Biochemical and Biophysical Research Communications 461 (2015) 354–360

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Strenuous exercise induces mitochondrial damage in skeletal muscle of old mice

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ARTICLE INFO

Article history:
Received 31 March 2015
Available online 15 April 2015

Keywords:
Mitochondria
Inflammation
Exercise
Redox signals
Skeletal muscle

ABSTRACT

Strenuous exercise is known to cause excessive ROS generation and inflammation. However, the mechanisms responsible for the regulation of mitochondrial integrity in the senescent muscle during high-intensity exercise (HE) are not well studied. Here, we show that HE suppresses up-regulation of mitochondrial function despite increase in mitochondrial copy number, following excessive ROS production, proinflammatory cytokines and NFκB activation. Moreover, HE in the old group resulted in the decreasing of both fusion (Mfn2) and fission (Drp1) proteins that may contribute to alteration of mitochondrial morphology. This study suggests that strenuous exercise does not reverse age-related mitochondrial damage and dysfunction by the increased ROS and inflammation.

1. Introduction

Skeletal muscle aging is associated with a decline in muscle mass and strength, which can be exacerbated by mitochondrial dysfunction [1]. Due to its vital role in the regulation of energy metabolism, intracellular signaling and apoptosis [2–4], maintaining mitochondrial function has been a key strategy to preserve muscle integrity and function during aging. However, mitochondrial morphological alterations and dysfunction increase with age [5], following which several adverse incidents such as a higher level of mitochondrial DNA (mtDNA) mutation, apoptosis and reactive oxygen species (ROS) induced oxidative damage take place [6–8]. Signaling cascades affecting the phenotypical changes of mitochondria are driven by some key pathways of mitochondrial biogenesis, dynamics and autophagy, thereby maintaining or changing mitochondrial function [9,10]. Upon the above multiple mechanisms, calorie restriction (CR), resveratrol or antioxidant supplementation and exercise have been introduced as therapeutic applications to ameliorate mitochondrial dysfunction in skeletal muscles [11–14], although there is still a controversy as to whether the above mentioned treatments always have beneficial effects [15,16]. Nevertheless, it has been generally believed that exercise is securely linked to muscle metabolic adaptations including enhanced mitochondrial function [17,18]. Indeed, most studies performed with non-senescent muscles have reported that increases in mitochondrial biogenesis signals and functions occur widely in response to various types, durations and intensities of exercise, importantly, not limited to any specific forms of exercise [19–21]. However, since excess inflammatory cytokines and oxidative stress may trigger mitochondrial deterioration and malfunction [22–24], it has been questioned whether these beneficial effects of exercise on mitochondria can also be observed in senescent muscles, which are more vulnerable to inflammation and ROS-induced oxidative damage. Moreover, strenuous exercise can exacerbate disruption of the cellular environment by increased oxidative damage and inflammatory process [25–27], which may have a negative synergic impact on the senescent muscle. Thus, upon the pathophysiological traits of skeletal muscle aging, we hypothesized there would be an adverse effect of exercise on mitochondrial function in the aged muscle due to the disturbance of cellular homeostasis by strenuous exercise, which may hamper pathways for maintaining mitochondrial integrity. In the current study, we aimed to investigate the link between strenuous exercise

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http://dx.doi.org/10.1016/j.bbrc.2015.04.038
0006-291X/

and mitochondrial function in mouse skeletal muscle following treadmill exercise.

2. Material and methods

2.1. Animals, experimental design

All experimental protocols were carried out in accordance with the National Institutes of Health Guidelines for animal research (Guide for the Care and Use of Laboratory Animal) and approved by the Animal Care and Use Committee at Hankuk University of Foreign Studies, South Korea. Male C57BL/6 mice at age 2 months (young, Y) and 24 months (old, O) were housed in temperature-controlled rooms (22 °C), on a reverse 12-h light/dark cycle. After a 1-week acclimation, mice were randomly assigned to six groups: young and non-exercise (Y-NE, $N = 10$), young and low intensity treadmill exercise (Y-LE, $N = 10$), young and high intensity treadmill exercise (Y-HE, $N = 10$), old and non-exercise (O-NE, $N = 10$), old and low intensity treadmill exercise (O-LE, $N = 10$) or old and high intensity treadmill exercise (O-HE, $N = 10$).

2.2. Treadmill exercise training

Animals from the exercise groups were subjected to 5 days of exercise regimen on a treadmill, while control animals were exposed to daily handling and spent the same time on a treadmill. Exercise intensity and duration were gradually increased during the first week of exercise training from 5 min at a speed of 4 m/min for aged mice and 6 m/min for young animals to a regular regimen and then increasing the speed 1 m/min per minute until exhaustion. Starting from the second week, for 5 days at 50 min a day, mice of the LE and HE groups ran on a motor-driven treadmill at 35% and 70%, respectively, of the speed at which the mice reached exhaustion. Thus, the Y-LE and Y-HE mice were run at 11.9 and 23.8 m/min, respectively and the O-LE and O-HE were run at 8.8 and 17.45 m/min, respectively.

