after AICAR treatment. Values are the means ± SE; n = 12 muscles per group. *$P < .05$ vs pre.

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 ($\mu mol\ L^{-1}\ g^{-1}\ min^{-1}$)	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

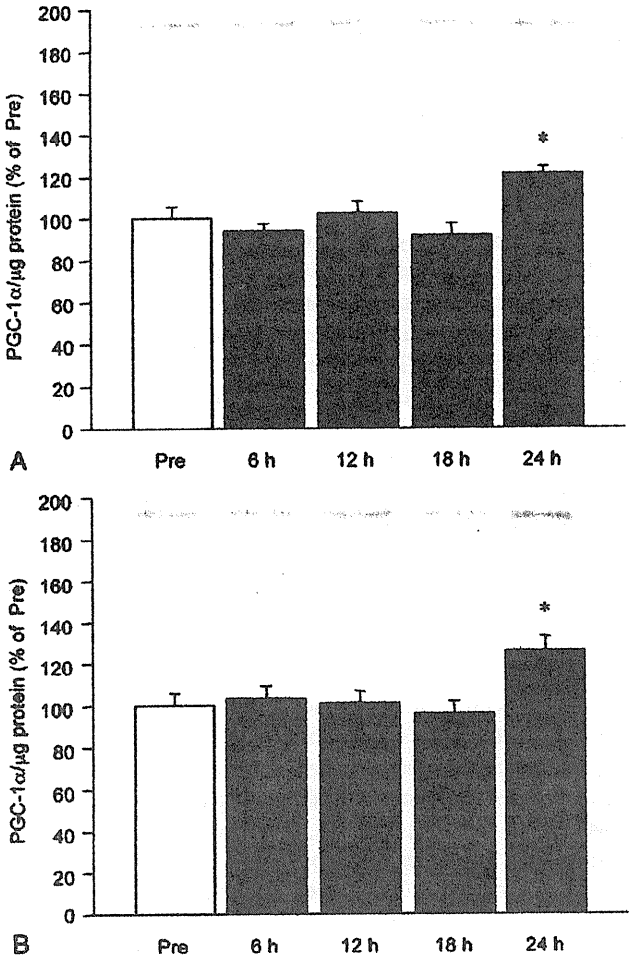

Fig. 4. PGC-1α protein expression in the soleus (A) and EDL (B) muscles before and 6, 12, 18, and 24 hours after AICAR treatment. Values are the means ± SE; n = 12 muscles per group. *$P < .05$ vs pre.

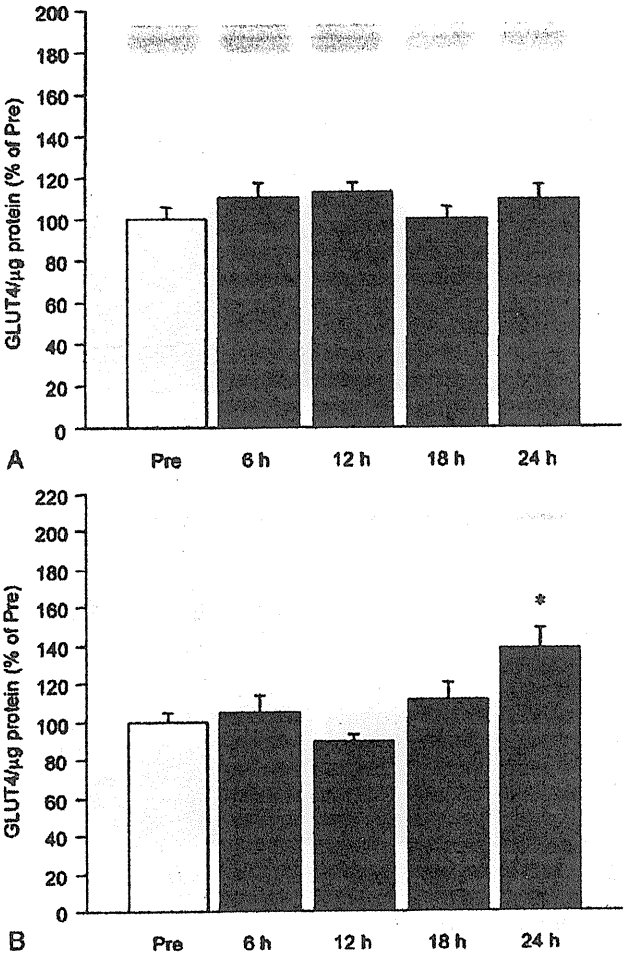

Fig. 5. GLUT4 protein expression in the soleus (A) and EDL (B) muscles before and 6, 12, 18, and 24 hours after AICAR treatment. Values are the means ± SE; n = 12 muscles per group. *$P < .05$ vs pre.

significantly higher than that in the saline treatment (Table 1, $P < .05$).

