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Endurance exercise increases the SIRT1 and peroxisome proliferator-activated receptor γ coactivator-1α protein expressions in rat skeletal muscle

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Abstract

Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is considered to play a pivotal role in the exercise-induced metabolic adaptation of skeletal muscle. Although the oxidized form of nicotinamide adenine dinucloetide (NAD^+)-dependent histone deacetylase SIRT1 has been shown to mediate PGC-1α–induced metabolic adaptation, the effect of endurance exercise on the SIRT1 protein remains to be elucidated. The purposes of this study were (1) to investigate the distribution of SIRT1 and PGC-1α proteins in skeletal muscle and (2) to examine the effects of acute endurance exercise and low- or high-intensity exercise training on SIRT1 and PGC-1α protein expressions and on the metabolic components in rat skeletal muscle. Both the SIRT1 and PGC-1α proteins preferentially accumulate in red oxidative muscles. Acute endurance exercise on a motor-driven treadmill (20 m/min, 18.5% incline, 45 minutes) increases the PGC-1α protein expression at 18 hours after exercise and the SIRT1 protein expression at 2 hours after exercise in the soleus muscle. In the training experiment, the rats were divided into control, low-intensity (20 m/min, 18.5% incline, 90 min/d), and high-intensity (30 m/min, 18.5% incline, 60 min/d) training groups. After 14 days of training, the SIRT1 and PGC-1α proteins, hexokinase activity, mitochondrial proteins and enzyme activities, and glucose transporter 4 protein in the soleus muscle were increased by both trainings. In the plantaris muscle, SIRT1, hexokinase activity, mitochondrial proteins and enzyme activities, and glucose transporter 4 were increased by high-intensity training whereas the PGC-1α was not. These results suggest that endurance exercise increases the skeletal muscle SIRT1 protein content. In addition, the findings also raise the possibility that the SIRT1 protein expression may play a potentially important role in such adaptations, whereas an increase in the PGC-1α protein expression is not necessary for such adaptations.

1. Introduction

Endurance exercise training has a great impact on the metabolic characteristics including mitochondrial biogenesis and glucose transporter 4 (GLUT4) expression in skeletal muscle [1]. Despite the fact that such adaptations have been analyzed for several decades, the mechanisms of such adaptations remain to be fully elucidated.

Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is a transcriptional coactivator that is considered to be a key regulator of skeletal muscle adaptations. PGC-1α was first determined to be a cold-inducible factor in brown adipocyte and skeletal muscle [2]. PGC-1α stimulates mitochondrial biogenesis and GLUT4 expression via coactivating several transcriptional factors such as nuclear respiratory factor 1 (NRF-1), peroxisome proliferator–activated receptors (PPARs), and myocyte enhancer factor 2 (MEF2) [3]. PGC-1α mRNA and protein expression were increased by acute endurance exercise [4-7] and endurance exercise training [8,9], thus suggesting that PGC-1α was a possible regulator of metabolic adaptations with endurance exercise.

PGC-1α mRNA expression was enhanced by 5′-adenosine monophosphate–activated protein kinase (AMPK) activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofranoside (AICAR) in C2C12 myotubes [10]. Incubation with AICAR increased both the PGC-1α mRNA and protein

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expressions in rat epitrochlearis muscle [6,7]. In addition, in vivo treatment with AICAR [11] or the antihyperglycemic drug metformin [12], which also activated AMPK [12], also increased the skeletal muscle PGC-1α protein expression with a concomitant increase in the mitochondrial enzyme activities and cytochrome C protein expression. Therefore, AMPK was a potential regulator of the PGC-1α expression and mitochondrial biogenesis. The PGC-1α gene promoter activity was stimulated by the activation of p38 mitogen-activated protein kinase (p38 MAPK) in C2C12 myotubes [13]. Peroxisome proliferator-activated receptor γ coactivator-1α protein expression was also induced with a parallel increase in the cytochrome oxidase IV protein expression in constitutively active activator of p38 MAPK, mitogen-activated protein kinase kinase 6E (MAPKK6E) transgenic mice [13], thus suggesting p38 MAPK to be another regulator of PGC-1α expression and mitochondrial biogenesis. Both AMPK [14-16] and p38 MAPK [13,16,17] in skeletal muscle were activated by contractile activity and endurance exercise. Collectively, these data raise the possibility that the metabolic adaptations resulting from endurance exercise training result at least in part via an increased PGC-1α protein expression through the AMPK and p38 MAPK pathways.

Silent information regulator 2 is an oxidized form of nicotinamide adenine dinucleotide (NAD^+)-dependent histone deacetylase that is required for longevity in *Caenorhabditis elegans* [18] and *Saccharomyces cerevisiae* [19] in response to energy restriction. In mammals, the silent information regulator 2 ortholog, SIRT1 functionally interacts and deacetylates several proteins [20]. SIRT1 also deacetylates and functionally activates PGC-1α [21,22]. SIRT1 is required for PGC-1α–induced up-regulation of mitochondrial biogenesis in skeletal muscle cells [21,23]. SIRT1 plays a role in muscle gene expression in the modulation of the cytosolic NAD^+-to-NADH (reduced form of nicotinamide adenine dinucleotide) ratio [24]. Because the cytosolic NAD^+-to-NADH ratio changes during muscle contraction [25], it is possible that SIRT1 contributes to skeletal muscle adaptations with endurance exercise. We therefore hypothesized that SIRT1 protein expression increased after endurance exercise to facilitate such metabolic adaptation.

The purposes of the present study were (1) to investigate the distribution of SIRT1 and PGC-1α proteins in skeletal muscles with various metabolic capacities, and the relationship between these protein expressions and the metabolic components, and (2) to examine the effects of acute endurance exercise and low- or high-intensity exercise training on SIRT1 and PGC-1α protein expressions and other metabolic components in skeletal muscle.

2. Materials and methods

2.1. Animals

Seven-week-old male Wistar rats with a body weight of 200 to 220 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-controlled (22°C ± 2°C) and humidity-controlled (60% ± 5%) room with a 12-hour light (7:00 AM to 7:00 PM) and 12-hour dark (7:00 PM to 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. Distribution and correlation study

Rats (n = 6) were anesthetized with pentobarbital sodium (60 mg/kg body weight IP). The soleus, red and white gastrocnemius, plantaris, adductor longus, biceps brachii, and diaphragm muscles were rapidly dissected and immediately frozen in liquid nitrogen. These tissue specimens were stored at −80°C until analysis.

The frozen samples were homogenized with Polytron-type homogenizer in ice-cold homogenizing buffer (1:20 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, Tokyo, Japan], pH 7.4). The homogenate was centrifuged at 15 000*g* (4°C) for 25 minutes. The protein concentration of the supernatant was determined by the use of a protein determination kit (Bio-Rad, Richmond, CA). The muscle homogenate was used for enzymatic assays including citrate synthase (CS) and β-hydroxyacyl-coenzyme A (CoA) dehydrogenase (βHAD) activity, and Western blotting was performed to determine the SIRT1, PGC-1α, cytochrome C, and GLUT4 protein contents. For Western blotting, the muscle protein homogenate was solubilized in sample loading buffer (50 mmol/L Tris-HCl pH 6.8, 2% sodium dodecylsulfate, 10% glycerol, 5% β-mercaptoethanol, and 0.005% bromophenol blue).

