Muscle contractile activity regulates Sirt3 protein expression in rat skeletal muscles

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Submitted 31 March 2009; accepted in final form 9 April 2010

Hokari F, Kawasaki E, Sakai A, Koshinaka K, Sakuma K, Kawanaka K. Muscle contractile activity regulates Sirt3 protein expression in rat skeletal muscles. J Appl Physiol 109: 332–340, 2010. First published April 22, 2010; doi:10.1152/japplphysiol.00335.2009.—Sirt3, a member of the sirtuin family, is known to control cellular mitochondrial function. Furthermore, because sirtuins require NAD for their deacetylase activity, nicotinamide phosphoribosyltransferase (Nampt), which is a rate-limiting enzyme in the intracellular NAD biosynthetic pathway, influences their activity. We examined the effects of exercise training and normal postural contractile activity on Sirt3 and Nampt protein expression in rat skeletal muscles. Male rats were trained by treadmill running at 20 m/min, 60 min/day, 7 days/wk for 4 wk. This treadmill training program increased the Sirt3 protein expression in the soleus and plantaris muscles by 49% and 41%, respectively (P < 0.05). Moreover, a 4-wk voluntary wheel-running program also induced 66% and 95% increases in Sirt3 protein in the plantaris and triceps muscles of rats, respectively (P < 0.05). Treadmill-running and voluntary running training induced no significant changes in Nampt protein expression in skeletal muscles. In resting rats, the soleus muscle, which is recruited during normal postural activity, possessed the greatest expression levels of the Sirt3 and Nampt proteins, followed by the plantaris and triceps muscles. Furthermore, the Sirt3, but not Nampt, protein level was reduced in the soleus muscles from immobilized hindlimbs compared with that shown in the contralateral control muscle. These results demonstrated that 1) Sirt3 protein expression is upregulated by exercise training in skeletal muscles and 2) local postural contractile activity plays an important role in maintaining a high level of Sirt3 protein expression in postural muscle.

MITOCHONDRIA IN SKELETAL MUSCLES are intimately linked to proper muscle function, as these organelles constitute the main energy supply in contracting muscles. Decrements in the oxidative capacity of aged skeletal muscle are associated with impairment of mitochondrial function, such as reduced electron transport chain complex activity (11, 16, 37) or ATP synthesis (14). Results from other studies suggest that mitochondrial dysfunction is likely to be involved in sarcopenia, a condition characterized by an age-related loss of muscle mass, either via decrements in energy supply or via mitochondrially mediated apoptosis (7, 9, 13, 25, 38). On the other hand, exercise is known to enhance mitochondrial oxidative capacity in skeletal muscle (18, 19) and to be effective for the prevention of mitochondrially mediated apoptosis (1).

The sirtuins are a conserved family of proteins possessing NAD-dependent deacetylase activity. Sirtuins mediate the longevity-promoting effects of calorie restriction in yeast, worms, flies, and mice (10). There are seven mammalian sirtuin homologs (Sirt1 to Sirt7), and at least three sirtuins (Sirt3, Sirt4, and Sirt5) appear to be localized primarily in the mitochondria (2, 28, 31–33). Ahn et al. (2) demonstrated that mitochondria from Sirt3-deficient mice display a selective inhibition of the electron-transport chain, suggesting that Sirt3 plays a role as a regulator of mitochondrial oxidative capacity. Furthermore, increased expression of Sirt3 in adipocytes was found to be capable of inducing the expression of genes involved in mitochondrial oxidative capacity (33). Moreover, mitochondrially localized Sirt3 has been linked to protection against mitochondrially mediated apoptosis (34, 39). These results raised the possibility that exercise-induced enhancement of mitochondrial oxidative capacity and prevention of mitochondrially mediated apoptosis in skeletal muscle are due to increased Sirt3 protein expression. It was recently reported that Sirt3 protein expression was higher in the skeletal muscles of endurance-trained individuals than in those of sedentary individuals (23). However, because this previous study was designed as a cross-sectional comparison of sedentary and endurance-trained subjects, the higher expression of Sirt3 in the muscles of trained individuals might be due to genetic or life-style-related factors.

