IVYSPRING INTERNATIONAL PUBLISHER

International Journal of Medical Sciences

2016; 13(4): 260-270. doi: 10.7150/ijms.14586

Research Paper

Effect of Exercise Training on Skeletal Muscle SIRT1 and PGC-1α Expression Levels in Rats of Different Age

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Received: 2015.12.02; Accepted: 2016.02.24; Published: 2016.03.16

Abstract

The protein deacetylase sirtuin 1 (SIRT1) and activate peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) pathway drives the muscular fiber-type switching, and can directly regulate the biophysiological functions of skeletal muscle. To investigate whether 12-week swimming exercise training modulates the SIRT1/PGC-1α pathway associated proteins expression in rats of different age. Male 3-month-old (3M), 12-month-old (12M) and 18-month-old (18M) Sprague-Dawley rats were used and assigned to sedentary control (C) or 12-week swimming exercise training (E) and divided into six groups: 3MC (*n* = 8), 12MC (*n* = 6), 18MC (*n* = 8), 3ME (*n* = 8), 12ME (*n* = 5) and 18ME (*n* = 6). Body weight, muscle weight, epididymal fat mass and muscle morphology were performed at the end of the experiment. The protein levels of SIRT1, PGC-1α, AMPK and FOXO3a in the gastrocnemius and soleus muscles were examined. The SIRT1, PGC-1α and AMPK levels in the gastrocnemius and soleus muscles were up-regulated in the three exercise training groups than three control groups. The FOXO3a level in the 12ME group significantly increased in the gastrocnemius muscles than 12MC group, but significantly decreased in the soleus muscles. In 3-, 12- and 18-month-old rats with and without exercise, there was a significant main effect of exercise on PGC-1α, AMPK and FOXO3a in the gastrocnemius muscles, and SIRT1, PGC-1α and AMPK in the soleus muscles. Our result suggests that swimming training can regulate the SIRT1/PGC-1α, AMPK and FOXO3a proteins expression of the soleus muscles in aged rats.

Key words: exercise training, aging, skeletal muscle, SIRT1, PGC-1α.

Introduction

Physical exercise enhances or maintains physical fitness and health. Regular physical exercise helps to improve human physiological function [1, 2], and prevent the metabolic syndrome, heart disease, cardiovascular disease, hypertension, Type 2 diabetes, obesity and so on [3, 4]. Childhood obesity is a growing global problem, and physical exercise may help decrease some of the effects of childhood and adult obesity. It has been believed that exercise is an efficient non-pharmacological intervention for human health.

Physical exercises are generally grouped into aerobic exercise, anaerobic exercise and flexibility exercise. Swimming exercise training is an aerobic exercise that uses large-muscle groups and causes your body to use more oxygen than it would while resting. Exercise demands a greater supply of energy [5]. Different types of exercises elicit varied responses from various substrates including glucose, lactate and pyruvate in the blood that may be due to changes in the effect of stress imposed on the individual organs [6, 7]. Swimming has been considered as a suitable model of endurance exercise training [8]. Ravi Kiran *et al*. [9] showed swimming exercise training significantly increased superoxide dismutase (Mn-SOD), and reduced lipid peroxidation products,

malondialdehyde (MDA) and lipofuscin in the left and right ventricles.

SIRT1 is an enzyme that deacetylates FOXO3a and NF-κB [10-12]. FOXO3a and NF-κB deacetylation causes their transcription to fail and inhibits the downstream regulation of cell death by inflammation proteins [13]. Thus, SIRT1 activation could promote cell survival. Zarzuelo *et al*. [14] showed that the appropriate long-term exercise training can protect the heart through SIRT1 activation and reducing ROS. Ferrer *et al*. [15] reported that the SIRT3 and PGC-1α increases in white blood cells to activate the antioxidant response after intense swimming. In addition, SIRT3 and PGC-1α in human skeletal muscle decreased with age and correlate with a sedentary proteomic profile found in people with decreased metabolic output [16]. With exercise, however, Palacios *et al*. [17] observed that the effect is reversed.

The purpose of the present study was to examine the effects of swim exercise training at 40 min/d for 12 weeks on SIRT1, PGC-1α, AMPK and FOXO3a in adult (6-month-old), middle-aged (12-month-old) and old-aged rats (18-month-old).

Materials and methods

Animals and experiment design

Specific pathogen-free female Sprague Dawley (SD) rats were purchased from BioLASCO (A Charles River Licensee Corp., Yi-Lan, Taiwan). All animals were fed a chow diet (No. 5001; PMI Nutrition International, Brentwood, MO, USA), distilled water *ad libitum*, housed at room temperature (23±2°C) and humidity-controlled (70 ± 10%) with a 12-h light/12-h dark cycle. Fig. 1 denotes the categorization of rats into groups and subgroups. In brief, rats were randomly assigned to one of three groups i.e. 3-, 12- and 18-month-old, and two sub groups with or without swim exercise training intervention. Thus, SD rats were assigned into sedentary control (C) or a 12-week swimming exercise training (E), and divided into six groups: (1) 3-month-old rats without swim exercise training (3MC; n = 8); (2) 3-month-old rats with swim exercise training (3ME; n = 8); (3) 12-month-old rats without swim exercise training (12MC; n = 6); (4) 12-month-old rats with swim exercise training (12ME; n = 5); (5) 18-month-old rats without swim exercise training (18MC; n = 8); (6) 18-month-old rats with swim exercise training (18ME; n = 6). Animals were anesthetized with Zoletil/Xylazine and sacrificed after 12-week swimming exercise training. Body weight, muscle weight, epididymal fat mass and muscle morphology were performed at the end of the experiment. The gastrocnemius and soleus muscles were carefully harvested, rinsed in ice-cold normal saline, blotted dry and stored at -80°C for further analysis. All animal experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Tunghai University, and the study conformed to the guidelines of the protocol IACUC-98-27 approved by the IACUC ethics committee.

Exercise training program

Swim exercise training was similar to earlier protocols with minor modifications [9]. In brief, rats were made to exercise in groups of three in a plastic tank (diameter: 48 cm) filled with water to a height of 50 cm at 35±1°C. Animals were trained daily between AM 10:00 and PM 12:30. The pre-training period lasted for three-weeks (the first weeks lasted only 10 min, the second weeks lasted only 20 min, and the third weeks lasted only 30 min), and the animals were exercised in 40 min/day, 5 days/week for 4-12 weeks (Fig. 1B). At the completion of exercise, rats were towel-dried and returned to their respective cages. No deaths occurred during or after exercise in any groups. Sedentary control group of rats were confined to stand in groups of three in a plastic tank (diameter: 48 cm) filled with water to a height of 5 cm at 35±1°C. The body weight of all groups was monitored recorded weekly.