2.3. Acute endurance exercise

The rats were randomly assigned to control (n = 12) and acute endurance exercise (n = 72) groups. All rats were accustomed to running (10-20 m/min for 30 min/d) on a motor-driven treadmill at a 0% grade for 3 days. About 24 hours after the last running for accustoming, the rats of the endurance exercise groups ran on a motor-driven treadmill for 45 minutes at a speed of 20 m/min at an 18.5% incline. The rats were anesthetized with pentobarbital sodium (60 mg/kg body weight IP), and the soleus and plantaris muscles were rapidly dissected out immediately (n = 12), and at 1 (n = 12), 2 (n = 12), 6 (n = 12), 18 (n = 12), and 24 (n = 12) hours after the running exercise. The control rats were also anesthetized and the soleus and plantaris muscles were dissected out. The muscles were frozen in liquid nitrogen and stored at −80°C until the analyses were performed. These frozen samples were prepared according to the protocol for the distribution and

correlation study associated with Western blotting to determine the SIRT1 and PGC-1α protein expressions.

2.4. Endurance training

The rats were divided into a sedentary control group (Con, n = 13), a low-intensity training group (Low, n = 13), or a high-intensity training group (High, n = 12). The rats of the Low and High groups were accustomed to running (10-20 m/min for 30 min/d) for 3 days on a motor-driven treadmill at a 0% grade. The rats trained daily for 14 days. At the first day of training, the rats of both groups ran for 45 minutes at a speed of 20 m/min at an 18.5% incline. In the rats of the Low group, the running duration was gradually increased to 90 minutes during the first 7 days and maintained for the following 7 days. In the rats of the High group, both the running duration and the speed were gradually increased to 60 minutes and 30 m/min, respectively, during the first 7 days and then they were maintained for the following 7 days. Thereafter, the total running distance was consistent between the groups whereas the exercise intensity and running speed differed. About 18 hours after the last exercise, the rats that fed freely were anesthetized with pentobarbital sodium (60 mg/kg body weight IP). The soleus and plantaris muscles were rapidly dissected, frozen in liquid nitrogen, and stored at −80°C until the analyses were performed. These frozen samples were prepared according to the protocol for the distribution and correlation study for enzymatic assays including CS, malate dehydrogenase (MDH), βHAD, hexokinase (HK), pyruvate kinase (PK), and lactate dehydrogenase (LDH) and Western blotting for determining the SIRT1, PGC-1α, cytochrome C, medium chain fatty acyl-CoA dehydrogenase (MCAD) and GLUT4 protein contents.

2.5. Food restriction

The endurance-trained rats especially those in the High group showed a significantly lower body mass than did the control rats (see Results). To examine the effect of a lower body mass for the skeletal muscle components, the body masses of 6 male rats were matched to the mean body mass of the High group by food restriction. Another group of age- and body mass–matched 6 male rats fed ad libitum was established as a control group. All rats in both groups were 7 weeks old with a body weight of 200 to 220 g. After a 14-day food restriction period, the rats were anesthetized with pentobarbital sodium (60 mg/kg body weight IP). The soleus and plantaris muscles were rapidly dissected, frozen in liquid nitrogen, and stored at −80°C until the analyses were performed. The muscle dissection was performed at fed state. These frozen samples were prepared according to the protocol for the distribution and correlation study for enzymatic assays including CS, MDH, βHAD, and LDH, and Western blotting was performed to determine the SIRT1, PGC-1α, cytochrome C, and GLUT4 protein contents.

2.6. Gel electrophoresis and Western blotting

The proteins (20 μg) of these homogenates were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis using 7.5% (SIRT1 and PGC-1α), 10% (GLUT4 and MCAD), and 15% (cytochrome C) resolving gels. The proteins separated by sodium dodecylsulfate-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 PGC-1α (1:500 dilution, AB3242, Chemicon International, Temecula, CA), Sir2 (1:1000 dilution, no. 07-131, Upstate Biotechnology, Lake Placid, NY) or GLUT4 (1:8000 dilution, AB1346, Chemicon International), rabbit polyclonal antiserum to MCAD (1:200 dilution, Cayman Chemical Company, Ann Arbor, MI), or mouse monoclonal antibody to cytochrome C (1:500 dilution, ANN0012, clone 7H8.2C12, Biosource, Camarillo, CA) overnight at 4°C, and then was incubated with biotinylated antirabbit/mouse immunoglobulin G (1:1000 dilution, BA-1400, Vector 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 NIH Image 1.62 software package (National Institutes of Health, Bethesda, MD).

2.7. Enzyme assay

The enzyme activities were measured spectrophotometrically. All enzymatic assays were carried out at 30°C using saturating concentrations of substrates and cofactors as determined in preliminary analyses. Citrate synthase activities were measured at 412 nm to detect the transfer of sulfhydryl groups to 5,5′-dithiobis(2-nitrobenzonic acid) (DTNB). The extinction coefficient for DTNB, which is a reference of CS activity, was 13.6. Malate dehydrogenase, βHAD (β oxidation), HK (mobilization of blood glucose), PK (glycolysis), and LDH (anaerobic glycolysis) activities were measured at 340 nm by following the production or disappearance of NADH or NADPH. The extinction coefficient for NAD(P)H, which are references of these enzyme activities, was 6.22.

For the CS (EC 4.1.3.7) assay, 100 mmol/L Tris-HCl, 0.1 mmol/L DTNB, 0.3 mmol/L acetyl-CoA, 3.33 mmol/L K_2HPO_4, and 0.5 mmol/L oxalacetate (omitted for the measurement of nonspecific activity), pH 8.0, were used.

For the MDH (EC 1.1.1.37) assay, 50 mmol/L Tris-HCl, 0.28 mmol/L NADH, and 0.5 mmol/L oxalacetate (omitted for the measurement of nonspecific activity), pH 7.6, were used.

For the βHAD (EC 1.1.1.35) assay, 100 mmol/L Tris-HCl, 0.28 mmol/L NADH, 5 mmol/L EDTA, and 0.05 mmol/L

acetoacetyl-CoA (omitted for the measurement of nonspecific activity), pH 6.9, were used.

For the HK (EC 2.7.1.1) assay, 100 mmol/L Tris-HCl, 0.4 mmol/L 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 ATP (omitted for the measurement of nonspecific activity), pH 7.0, were used.

For the PK (EC 2.7.1.40) assay, 50 mmol/L Tris-HCl, 0.1 mmol/L KCl, 10 mmol/L $MgCl_2$, 0.28 mmol/L NADH, 1.5 mmol/L ADP, 6 U/mL LDH, and 5 mmol/L phospho*enol*-pyruvate (omitted for the measurement of nonspecific activity), pH 7.6, were used.

For the LDH (EC 1.1.1.27) assay, 50 mmol/L Tris-HCl, 0.28 mmol/L NADH, and 2.4 mmol/L pyruvic acid (omitted for the measurement of nonspecific activity), pH 7.6, were used.

2.8. Statistical analysis

All data are expressed as the means ± SE. The relationship between the SIRT1 or PGC-1α and other metabolic characteristics including CS and βHAD activities and cytochrome C and GLUT4 protein content were ascertained using Pearson correlation coefficients. To compare among the 7 muscles, to estimate the time course of the SIRT1 and PGC-1α protein expressions with acute exercise, and to compare the findings between the Con and the Low or High groups, we used the 1-way analysis of variance. Tukey-Kramer post hoc test was conducted if the analysis of variance indicated a significant difference. An unpaired *t* test was used to compare between the ad libitum and food restriction groups. A value of $P < .05$ was considered to be significant.