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.

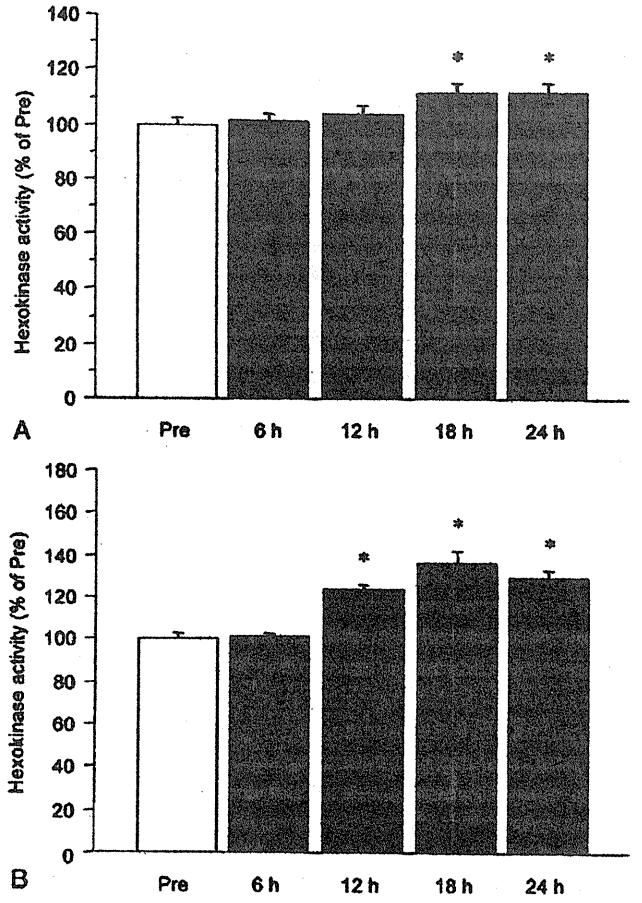

Fig. 6. Hexokinase activity in the soleus (A) and EDL (B) muscles before and 6, 12, 18, and 24 hours after AICAR treatment. Values are the means ± SE; n = 12 muscles per group. *$P < .05$ vs pre.

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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Research article

Different circulating brain-derived neurotrophic factor responses to acute exercise between physically active and sedentary subjects

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Running Head: Circulating BDNF and acute exercise

Abstract

Although circulating brain-derived neurotrophic factor (BDNF) level is affected by both acute and chronic physical activity, the interaction of acute and chronic physical activity was still unclear. In this study, we compared the serum and plasma BDNF responses to maximal and submaximal acute exercises between physically active and sedentary subjects. Eight active and 8 sedentary female subjects participated in the present study. Both groups performed 3 exercise tests with different intensities, i.e. 100% (maximal), 60% (moderate) and 40% (low) of their peak oxygen uptake. In each exercise test, blood samples were taken at the baseline and immediately, 30 and 60 min after the test. The serum BDNF concentration was found to significantly increase immediately after maximal and moderate exercise tests in both groups. In maximal exercise test, the pattern of change in the serum BDNF concentration was different between the groups. While the serum BDNF level for the sedentary group returned to the baseline level during the recovery phase, the BDNF levels for the active group decreased below the baseline level after the maximal exercise test. No group differences were observed in the pattern of plasma BDNF change for all exercise tests. These findings suggest that regular exercise facilitates the utilization of circulating BDNF during and/or after acute exercise with maximal intensity.

Key words: Serum BDNF, plasma BDNF, acute exercise.