Because the sirtuin protein family requires NAD for its deacetylase activity, the regulation of intracellular NAD biosynthesis has attracted attention. In cellular NAD biosynthesis pathways, nicotinamide is converted into nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (Nampt). NMN is then directly synthesized into NAD by nicotinamide/nicotinic acid mononucleotide adenyltransferase (Nmnat). Furthermore, evidence has also shown that increased dosage of Nampt, but not Nmnat, increases the total cellular NAD level and enhances the activity of sirtuins (29). Thus Nampt is the rate-limiting enzyme in the mammalian NAD biosynthesis pathway. A previous study has shown that overexpression of Nampt increases the total cellular NAD level and enhances the activity of sirtuins in mouse fibroblasts (29), suggesting the possibility that exercise may increase muscle mitochondrial function by increasing Nampt protein expression and subsequent sirtuin activation.

In our present study, we examined the effects of endurance exercise training in experimental animals on Sirt3 and Nampt protein expression in various types of skeletal muscles. Furthermore, we examined the differences in these protein expression levels between red and white muscle fiber types in resting animals. In addition, we tested whether local postural contrac-
tile activity regulates Sirt3 and Nampt protein expression in postural muscle by using a one-legged prolonged immobilization model.

MATERIALS AND METHODS

Materials

Antibody against Sirt1 was obtained from Upstate Biotechnology (Chicago, IL). Anti-Sirt3 antibody was obtained from Cell Signaling Technology (Beverly, MA). Anti-Nampt antibody was obtained from Bethyl Laboratories (Montgomery, TX). Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) antibody was obtained from CalBiochem (San Diego, CA). Cytochrome-c oxidase subunit I (COXI) antibody was obtained from Invitrogen (Eugene, OR). Horse-radish peroxidase-conjugated anti-rabbit IgG and enhanced chemiluminescence reagents (ECL and ECL plus) were obtained from Amersham Biosciences (Buckinghamshire, UK). All other reagents were obtained from Sigma (St. Louis, MO).

Animals

This research was approved by the Animal Studies Committee of Niigata University of Health and Welfare. Male Wistar rats weighing ~120–130 g were obtained from CLEA Japan (Tokyo, Japan).

Treadmill-Running Protocol

For the first set of experiments, rats (130–150 g) were assigned to either an untrained-sedentary control group or a treadmill-running training group. Rats were trained for a total of 4 wk by having them run on a motorized treadmill without a grade. The speed and duration of their running were increased to 20 m/min and 60 min/day, respectively, during the 1st wk, and they were then made to exercise at 20 m/min for 60 min, 7 days/wk for 3 wk. All rats were maintained in individual cages and fed a powdered standard rodent chow diet and water ad libitum. Their food intake was measured over the period of the experiment. All rats were fasted for ~16 h before the muscle sampling. Between 12:00 PM and 4:00 PM (~21 h after the last exercise bout for the trained rats), the rats were anesthetized with pentobarbital sodium (5 mg/100 g body wt). Then, their soleus, plantaris, and triceps muscles were dissected and clamp-frozen in liquid nitrogen for subsequent measurement of Sirt1, Sirt3, Nampt, PGC-1α, and COXI protein concentrations. The muscles from the contralateral side of the same animals were used for the measurement of Sirt1, Sirt3, and Nampt mRNA expression. Soleus muscle was chosen because this muscle, consisting of predominantly type I fibers, plays a crucial role in normal postural activity in rats housed in standard laboratory cages (3, 17).