Gross and histological evaluation of the gastrocnemius and soleus muscles

The gastrocnemius and soleus muscles were fixed in 10% formalin, embedded in paraffin and cut into 4-μm thick slices as per our previous study. Tissue sections were stained with Hematoxylin and Eosin (H&E), and examined using a light microscope equipped with a CCD camera (Olympus BX50; Olympus Co., Ltd., Tokyo, Japan). The total muscle area of each section was highlighted and the total number of pixels was recorded.

Western blot analysis

Expressions of the gastrocnemius and soleus muscle proteins were measured by western blot. The gastrocnemius and soleus muscles were homogenized in 500 μl of homogenization buffer (5 mM Tris-HCl pH 7.4, 0.15 M NaCl, 1% NP40, 0.25% Sodium deoxycholate, 5 mM EDTA, and 1 mM ethylene glycol-bis(2-aminoethyl-ether)-N, N, N, N-tetraacetic acid). The homogenates were centrifuged at 13,200 *g* for 40 minutes at 4°C. Protein (50 μg) was then separated by SDS-PAGE in 8% polyacrylamide and electrotransferred to polyvinylidene difluoride membranes. The membranes were incubated in blocking solution (5% milk) at room temperature for 2

hours. The membranes were then incubated with primary antibody including SIRT1 (sc-74465, Santa Cruz, USA), PGC-1α (#516557, Calbiochem, USA), FOXO3a (# 2497, Cell Signaling, USA), AMPK (sc-33524, Santa Cruz, USA), and α-tubulin (sc-74465, Santa Cruz, USA) overnight at 4°C. After washing, the membranes were incubated with a goat anti-rabbit (Santa Cruz, USA) or goat anti-mouse IgG (Santa Cruz, USA) peroxidase-conjugated secondary antibody directed against the primary antibody. The membranes were developed by an enhanced chemiluminescence western blot detection system.

Statistical analysis

Data were expressed as mean ± SEM. Results were analyzed by one-way analysis of variance (ANOVA, Scheffe's method). A value of $P < 0.05$ was considered to indicate statistical significance.

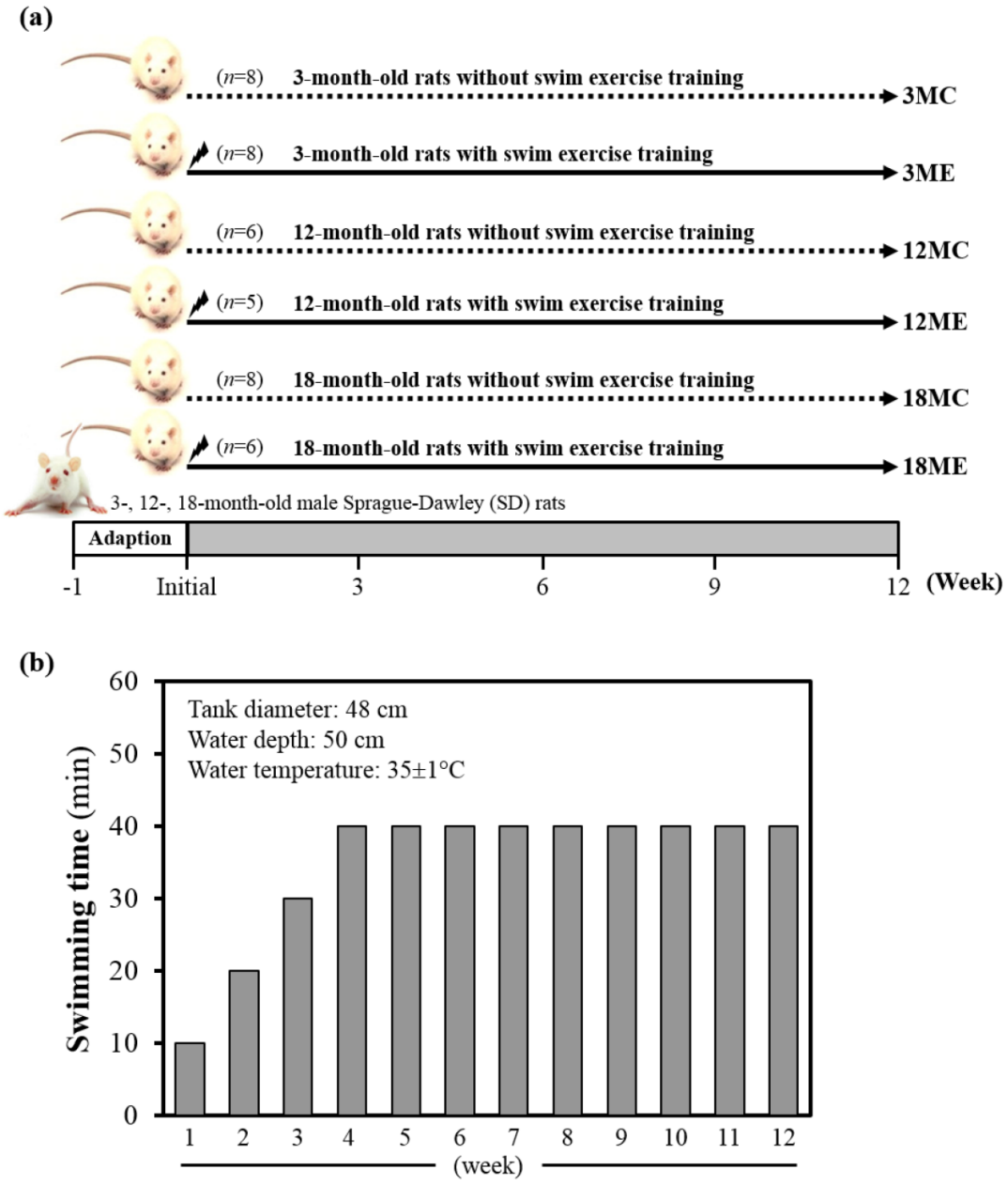

Figure 1. Experimental design (**a**) and protocol for 12-wk swim exercise training (**b**). Male 3-month-old (3M), 12-month-old (12M) and 18-month-old (18M) Sprague-Dawley rats were used for this study, assigned to sedentary control (C) or 12-week swimming exercise training (E) and divided into six groups: which were respectively designated the 3MC (n = 8), 12MC (n = 6), 18MC (n = 8), 3ME (n = 8), 12ME (n = 5) and 18ME (n = 6). The pre-training period lasted for three-weeks (the first weeks lasted only 10 min, the second weeks lasted only 20 min, and the third weeks lasted only 30 min), and the rats were exercised in 40 min/day, 5 days/week for 4-12 weeks.