Introduction

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors. In addition to its neurotrophic and synaptotrophic actions, such as the promotion of growth and survival of neurons (Aloe and Calza, 2004; Thoenen, 1995) and learning and memory (Ma et al., 1998), BDNF may play important metabotrophic roles such as the regulation of food intake (Xu et al., 2003), glucose and lipid metabolism, and energy homeostasis (Chaldakov, 2011; Nakagawa et al., 2000; Noble et al., 2011; Tsuchida et al., 2002). BDNF is present in the nervous system and peripheral tissues, and is also found in blood (Fujimura et al., 2002; Radka et al., 1996; Rosenfeld et al., 1995). Chronic treatment with subcutaneous BDNF administration significantly decreased food intake and improved the glucose uptake in skeletal muscle (Yamanaka et al., 2007) in diabetic mice, and increased glucose transporter 4 expression in normal mice (Suwa et al., 2010). In humans, the level of circulating BDNF is associated with depression (Duman, 2004), Alzheimer's disease (Tapia-Arancibia et al., 2008), obesity (Suwa et al., 2006), glucose and lipid metabolism (Levinger et al., 2008; Suwa et al., 2006), type 2 diabetes mellitus (Suwa et al., 2006) and metabolic syndrome (Chaldakov et al., 2004). Although it has been generally accepted that the neurotrophins act by paracrine or autocrine mechanisms (Davies, 1996), evidence also indicates that circulating BDNF may exert endocrine action to reveal or execute physiologic functioning.

BDNF is present in human serum and plasma, and is much more concentrated in the serum (Radka et al., 1996). Because more than 90% of blood BDNF is stored in the platelets and is released during the clotting process (Fujimura et al., 2002), serum BDNF seems to reflect both the platelet-stored BDNF and the freely-circulating BDNF in the blood, while plasma BDNF seems to reflect only the freely-circulating *BDNF* (Lommatzsch et al., 2005).

Regular exercise is well known to have many health benefits, including the prevention and improvement of obesity (Wing and Hill, 2001), type 2 diabetes mellitus (Orozco et al., 2008) and Alzheimer's disease (Heyn et al., 2004). Several animal studies have shown that $mRNA^{BDNF}$ and BDNF protein levels increase with acute and chronic voluntary wheel running in the hippocampus (Neeper et al., 1996; Gomez-Pinilla et al., 2011), and improved learning and memory (Vaynman et al., 2004). In addition, the $mRNA^{BDNF}$ and BDNF protein expression levels in skeletal muscle have been shown to be enhanced in response to muscle contraction, which is associated with enhanced lipid oxidation (Matthews et al., 2009). Collectively, these results raise the possibility that BDNF mediates, at least in part, the adaptation to exercise.

There have been several studies examining circulating BDNF responses to acute endurance exercise (Ferris et al., 2007; Gold et al., 2003; Gustafsson et al., 2009; Matthews et al., 2009; Rasmussen et al., 2009; Rojas Vega et al., 2006; Zoladz et al., 2008). In the majority of these studies, serum (Ferris et al., 2007; Gold et al., 2003; Matthews et al., 2009; Rojas Vega et al., 2006) and plasma (Gustafsson et al., 2009; Rasmussen et al., 2009) BDNF levels increased following acute exercise. On the other hand, we (Nofuji et al., 2008) and Chan et al. (2008) showed that regular physical activity affected the resting serum BDNF level. Therefore, it appears that the circulating BDNF level is affected by both acute and chronic

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physical activity. However, the interaction of acute and chronic physical activity was still unclear.

Therefore, the aim of this study was to clarify the effect of chronic physical activity on the circulating BDNF responses to acute exercise. In the present study, we simultaneously measured the serum and plasma BDNF concentrations before and after three exercise tests with different intensities for the physically active and sedentary subjects.

Methods

Subjects

Eight physically active and 8 sedentary female Japanese subjects participated in this study. "Active" was defined as performing regular sports activities more than 3 times per week for more than 3 years. The active group included distance runners (n = 3), basketball players (n = 3), and badminton players (n = 2). The sedentary subjects had not performed any regular exercise for at least 1 year. All participants were non-smokers, free from any diseases, and not taking any medications. This study was conducted in accordance with the Declaration of Helsinki, and approved by the ethics committee of the Institute of Health Science, Kyushu University, Fukuoka, Japan. Written informed consent was obtained from all participants prior to their participation.