Immobilization Protocol

For the third set of experiments, rats (130–150 g) were assigned to either a naive control group or an immobilization group. Rats in the immobilization group were subjected to unilateral hindlimb immobilization. A plaster cast (Castlight, ALCARE, Tokyo, Japan) was applied to the left hindlimb of rats. The leg was immobilized at the plantar flexion position. After the casting, the rats were housed individually. Immobilization was imposed for 3 or 14 days. All rats were maintained in individual cages and fed a standard rodent chow diet and water ad libitum until sampling of muscles. For muscle sampling, the casts were removed under pentobarbital sodium anesthesia (5 mg/100 g body wt), and soleus muscles from both the immobilized and contralateral hindlimb were dissected and clamp-frozen in liquid nitrogen for subsequent measurement of Sirt1, Sirt3, and Nampt protein concentrations. Soleus muscle was chosen because this muscle, consisting of predominantly type I fibers, plays a crucial role in normal postural activity in rats housed in standard laboratory cages (3, 17).

Western Blot Analysis

The muscles were homogenized in ice-cold buffer containing 50 mM HEPES (pH 7.4), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl2, 1 mM EDTA, 10 mM Na4P2O7, 100 mM NaF, 2 mM Na3VO4, 2 mM PMSF, aprotinin (10 μg/ml), leupeptin (10 μg/ml), and pepstatin (0.5 μg/ml) (24). The homogenates were then rotated end-over-end at 4°C for 60 min and centrifuged at 4,000 g for 30 min at 4°C. Aliquots of the supernatants were treated with 2× Laemmli sample buffer containing 100 mM dithiothreitol. For the measurement of Sirt1 and PGC-1α protein concentrations, the samples containing 15–25 μg of total protein were run on 7% SDS-PAGE; for the measurement of Nampt and COXI protein concentrations, the samples containing 15–25 μg of total protein were run on 10% SDS-PAGE; and for the measurement of Sirt3 protein concentration, the samples containing 15–25 μg of total protein were run on 15% SDS-PAGE. The resolved proteins were then transferred to polyvinylidene difluoride membranes and blocked in 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween 10 (TBST), pH 7.5. After membranes were blocked, the membranes were rinsed in TBST, incubated overnight with the appropriate antibody at 4°C, rinsed in TBST, and incubated for 120 min with horseradish peroxidase-conjugated anti-rabbit IgG. Antibody-bound protein was reacted with enhanced chemiluminescence (ECL or ECL plus; Amersham), and the light produced by this enhanced chemiluminescence was detected by a short exposure to blue light-sensitive autoradiography film (Hyperfilm; Amersham), with the intensity of the bands being quantified by densitometry.
mRNA Expression

The muscles were homogenized in 600 μl of RNAgents (Promega, Madison, WI) using a Polytron homogenizer (Polytron PT2100, Kinematica). RNA was isolated according to the manufacturer’s instructions, and then the purified RNA was reverse transcribed with a reverse transcription system (Promega). Measurement of reverse-transcribed cDNA was carried out with Power SYBR green PCR Master Mix (Applied Biosystems, Foster City, CA) with primers to Sirt1 (forward: GGACAGATGCAGTGCAAG; reverse: CACTCCGGTCTGTCAATCATCA), Sirt3 (forward: CCGCCGCCGTGCTGT; reverse: CTCCTCCAAGAAGACATATCA), Nampt (forward: CTGGAATCGGCTGCACT; reverse: AGTTCTCAGAGAGGGAACTTCTTG), and GAPDH (forward: GACAACTTCGCAATCGTGAA; reverse: ATGCCGGATGATTCTCGG). Fluorescence was monitored during PCR by an ABI7300 sequence detection system (Applied Biosystems). PCR data were then normalized to GAPDH expression.

Statistical Analysis

Data are expressed as means ± SE. Differences were determined using an unpaired Student’s t-test. To show the difference between muscle types, we used one-way ANOVA with a subsequent Fisher’s least significant difference method. Differences between groups were considered statistically significant at P < 0.05.

RESULTS

Adaptations to Treadmill-Running Training

Body weight and energy intake. Four weeks of treadmill-running training significantly reduced the body weight in rats [320 ± 8 g (n = 7) for sedentary control and 295 ± 5 g (n = 8) for trained rats] (P < 0.05). Treadmill-running training also significantly reduced the daily energy intake in rats over the 4-wk training period [97.6 ± 3.1 kcal/day (n = 7) for sedentary control and 86.2 ± 2.3 kcal/day (n = 8) for trained animals] (P < 0.05).