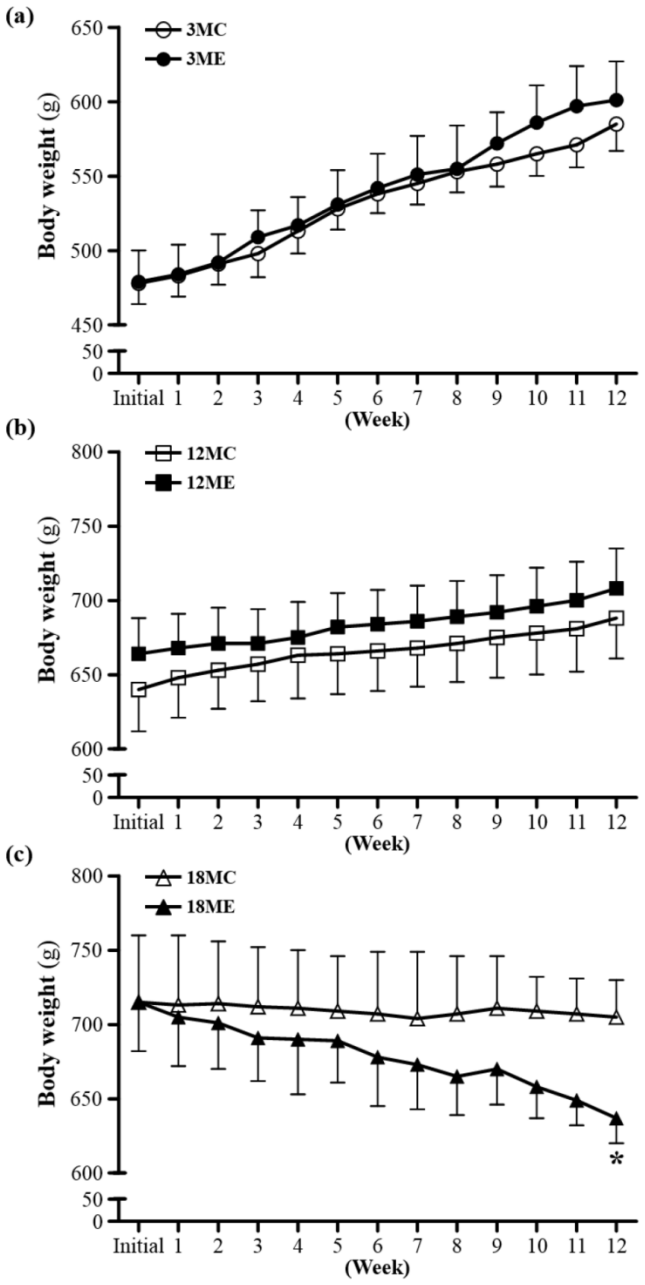

Figure 2. Body weights over the course of 12 weeks. 3MC; male 3-month-old SD rats without swimming exercise training, 3ME; male 3-month-old SD rats with swimming exercise training, 12MC; male 12-month-old SD rats without swimming exercise training, 12ME; male 12-month-old SD rats with swimming exercise training, 18MC; male 18-month-old SD rats without swimming exercise training, 18ME; male 18-month-old SD rats with swimming exercise training. Data are mean ± SEM. * indicated significant difference at $P < 0.05$ by one-way ANOVA.

Results

Effect of exercise training on body weight

The rats of the two experimental groups at the same age had the similar initial body weights. To examine whether swim exercise training could increase or decrease body weight was recorded weekly (Fig. 2). After swim exercise training for 40 min/d, 5 days/week, for 12 weeks, there was no difference found in body weight between 3MC group and 3ME group, or 12MC group and 12ME group. In addition, it is interesting that the 18-month-old rats after swim exercise training showed a significant decrease in body weight by 9.6% (637 ± 17 g) relative to the rats without swim exercise training (705 ± 25 g) ($P = 0.0486$). Numerous studies have shown that male rats subjected to a program of regularly performed endurance exercise gain weight more slowly and have significantly lower final body weights than freely eating sedentary controls [18].

Effect of exercise training on epididymal fat pad

The epididymal fat pad (EFP) weights at the end of the study were shown in Table 1. EFP mass was slightly lowered in 3-, 12- or 18-month-old rats for swim exercise training compared to 3-, 12- or 18- rats without swim exercise training by 2.3%, 15.7% or 43.8% ($P = 0.0868$), respectively. In addition, the relative weight of EFP was slightly decreased for swim exercise training than the rats without swim exercise training. In early life of rats, the fat accumulates in EFP as a result of an increase in cell number and cell size [19, 20]. At approximately 15-week-old, cell number becomes fixed in this depot, and only cell size changes with further increases in adiposity [19, 20]. These results showed that exercise caused a reduction of EFP in 18-month-old and revealed that exercise retards the rate at which adipose tissue cells accumulate or enlarge, or both.

Effect of exercise training on muscles

The mass of the gastrocnemius muscles was no significant difference in 3MC group and 3ME group, 12MC group and 12ME group, or 18MC group and 18ME group (Table 1). The representative pictures of the gastrocnemius muscle fibers in each group were shown in Fig. 3. In the swim exercise training groups, there were increased in the gastrocnemius muscle length and area when compared with those without swim exercise training. The fiber length of the gastrocnemius muscles was significantly increased by 23%, 22% and 31%, respectively, in the 3ME group (66.1 ± 1.4 μm), 12ME group (69.0 ± 1.3 μm) and 18ME group (67.0 ± 1.5 μm) when compared with 3MC group (53.7 ± 1.5 μm), 12MC group (56.6 ± 1.4 μm) and 18MC group (51.3 ± 0.8 μm) ($P < 0.05$). And the area of the gastrocnemius muscles was significantly increased by 40%, 44% and 86%, respectively, in the 3ME group (21903 ± 827 μm^2), 12ME group (24164 ± 771 μm^2) and 18ME group (23726 ± 2282 μm^2) when compared with 3MC group (15643 ± 664 μm^2), 12MC group (16740 ± 720 μm^2) and 18MC group (12760 ± 360

μm²) ($P < 0.05$). In this study, an obvious increase in fiber size of the gastrocnemius muscles in the swim exercise training groups was observed.