Exercise tests

All subjects performed 3 different exercise tests in 3 separate days. At Day1, they performed the graded exercise test (maximal) to determine their volume of peak oxygen uptake (VO_2peak). After 15 min of seated rest, the subjects started pedaling at 0 W (for the sedentary group) or 30 W (for the active group). The workload was increased by 30 W in every 4 min until a 4.0 $mmol \cdot L^{-1}$ of blood lactate level was obtained. After that, the workload was increased by 15 W in every 1 min until exhaustion. "The blood for measuring the lactate concentration was obtained from an ear lobe and blood lactate level was measured using the Lactate Pro instrument (Lactate Pro LT-1710, ARKRAY, Kyoto, Japan) in every 3 min and immediately after exercise test." The heart rate (HR) was monitored using an electrocardiogram telemetry system (DS-3140, Fukuda Denshi, Tokyo, Japan). The VO_2 peak was defined as the highest VO_2 obtained during a maximal exercise test.

Two submaximal exercise tests were conducted at Day2 or 3 in random order. Trials consisted of a 30-min cycle ergometry (Monark 828E) at a constant load of 60% (moderate) or 40% (low) of the subject's VO_2peak, preceded by a 15 min of seated rest. The HR and VO_2 were recorded during each exercise test.

The subjects were instructed to refrain from heavy exercise the day before each exercise test. All exercise tests were conducted at 9:00-10:30 to diminish the effect of circadian changes in circulating BDNF levels (Piccinni et al., 2008).

Physical activity level

The daily physical activity level was evaluated with an accelerometer (Lifecorder, Suzuken Co., Nagoya, Japan). This device comprises an acceleration sensor, an amplifier, a microprocessor and memory, and was employed to ensure different physical activity levels between the two groups. All participants attached the accelerometer for 1 week just before the Day1.

Anthropometric measurements

Anthropometric measurements were conducted at Day1. The percentage of body fat was measured by bioelectrical impedance analysis device (Tanita, Tokyo, Japan).

Blood collection and biochemical analysis

In each exercise test, blood samples were taken from an antecubital vein in a sitting position at the baseline time, and immediately, 30 min and 60 min after the exercise. The blood samples were drawn into additive-free containers (serum) or heparinized containers (plasma). After kept at room temperature for 1 hour, the serum samples were centrifuged at 2000 × *g* for 10 min at 4°C. Plasma samples were immediately centrifuged. Supernatants were stored at -80°C until the analyses were performed. The serum and plasma BDNF concentrations were measured using an enzyme-linked immunoassay (ELISA) kit (Promega, Madison, WI).

Statistical analysis

The anthropometric measurements and physiological responses to maximal exercise tests between the active and sedentary groups were compared using Student's unpaired t-test. The comparisons of physical responses during moderate and low exercise tests in each group and serum BDNF level at rest between the groups were performed using the paired t-test. The changes in BDNF responses were assessed by two-way (4 time point × 2 groups) repeated measures analysis of variance (ANOVA). If an interaction was significant, one-way ANOVA was performed. A Dunnett's test was employed for all post-hoc tests. The alpha-level was set at 0.05.

Results

Characteristics of the subjects

The subject characteristics are summarized in Table 1. There were no significant differences in any anthropometric variables between the two groups. The daily physical activity level was significantly higher in the active group compared to the sedentary group ($p < 0.05$).

Table 1. Characteristics of the subjects. The data are expressed as the means (± SD).

	Sedentary	Active
Age (years)	22.8 (1.9)	21.6 (3.0)
Height (m)	1.59 (.06)	162.9 (6.8)
Weight (kg)	50.8 (6.7)	54.5 (7.5)
Body mass index ($kg \cdot m^{-2}$)	20.0 (2.0)	20.5 (1.9)
Body fat (%)	23.6 (5.9)	21.8 (2.0)
Total energy expenditure ($kJ \cdot day^{-1}$)	7451 (793)	8749 (842)**
Moving-related energy expenditure ($kJ \cdot day^{-1}$)	1115 (379)	1970 (640)**
Step count ($steps \cdot day^{-1}$)	10890 (2950)	14961 (4188)*

* $p < 0.05$, ** $p < 0.01$

Table 2. Physical parameters at the end of the low and moderate exercise tests. The data are expressed as the means (± SD).

