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Exercise training attenuates aging-associated mitochondrial dysfunction in rat skeletal muscle: Role of PGC-1α

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ABSTRACT

Aged skeletal muscle demonstrates declines in muscle mass and deterioration of mitochondrial content and function. Peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α) plays an important role in promoting muscle mitochondrial biogenesis in response to exercise training, but its role in senescent muscle is not clear. In the present study we hypothesize that a downregulation of the PGC-1α signaling pathway contributes to mitochondrial deterioration in aged muscle whereas endurance training ameliorates the deficits. Three groups of Fischer 344/BNF1 rats were used: young, sedentary (Y, 4 mo); old, sedentary (O, 22 mo); and old trained (OT, 22 mo), subjected to treadmill running at 17.5 m/min, 10% grade for 45 min/day, 5 days/week for 12-weeks. PGC-1α mRNA and nuclear PGC-1α protein content in the soleus muscle were both decreased in O vs. Y rats, whereas OT rats showed a 2.3 and 1.8-fold higher PGC-1α content than O and Y rats, respectively ($P<0.01$). Mitochondrial transcription factor A (Tfam), cytochrome c (Cyt c) and mitochondrial (mt) DNA contents were significantly decreased in O vs. Y rats, but elevated by 2.2 ($P<0.01$), 1.4 ($P<0.05$) and 2.4-fold ($P<0.01$), respectively, in OT vs. O rats. In addition, Tfam and mtDNA showed 1.6 and 1.8-fold ($P<0.01$) higher levels, respectively, in OT vs. Y rats. These adaptations were accompanied by significant increases in the expression of the phosphorylated form of AMP-activated kinase (AMPK)($P<0.01$), p38 mitogen-activated kinase (MAPK)($P<0.05$) and silent mating type information regulator 2 homolog 1 (SIRT1) ($P<0.01$) in OT rats. Furthermore, OT rats showed great levels of phosphorylation in cAMP responsive element binding protein (p-CREB) and DNA binding compared to O and Y rats. These data indicate that endurance training can attenuate aging-associated decline in mitochondrial protein synthesis in skeletal muscle partly due to upregulation of PGC-1α signaling.

Key words: aging; exercise; mitochondria; PGC-1**α;** skeletal muscle

I. Introduction

Mitochondria, as dynamic organelles in the cells, have a critical function to regulate energy metabolism, intracellular signaling and apoptosis (Finkel and Holbrook, 2000; Wallace, 2005; Ryan and Hoogenraad, 2007). Reduction in mitochondrial activity and numbers is implicated in aging and age-related diseases such as neurodegenerative diseases, cancer and diabetes (Wallace, 2005). Thus, mitochondria have become one of the most studied organelles in aging research (Merry, 2004; Hunt et al., 2006; Navarro and Boveris, 2007). Among various theories postulated to explain the fundamental mechanisms underlying biological aging, free radical theory of aging and mitochondrial theory of aging both emphasize the role of reactive oxygen species (ROS) produced from the electron transport chain (ETC) as an important etiological mechanism for mammalian aging (Harman, 1956; Finkel and Holbrook, 2000; Wallace, 2005).

Mitochondria undergo constant biogenesis controlled primarily by the gene expression and post-translational modification of peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α) (Wu et al, 1999). Coactivation of PGC-1α induces nuclear respiratory factors (NRF-1 and -2), which promote the expression of most nuclear - encoding mitochondrial proteins, as well as mitochondrial transcription factor A (Tfam) that directly stimulates mitochondrial DNA replication and transcription (Puigserver and Spiegelman, 2003; Kelly and Scarpulla, 2004; Lin et. al., 2005). This process is not only important for the organelle to adapt to functional demands such as energy output and thermogenesis, but also regulates other vital cellular events such as mitochondrial fusion and fission (Chan 2006), antioxidant defense (St-

Pierre et al., 2006) and apoptosis (Dirks and Leeuwenburgh, 2002). Thus, it is not surprising that the PGC-1α activated signal transduction pathway may have a significant impact on aging. On the other hand, aging is known to alter several important mechanisms that may affect PGC-1α expression. For example, ROS generation is increased in aged skeletal muscle that could activate the redox-sensitive signaling pathway nuclear factor (NF) κB, a negative regulator of PGC-1α (Alvarez-Guardia et al., 2010). It has also been demonstrated that PGC-1α and PGC-1β both can reduce phosphorylation of NFκB subunit p65 and weaken its transcriptional potential (Eisele et al., 2012). Furthermore, several upstream enzymes known to activate the PGC-1α pathway such as p38 mitogen-activated protein kinase (p38 MAPK), AMP-activated protein kinase (AMPK) and silent mating type information regulator 2 homolog 1 (SIRT1) are redox-sensitive. AMPK and SIRT1 have been reported to directly affect PGC-1α activity through phosphorylation and deacetylation, respectively (Cantó and Auwerx, 2009). Skeletal muscle contraction at high intensity increases AMP/ATP ratio and Ca^{2+} flux thus causing upregulation of PGC-1α by the activation of AMPK, calcium/calmodulin-dependent protein kinase (CaMK), and calcineurin A (Wu et al., 2002). AMPK and p38 MAPK regulate transcriptional activity of PGC-1α via bindings of the promoter region by upstream stimulatory factor-1 (USF-1), and cAMP response element binding protein (CREB)/activating transcription factor-2 (ATF-2) (Irrcher et al., 2008; Akimoto et al., 2004; Akimoto et al., 2005). SIRT1 is a member of the sirtuin family of proteins (known as NAD-dependent deacetylase) and is implicated in a wide range of cellular function including cellular differentiation, aging, neural- and cardio-protection, and skeletal muscle metabolism (Anastasiou and Krek, 2006; Dali-Youcef et al., 2007). Within skeletal muscle, SIRT1 appears to contribute to the regulation of metabolism through a pathway in which it deacetylates and activates PGC-1α (Rodgers et al., 2008).