Sirt1, Sirt3, and Nampt protein. As shown in Fig. 1, A and B, treadmill-running training increased the Sirt3 protein levels by 49% and 41% in the soleus and plantaris muscles, respectively (P < 0.05). Treadmill-running training did not increase the Sirt1 and Nampt protein levels in either the soleus or plantaris muscle (Fig. 1, A and B) and did not affect Sirt1, Sirt3, or Nampt protein levels in the triceps muscle (Fig. 1C).

To examine the possibility that Sirt1, Sirt3, and Nampt protein levels were acutely increased after the cessation of exercise on each day during the training period, we examined the effects of a single bout of treadmill running on the levels of these proteins in the soleus muscle. The single bout of treadmill running had no significant effect on Sirt1, Sirt3, or Nampt protein levels in the soleus muscle at 8 h after the cessation of running (Fig. 2). Thus it is very likely that increased Sirt3 protein expression in skeletal muscles of treadmill runners is an effect of chronic exercise.

Sirt1, Sirt3, and Nampt mRNA. As shown in Table 1, treadmill-running training did not increase Sirt1, Sirt3, or Nampt mRNA levels in the soleus muscle.

We also examined the effects of a bout of treadmill running on Sirt1, Sirt3, and Nampt mRNA levels in the soleus muscle at 2, 4, and 8 h after running. Sirt1 mRNA levels in the muscle from running animals were 51% greater than those in the sedentary control muscle at 4 h after the cessation of running (Table 2; P < 0.05). In running animals, Sirt1 mRNA levels returned to control levels at 8 h after the cessation of running.
Adaptations to Voluntary Wheel-Running Training

Body weight and energy intake. The progression of voluntary wheel-running activity is shown in Fig. 3. Four weeks of voluntary wheel-running training significantly reduced the body weight in rats [307 ± 4 g (n = 7) for the sedentary controls and 273 ± 7 g (n = 7) for the voluntary runners] (P < 0.05). Voluntary wheel-running training did not affect the daily energy intake in rats over the 4-wk training period (Table 2). The single bout of treadmill running had no effect on Sirt3 mRNA level in the soleus muscle at 4 and 8 h after the cessation of running (Table 2). Nampt mRNA level in the runner’s muscle was 66% lower than that in the control muscle at 4 h after the cessation of running (Table 2, P < 0.05). Nampt mRNA level in the runner’s muscle was 32% lower than that in the control muscle at 8 h after the cessation of running (Table 2). Nampt protein level in the runner’s muscle was 32% lower than that in the control muscle at 8 h after the cessation of running (Table 2, P < 0.05). Nampt protein levels are expressed as means ± SE for 6–8 muscles. Rats were divided into a resting control group and a treadmill-running (acute treadmill) group. The mean values for Sirt1, Sirt3, and Nampt in the resting control muscle were set at 1.0. Top: representative Western blots. Relative amounts of Sirt1, Sirt3, and Nampt protein levels are expressed as means ± SE; n = 6–7.

Table 1. Sirt1, Sirt3, and Nampt mRNA levels in rat soleus, plantaris, and triceps muscles in sedentary control and treadmill-running-trained rats

<table>
<thead>
<tr>
<th></th>
<th>Soles</th>
<th>Plantaris</th>
<th>Triceps</th>
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<tbody>
<tr>
<td>Sirt1</td>
<td>1.00 ± 0.15</td>
<td>1.00 ± 0.14</td>
<td>1.00 ± 0.10</td>
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<tr>
<td>Sirt3</td>
<td>0.91 ± 0.08</td>
<td>0.98 ± 0.09</td>
<td>0.81 ± 0.02</td>
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<tr>
<td>Nampt</td>
<td>1.00 ± 0.10</td>
<td>1.00 ± 0.14</td>
<td>1.00 ± 0.10</td>
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Values (relative amounts of mRNA levels) are means ± SE for 6–8 muscles. Nampt, nicotinamide phosphoribosyltransferase.