	Low exercise		Moderate exercise	
	Sedentary	Active	Sedentary	Active
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	10.8 (2.6)*	14.7 (2.4)*	20.0 (4.4)	22.9 (2.7)
$\%VO_2$ (%)	30.8 (5.6)*	35.2 (8.0)*	58.4 (8.9)	54.8 (5.1)
Heart rate (bpm)	99 (12)*	100 (13)*	141 (16)	130 (14)
Workload (W)	39 (11)*	66 (8)*	77(16)	107 (12)

* Significantly different from the moderate exercise ($p < 0.05$)

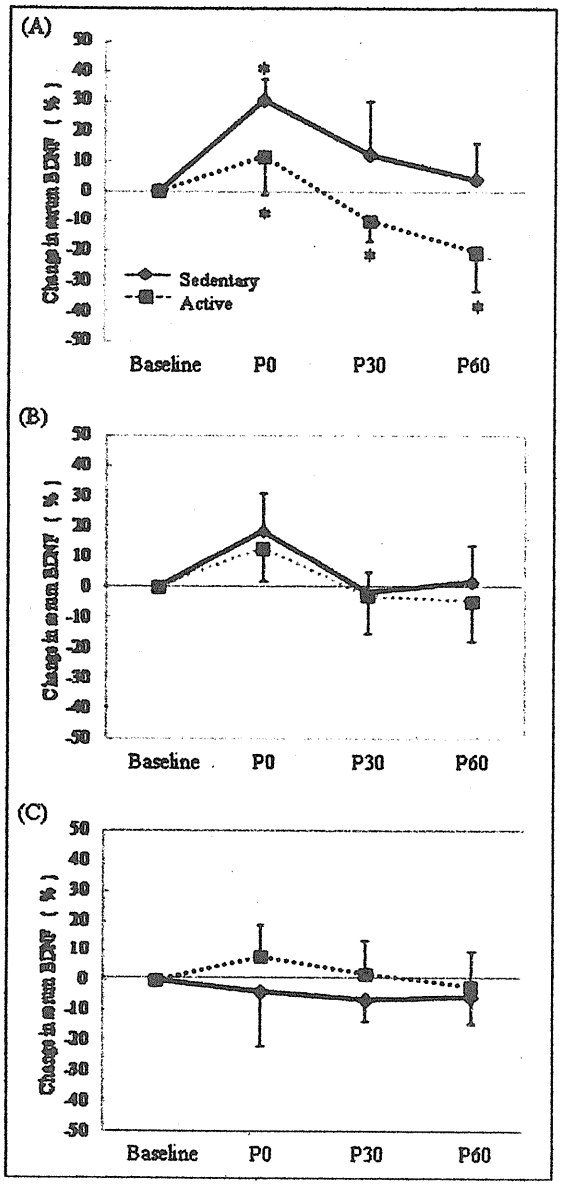

Figure1. Level of serum BDNF concentrations before maximal (A), moderate (B), or low (C) exercise tests (baseline), immediately after (P0), 30 min after (P30), and 60 min after (P60) the exercise session. The data are expressed as the means ± SD. * $p < 0.05$ vs. baseline. The changes in BDNF responses for the groups were assessed by two-way repeated ANOVA. As an interaction and main effect of time were significant, one-way ANOVA followed by a Dunnett's post-hoc test was performed.

Physical parameters in the exercise test

The VO_2peak and workload at the end of maximal exercise in the active group (42.3 ± 4.5 $ml \cdot kg^{-1} \cdot min^{-1}$, 199 ± 16 W, respectively) was significantly higher than that in the sedentary group (34.7 ± 4.0 $ml \cdot kg^{-1} \cdot min^{-1}$, 147 ± 16 W, respectively, < 0.01). There were no significant differences in the HR (Sedentary 183 ± 5 bpm, Active 179 ± 12 bpm, $p = 0.45$) and blood lactate level (Sedentary 9.6 ± 0.8 $mmol \cdot L^{-1}$, Active 8.6 ± 1.4 $mmol \cdot L^{-1}$, $p = 0.14$) between the groups at the end of the maximal exercise test. Table 2 shows the physical parameters for two submaximal exercise tests. All parameters were significantly higher at the moderate exercise test than at the low exercise test. There was no group difference in the average BDNF level at rest (Sedentary; 11.9 $ng \cdot ml^{-1}$, Active; 12.5 $ng \cdot ml^{-1}$, $p = 0.49$).