The mass of the soleus muscles were no significant difference in the 3MC, 3ME, 12MC, 12ME, 18MC and 18ME groups (Table 1), but the soleus muscle length and area were increased in the swim exercise training groups when compared with those without swim exercise training (Fig. 4). The fiber length of the soleus muscles was significantly increased by 8%, 32% and 13%, respectively, in the 3ME group (55.3 ± 0.9 μm), 12ME group (69.2 ± 1.5 μm) and 18ME group (71.4 ± 1.2 μm) when compared with 3MC group (51.3 ± 0.8 μm), 12MC group (52.4 ± 0.9 μm) and 18MC group (63.2 ± 1.4 μm) ($P < 0.05$). And the area of s the soleus muscles was significantly increased by 63% and 28%, respectively, in the 12ME group (23018 ± 743 μm²) and 18ME group (25636 ± 1620 μm²) when compared with 12MC group (14132 ± 381 μm²) and 18MC group (20048 ± 683 μm²) ($P < 0.05$). In this study, an increase in fiber size of the soleus muscles was observed and the results were the same with the data of the gastrocnemius muscles [21]. Kraemer *et al*. [21] showed that the increase of fiber size leads to the increase of muscle force-generating potential. Therefore, swim exercise training rats may increase muscle force-generating potential.

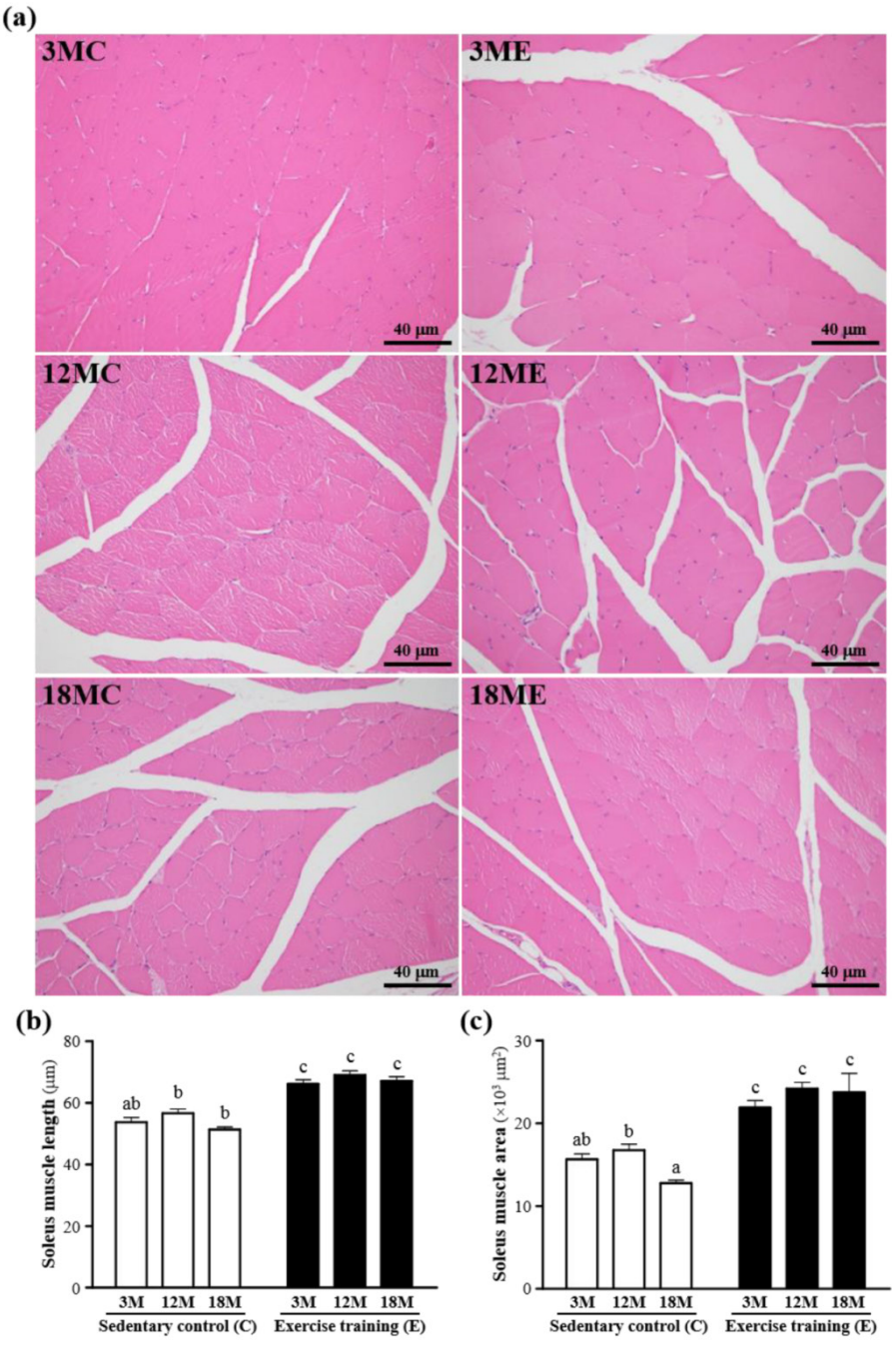

Figure 3. Effect of exercise training in the gastrocnemius muscles. **a**; the hematoxylin-eosin (H&E) staining of histologically sectioned the gastrocnemius muscles. **b**; the fiber length of the gastrocnemius muscles. **c**; the fiber area of the gastrocnemius muscles. 3MC; male 3-month-old SD rats without swimming exercise training, 3ME; male 3-month-old SD rats with swimming exercise training, 12MC; male 12-month-old SD rats without swimming exercise training, 12ME; male 12-month-old SD rats with swimming exercise training, 18MC; male 18-month-old SD rats without swimming exercise training, 18ME; male 18-month-old SD rats with swimming exercise training. Data are mean ± SEM. Different letters indicated significant difference at $P < 0.05$ by one-way ANOVA.

Table 1. General characteristics of the experimental groups

Characteristic	3MC	3ME	12MC	12ME	18MC	18ME
Initial BW (g)	478±14	479±21	640±28	664±24	715±45	715±33
Final BW (g)	585±18	601±26	688±27	708±27	705±25	637±17*
Gastrocnemius (g)	2.82±0.18	2.93±0.10	2.67±0.15	2.44±0.09	2.79±0.14	2.84±0.17
Soleus (g)	0.25±0.03	0.22±0.01	0.26±0.03	0.27±0.03	0.24±0.01	0.25±0.01
EFP (g)	4.69±0.62	4.80±0.66	5.62±0.67	4.74±0.42	8.06±1.69	4.53±0.55
Relative EFP (%)	0.80±0.10	0.78±0.09	0.82±0.09	0.67±0.05	1.15±0.26	0.72±0.10

EFP, epididymal fat pad; Relative EFP (%), epididymal fat pad weight/body weight×100%.
Data are mean ± SEM. *, differ significantly at $P < 0.05$ by Student's *t* Test between same age in the same line.