It is well known that prolonged exercise training increases PGC-1α, NRF-1, and Tfam protein contents (Baar et al., 2002; Irrcher et al., 2003; Gordon et al., 2001) and consequently enhances mitochondrial adaptation marked by elevated respiratory activity (oxygen consumption), increased expression of Krebs cycle and ETC enzymes, enhanced fatty acid oxidation and mitochondrial morphological changes (Wu et al., 1999). Despite the fact that aging decreases mitochondrial content and function, the effects of aging on PGC-1α signaling pathway and mitochondrial biogenesis in skeletal muscle have not been fully investigated. Furthermore, whether these age-related alterations could be reversed by endurance training is largely unknown. In the present study we provided evidence that chronic exercise is a powerful means to reduce age-associated deterioration of PGC-1α signaling and mitochondrial dysfunction.

II. Materials and methods

Animals

Male Fisher 344 × Brown Norway F_1 hybrid rats (F344BN) at age of 4 months (young, Y) and 22 months (old, O) were obtained from the National Institute on Aging colony, maintained by Harlan Sprague–Dawley (Indianapolis, IN). After arrival, the rats were housed individually in the animal facilities at the University of Wisconsin–Madison in a temperature-controlled room (22°C) on a reverse 12-h light/dark cycle. Animals were fed a chow diet and tap water ad libitum. The animal use protocol was approved by the University of Wisconsin–Madison Research Animal Resource Center. After two weeks of acclimation the old rats were divided into two groups, trained and sedentary. The trained rats ran on a motor-driven treadmill at 17.5 m/min, 10% grade for 45 min/day, 5 days/week for 12 weeks (OT, n=4), whereas sedentary rats were exposed to daily handling and treadmill walking at low speed (5 m/min) for 15 min/day during the entire training period (O, n=4). Young rats (Y, n=5) remained sedentary

during the experimental period and treated the same way as the O rats. At the end of the training regimen, Y rats and O/OT rats reached the age of 7 month and 25 month of age, respectively.

Tissue Preparation

In the end of the 12 week training and 48 hours after the last training session, the rats were euthanized by decapitation. After exsanguination, the soleus muscle of one leg was immediately excised, freeze-clamped between aluminum tongs precooled with liquid nitrogen, and stored at −80°C for protein and mRNA assays. One portion of the muscle was weighed and submerged in ice-cold 0.1 M K_2HPO_4–KH_2PO_4 buffer (wt/vol of 1 g/10 ml), minced, and homogenized at 0–4°C with a tissue grinder (Tekmar, Germany) for 15s in an ice-cold buffer in the presence of protease inhibitor cocktails (Roche, IN, USA). The nuclear protein extraction (NE-PER) kit (Thermo Scientific, IL, USA) was used according to the manufacturer's description to separate nuclear and cytosolic extracts and protease inhibitor cocktails (Roche, IN, USA) were added during nuclear and cytoplasmic extraction. Mitochondria were isolated via differential centrifugation according to the manufacturer's instructions (Thermo Scientific, IL, USA). Briefly, tissues were washed with PBS and dounce-homogenized on ice. Mitochondrial isolation reagent C was added and centrifuged at 700g for 10 minutes at 4°C. The pellets containing nucleus and cell debris were discarded and the supernatant was transferred to a new tube and centrifuged at 3,000g for 15 minutes at 4°C to obtain mitochondrial pellets.

Western blot analysis

After homogenization, the muscle samples were electrophoresed in 10 or 12% SDS polyacrylamide gels, electroblotted onto a PVDF membrane, and incubated with the appropriate IgG fractions in PBS containing 5% nonfat dry milk or bovine serum albumin with 0.2% Tween 20 (Sigma, St. Louis, MO, USA). Antibody against PGC-1α (ST1202, ~113kD) and SIRT 1

were purchased from Calbiochem (Gibbstown, NJ, USA). Antibodies against VDAC (porin), Histone H2B and Tfam were purchased from Abcam (Cambridge, MA, USA). Antibodies against pan-actin, CREB, p-CREB (Ser133), AMPKα, p-AMPK (Thr172) and p-p38MAPK (Thr180/Tyr182) were purchased from Cell Signaling Technology (Beverly, MA, USA). After an overnight incubation, the blots were washed for 10 minutes, three times with a wash buffer (TBS–0.5% Tween 20) at room temperature and further incubated for 1 h with a secondary horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse antibody (Santa Cruz Biotechnology, CA, USA). The blots were washed three times as above and developed by using the enhanced chemiluminescence procedure as specified by the manufacturer (Amersham ECL Plus, USA). NIH ImageJ (http://rsb.info.nih.gov/ij/index.html) program was used to compare the density of bands on western blot.