3. Results

3.1. SIRT1 protein distribution and relation of metabolic components

Fig. 1A shows the SIRT1 protein content in 7 skeletal muscles. An immunoreactive band of 110 kd was detected on Western blots. The SIRT1 protein content in the red oxidative muscles seemed higher than that in the white glycolytic muscles. Fig. 1B to E indicates the associations between the mean value of the SIRT1 protein content and the metabolic components. The SIRT1 protein expression was highly correlated with the βHAD activity ($r = 0.829$, $P < .05$, Fig. 1C), the cytochrome C protein expression ($r = 0.777$, $P < .05$, Fig. 1D), and the GLUT4 protein expression ($r = 0.924$, $P < .01$, Fig. 1E). The SIRT1 protein expression was not associated with the CS activity ($r = 0.637$, $P = .132$, Fig. 1B). The SIRT1 protein expression tended to be associated with the PGC-1α protein expression, but the correlation coefficient did not reach statistical significance ($r = 0.732$, $P = .06$). When such relationships in all 42 muscles were estimated, the SIRT1 protein expression correlated significantly with the CS activity ($r = 0.566$, $P < .001$), the βHAD activity ($r = 0.630$, $P < .001$), the cytochrome C protein expression ($r = 0.720$, $P < .001$), the GLUT4 protein expression ($r = 0.778$, $P < .001$), and the PGC-1α protein expression ($r = 0.611$, $P < .001$).

3.2. Peroxisome proliferator-activated receptor γ coactivator-1α protein distribution and relationship to metabolic components

Fig. 2A shows the PGC-1α protein contents in 7 skeletal muscles. The PGC-1α protein content in the red oxidative muscles seemed higher than that in the white glycolytic muscles. Fig. 2B to E shows the associations between the mean value of the PGC-1α protein content and the metabolic components including the CS (Fig. 2B) and βHAD (Fig. 2C) activities and the cytochrome C (Fig. 2D) and GLUT4 (Fig. 2E) protein contents. The PGC-1α protein expression correlated highly with the CS activity ($r = 0.926$, $P < .01$), the βHAD activity ($r = 0.929$, $P < .001$), and the cytochrome C protein expression ($r = 0.948$, $P < .001$). The PGC-1α protein expression tended to be associated with the GLUT4 protein expression, but the correlation coefficient did not reach statistical significance ($r = 0.707$, $P = .08$). When such relationships in all 42 muscles were estimated, the PGC-1α protein expression was significantly correlated with the CS activity ($r = 0.859$, $P < .001$), the βHAD activity ($r = 0.858$, $P < .001$), the cytochrome C protein expression ($r = 0.884$, $P < .001$), and the GLUT4 protein expression ($r = 0.681$, $P < .001$).

3.3. Effect of acute endurance exercise on the SIRT1 and PGC-1α protein expression

Fig. 3 shows the change in the SIRT1 protein expression after a single bout of treadmill running. In the soleus muscle, the SIRT1 protein increased at 2 hours after the exercise from the pretrial period (Fig. 3A, +18% from pre, $P < .05$). In the plantaris muscle, no changes were observed after the exercise (Fig. 3B).

Fig. 4 shows the change of the PGC-1α protein expression after a single bout of treadmill running. In the soleus muscle, the PGC-1α protein increased at 18 hours after the exercise from the pretrial period (Fig. 4A, +29% from pre, $P < .05$). In the plantaris muscle, no changes were observed after the exercise (Fig. 4B).

3.4. Effect of endurance exercise training

The body mass of the Con, Low, and High groups on the first day of the training did not differ among the groups (211 ± 1, 214 ± 1, and 212 ± 1 g, respectively). On the day of the muscle dissection, the body mass of the 3 groups differed one from another and the rank order of the body mass was Con > Low > High (303 ± 6g, 273 ± 4g, and 255 ± 4g, respectively, $P < .05$).

Fig. 5 showed the SIRT1 (Fig. 5A) and PGC-1α (Fig. 5B) protein expressions in the Con, Low, and High groups. The SIRT1 protein expressions in the soleus muscle significantly

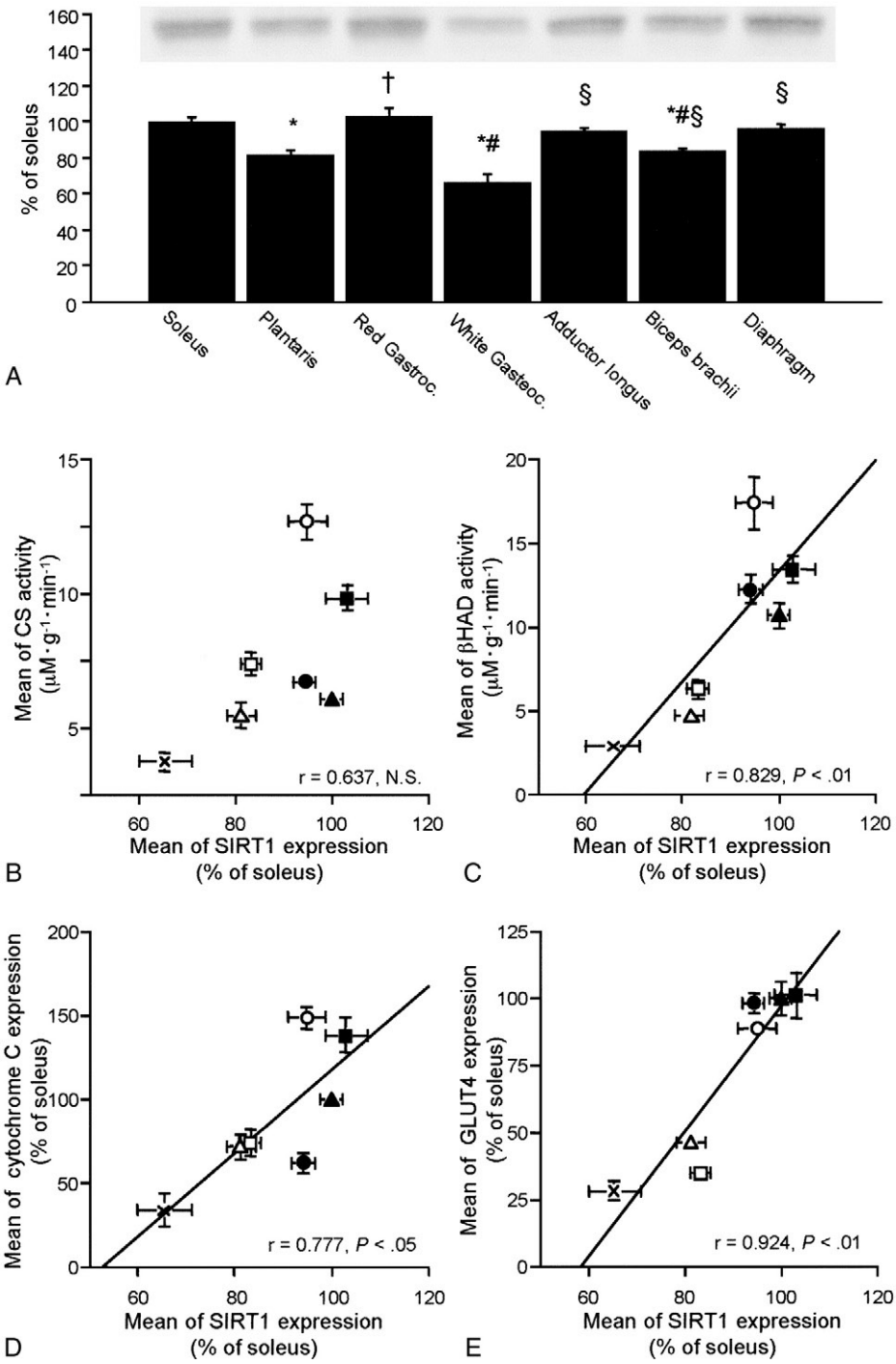

Fig. 1. SIRT1 protein expression in skeletal muscles (A) and relationship between SIRT1 protein expression and CS activity (B), βHAD activity (C), cytochrome C protein expression (D), or GLUT4 protein expression (E) in soleus (filled triangle), plantaris (open triangle), red gastrocnemius (filled square), white gastrocnemius (cross), adductor longus (filled circle), biceps brachii (open square), and diaphragm (open circle) muscles. Values are the means ± SE; n = 6 muscles per group. *$P < .05$ vs soleus; †$P < .05$ vs plantaris; #$P < .05$ vs red gastrocnemius; §$P < .05$ vs white gastrocnemius.