2.3. Western blot analysis

Western blot was performed as described previously [28] using the following antibodies: anti-p-IκBα (Ser^{32} and Ser^{36}, #9246), IκBα (#9242) and Beclin-1 (#3495) (Cell Signaling Technology); anti-α-tubulin (loading control, ab18251), Tfam (#ab131607), histone H2B (nuclear loading control, ab1790), LC3B (#ab48394), p62 (#ab56416), Mfn1 (#ab126575) and Mfn2 (#ab50843) (Abcam); anti-Drp1 (#sc-21804) (Santa Cruz Biotechnology) and anti-PGC-1α (ST1202) (Calbiochem).

2.4. RNA extraction and RT-qPCR

Quantification of monocyte chemoattractant protein (MCP)-1 mRNA, which was normalized to the internal control glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA was carried out using the following primers; MCP-1 forward primer, 5′-ATTGGGATCATCTTGCTGGT-3′, reverse primer: 5′-CCTGCTGTTCACAGTTGCC-3′; GAPDH forward primer, 5′-CGTCCCGTAGACAAAATGGT-3′, reverse primer, 5′-TTGATGGCAACAATCTCCAC-3′.

2.5. Mitochondrial isolation, mitochondrial ATP production rate and ROS generation

Mitochondria were isolated according to the manufacturer's instructions (Mitochondria Isolation Kit for Tissue, Thermo Scientific) and measurements of the mitochondrial ATP production rate and mitochondrial ROS production were carried out as previously described [28].

2.6. Quantitative analysis of mitochondrial DNA (mtDNA)

Mitochondria were lysed in the presence of 0.5% SDS and 0.2 mg/ml proteinase K in 10 mM Tris–HCl, 0.15 M NaCl, and 0.005 M EDTA. mtDNA was then purified by DNA purification kit (Qiagen). Quantification of relative copy number differences was carried out as previously described [28].

2.7. Pro-inflammatory cytokines and NFκB DNA binding

TNF-α, IL-6 (BD bioscience), IL-1β (Abcam) and NFκB DNA binding (Thermo Scientific) were measured according to the manufacturer's instructions in muscle homogenate.

2.8. Measurement of reduced and oxidized glutathione levels and enzyme activities

Glutathione assay kit (Cayman Chemical Company) was used to measure the reduced glutathione (GSH) and oxidized glutathione (GSSG) levels in muscle. Citrate synthase (CS) and COX activities were measured as recommended by the manufacture's protocol (Sigma–Aldrich).

2.9. Statistical analysis

Experimental data were expressed as mean ± SE and group comparisons were made by one-way or two-way ANOVA with Tukey's HSD *post hoc* test.

3. Results

To test whether aging and/or exercise intensity affect skeletal muscle inflammation, we examined pro-inflammatory markers, TNF-α, IL-1β and MCP-1 in both young (Y) and old (O) mouse TA muscles. The old non-exercise (O-NE) mice showed a 61% increase in TNF-α compared to the young control mice (Y-NE) ($P < 0.05$). Furthermore, HE increased the TNF-α significantly in both the young (52%, $P < 0.05$) and old (101%, $P < 0.05$) groups, whereas LE did not affect it (Fig. 1A). The basal level of IL-1β in the O-NE group was 2.3-fold higher than in the Y-NE group ($P < 0.05$). There was a 69% increase in IL-1β with HE in the old group ($P < 0.05$), whereas both LE and HE did not affect the IL-1β levels in the young group (Fig. 1B). The MCP-1 mRNA increased 2.7-fold in the O-NE vs. the Y-NE groups. Similarly to TNF-α and IL-1β, LE did not change the MCP-1 mRNA in both the young and old groups. However, there was a 70–80% increase in MCP-1 mRNAs by HE in both the young and old groups ($P < 0.05$) (Fig. 1C).

The mitochondrial ROS production rate remained unchanged with aging, however LE increased the ROS generation significantly in both young (34%, $P < 0.05$) and old (37%, $P < 0.05$) groups compared to their corresponding NE controls. Notably, HE enhanced the mitochondrial ROS production rate dramatically in the old group vs. the O-NE (1.3-fold, $P < 0.01$), significantly, although there was a 31% increase in ROS with HE in the young group ($P < 0.05$) (Fig. 1D). Redox status, expressed as the GSSG/GSH ratio, was determined as a marker for oxidative stress. There was a 48% increase in GSSG/GSH in the O-NE vs. the Y-NE group. Both LE and HE in young mice did not affect the ratio; however, in the old groups, the ratio in the HE was higher than in the NE and LE groups by 45% ($P < 0.05$) and 32% ($P < 0.05$), respectively. Furthermore, GSSG/GSH levels in the old group were significantly higher than in the corresponding young control ($P < 0.05$) (Fig. 1E). The oxidative