Table 2. Sirt1, Sirt3, and Nampt mRNA levels in rat soleus muscle at 8 h after cessation of a single bout of treadmill running

<table>
<thead>
<tr>
<th></th>
<th>Soles</th>
<th>Plantaris</th>
<th>Triceps</th>
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<tbody>
<tr>
<td>Sirt1</td>
<td>1.00 ± 0.06</td>
<td>1.00 ± 0.06</td>
<td>1.00 ± 0.09</td>
</tr>
<tr>
<td>Sirt3</td>
<td>0.89 ± 0.05</td>
<td>0.84 ± 0.03</td>
<td>0.85 ± 0.12</td>
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Results at 2 h

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<th></th>
<th>Soles</th>
<th>Plantaris</th>
<th>Triceps</th>
</tr>
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<tbody>
<tr>
<td>Sirt1</td>
<td>1.00 ± 0.10</td>
<td>1.00 ± 0.08</td>
<td>1.00 ± 0.16</td>
</tr>
<tr>
<td>Sirt3</td>
<td>1.51 ± 0.12*</td>
<td>0.89 ± 0.01</td>
<td>0.34 ± 0.08*</td>
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Results at 4 h

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<thead>
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<th></th>
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<th>Plantaris</th>
<th>Triceps</th>
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<tbody>
<tr>
<td>Sirt1</td>
<td>1.00 ± 0.06</td>
<td>1.00 ± 0.04</td>
<td>1.00 ± 0.03</td>
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<tr>
<td>Sirt3</td>
<td>1.12 ± 0.07</td>
<td>1.15 ± 0.07</td>
<td>0.68 ± 0.07*</td>
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Results at 8 h

Values (relative amounts of mRNA levels) are means ± SE for 5–8 muscles. Rats were divided into a resting control group and a treadmill-running group. *P < 0.05 vs. resting control results.

Table 3. PGC-1α and COXI protein levels in rat soleus, plantaris, and triceps muscles in sedentary control and treadmill-running-trained rats

<table>
<thead>
<tr>
<th></th>
<th>PGC-1α</th>
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<tbody>
<tr>
<td>Soles</td>
<td>Sedentary control</td>
<td>1.00 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Treadmill training</td>
<td>1.10 ± 0.05</td>
</tr>
<tr>
<td>Plantaris</td>
<td>Sedentary control</td>
<td>1.00 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Treadmill training</td>
<td>1.04 ± 0.01</td>
</tr>
<tr>
<td>Triceps</td>
<td>Sedentary control</td>
<td>1.00 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>Treadmill training</td>
<td>1.00 ± 0.03</td>
</tr>
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</table>

Values (relative amounts of protein levels) are means ± SE for 5–8 muscles. PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; COXI, cytochrome-c oxidase subunit I.
Difference Between Muscles of Different Types

We used resting sedentary rats to examine the difference in Sirt1, Sirt3, and Nampt protein levels between muscle types. As shown in Fig. 5A, Sirt1 protein levels in both the plantaris and triceps muscles were significantly lower than those in the soleus muscle ($P < 0.05$). As shown in Fig. 5B, Sirt3 protein levels in both the plantaris and triceps muscles were significantly lower than levels in the soleus muscle ($P < 0.05$), and Sirt3 protein level in the triceps muscle was significantly lower than that in the plantaris muscle ($P < 0.05$). As shown in Fig. 5C, Nampt protein level in the triceps muscle was significantly lower than that in the soleus muscle ($P < 0.05$). Thus, in sedentary control rats, the soleus possessed the greatest expression levels of Sirt1, Sirt3, and Nampt proteins, followed by the plantaris and the triceps muscles.