RNA extraction and real time qPCR

Total RNA was extracted according to the manufacturer's instructions (RNeasy® Mini Kit, Qiagen, Valencia, CA, USA). The RNA quantification and purity were estimated by Spectrophotometer absorption readings at 260 and 280 nm, and Complementary DNA was synthesized by SuperScript II Reverse Transcriptase kit (Invitrogen, Grand Island, NY, USA). PGC-1α, CREB, AMPK and Tfam mRNA levels were quantified using TaqMan Real-Time PCR with a StepOnePlus real time PCR system (Applied Biosystems). Serial dilutions of pooled muscle cDNA were used for the generation of standard curves for each TaqMan gene expression assay using the following Applied Biosystems identification numbers: PGC-1α, Rn00580241_m1; CREB, Rn00578828_g1; AMPKα2, Rn00576935_m1; Tfam, Rn00580051_m1; Bax, Mm00432051_m1; Bcl2, Mm00477631_m1 and Caspase 3,

Mm01195085_m1 in addition to the endogenous control (GAPDH, Rn01775763_g1). Each sample was run in triplicate and relative quantification was measured.

Quantitative Analysis of Mitochondrial DNA (mtDNA)

The mtDNA was purified by DNA purification kit (Qiagen, MD, USA). Total DNA was isolated using standard protocols. To quantify the amount of mtDNA present per nuclear genome, we used the following primers: mtDNA forward primer, R-CYTB-F (5′-CCC CAG AGG ATT AAA CTC CAA CGC A-3′) and mtDNA reverse primer, R-CYTB-R (5′-GGG TGG GGT CAG GGG GT-3′). To quantify nuclear DNA (nDNA), we used a primer set that detects the Pecam gene on chromosome 6: nuclear DNA forward primer, R-BDNF-genome-exon-IV-F (5′-TTG GGA TGG GAA AGA TGG G-3′) and nuclear DNA reverse primer, R-BDNF-genome-exon-IV-R (5′- CAG AGT AGG AGG GAA CAA GTG TGA C-3′) (Koltai et al., 2012). Quantification of relative copy number differences was carried out using analysis of the difference in threshold amplification between mtDNA and nuclear DNA (delta delta C(t) method).

Electrophoretic Mobility Shift Assay (EMSA)

CREB binding in the nuclear extract of the soleus muscle was measured by EMSA analysis. The following single-stranded oligonucleotides were obtained from a labeling kit manufactured by Santa Cruz Bio Technology, INC. : CREB 5' – AGA GAT TGC CTG ACG TCA GAG AGC TAG – 3', 3' – TCT CTA ACG GAC TGC AGT CTC TCG ATC – 5'. Briefly, the double-stranded oligonucleotides were subjected to labeling with terminal transferase, which adds a single digoxigenin-11–ddUTP (DIG) moiety to the 3′ end of the oligonucleotides, using the DIG Gel Shift Kit (Roche Applied Science, Germany). The labeled DNA fragment containing the sequence of interest was mixed on ice with the muscle nuclear extract (40 μg of protein) and binding buffer [100 mM Hepes, pH 7.6, 5 mM EDTA, 50 mM $(NH_4)_2SO_4$, 5 mM

DTT, Tween 20, 1% (wt/vol), 150 mM KCl]. The digoxigenin-labeled probes were subsequently detected by an enzyme immunoassay using anti-digoxigenin Fab fragments conjugated with alkaline phosphatase and the chemiluminescent substrate CSPD (Roche Applied Science, Germany). The chemiluminescent signals were recorded by exposure to X-ray film for 40–60 min.

Statistical Analysis

Experimental data were expressed as mean ± SE and group comparisons were made by one-way ANOVA. When a significant main effect was found ($P<0.05$), Tukey-Kramer method was used as a post-hoc test to compare difference between groups.

III. Results

The body weight was 50% heavier in O vs. Y rats ($P<0.01$) (**Table 1**). Twelve weeks of endurance training did not significantly affect the body weight in OT rats. The soleus muscle weight was 7% ($P<0.05$), but the ratio of soleus muscle to body weight was decreased by 30% in O vs. Y rats ($P<0.01$). OT rats showed a 6% increase in muscle weight ($P<0.05$) and the soleus/body weight ratio was increased by 11% ($P<0.05$) in OT vs. O rats.

To assess endurance training induced changes of mitochondrial biogenic pathway in O rats, we first investigated whether exercise training affects PGC-1α levels in the soleus muscle (**Fig. 1**). PGC-1α mRNA expression was decreased 35% in O rats compared to Y rats ($P < 0.01$), but no difference was seen between OT and Y rats. Nuclear PGC-1α protein content was 19% lower in O vs. Y rats ($P<0.05$), whereas OT rats showed a 2.3-fold higher ($P<0.01$) PGC-1α content than the O rats. Thus, age-associated downregulation of nuclear PGC-1α was completely abolished and dramatically boosted after a 12-wk treadmill running regimen.