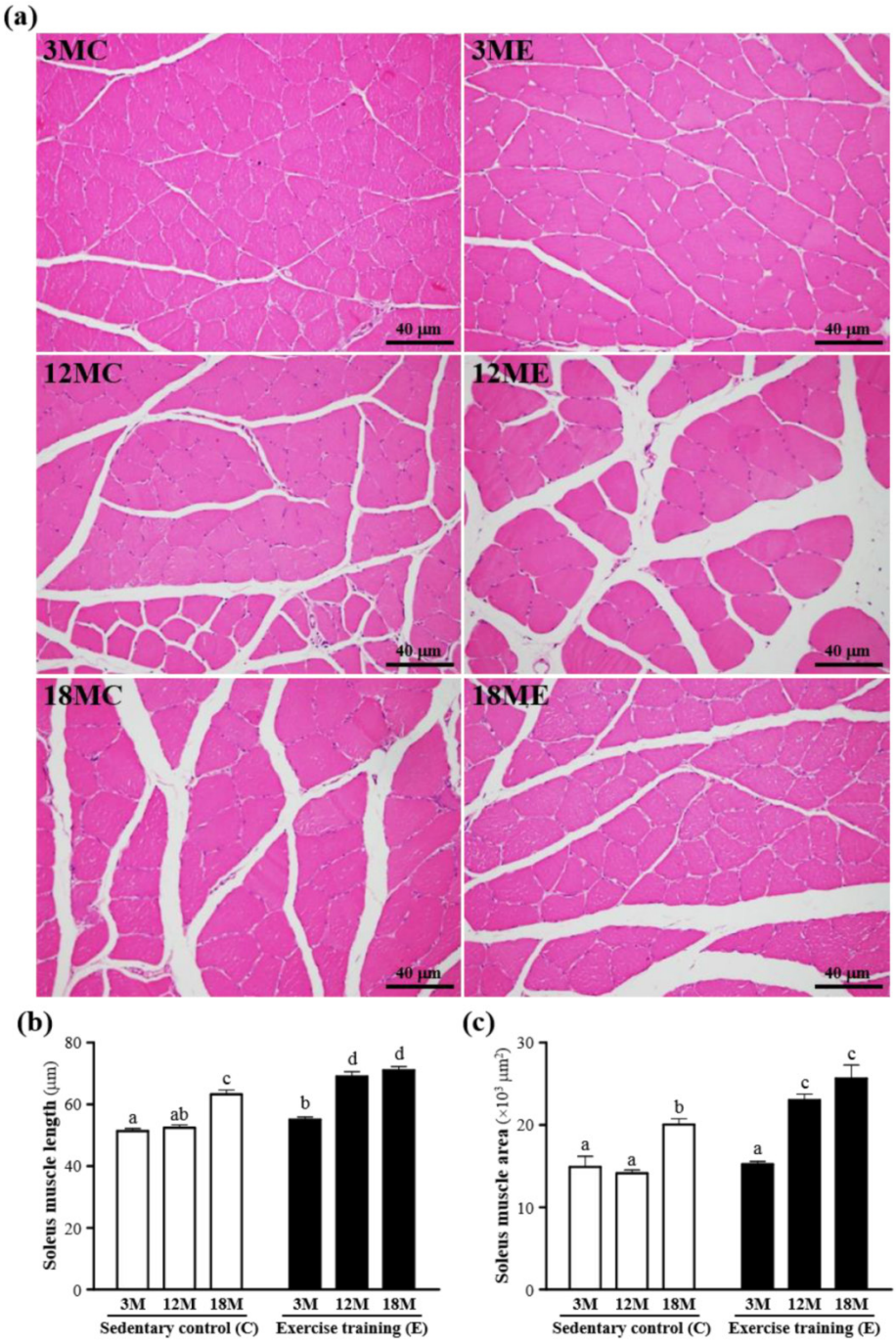

Figure 4. Effect of exercise training in the soleus muscles. **a**; the hematoxylin-eosin (H&E) staining of histologically sectioned the soleus muscles. **b**; the fiber length of the soleus muscles. **c**; the fiber area of the soleus muscles. 3MC; male 3-month-old SD rats without swimming exercise training, 3ME; male 3-month-old SD rats with swimming exercise training, 12MC; male 12-month-old SD rats without swimming exercise training, 12ME; male 12-month-old SD rats with swimming exercise training, 18MC; male 18-month-old SD rats without swimming exercise training, 18ME; male 18-month-old SD rats with swimming exercise training. Data are mean ± SEM. Different letters indicated significant difference at $P < 0.05$ by one-way ANOVA.

In addition, Fig. 3 and 4 also showed that exercise training increased capillary density with neocapillarization in the gastrocnemius and soleus muscles. Angiogenesis induced by exercise has been reported to cooperate with increasing expression of angiogenic factors [22]. Lloyd *et al*. [23] showed that the treadmill exercise training induced angiogenesis in the gastrocnemius muscles, which might be related with activation of angiopoietin and VEGF. Iemitsu *et al*. [24] also exhibited that the swimming exercise training improved aging-induced reduction of cardiac capillary density, and a decrease in expression of VEGF and its receptors, Flt-1 and Flk-1, in the heart.

Effect of exercise training on SIRT1, PGC-1α, AMPK and FOXO3a

Fig. 5 showed a representative western blot of SIRT1, PGC-1α, AMPK and FOXO3a levels in the gastrocnemius muscles. The SIRT1 of the gastrocnemius muscles in the 3ME and 12ME groups with the ratios of 1.27 ± 0.16 and 1.70 ± 0.34 showed slightly increased by 27% and 23%, respectively, relative to those observed in 3MC group (1.00 ± 0.25) and 12MC group (1.38 ± 0.19). The groups of 3ME (1.05 ± 0.07), 12ME (1.34 ± 0.63) and 18ME (1.64 ± 0.21) had increased the gastrocnemius PGC-1α levels by 5%, 38% and 48% ($P < 0.05$), respectively, relative to those observed in 3MC (1.00 ± 0.13), 12MC (0.98 ± 0.27) and 18MC (1.11 ± 0.13) groups. The FOXO3a level of the gastrocnemius muscles in 3ME group (1.30 ± 0.16) was slightly increased by 30% than 3MC group (1.00 ± 0.26), and the groups of 12ME (2.15 ± 0.28) and 18ME (1.27 ± 0.11) had significantly increased FOXO3a levels by 62% and 66% ($P < 0.05$), respectively, relative to those observed in 12MC (1.33 ± 0.44) and 18MC (0.77 ± 0.22) groups. The AMPK of the gastrocnemius muscles in the 3ME, 12ME and 18ME groups showed slightly increased by 62%, 14% and 74%, respectively, relative to those observed in 3MC, 12MC and 18MC groups. But there was no significant difference among each group in the AMPK levels of the gastrocnemius muscles. There was a significant main effect of exercise on PGC-1α ($P = 0.0088$) and FOXO3a ($P < 0.0001$) in the gastrocnemius muscles, but there was no effect of exercise on SIRT1 ($P = 0.1052$) and AMPK ($P = 0.1494$). Calculated SIRT1 ($P = 0.0005$), PGC-1α ($P = 0.0446$) and FOXO3a ($P < 0.0001$) have significant difference on different ages, but AMPK ($P = 0.7084$) did not differ among different ages. There was no significant interaction (age × exercise) for SIRT1 ($P = 0.1191$), PGC-1α ($P = 0.1897$), AMPK ($P = 0.7527$) and FOXO3a ($P = 0.0926$) (Table 2).