increased in both the Low (+12%, $P < .05$) and the High (+19%, $P < .05$) groups. In the plantaris muscle, the SIRT1 protein expressions significantly increased in the High group (+14%, $P < .05$). The PGC-1α protein expression significantly increased in both the Low (+23%, $P < .05$) and the High (+20%, $P < .05$) groups in the soleus muscle. On the other hand, the PGC-1α protein expression was not altered by endurance exercise training in the plantaris muscle.

Fig. 6 showed the metabolic enzyme activities in the Con, Low, and High groups. The CS activities of the Low and High groups in the soleus muscle were significantly higher than that of the Con group (+29%, $P < .05$ and +30%, $P <$

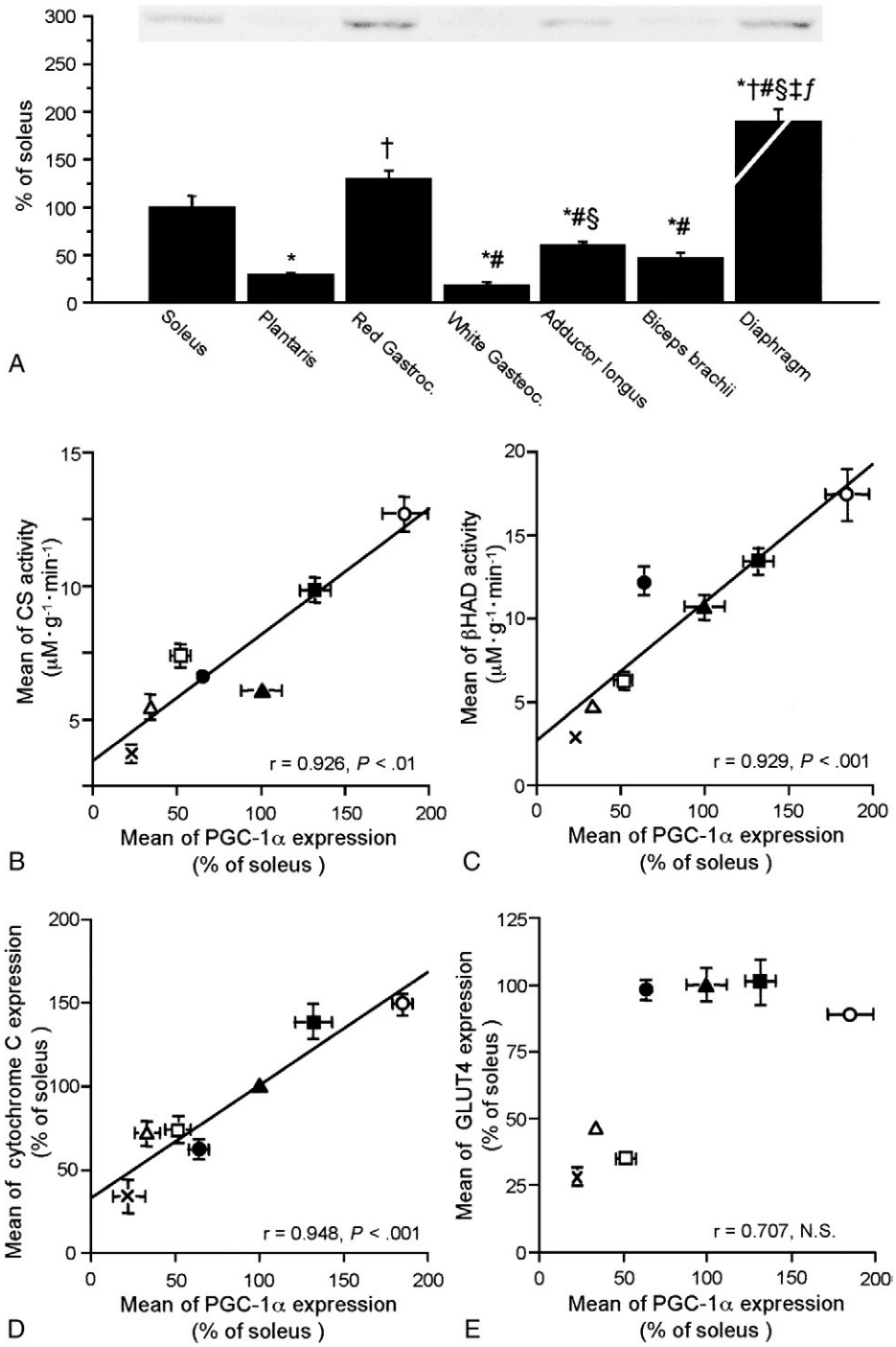

Fig. 2. Peroxisome proliferator-activated receptor γ coactivator-1α protein expression in skeletal muscles (A) and relationship between PGC-1α protein expression and CS activity (B), βHAD activity (C), cytochrome C protein expression (D), or GLUT4 protein expression (E) in soleus (filled triangle), plantaris (open triangle), red gastrocnemius (filled square), white gastrocnemius (cross), adductor longus (filled circle), biceps brachii (open square), and diaphragm (open circle) muscles. Values are the means ± SE; n = 6 muscles per group. *$P < .05$ vs soleus; †$P < .05$ vs plantaris; #$P < .05$ vs red gastrocnemius; §$P < .05$ vs white gastrocnemius; ‡$P < .05$ vs adductor longus; $fP < .05$ vs biceps brachii.

.05, respectively) (Fig. 6A). In the plantaris muscle, the CS activity of the High group was significantly higher than that of the Con group (+16%, $P < .05$) (Fig. 6A). The MDH activities of the Low and High groups in the soleus muscle were significantly higher than that of the Con group (+15%, $P < .05$ and +12%, $P < .05$, respectively) (Fig. 6B). In the plantaris muscle, the MDH activity of the High group was significantly higher than that of the Con group (+13%, $P < .05$) (Fig. 6B). The βHAD activities of the Low and High groups in the soleus muscle were significantly higher than