Since Tfam is directly controlled by PGC-1α signaling, it is not surprising to see that both Tfam mRNA and protein content were significantly decreased in the O rats (P<0.01)(**Fig. 2a, b**). In the OT rats, Tfam mRNA level was not significantly different from O rats, whereas Tfam protein content was elevated by two-fold (P<0.01) compared to O rats and 63% higher (P<0.01) than Y rats. The nuclear-encoded mitochondrial protein marker Cyt c level was reduced by ~50% in O rats (P<0.01). The OT rats showed 40% higher (P<0.05) Cyt c content than O rats, though it was still significantly lower than Y rats (P<0.01) (**Fig. 2c**). The ratio mtDNA: nDNA was used to assess mitochondrial proliferation in the current study. As shown in **Fig. 2d**, the ratio was decreased by 26% (P<0.05) in O vs. Y rats, whereas in the OT rats mtDNA /nDNA ratio was elevated 2.4 fold (P<0.01) by training compared to O rats.

Repetitive muscle contraction increases AMP:ATP and NAD/NADH ratios, two metabolic status changes known to activate AMPK and SIRT1, respectively, causing transcriptional activation and modification of the PGC-1α. Therefore, the activity of AMPK, p-p38MAPK and the expression of SIRT1 were assessed. There was no difference in AMPK mRNA level among the three groups of rats; however, AMPK protein content was decreased significantly in both O and OT compared to Y rats (P<0.01) with no difference between OT vs. O rats (**Fig. 3a**). Protein content of phosphorylated (p)-AMPK (the active form) was not affected by aging, but training increased p-AMPK content by 35% (P<0.05), comparing OT vs. O or Y rats (**Fig. 3b**). The active p-p38MAPK content was not different between O and Y rats, but OT showed a 80 and 50% higher p-p38MAPK content than Y and O rats, respectively (P<0.05)(**Fig. 3c**). Similar to p-AMPK and p-p38MAPK, there were no significant changes in SIRT1 protein content between Y and O groups, but its level was increased by 138% and 68% in OT vs. Y and O rats, respectively (P<0.01)(**Fig. 3d)**.

Since CREB activity plays an important role in regulating PGC-1α transcription, we measured CREB mRNA and protein levels as well as DNA binding in the present study. Aging had no significant effect on CREB mRNA or basal protein content (**Fig. 4a, b).** p-CREB content was 50% ($P<0.01$) lower in O vs. Y rats, whereas OT showed a 38% ($P<0.05$) higher p-CREB content than O rats, though it is still significantly lower than Y ($P<0.01$) (**Fig. 4b**). CREB binding was dramatically decreased in O vs. Y rats ($P<0.01$)(**Fig. 4c**). Training completely reversed the age difference and elevated CREB binding by almost 10-fold in OT muscle ($P<0.01$).

The effects of aging and endurance training on apoptotic potential was assessed by measuring the mRNA abundance of pro-apoptotic factor Bax and anti-apoptotic factor Bcl2, as well as the key enzyme caspase-3. O rats showed almost a two-fold higher ($P<0.01$) Bax mRNA level compared to Y rats, whereas it was reduced by 23% ($P<0.05$) in OT vs. O rats (**Figure 5a**). Bcl2 mRNA level was 26% lower ($P<0.05$) comparing O vs. Y rats, and training completely wiped out this age difference (**Figure 5b**). Thus, while Bax/Bcl2 ratio was elevated by 2.5 fold in O rats, it was reduced by 43% ($P<0.01$) in OT rats (**Figure 5c**). Caspase-3 mRNA content in O rats was 72% higher than that in Y rats ($P<0.01$), whereas in OT rats it was 24% lower ($P<0.05$) than in O rats **(Figure 5d)**.

IV. Discussion

Sarcopenia is characterized by loss of skeletal muscle mass and strength at old age and reduced physical activity is considered a significant risk factor (Abate et al. 2007). Recent research showed that transgenic mice over-expressing PGC-1α displayed significant resilience against age-associated mitochondrial deterioration, inflammation and apoptosis, as well as whole

body health indices, highlighting the role of this master transcription cofactor in regulating biological aging (Wenz et al. 2009). Over the past decade a large number of studies using both rodent model and human subjects have confirmed that exercise is a powerful intervention to upregulate mitochondrial biogenesis (Barr et al. 2002, Pilegaard et al. 2003, Norrbom et al. 2004, Akimotor et al, 2005). However, only sparse studies have investigated whether exercise training can prevent or reverse age-related mitochondrial declination and the role of PGC-1α signaling in this adaptation (Short et al. 2005).

Effect of aging on mitochondrial biogenesis

The mechanism for age-associated decline of mitochondrial biogenesis and dysfunction is still elusive and under intense investigation (Barja 2013). Our data demonstrated that aging has a significant impact on PGC-1α signaling pathway evidenced by a large decrease in PGC-1α mRNA and protein content, as well as Tfam level, a critical nuclear protein for mtDNA maintenance and replication (Scarpulla, 2008). Supporting data also came from the decreases of Cyt c protein content and mtDNA/nDNA ratio in the old rats. These findings are consistent with previous reports by Vina et al. (2009) and others showing decreased mtDNA and mitochondrial protein expression with aging (Short et al., 2005; Koltai et al., 2012). Reduced mitochondrial biogenesis and turnover may result in accumulation of old, damaged mitochondrial and explain the functional deterioration seen in senescent muscles.