Adaptations to Immobilization

The weights of soleus muscles from 3-day-immobilized hindlimbs were 27% ($P < 0.05$) smaller than those from contralateral control legs [57.5 ± 1.0 mg ($n = 7$) for contralateral control muscle and 41.8 ± 1.2 mg ($n = 7$) for immobilized muscle]. The weights of the soleus muscles from hindlimbs immobilized for 14 days were 55% ($P < 0.05$) smaller than those from contralateral control legs [88.8 ± 3.5 mg ($n = 7$) for contralateral control muscle and 39.6 ± 3.2 mg ($n = 8$) for immobilized muscle]. There was no difference in the weights of the soleus muscles between the leg of naive control rats and the contralateral noncasted leg of rats immobilized for 14 days [90.7 ± 1.5 mg ($n = 8$) for muscles of naive controls and 88.8 ± 3.5 mg ($n = 7$) for muscles of contralateral noncasted legs]. This result indicates that unilateral immobilization did not cause overuse in the soleus muscle of the contralateral noncasted leg. Consequently, the contralateral hindlimb serves as the control in our study.

As shown in Fig. 6B, Sirt3 protein level was decreased by 17% in the soleus muscle from rats immobilized for 14 days relative to the contralateral control muscle ($P < 0.05$). Sirt1 and Nampt protein levels were not reduced in the soleus muscle from rats immobilized for 14 days (Fig. 6B). There were no differences in Sirt3 protein level in the soleus muscles between the leg of naive control rats and the contralateral noncasted leg of rats immobilized for 14 days [88.8 ± 3.5 mg ($n = 7$) for muscles of contralateral control muscle and 39.6 ± 3.2 mg ($n = 8$) for immobilized muscle]. This result indicates that unilateral immobilization did not cause overuse in the soleus muscle of the contralateral noncasted leg. Consequently, the contralateral hindlimb serves as the control in our study.

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between the leg of naive control rats and the contralateral noncasted legs of immobilized rats [1.00 ± 0.07 arbitrary units (n = 8) for muscles of naive controls and 1.04 ± 0.08 arbitrary units (n = 8) for muscles of contralateral noncasted legs].

**DISCUSSION**

In the present study, endurance treadmill-running training increased the Sirt3 protein expression levels in rat soleus and plantaris muscles (Fig. 1, A and B), whereas voluntary wheel-running training increased Sirt3 protein levels in rat plantaris and triceps muscles (Fig. 4, B and C). This evidence clearly showed that Sirt3 protein expression is upregulated by exercise training in rat skeletal muscles. Furthermore, in the present study, one side of the rat hindlimb was immobilized for 14 days. The Sirt3 protein level was reduced in the soleus muscle from the immobilized hindlimb compared with that shown from contralateral controls. Thus Sirt3 protein expression in rat soleus muscle is downregulated by the loss of postural support.

In the present study, energy intake during the experimental period was reduced in treadmill-running animals compared with that shown in sedentary controls (see RESULTS). Furthermore, energy intake was not increased in voluntary running animals (see RESULTS), showing that these rats did not increase their energy intake to compensate for the increased energy expenditure caused by voluntary running. As a result, both treadmill- and voluntary running rats resemble calorie-restricted rats in that they have a decreased availability of energy for daily living. A previous study demonstrated that gene expression of Sirt3 is upregulated in both white and brown adipose tissues in response to calorie restriction (33). Therefore, we initially hypothesized that the exercise-induced increase in Sirt3 protein expression in skeletal muscles is due to calorie restriction. However, this possibility seems to be unlikely. The present study demonstrated that Sirt3 protein expression was downregulated in immobilized soleus muscle
The intriguing question is, what happens in skeletal muscles as a consequence of the increased Sirt3 protein expression in response to exercise? Initially, we hypothesized that exercise-induced mitochondrial biogenesis is mediated by the increased Sirt3 protein expression. In our present study, voluntary wheel-running training increased or tended to increase PGC-1α and COXI protein levels in the plantaris and triceps muscles, respectively, with the increase in Sirt3 protein level (Fig. 4, Table 5). On the other hand, voluntary wheel-running training did not cause an increase in PGC-1α, COXI, or Sirt3 in the soleus muscle (Fig. 4, Table 5). Thus, in voluntary wheel runners, the increase in Sirt3 protein was limited to muscles in which the expression of mitochondrial biogenesis marker proteins was increased. Given the previous evidence that constitutive expression of Sirt3 promotes PGC-1α expression in brown adipocytes (33), our present results from voluntary wheel runners may support our hypothesis that mitochondrial biogenesis in exercised muscles is mediated by the increased Sirt3 protein expression. However, in our present study, treadmill-running training increased Sirt3 protein in the soleus and plantaris muscles, although mitochondrial biogenesis markers, i.e., PGC-1α and COXI, were not increased in these muscles (Fig. 1, Table 3). Thus it is unlikely that exercise-induced mitochondrial biogenesis is always mediated by the increased Sirt3 protein expression.