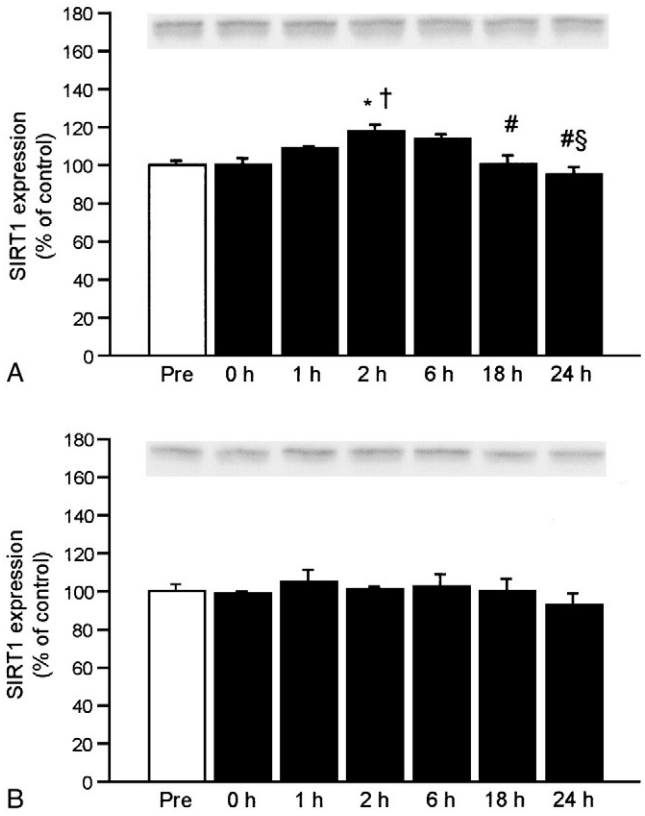

Fig. 3. SIRT1 protein expression in the soleus (A) and plantaris (B) muscles before, immediately (0 hour), and 1, 2, 6, 18, and 24 hours after an acute endurance exercise. Values are the means ± SE; n = 12 muscles per group. One-way analyses of variance indicated significance in both proteins ($P < .0001$). *$P < .05$ vs pre; †$P < .05$ vs 0 hour; #$P < .05$ vs 2 hours; §$P < .05$ vs 6 hours.

that of the Con group (+33%, $P < .05$ and +29%, $P < .05$, respectively) (Fig. 6C). In the plantaris muscle, the endurance exercise training did not affect the βHAD activity (Fig. 6C). The HK activities of the Low and High groups in the soleus muscle were significantly higher than that of the Con group (+85%, $P < .05$ and +86%, $P < .05$, respectively) (Fig. 6D). In the plantaris muscle, the HK activity of the High group was significantly higher than that of the Con group (+54%, $P < .05$) (Fig. 6D). The endurance exercise training did not affect the PK activity (Fig. 6E). The LDH activity of the High group in the plantaris muscle was significantly lower than that of the Con group (−7%, $P < .05$) (Fig. 6F).

Fig. 7 showed the protein expression of the metabolic components in the Con, Low, and High groups. The cytochrome C protein expression of the Low and High groups in the soleus muscle were significantly higher than that of the Con group (+58%, $P < .05$ and +49%, $P < .05$, respectively) (Fig. 7A). In the plantaris muscle, the cytochrome C protein expression of the High group was significantly higher than that of the Con (+27%, $P < .05$) group (Fig. 7A). The MCAD protein expression of the Low and High groups in the soleus muscle was significantly higher than that of the Con group (+38%, $P < .05$ and +32%, $P < .05$, respectively) (Fig. 7B). In the plantaris muscle, the MCAD protein expression of the High group was significantly higher than that of the Con group (+37%, $P < .05$) (Fig. 7B). The GLUT4 protein expression of the Low and High groups in the soleus muscle was significantly higher than that of the Con group (+96%, $P < .05$ and +116%, $P < .05$, respectively) (Fig. 7C). In the plantaris muscle, the GLUT4 protein expression of the Low and High groups was significantly higher than that of the Con group (+63%, $P < .05$ and +99%, $P < .05$, respectively) (Fig. 7C).

3.5. Effect of weight reduction by food restriction

The body mass of the food-restricted rats was significantly lower than that of the *ad libitum* fed rats (254 ± 8 vs 302 ± 4 g, respectively, $P < .001$). The total food intake for 14 days in the food-restricted rats was ~ 65% of that for *ad libitum* fed rats. As shown in Table 1, no metabolic components were altered by food restriction. These results suggest that the changes of the SIRT1 and PGC-1α protein content and in the metabolic components in the skeletal

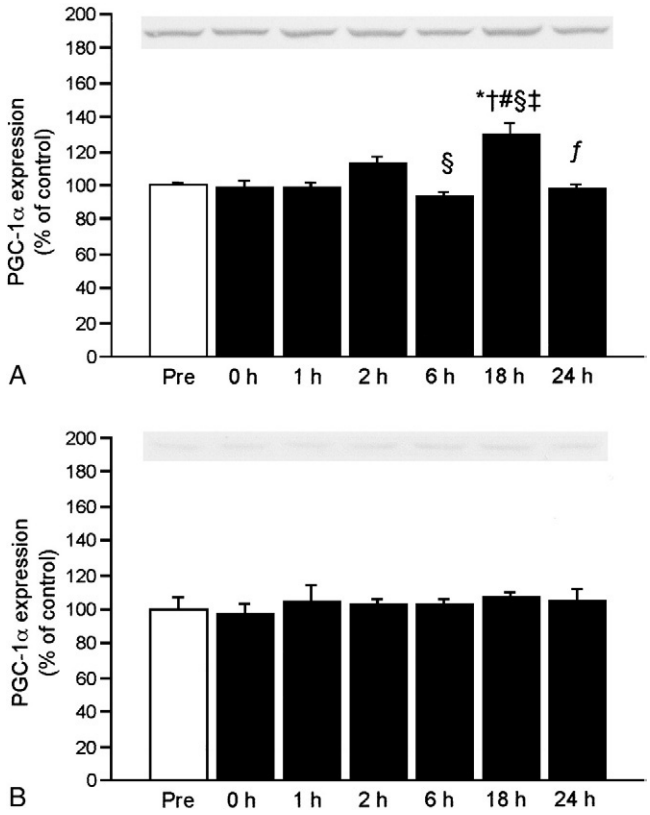

Fig. 4. Peroxisome proliferator-activated receptor γ coactivator-1α protein expression in the soleus (A) and plantaris (B) muscles before, immediately (0 hour), and 1, 2, 6, 18, and 24 hours after an acute endurance exercise. Values are the means ± SE; n = 12 muscles per group. One-way analyses of variance indicated significance in both proteins ($P < .0001$). *$P < .05$ vs pre; †$P < .05$ vs 0 hour; #$P < .05$ vs 1 hour; §$P < .05$ vs 2 hours; ‡$P < .05$ vs 6 hours; $fP < .05$ vs 18 hours.