Table 2. Effect of age and exercise training on SIRT1, PGC-1α, AMPK and FOXO3a.

Group	3-month-old	12-month-old	18-month-old	Effect	*P*
SIRT1 of gastrocnemius				Age	0.0005
Sedentary	1.00 ± 0.10	1.38 ± 0.08	1.79 ± 0.17	Exercise	0.1052
Swimming exercise training	1.27 ± 0.07	1.70 ± 0.14	1.67 ± 0.02	Age x Exercise	0.1191
PGC-1α of gastrocnemius				Age	0.0446
Sedentary	1.00 ± 0.05	0.98 ± 0.11	1.11 ± 0.05	Exercise	0.0088
Swimming exercise training	1.05 ± 0.03	1.34 ± 0.26	1.64 ± 0.09	Age x Exercise	0.1897
AMPK of gastrocnemius				Age	0.7084
Sedentary	1.00 ± 0.19	0.99 ± 0.17	0.77 ± 0.06	Exercise	0.1494
Swimming exercise training	1.62 ± 0.60	1.13 ± 0.13	1.34 ± 0.54	Age x Exercise	0.7527
FOXO3a of gastrocnemius				Age	<0.0001
Sedentary	1.00 ± 0.11	1.33 ± 0.18	0.77 ± 0.09	Exercise	<0.0001
Swimming exercise training	1.30 ± 0.07	2.15 ± 0.11	1.27 ± 0.05	Age x Exercise	0.0926
SIRT1 of soleus				Age	0.0023
Sedentary	1.00 ± 0.01	1.42 ± 0.07	1.75 ± 0.15	Exercise	0.0081
Swimming exercise training	1.45 ± 0.05	1.85 ± 0.20	1.92 ± 0.20	Age x Exercise	0.5521
PGC-1α of soleus				Age	0.2001
Sedentary	1.00 ± 0.04	1.07 ± 0.10	0.91 ± 0.01	Exercise	0.0029
Swimming exercise training	1.04 ± 0.00	1.20 ± 0.07	1.30 ± 0.08	Age x Exercise	0.0391
AMPK of soleus				Age	0.1422
Sedentary	1.00 ± 0.02	1.37 ± 0.25	1.07 ± 0.02	Exercise	0.0496
Swimming exercise training	1.56 ± 0.12	1.35 ± 0.07	1.16 ± 0.02	Age x Exercise	0.0657
FOXO3a of soleus				Age	0.1770
Sedentary	1.00 ± 0.05	1.71 ± 0.17	1.08 ± 0.10	Exercise	0.2693
Swimming exercise training	1.38 ± 0.44	1.00 ± 0.08	0.84 ± 0.02	Age x Exercise	0.0534

Data are mean ± SEM.