Besides mitochondrial biogenesis, the results of a previous study demonstrated a role for Sirt3 in the mitochondrial electron transport. Ahn et al. (2) reported that Sirt3 can physically interact with the 39-kDa protein NDUFA9, which is one of the known subunits of complex I of the mitochondrial electron transport chain. They also demonstrated that mitochondria from Sirt3-deficient mice display a selective inhibition of complex I activity (2). Therefore, it is possible that mitochondrial electron transport chain activity in skeletal muscles is upregulated as a result of the exercise-induced increase in Sirt3 protein expression.

Mitochondria are the major sites for reactive oxygen species production, which causes oxidative damage. Exercise-induced increases in Sirt3 may play a key role in protecting skeletal muscle cells from oxidative damage and/or oxidative stress-mediated apoptosis. Jacobs et al. (20) demonstrated that Sirt3 forms a physical interaction with the transcription factor FOXO3a and that its overexpression increases FOXO3a-dependent gene expression of manganese superoxide dismutase, which can serve to neutralize superoxide. Thus an exercise-induced increase in Sirt3 may reduce the production of reactive oxygen species and play a role in protecting skeletal muscle cells from oxidative stress. The results of another study demonstrated that oxidative stress causes the translocation of the proapoptotic protein Bax from the cytoplasm to mitochondria and facilitates the release of apoptosis-inducing factor, resulting in stress-induced apoptosis (27). Moreover, Sundaresan et al. (34) identified Ku70 as a new target of Sirt3 deacetylase and demonstrated that deacetylation of Ku70 by Sirt3 promotes the physical association of Ku70 with Bax in cardiomyocytes, and this makes cells resistant to Bax-mediated cell apoptosis during oxidative stress. Given these roles for Sirt3 and previous reported evidence that chronic contractile activity can exert a protective effect on mitochondrially mediated apoptosis in skeletal muscle (1), it is possible that the exercise-induced increase in Sirt3 protein expression protects skeletal muscles from oxidative stress-mediated apoptosis. These possibilities described above await further experimental exploration.
In the present study, neither treadmill- nor voluntary wheel-running training increased the rats’ Sirt3 mRNA level (Tables 1 and 4), although exercise training increased Sirt3 protein expression (Figs. 1 and 4). Moreover, Sirt3 mRNA remained unchanged in the soleus muscle at 2, 4, and 8 h after the cessation of acute treadmill running (Table 2). Thus, it is likely that the exercise-induced increase in Sirt3 protein is independent of a change in Sirt3 mRNA level. Our present findings imply that enhanced translation process plays an important part in the exercise-induced increase in Sirt3 protein. For mRNA to be translated into protein, it must first associate with ribosomes to form translationally competent structures referred to as polysomes. Stimulation of translation initiation results in an increase in the proportion of ribosomal subunits in polysomes (6). Baar and Esser (4) showed that, in skeletal muscle, ribosomal subunits redistribute into polysomes 6 h after electrical stimulation, indicating that translation initiation is enhanced after muscle contractile activity. This mechanism might be the case for the exercise-induced increase in Sirt3 protein expression without the increase in mRNA level.