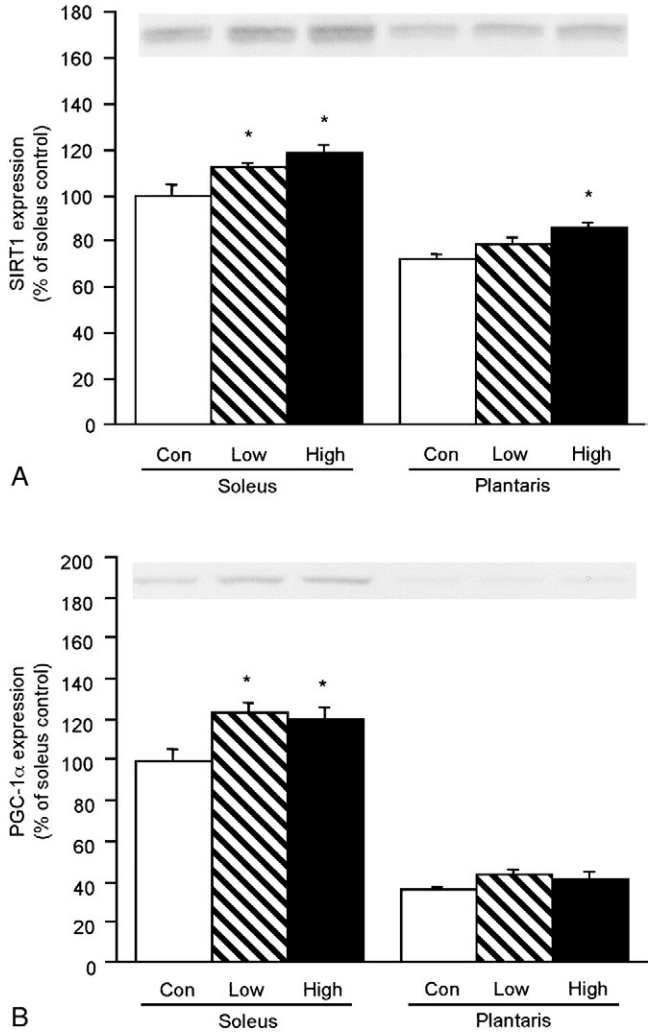

Fig. 5. SIRT1 (A) and PGC-1α (B) protein content of the soleus and plantaris muscles in the Con, Low, and High groups. Data are expressed as the means ± SE; n = 12 to 13 muscles per group. *$P < .05$ vs Con group.

muscle with endurance training are independent of the body weight reduction.

4. Discussion

In this study, we demonstrated that both the SIRT1 and PGC-1α protein expression was higher in the red, slow-twitch, and oxidative muscles than in the white, fast-twitch and glycolytic muscles and correlated highly with the mitochondrial components. It is therefore possible that both SIRT1 and PGC-1α play a role in maintaining the muscle fiber type–specific manner of mitochondrial oxidative capacity. SIRT1 deacetylates and activates PGC-1α [21,22], which can coactivate various transcriptional factors including NRF-1 and NRF-2 and peroxisome proliferator–activated receptors α, γ, and δ [3]. Because such transcriptional factors regulate the genes involved in mitochondrial fatty acid oxidation, in the TCA cycle, and in the respiratory chain [26-28], such functions of SIRT1 and PGC-1α may at least partially lead to the association between these protein expressions and the mitochondrial biogenesis observed in the current study.

The PGC-1α was involved in mitochondrial biogenesis [3], and also its expression increased by endurance exercise in the skeletal muscle [4-8]. Based on previous studies, it has been speculated that PGC-1α may possibly play a role in the endurance exercise-induced enhancement of mitochondrial biogenesis. In the current study, the main effect of high-intensity endurance exercise training for the fast-twitch plantaris muscle was an increase of the mitochondrial components as well as in the slow-twitch soleus muscle. On the other hand, in the plantaris muscle, the PGC-1α protein expression was not altered by endurance exercise training in the High group. This phenomenon does not imply that any types of endurance exercise fail to affect for PGC-1α protein expression in fast-twitch glycolytic muscles. Previous studies have demonstrated that both severe intermittent exercise and 2 sessions of 3 hours swimming and running increased the PGC-1α protein expression in the fast-twitch glycolytic muscles [4,6,7,9]. Collectively, these results suggest that severe endurance exercise can enhance the PGC-1α protein expression in the fast-twitch glycolytic muscles. We speculate that the high-intensity running training performed in this study was not sufficient to enhance the PGC-1α expression in the plantaris muscle.

Importantly, the high-intensity running training increased the mitochondrial components except for the βHAD activity without any changes of the PGC-1α protein expression in the plantaris muscle. The results agree with a previous report that stated that the mitochondrial proteins and the GLUT4 protein in the tibialis anterior muscle of wheel running mice increased without any changes of the PGC-1α protein expression [29]. These results imply that a change in the PGC-1α protein expression level is not necessary for the enhancement of mitochondrial biogenesis by endurance exercise training.

The results of this study did not rule out the possible role of PGC-1α in such adaptations by endurance training. Presumably, the posttranslational regulation of PGC-1α may affect such adaptations. Endurance exercise induced an increase in the PGC-1 protein content in the nucleus without causing any change in the total PGC-1α protein level immediately after 2 hours of swimming in rats [30]. Peroxisome proliferator-activated receptor γ coactivator-1α was phosphorylated by upstream kinases such as p38 MAPK and AMPK [31,32]. The phosphorylation of PGC-1α was required for the PGC-1α–dependent induction of the PGC-1α promoter [31]. Because both p38 MAPK and AMPK in skeletal muscle were activated by exercise [12,14,15,17,30], it is very likely that endurance exercise enhances the PGC-1α phosphorylation via p38 MAPK and AMPK activation and after metabolic adaptations in skeletal muscle. The deacetylation of PGC-1α by SIRT1 also associated with metabolic adaptations in skeletal muscle [21,23]. The deacetylation activity of SIRT1 in the skeletal muscle is considered to