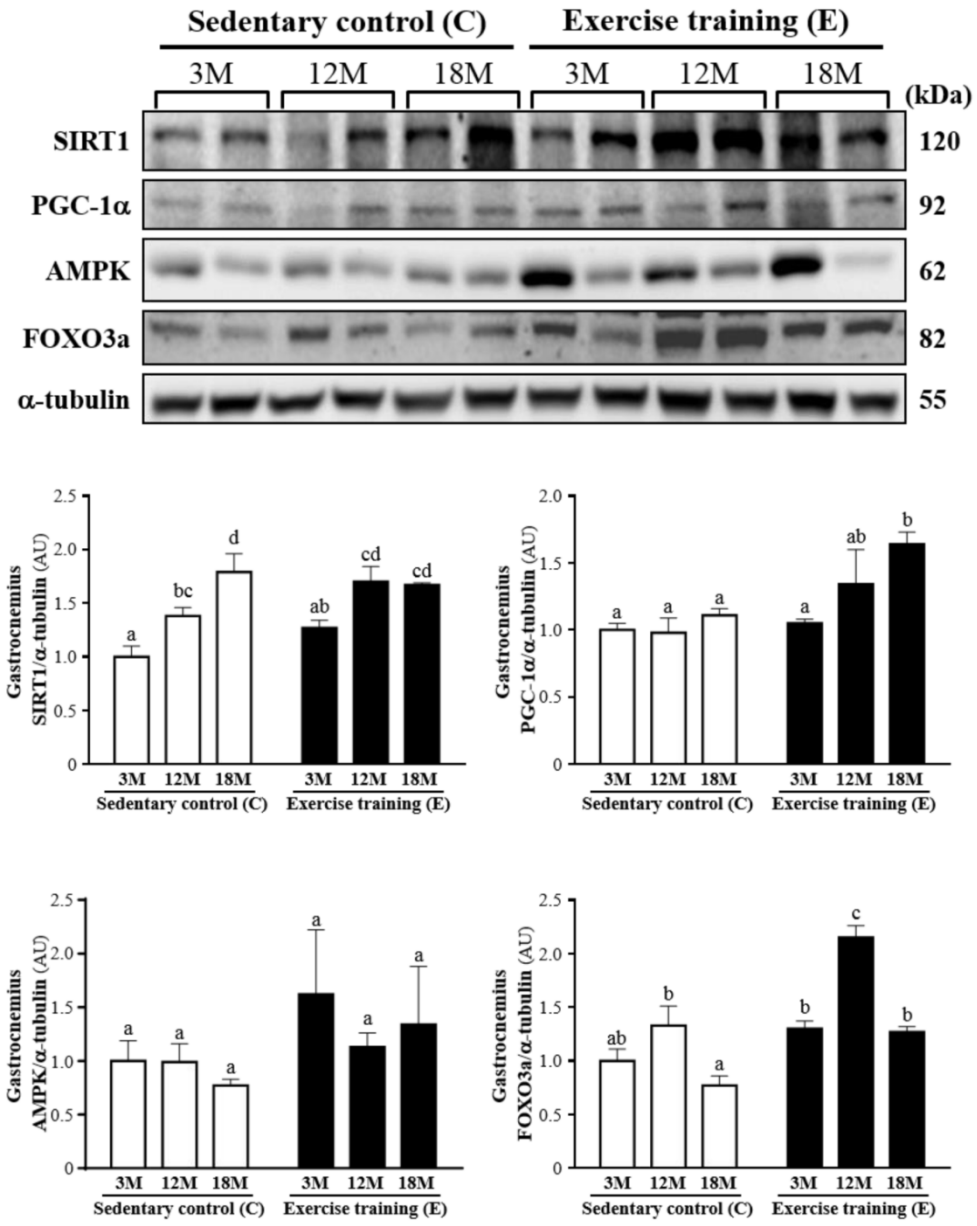

Figure 5 Protein expression levels of SIRT1, PGC-1α, AMPK and FOXO3a in the gastrocnemius muscles as measured by Western blot. α-tubulin was used an internal control. Data were expressed as mean ± SEM of eight rats. 3MC; male 3-month-old SD rats without swimming exercise training, 3ME; male 3-month-old SD rats with swimming exercise training, 12MC; male 12-month-old SD rats without swimming exercise training, 12ME; male 12-month-old SD rats with swimming exercise training, 18MC; male 18-month-old SD rats without swimming exercise training, 18ME; male 18-month-old SD rats with swimming exercise training. Different letters indicated significant difference at $P < 0.05$ by one-way ANOVA.

The western blot of SIRT1, PGC-1α, AMPK and FOXO3a levels in the soleus muscles was shown in Fig. 6. SIRT1 was significantly increased by 45% ($P < 0.05$), 30% ($P < 0.05$) and 10%, respectively, in 3ME group (1.45 ± 0.13), 12ME group (1.85 ± 0.48) and 18ME group (1.92 ± 0.50) when compared with 3MC group (1.00 ± 0.02), 12MC group (1.42 ± 0.18) and 18MC group (1.75 ± 0.36). And PGC-1α was increased by 4%, 13% and 43% ($P < 0.05$), respectively, in the 3ME group (1.04 ± 0.13), 12ME group (1.20 ± 0.17) and 18ME group (1.30 ± 0.20) when compared with 3MC group (1.00 ± 0.10), 12MC group (1.07 ± 0.25) and 18MC group (0.91 ± 0.03) ($P < 0.05$). It is interesting, the FOXO3a levels of the soleus muscles in the 12MC group (1.71 ± 0.41) were significantly higher than 12ME group (1.00 ± 0.19) ($P < 0.05$). The AMPK level of the soleus muscles in the 3ME group (1.56 ± 0.29) was significantly increased by 56% than 3MC group (1.00 ± 0.06) ($P < 0.05$). By directly observing SIRT1, PGC-1α, AMPK and FOXO3a levels in 3-, 12- and 18-month-old rats with and without exercise, there was a significant main effect of exercise on SIRT1 ($P = 0.0081$), PGC-1α ($P = 0.0029$) and AMPK ($P = 0.0496$) in the soleus muscles. Calculated SIRT1 ($P = 0.0023$)

has significant difference on different ages, but PGC-1α (P = 0.2001), AMPK (P = 0.1422) and FOXO3a (P = 0.1770) did not differ among different ages. There was no significantly interaction (age × exercise) for SIRT1 (P = 0.5521), AMPK (P = 0.0657) and FOXO3a (P = 0.0534), but there was significantly interaction (age × exercise) for PGC-1α (P = 0.0391).

Comment

We conducted a series of experiments to characterize the effects of swim exercise training and age on SIRT1, PGC-1α, AMPK and FOXO3a. The major findings were that (i) 40 min/d of swim exercise significantly decreased body weight in 18-month-old rats, but not in 3- and 12-month-old rats; (ii) 40 min/d of swim exercise significantly increased fiber length and area of the gastrocnemius and soleus muscles, regardless of age; and (iii) rats with exercise compared to rats without exercise at the same age consistently had higher protein expressions of SIRT1, PGC-1α and AMPK in the gastrocnemius and soleus muscles.