Among the upstream enzymes and transcription factors known to control PGC-1α gene expression and activity, such as AMPK, p38MAPK, SIRT1 and CREB, we found that AMPK and p-CREB levels showed a significant age-related reduction, along with a dramatically reduced CREB-DNA binding (**Fig. 4b,c**). Activation of CREB by both phosphorylation and DNA binding plays a crucial rule in transcriptional activation of PGC-1α (Handschin and

Spiegelman, 2006). Thus, its decline could be a main reason for decreased PGC-1α mRNA and protein expression at old age. As an important regulator of PGC-1α, AMPK protein content was markedly decreased with aging, whereas p-AMPK as a portion of total (non-phosphorylated) AMPK was increased (**Fig. 4b**). AMPK activation is thought to inhibit protein synthesis and play a role in age-related muscle loss (Thomson and Gordon 2005). Our findings agreed with those reported by Thomson and Gordon (2005) in soleus muscle, but differed from Reznick et al. (2007) showing no age-related changes in AMPK in extensor digitorum longus (EDL) muscle. Since AMPK response to aging is muscle fiber specific and each AMPK subunit (AMPKα1 and AMPKα2) also showed differential response to aging (Thomson and Gordon 2005, Qiang et al. 2007), more work is needed to clarify the role of this enzyme in muscle aging.

Senescent skeletal muscle undergoes enhanced apoptosis in which mitochondria play a critical role (Dirks and Leeuwenburgh, 2002). PGC-1α has been suggested to have anti-apoptotic effects and as such, age-associated decline in PGC-1α pathway activity may impact on this degradative process in terms of both muscle fiber size and number (Adhihetty et al. 2009). We found that in the soleus muscle of aged rats apoptotic potential as indicated by the Bax/Bcl2 mRNA ratio was dramatically increased along with elevated caspase-3 mRNA level (Figure 5). These changes in mRNA levels were expected to influence their respective protein levels and could be another factor to explain the decreased mitochondrial cytochrome c (Cyt c) content. Enhanced degradation of muscle fibers via apoptosis could also be a potential reason for soleus muscle weight loss in older rats.

Effect of Training on PGC-1α Controlled Mitochondrial Biogenesis

A major finding in the current study is that endurance training dramatically increased PGC-1α controlled muscle mitochondrial protein levels in old rats. Since many of the age-related

changes reported here already occurred when rats started training at 22 months of age, our finding suggests that muscle contraction is powerful enough to not only prevent but also reverse some of the important metabolic deteriorations seen at old age.

First, training resulted in more than a two-fold increase in the protein contents of PGC-1α and Tfam, the two key regulators of mitochondrial biogenesis in the soleus muscle of old rats. To our knowledge this has never been reported before. Secondly, training mitigated decreases of PGC-1α and Tfam mRNA abundance with aging, indicating that transcription and/or mRNA stability of the two gene products was enhanced with regular chronic exercise. The finding that Cyt c content and mtDNA/nDNA ratio (the indicator of mitochondrial replication) were significantly elevated in the trained old rats might be the result of these adaptations.

It is well known that PGC-1α signaling pathway is redox-sensitive and that p38MAPK plays an important role in transferring the redox signal to activate CREB/ATF-2 binding to the PGC-1α gene promoter (Akimoto et al., 2004, Scarpulla, 2008). Post-translational modification of PGC-1α by p38MAPK and SIRT1 also plays an important role in enhancing its half-life and thus its transcriptional coactivation (Puigserver et al., 2001, Nemoto et al., 2005). In the present study 12 weeks of training significantly increases p-p38MAPK expression in old rats. This could in part explain the increased CREB phosphorylation and DNA binding in these animals. Increased p-38MAPK alone probably did not account for the dramatically elevated CREB binding with training (**Fig 4c**). The content of p-AMPK, another upstream enzyme that regulates CREB activity, also increased with training in old rats. It is noteworthy that we used Ser133 phosphorylated CREB antibody to detect its phosphorylation status, as this site represents a major DNA binding domain. However, CREB contains other phosphorylation sites such as

Ser142 and Ser143. Thus, the high level of CREB –DNA binding found in our study may not represent the magnitude of increase in CREB binding in vivo.

SIRT1 has been shown to interact with ERR-α and activate PGC-1α by deacetylation (Nemoto et al., 2005, Wilson et al., 2010). Furthermore, aging has been shown to upregulate the expression of SIRT1 in the rat skeletal muscle, although the specific activity of the enzyme did not change with age (Koltai et al., 2010). The aging effect on SIRT1 expression seems to be tissue-specific and the mechanism that affects SIRT1 gene expression in aged organism is still not clear (Kwon and Ott, 2008). In this study, SIRT1 content was not affected by animal age but was significantly increased with endurance training in old rats. Increased SIRT1activity can prevent PGC-1α acetylation and thus enhance its DNA binding. This new finding may represent another potential mechanism that explains how aged muscle can still respond to exercise stimulus and increase mitochondrial protein synthesis.