We found that, in resting sedentary rats, Sirt3 protein expression was highest in the soleus, which consists of >80% type I fibers (3), and lowest in the triceps, which consists of 70% type IIB fibers (26) (Fig. 5). Type I muscle fibers are heavily recruited (~7 h/day) in rats housed in standard laboratory cages, whereas type IIB fibers are rarely recruited (~2 min/day) (17). Therefore, we hypothesize that the difference in the protein expression level of Sirt3 between different kinds of muscles can be attributed to the daily postural contractile activity level. This hypothesis is supported by our present finding that the loss of postural support due to 14-day hindlimb immobilization reduces the Sirt3 protein level in the soleus muscle (Fig. 6B).

Treatment of mice with resveratrol, which is a specific activator of Sirt1, results in mitochondrial biogenesis in skeletal muscles (5, 22). Furthermore, Suwa et al. (35) demonstrated that treadmill-running training increased the Sirt1 protein level in rat skeletal muscles. These experimental findings led us to hypothesize that exercise training induces mitochondrial biogenesis in skeletal muscles through increased Sirt1 expression. However, unexpectedly, in the present study, voluntary wheel-running training did not increase the Sirt1 protein level in skeletal muscles, whereas the expressions of mitochondrial biogenesis marker proteins PGC-1α and COX1 were increased in the plantaris muscles (Table 5). Although we cannot explain this discrepancy between our results and those of Suwa et al., it is at least suggested that increased mitochondrial biogenesis in skeletal muscles of our voluntary wheel runners was not mediated by the increased Sirt1 expression. The results of a recent study demonstrated that AMP-activated protein kinase enhances Sirt1 activity by increasing cellular NAD levels in mouse skeletal muscles, resulting in the deacetylation of PGC-1α and the promotion of mitochondrial biogenesis (8). Because exercise is a significant physiological stimulus known to activate AMP-activated protein kinase in skeletal muscles (15), it is possible that exercise increases mitochondrial biogenesis in skeletal muscles through the increased Sirt1 intrinsic activity but not through the increased expression level.

The sirtuin proteins, e.g., Sirt1 and Sirt3, require NAD for their deacetylase activity. Furthermore, evidence that Nampt is the rate-limiting component in the mammalian intracellular NAD biosynthesis pathway was previously shown (29). Moreover, we demonstrated that, in resting rats, the Nampt protein level was highest in a red muscle (soleus) with a high amount of daily contractile activity and was lowest in a white muscle (triceps) with a low amount of contractile activity (Fig. 5). Therefore, we hypothesized that exercise training increases Nampt protein expression as well as Sirt3 expression in skeletal muscles, thus increasing sirtuins activity and promoting mitochondrial function. However, unlike Sirt3, the Nampt protein expression level in rat skeletal muscles was not upregulated as a result of our exercise training program (Figs. 1 and 4). While our paper was in preparation, Costford et al. (12) published a study in which 3 wk of exercise training increased Nampt protein level in skeletal muscles of nonobese human subjects. We do not presently know the reason for this discrepancy between our results and those of Costford et al.; however, it might be possible that it is due to differences in age of subjects and/or exercise protocol. Because Koltai et al. (21) demonstrated that there was an age-related decrease in Nampt protein level in rat skeletal muscle, Nampt protein level is suspected to be very high even in sedentary skeletal muscles of young rats (9 wk), which were used in our present study. Therefore, it is possible that exercise might not increase Nampt protein level above young sedentary levels in our present study.

In summary, our present study demonstrated that endurance exercise training increased Sirt3 protein expression in rat skeletal muscles. Furthermore, we found that, in resting rats, Sirt3 protein expression was higher in the soleus muscle (red muscle) than in the triceps muscle (white muscle). In addition, our present study showed that Sirt3 protein expression was downregulated in immobilized soleus muscle compared with that shown in contralateral control muscle. These findings provide evidence that exercise training upregulates Sirt3 protein expression and that local postural contractile activity plays a crucial role in maintaining a high level of Sirt3 protein expression in postural muscle.

GRANTS

This research was supported by the Nakatomi Foundation (Tosu, Japan), a Grant-in-Aid from Niigata University of Health and Welfare, and Grant-in-Aid for Scientific Research (KAKENHI) (C) 18500518 from the Japan Society for the Promotion of Science.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

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