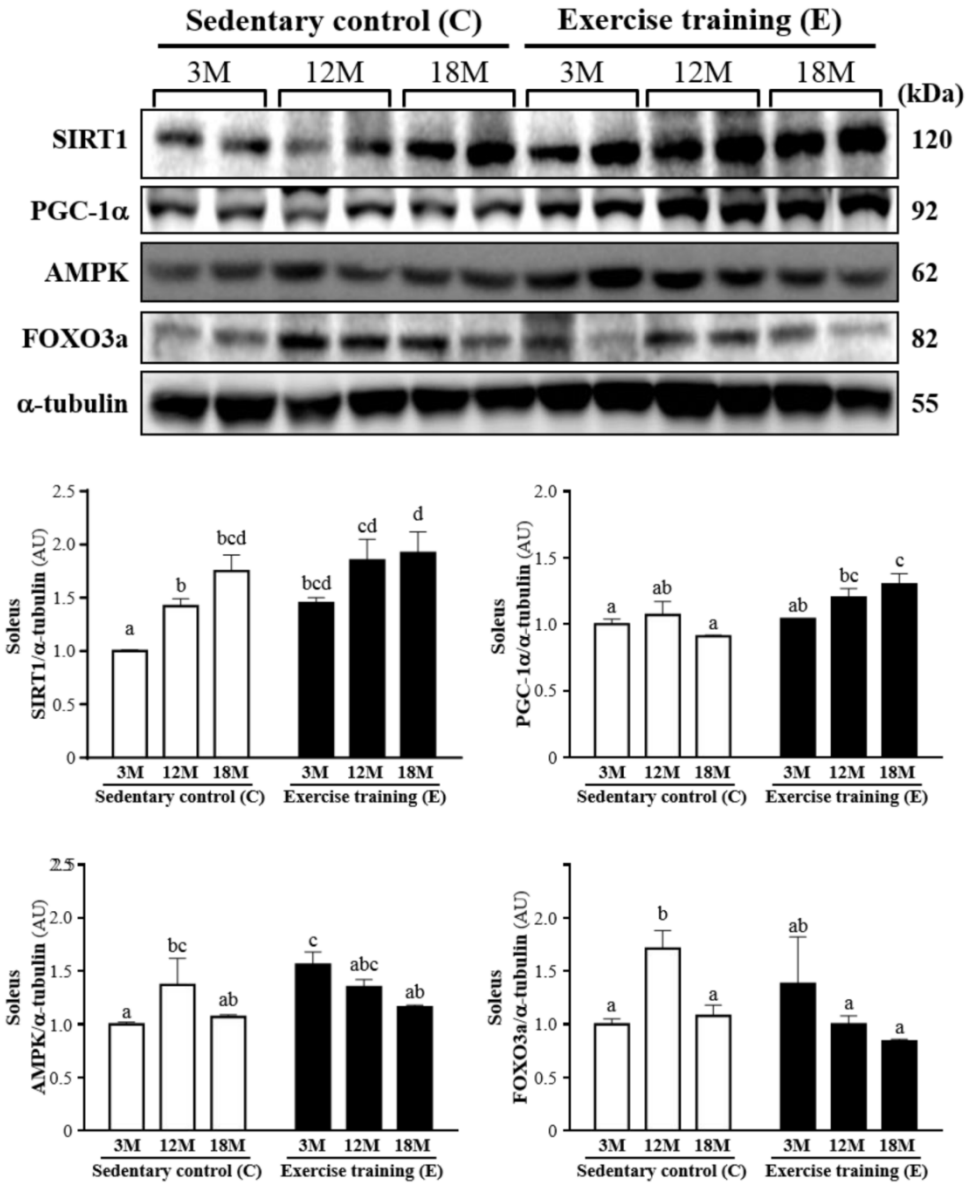

Figure 6 Protein expression levels of SIRT1, PGC-1α, AMPK and FOXO3a in the soleus muscles as measured by Western blot. α-tubulin was used an internal control. Data were expressed as mean ± SEM of eight rats. 3MC; male 3-month-old SD rats without swimming exercise training, 3ME; male 3-month-old SD rats with swimming exercise training, 12MC; male 12-month-old SD rats without swimming exercise training, 12ME; male 12-month-old SD rats with swimming exercise training, 18MC; male 18-month-old SD rats without swimming exercise training, 18ME; male 18-month-old SD rats with swimming exercise training. Different letters indicated significant difference at P < 0.05 by one-way ANOVA.

The SIRT1 of the gastrocnemius/soleus muscles in the exercise training groups showed increased when compared with the sedentary groups at 3-month- and 12-month-aged rats, except for 18-month-aged rats. These results showed that exercise has been considered a positive regulator in controlling SIRT1 expression at different age rats. In agreement with Falone *et al*. [25], exercise training enhances human SIRT1 expression in the hippocampus. SIRT1, which regulates diverse biological processes ranging from DNA repair and genome stability to glucose and lipid homeostasis, is an essential mediator of longevity in normal cells [26]. SIRT1 also plays a vital role in cellular physiological processes, including metabolism, and cell degeneration, growth and survival, and participates in an important function in regulating inflammation, such as the mitogen-activated protein kinase family (MAPKs) and NF-κB [27-29].

3ME, 12ME and 18ME groups had increased the gastrocnemius/soleus muscles PGC-1α levels by 5%/4%, 38%/13% and 48%/43%, respectively, relative to those observed in 3MC group, 12MC group and 18MC group. In agreement with previous studies, The mRNA and protein expression of PGC-1α were significantly increased by acute endurance exercise [30-33] and endurance exercise training [34, 35], thus suggesting that PGC-1α was a possible regulator of metabolic adaptations with endurance exercise.

The AMPK levels of gastrocnemius in the 3ME, 12ME and 18ME significantly increased by 62%, 14% and 74%, respectively, relative to those observed in 3MC group, 12MC group and 18MC group. In this study, we found that activation of AMPK may positively regulate SIRT1 and PGC-1α expression in muscles, thereby improving movement performance. Lezi *et al*. [36] exhibited that exercise training had higher SIRT1, PGC-1 and AMPK proteins in the liver and brain [36]. Both AMPK and p38 MAPK in muscle were activated by contractile activity and endurance exercise [37-41]. Collectively, these results increase the possibility that the metabolic adaptations resulting from endurance exercise training result at least in part *via* an increased PGC-1α protein through the AMPK and p38 MAPK pathways [42].

The FOXO3a levels of gastrocnemius in 12ME and 18 ME groups were significantly increased than 12MC and 18MC groups, but the FOXO3a level of the soleus muscles in the 12MC group was significantly higher than 12ME group. Moreover, SIRT1 regulates longevity factors and several factors by deacetylation of FOXO family [43], SIRT1 regulates age-related changes in different mechanisms including increasing mitochondriogenesis by modulating PGC-1α deacetylation, repressing oxidative stress survival response by FOXO family, reducing apoptosis and proliferation caused by p53 deacetylation and mitigating pro-inflammatory response by NF-κB activation [44, 45].

SIRT1, PGC-1α, AMPK and FOXO3a levels in 3-, 12- and 18-month-old rats with and without exercise, there was a significant main effect of exercise on PGC-1α, AMPK and FOXO3a in gastrocnemius muscles, and SIRT1, PGC-1α and AMPK in the soleus muscles. SIRT1 functionally deacetylates and activates PGC-1α [46, 47]. SIRT1 is a key regulator of mitochondrial biogenesis through the deacetylation of PGC-1α in skeletal muscle cells [46, 48]. SIRT1 plays a vital role in the modulation of the cytosolic NAD^+/NADH ratio in muscle gene expression [49]. SIRT1 contributes to skeletal muscle adaptations with endurance exercise that may be due to the cytosolic NAD^+/NADH ratio changes during muscle contraction [50]. Suwa *et al*. [42] therefore showed that SIRT1 has increased after endurance exercise to facilitate such metabolic adaptation. Palacios *et al*. [17] also showed that in the beginning of energy stress, AMPK acts as a sensor to allow the cell to interact efficiently with different energetic substrates. Hence, activation of SIRT1 involves the metabolic and transcriptional rearrangements which is an indirect sequence induced by AMPK activation. In addition to the ability to regulate Nampt expression, AMPK may also affect intracellular NAD+ levels, which further modulate SIRT1 downstream targets such as PGC-1α and FOXO1. Consequently, SIRT1 activation constitutes an indirect consequence of the metabolic and transcriptional rearrangements induced by AMPK activation.

In conclusion, the present study demonstrated that swimming exercise training at 40 min/d for 12 weeks can attenuate fiber size of muscles results in regulate the SIRT1, PGC-1α, AMPK and FOXO3a in muscles of different age rats. Therefore, the SIRT1/PGC-1α pathway can directly regulate the biophysiological functions of skeletal muscle.

Acknowledgments

This study was supported by the Ministry of Science and Technology of Taiwan (grants no. NSC-99-2410-H029-059-MY2 and MOST-103-2410-H-029-037 to Wan-Teng Lin). The authors are grateful to Miss Kai-Wen Chang and Dr. Wen-Ching Huang for technical assistance in animal experiments.

Competing Interests

The authors declare no competing interest.

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