Endurance training was shown to suppress muscle apoptotic potential with decreased Bax/Bcl2 mRNA ratio and caspase-3 mRNA level in old rats. PGC-1α is deemed essential in maintaining mitochondrial integrity and proper turnover with balanced biosynthesis and degradation. PGC-1α knockout mice showed enhanced muscle apoptotic activity (Adhibetty et al., 2009), whereas PGC-1α over-expression in transgenic mice increased resistance to nuclear cleavage and decreased caspase-3 activity in senescence (Wenz et al. 2009). Thus, the anti-apoptotic effects of training shown in the old rats were consistent with the protective effect of PGC-1α and reflect an additional adaptive response to increased muscle activity at old age.

In summary, while aging compromises several important nuclear proteins and enzymes involved in the PGC-1α signaling pathway, endurance training demonstrated powerful effects on these regulators that reversed or compensated for these degenerative changes in skeletal muscle.

Our data demonstrate that regular aerobic exercise is essential for the maintenance of muscle metabolic function at old age.

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Table 1. Soleus muscle weight of male F344BN Rats.

	Y	O	OT
Body weight (g)	374.72 ± 19.3	565.53 ± 26.4**	556.47 ± 44.5**
Soleus muscle weight (g)	0.15 ± 0.01	0.16 ± 0.01*	0.17 ± 0.01*†
Soleus to body weight (mg/g)	0.40 ± 0.03	0.28 ± 0.05**	0.31 ± 0.04**†

All values are mean ± SEM; Y, Young sedentary (4 mo); O, Old sedentary (22 mo); OT, Old trained (22 mo). *P<0.05; O or OT vs. Y. **P<0.01; O or OT vs. Y.

† P<0.05; OT vs. O.

Figure Legend

Figure 1. Relative abundance of PGC-1α mRNA analyzed by the real time PCR (a) and PGC-1α protein content in muscle nucleus measured by Western blot normalized with histone H2B content (b). Each bar represents mean ± SEM. Y, 4 month old rats (N=5); O, 22 month old rats (N=4); OT, 22 month old rats with 12 weeks of endurance training (N=4). *P<0.05, **P<0.01; O or OT vs. Y. †† P<0.01; OT vs. O.

Figure 2. Relative abundance of Tfam mRNA analyzed by the real time PCR (a), Tfam (b) and cytochrome c (c) protein contents in isolated mitochondria measured by Western blot normalized with porin content, and mitochondria DNA normalized with nuclear DNA content (mtDNA/nDNA, d). Each bar represents mean ± SEM. Y, 4 month old rats (N=5); O, 22 month old rats (N=4); OT, 22 month old rats with 12 weeks of endurance training (N=4). *P<0.05, **P<0.01; O or OT vs. Y. † P<0.05, †† P<0.01; OT vs. O.

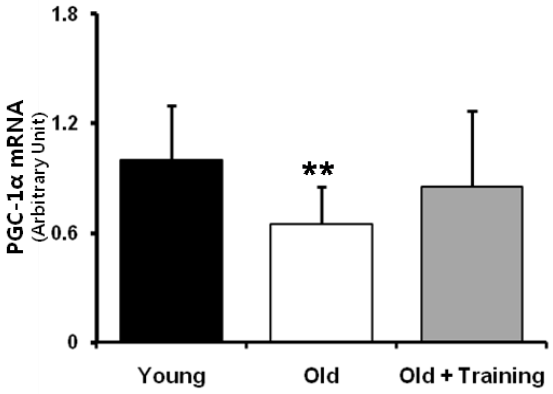
Figure 3. Relative abundance of AMPK analyzed by the real time PCR (a) and protein contents of AMPK and phosphorylated (p)-AMPK (b), p-p38MAPK (c), and SIRT1 (d) in rat soleus muscle measured by Western blot normalized with actin content. Each bar represents mean ± SEM. Y, 4 month old rats (N=5); O, 22 month old rats (N=4); OT, 22 month old rats with 12 weeks of endurance training (N=4). Each bar represents mean ± SEM. **P<0.01, O or OT vs. Y. † P<0.05, †† P<0.01; OT vs. O.

Figure 4. Relative abundance of CREB analyzed by the real time PCR (a), CREB and p-CREB protein content in rat soleus muscle measured by Western blot normalized with actin (b), and CREB nuclear binding measured by gel mobility shift assay (c). Each bar represents mean ± SEM. Y, 4 month old rats (N=5); O, 22 month old rats (N=4); OT, 22 month old rats with 12 weeks of endurance training (N=4). **P<0.01, O or OT vs. Y. †† P<0.01, OT vs. O.

Figure 5. Relative abundance of mRNA of Bax (a), Bcl2 (b), Bax/Bcl2 ratio (c) and Caspase-3 (d) analyzed by the real time PCR. Each bar represents mean ± SEM. Y, 4 month old rats (N=5); O, 22 month old rats (N=4); OT, 22 month old rats with 12 weeks of endurance training (N=4). *P<0.05, **P<0.01; O or OT vs. Y. † P<0.05, †† P<0.01; OT vs. O.