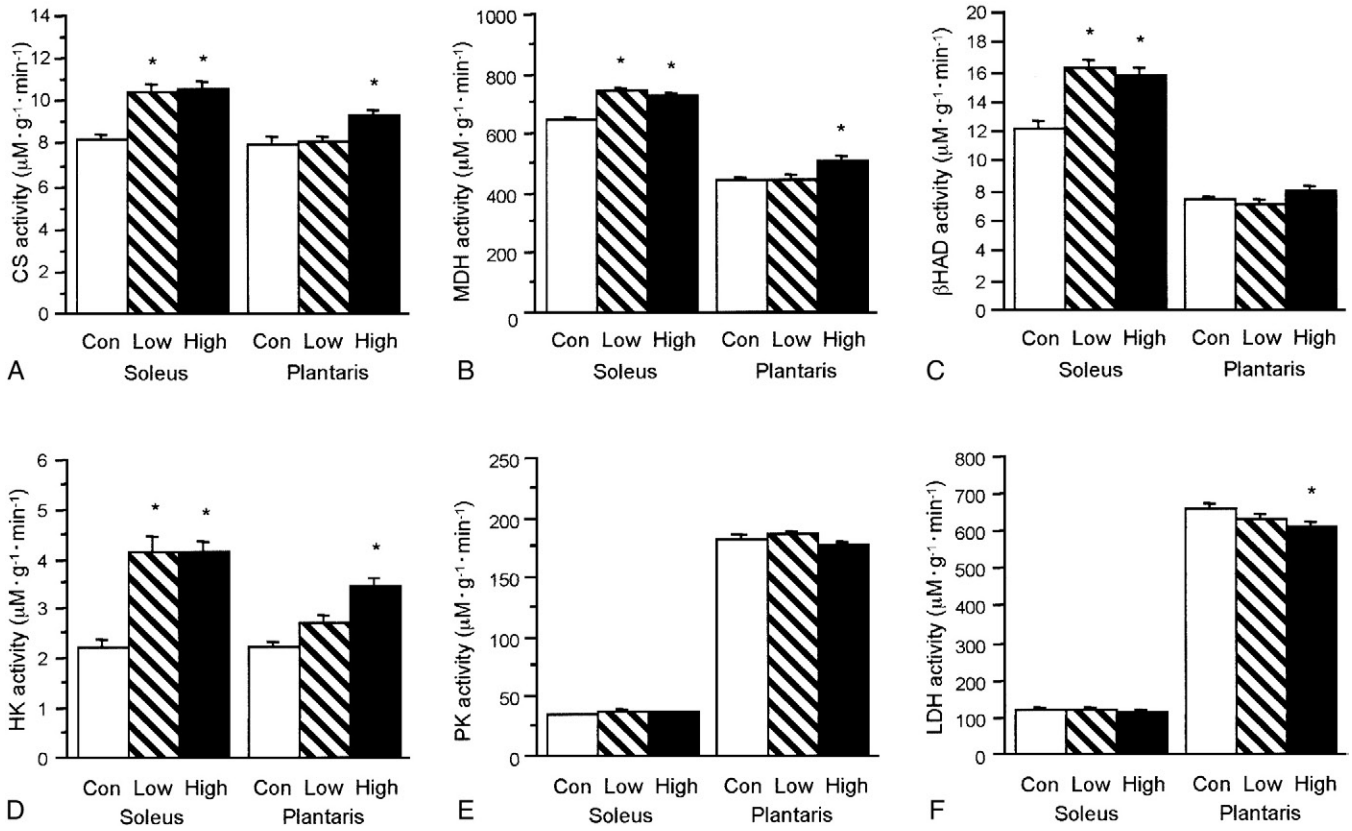

Fig. 6. Metabolic enzyme activities of soleus and plantaris muscles including CS (A), MDH (B), βHAD (C), HK (D), PK (E), and LDH (F) in the Con, Low, and High groups. Data are expressed as the means ± SE; n = 12 to 13 muscles per group. *$P < .05$ vs Con group.

increase during exercise [33], thus suggesting that such exercise promotes the deacetylation of PGC-1α. Collectively, it is possible that skeletal muscle metabolic adaptations with endurance training are associated with the posttranslational regulation of PGC-1α independent of the PGC-1α protein content.

The present study is the first to demonstrate that skeletal muscle SIRT1 protein expression increases with both acute endurance exercise and endurance training. Some previous studies demonstrated that SIRT1 plays an important role for metabolic adaptations including mitochondrial biogenesis, fatty acid oxidation, and glucose homeostasis through deacetylation of PGC-1α [21-23]. It is therefore very likely that increased SIRT1 protein expression by endurance exercise results in elevated SIRT1 deacetylase activity as well as causing an allosteric effect of an increased cytosolic NAD^+-to-NADH ratio and then at least in part contributes to the metabolic adaptations by activating the PGC-1α in skeletal muscle. In addition, the SIRT1 also interacts with several other proteins [20]. The SIRT1 regulates forkhead transcription factor FOXO1 transcriptional activity [34,35], which would contribute to the expression of genes involved in fatty acid oxidation [36]. The SIRT1 also interacts with p53 [20], which regulates mitochondrial respiration [37]. These observations raise the possibility that SIRT1 interacts and deacetylates other proteins, as well as PGC-1α, that regulates metabolic adaptations. Further experimentations are called for to clarify the mechanisms in the enhancement of skeletal muscle metabolic adaptations dependent on SIRT1 protein expression after endurance exercise.

A recent study in mice has shown that the activation of SIRT1 by resveratrol treatment enhances both the whole-body aerobic capacity and the endurance running performance, with a concomitant increase in the mitochondrial oxidative capacity of the skeletal muscle [21]. Furthermore, resveratrol treatment also improves the whole-body insulin sensitivity in high-fat diet–induced obese mice [21]. Therefore, the effects of increased SIRT1 activity by resveratrol treatment mimic the beneficial adaptations to endurance exercise training. Collectively, the results of this previous study [21] and those of the present study raise the possibility that skeletal muscle SIRT1 mediates the endurance exercise-induced metabolic adaptations in the skeletal muscle and improves endurance performance.

It has been postulated that an impaired mitochondrial oxidative capacity could be a direct cause of insulin resistance and type 2 diabetes mellitus [38-40]. Therefore, the enhancement of the mitochondrial capacity is potentially effective for ameliorating or preventing the insulin

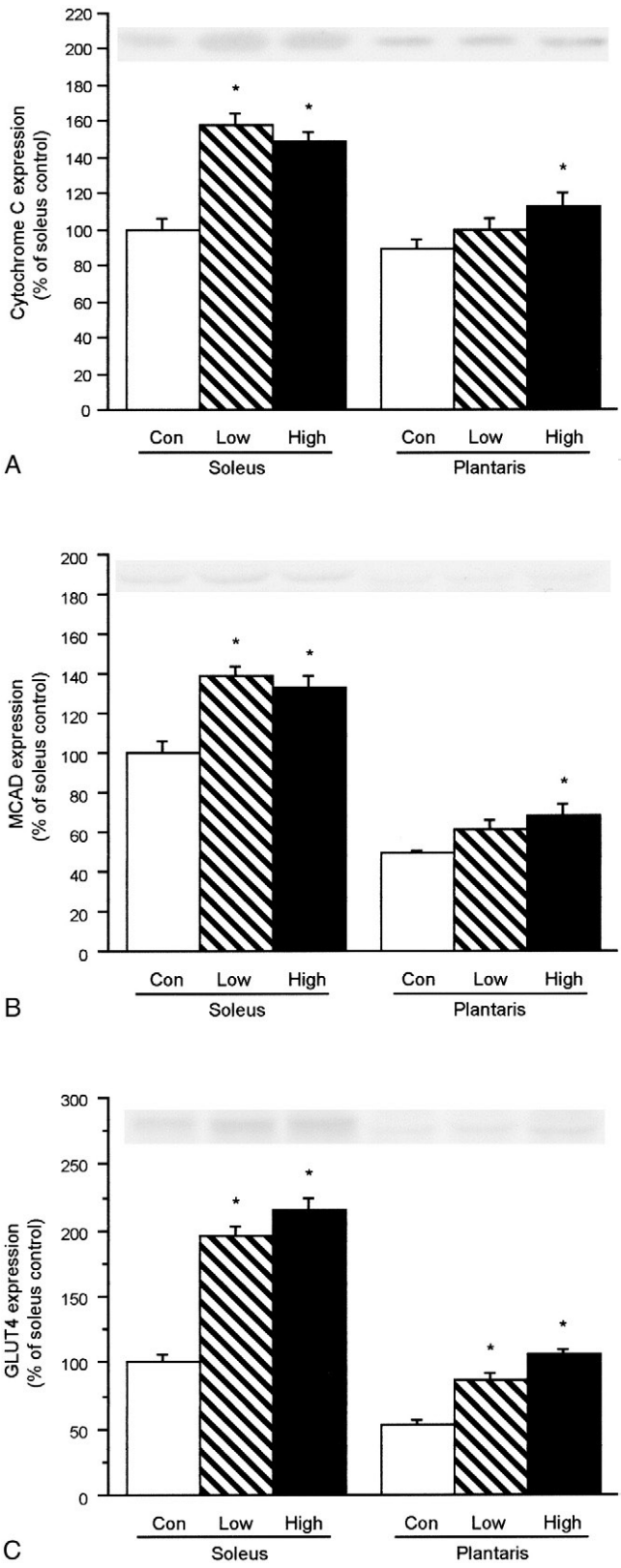

Fig. 7. Cytochrome C (A), MCAD (B), and GLUT4 (C) protein contents of the soleus and plantaris muscles in the Con, Low, and High groups. Data are expressed as the means ± SE; n = 12 to 13 muscles per group. *$P < .05$ vs Con group.

resistance or type 2 diabetes mellitus. Endurance exercise training is used as a first-line treatment of type 2 diabetes mellitus. It is well known that endurance exercise training improves whole-body insulin sensitivity with concomitant enhancement of skeletal muscle insulin-signaling cascade that leads to GLUT4 translocation to the plasma membrane [41], glucose uptake [41], and GLUT4 protein expression [42]. We herein demonstrated that endurance exercise training increased the SIRT1 protein expression, which directly regulates the skeletal muscle mitochondrial biogenesis [21,23]. In addition, a previous study demonstrated that increased expression of SIRT1 improved the insulin sensitivity in skeletal muscle in C2C12 myotubes by repressing protein-tyrosine phosphatase 1B expression [43], a well-known negative regulator of the insulin-signaling pathway [44]. Collectively these results raise the possibility that the effect of endurance exercise training for improving insulin sensitivity partially results from increased SIRT1 protein expression in skeletal muscle.