Change in the serum BDNF concentration

For the maximal exercise test, a two-way ANOVA for repeated measures on serum BDNF levels revealed significant interactions of the factors ($F(3, 42) = 7.01$, $p < 0.01$). A subsequent one-way ANOVA for repeated measures revealed a significant effect of time ($F(3, 45) = 24.8$, $p < 0.01$). The serum BDNF concentrations significantly increased immediately after the maximal exercise test in both groups (Sedentary; +30% $p < 0.01$, Active; +11% $p < 0.01$ vs. baseline, Figure 1A). While BDNF levels in the sedentary group returned to the baseline level during the recovery phase (30 min; +12% $p = 0.06$, 60 min; +4% $p = 0.80$, Figure 1A), the BDNF levels in the active group decreased below the baseline level (30 min; -15% $p < 0.01$, 60 min; -25% $p < 0.01$ vs. baseline, Figure 1A).

For the moderate exercise, neither interactions ($F(3, 42) = 0.68$, $p = 0.57$) nor the effect of groups ($F(1, 14) = 0.86$, $p = 0.37$) on the BDNF response was observed, although the effect of time was significant ($F(3, 42) = 18.7$, $p < 0.01$). The serum BDNF concentrations in both groups increased immediately after the exercise tests (+16%, $p < 0.01$ vs. baseline, Figure 1B) and returned to the baseline level during the recovery phase (30 min; -2% $p = 0.84$, 60 min; -2% $p = 0.94$ vs. baseline, Figure 1B).

The low exercise did not affect the BDNF concentration in either group (time × group; $F(3, 42) = 1.19$, $p = 0.33$, time; $F(3, 42) = 1.17$ $p = 0.33$, group; $F(1, 14) = 4.06$ $p = 0.06$, Figure 1C).

Change in the plasma BDNF concentration

For the maximal and moderate exercise test, interactions ($F(3, 42) = 1.85$, $p = 0.15$, $F(3, 42) = 1.19$, $p = 0.33$, respectively) nor the effect of groups ($F(1, 14) = 1.40$, $p = 0.26$, $F(1, 14) = 0.67$, $p = 0.43$, respectively) on the plasma BDNF response were detected. Although the effect of time were significant ($F(3, 42) = 4.24$, $p = 0.01$, $F(3, 42) = 5.40$, $p < 0.01$, respectively), a subsequent Dunnett's post-hoc test showed no significant difference in plasma BDNF between baseline and each time point

(maximal; 0min +33% p = 0.10, 30min +10% p = 0.87, 60min -9% p = 0.89 vs. baseline, Figure 2A, moderate; 0min +11% p = 0.58, 30min -11% p = 0.59, 60min -19% p = 0.17 vs. baseline, Figure 2B).

No interaction ($F(3, 42) = 0.65$, $p = 0.59$) or main effects (time; $F(3, 42) = 0.77$, $p = 0.52$, group; $F(1, 14) = 0.03$, $p = 0.86$, Figure 2C) were found in the low exercise.