Figure 1

a



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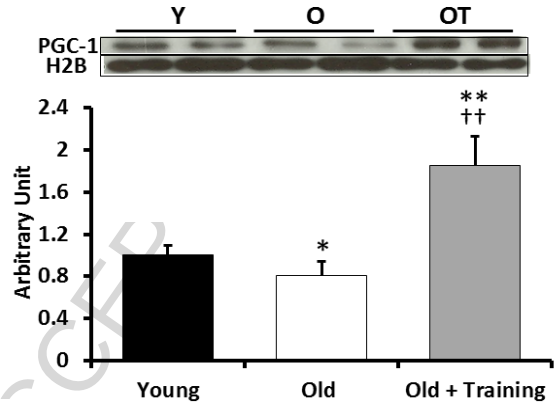

Figure 2

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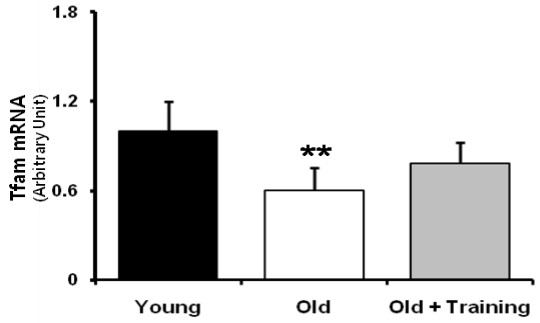

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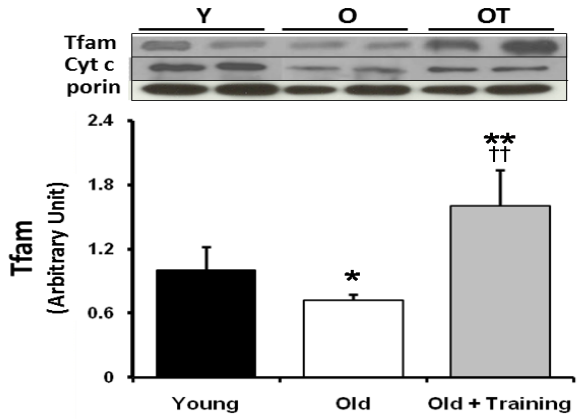

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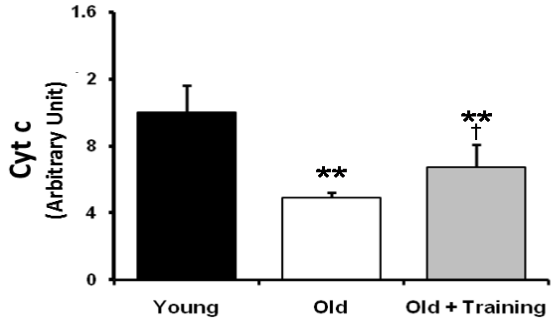

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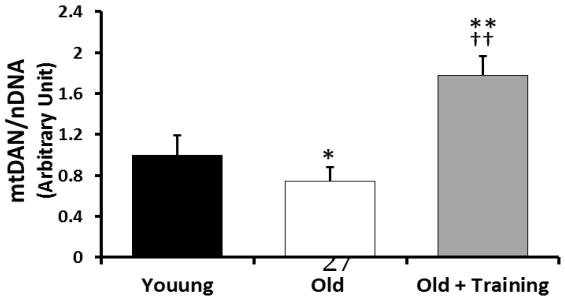

Figure 3

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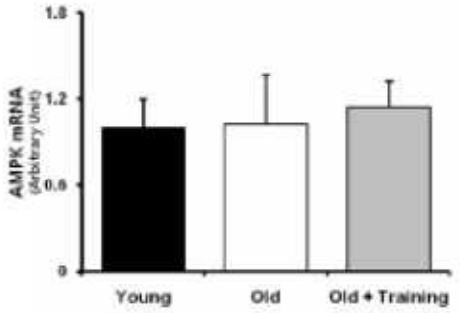

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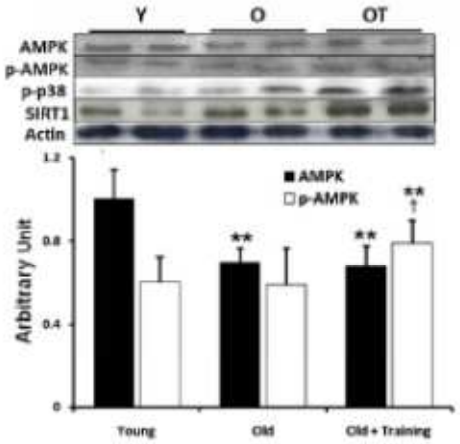

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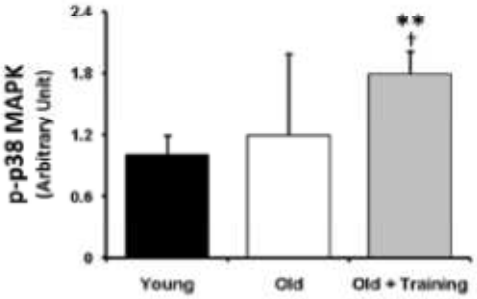

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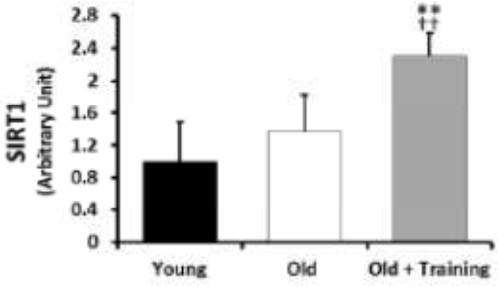