What could explain the mechanisms concerning the exercise-induced increase of the SIRT1 protein? One potential candidate is nitric oxide synthase (NOS). A previous study demonstrated that energy restriction in wild-type mice increased both the SIRT1 expression and mitochondrial biogenesis in several tissues, whereas such changes were largely diminished or did not occur in endothelial NOS null-mutant mice [45], thus suggesting that energy restriction–induced SIRT1 expression and mitochondrial biogenesis could in large part depend on NOS. Both the NOS activity and neuronal NOS (nNOS) protein expression in skeletal muscle is induced by muscle contraction with electrical stimulation in vivo [46]. Acute endurance exercise enhances the phosphorylation of nNOS in human skeletal muscle [14]. Three to four weeks of endurance swimming training to rats increases the Ca^{2+}-dependent NOS activity and the nNOS protein expression in the quadriceps femoris muscle [47]. Collectively, these results raise the possibility that endurance exercise increases skeletal muscle NOS activity and nNOS expression and then they can induce the SIRT1 expression. Another candidate is AMPK. Endurance exercise increases AMPK activity in skeletal muscle [14,15]. A single administration of AMPK activator AICAR to rats significantly increases the SIRT1 protein in the extensor digitorum longus muscle (Suwa M, Nakano H, and Kumagai S, unpublished data). It is therefore plausible that AMPK mediates the endurance exercise-induced SIRT1 protein expression in skeletal muscle.

In the current study we demonstrated that the increase in SIRT1 protein expression with acute exercise occurred before the onset of an increase in the PGC-1α protein expression in the soleus muscle. The SIRT1 protein expression significantly increased 2 hours after exercise and then completely returned to the baseline level by 18 hours after exercise, whereas the PGC-1α protein level significantly increased at 18 hours after exercise. Previous studies demonstrated the forced expression of PGC-1α using

Table 1
Effect of food restriction for 2 weeks on skeletal muscle protein expressions and metabolic enzymes activities

	Soleus muscle		Plantaris muscle	
	Ad libitum	Food restriction	Ad libitum	Food restriction
SIRTI (% of ad libitum)	100.0 ± 2.8	101.5 ± 2.2	100.0 ± 5.5	102.3 ± 3.4
PGC-1α (% of ad libitum)	100.0 ± 5.3	99.8 ± 2.6	100.0 ± 5.8	114.8 ± 7.0
Cytochrome C (% of ad libitum)	100.0 ± 14.8	93.3 ± 5.5	100.0 ± 11.3	114.3 ± 12.8
GLUT4 (% of ad libitum)	100.0 ± 2.1	99.4 ± 3.9	100.0 ± 7.4	106.8 ± 5.1
CS activity (μmol L^{-1} g^{-1} min^{-1})	11.31 ± 0.69	10.05 ± 0.41	9.22 ± 0.82	10.40 ± 0.47
MDH activity (μmol L^{-1} g^{-1} min^{-1})	743.8 ± 16.1	697.0 ± 19.0	412.3 ± 11.1	440.0 ± 29.4
βHAD activity (μmol L^{-1} g^{-1} min^{-1})	10.36 ± 0.73	9.12 ± 1.69	4.46 ± 0.35	5.52 ± 0.40
LDH activity (μmol L^{-1} g^{-1} min^{-1})	194.2 ± 8.2	189.9 ± 10.8	831.0 ± 20.9	863.6 ± 28.0

Data are expressed as mean ± SE; n = 6 muscles per group.

adenoviral system to increase the endogenous PGC-1α expression in cardiac myocytes [48] and C2C12 skeletal muscle cells [49], thus suggesting the existence of an autoregulatory mechanism on PGC-1α expression. The ectopic expression [23] and activation [21] of SIRT1 both up-regulated the PGC-1α expression in the mouse embryonic cells. Because SIRT1 deacetylation activity is required for the PGC-1α-mediated gene expressions [23], it is likely that endurance exercise promotes the deacetylation of PGC-1α by increasing both the SIRT1 expression and deacetylation activity, and thereafter, the PGC-1α protein expression increases as a result of the autoregulatory system of PGC-1α. Presumably, such mechanism is one of the possible causes that the increase in SIRT1 protein expression occurred before the onset of the increase in the PGC-1α protein expression after acute exercise.

A previous study demonstrated that the forced expression of PGC-1α increased the GLUT4 mRNA expression in cell culture [50]. The study also demonstrated that PGC-1α interacted with transcriptional factor MEF2C and PGC-1α coactivated GLUT4 promoter activity via the MEF2 enhancer element [50]. Collectively, it was considered that PGC-1α regulated GLUT4 expression through interaction with MEF2. On the contrary, we herein demonstrated that the association between the PGC-1α and GLUT4 protein expressions in the skeletal muscles seemed weaker than that between the PGC-1α and the mitochondrial components. Furthermore, endurance exercise training markedly increased the GLUT4 protein expression without any changes in the PGC-1α protein expression in the plantaris muscle in the current study. A previous study demonstrated that the acute endurance exercise increased the PGC-1α protein interaction with MEF2 and also with GLUT4 mRNA expression without any changes of the total or nuclear PGC-1α protein abundance [51]. In addition, mice overexpressing PGC-1α showed decreased GLUT4 expression in skeletal muscle [52]. Based on these findings, it might be suggested that although the PGC-1α regulates GLUT4 transcription via interaction with MEF2, the PGC-1α protein abundance is less important for GLUT4 expression under physiologic conditions, especially in exercise training.

The current study indicated that endurance training increased the skeletal muscle SIRT1 protein expression with a parallel increase in several mitochondrial components. On the other hand, the GLUT4 protein expression of the plantaris muscle in the Low group significantly increased without any changes in the SIRT1 protein expression. These results may thus imply that an increase in the SIRT1 expression is not required for the exercise-induced increase of the GLUT4 protein expression. It is possible that some other mechanism(s) may exist, which control GLUT4 regulation independent of mitochondrial biogenesis after exercise. More detailed analyses of GLUT4 regulation are therefore required to elucidate such exercise intensity-dependent metabolic adaptations.

In summary, our results suggest that the skeletal muscle SIRT1 and PGC-1α protein expressions were associated with mitochondrial oxidative capacity in basal conditions. The SIRT1 and PGC-1α protein expressions in soleus muscle increased at 18 and 2 hours after acute endurance exercise. The SIRT1 protein expression increased in parallel with the mitochondrial components with 2 weeks of endurance exercise training. On the other hand, although both the PGC-1α protein expression and the mitochondrial components increased with endurance training in the soleus muscle, the mitochondrial components increased in high-intensity endurance training without an increase of the PGC-1α protein expression in the plantaris muscle. These results may suggest that SIRT1 plays an important role in the adaptation of mitochondrial biogenesis by endurance training, whereas PGC-1α protein expression is not necessary. The relationship between PGC-1α and GLUT4 protein expression seemed weak, and PGC-1α protein abundance might not be important for endurance training–induced increase of GLUT4 expression.

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