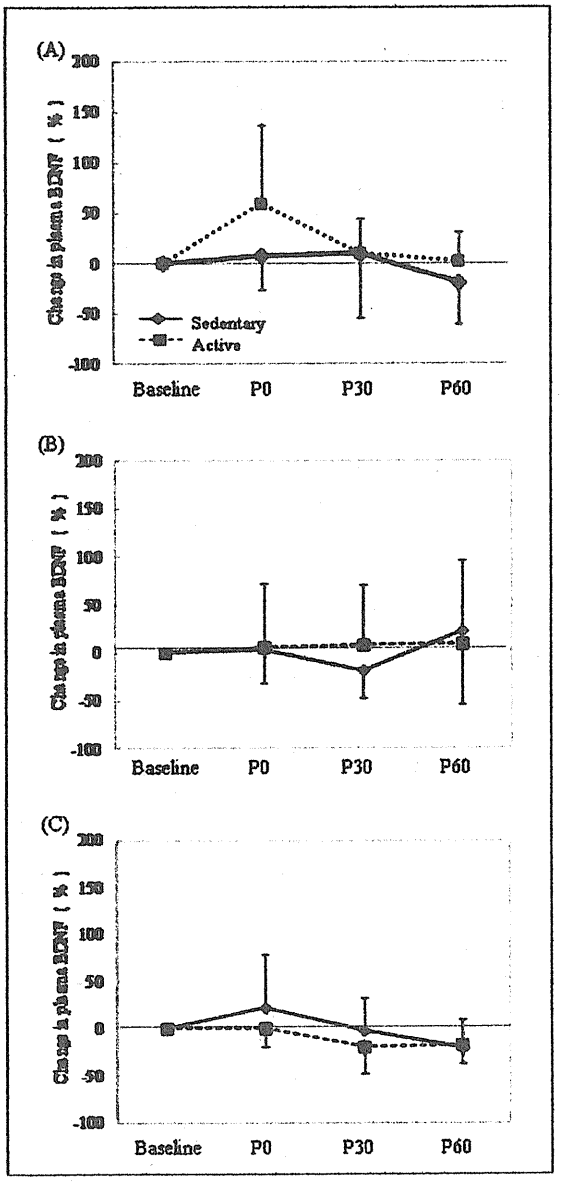

Figure 2. Level of plasma BDNF concentrations before maximal (A), moderate (B), or low (C) exercise tests (baseline), immediately after (P0), 30 min after (P30), and 60 min after (P60) the exercise session. The data are expressed as the means ± SD.

Discussion

We investigated the differences in the serum and plasma BDNF responses to acute maximal and submaximal exercises between the active and sedentary subjects. One of the novel findings of the present study was that the serum BDNF responses to maximal exercise were different between active and sedentary subjects. Especially, serum BDNF levels in the active group decreased below the baseline level during the recovery phase, while it was not the case in the sedentary group. A possible mechanism for this excessive reduction of serum BDNF in the active group is an enhanced utilization mediated by the upregulation of BDNF TrkB (tyrosine protein kinase) receptor in the peripheral tissues. Previous studies demonstrated that physical training increased the expression of TrkB in the spinal cord (Skup et al., 2000), brain (Widenfalk et al., 1999) and soleus muscle (Gómez-Pinilla et al., 2002) in rats. Although the physiological significance of the decreases in BDNF after exercise remains unknown, one of the possible roles of BDNF utilization is the repair of exercise-induced muscle damage. Ninety percent of circulating BDNF is stored in the platelets, where are also epidermal growth factor (EGF) (Oka and Orth, 1983), vascular endothelial growth factor (VEGF) (Tischer et al., 1989), and platelet-derived growth factor (PDGF) (Antoniades et al. 1979), all of which play a role in wound healing. In the current and previous studies (Ferris et al., 2007; Rojas Vega et al., 2006), serum BDNF increased with moderate- to high-intensity exercise, which has been shown to induce muscle damage (Kuipers, 1994). Therefore, it is possible that the increased BDNF during exercise contributes to the repair of skeletal muscle damaged. Although there are no direct reports demonstrating that circulating BDNF acts in the repair of exercise-induced muscle damage, BDNF treatment suppressed the release of creatine kinase and prostaglandin E2, which are common indicators of muscle cell damage in the rat muscle exposed to oxidative stress *in vivo* (Lian et al., 1998). Furthermore, the delayed regeneration of muscle fibers after injury was observed in muscle-specific BDNF knockout mice, suggesting that BDNF plays an important role in the regeneration of muscle fibers (Clow and Jasmin, 2010). Based on the potential wound-healing functions of BDNF, it is proposed that the utilization of serum BDNF during exercise may help muscle regeneration following exercise-induced damage and that the active group may have adapted to utilize circulating BDNF for the promotion of muscle repair.

Conclusion

In conclusion, the circulating BDNF responses to acute maximal exercise were different between active and sedentary groups. While serum BDNF levels in the sedentary group returned to the baseline level during the recovery phase, the BDNF levels in the active group decreased below the baseline level after high-intensity exercise. These results raise the possibility that regular exercise facilitates the utilization of circulating BDNF after acute exercise with maximal intensity. Limitations of this study were the small sample size. Additional studies with large sample size are called for. Likewise, further studies should clarify the mechanisms and physiological significance of the exercise-induced responses to circulating BDNF.

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Key points

- In maximal exercise test, the pattern of change in the serum BDNF concentration was different between the groups.
- While the serum BDNF level for the sedentary group returned to the baseline level during the recovery phase, the BDNF levels for the active group decreased below the baseline level after the maximal exercise test.
- No group differences were observed in the pattern of serum BDNF change for moderate or low exercise tests.
- No group differences were observed in the pattern of plasma BDNF change for all exercise tests.

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