Figure 4

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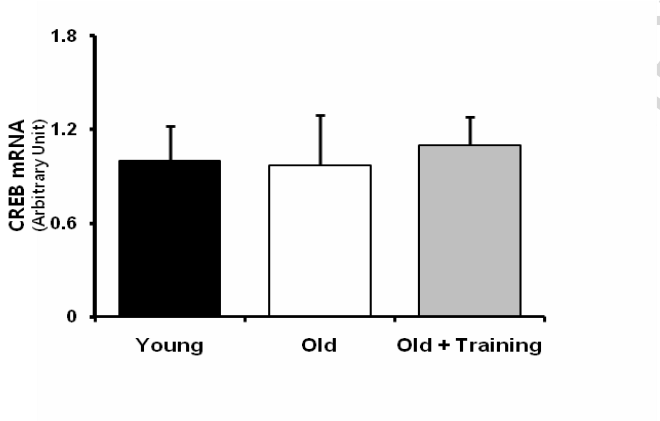

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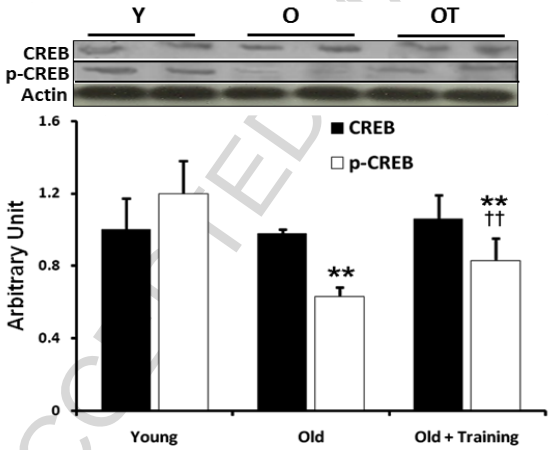

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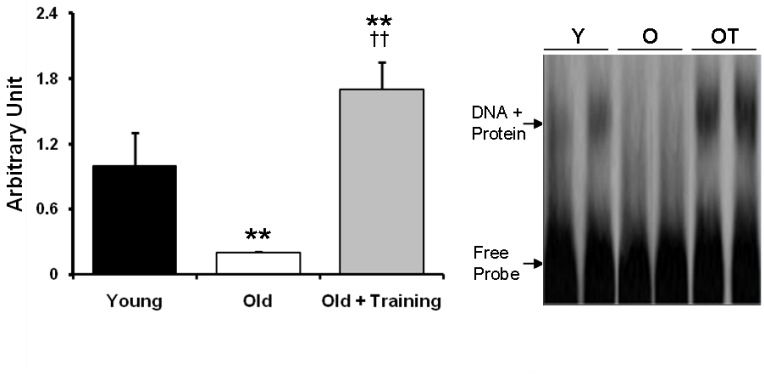

Figure 5

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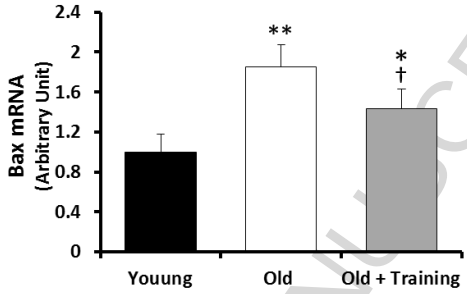

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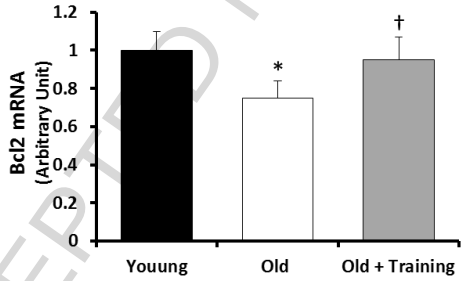

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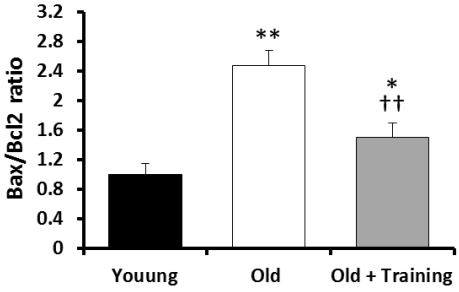

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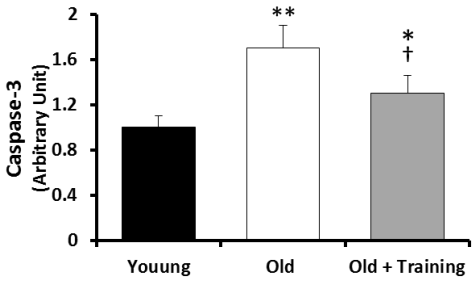

Highlights

- Aging attenuates PGC-1α pathways resulting in a deficit of mitochondrial content.
- Endurance training compensates for the decreases of PGC-1α pathways during aging.
- AMPK and p38MAPK play an important role in conferring the training effects.