Life Sciences 194 (2018) 98–103

Contents lists available at ScienceDirect

Life Sciences

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

The role of physical exercise on Sestrin1 and 2 accumulations in the skeletal muscle of mice

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ARTICLE INFO

Keywords:
Exercise
Sestrin1
Sestrin 2
Skeletal muscle

ABSTRACT

Aims: Sestrins, a class of stress-related proteins, is involved in the control of aging-induced organic dysfunctions and metabolic control. However, the factors that modulate the levels of Sestrins are poorly studied. Here, we evaluated the effects of acute and chronic aerobic exercise on Sestrin 1 (Sesn1) and Sesn2 protein contents in the skeletal muscle of mice.

Main methods: Male C57BL/6 J mice performed an acute or chronic (4 weeks) exercise protocols on a treadmill running at 60% of the peak workload. Then, the quadriceps muscle was removed and analyzed by Western blot. Bioinformatics analysis was also performed to evaluate *Sesn1* and *Sesn2* mRNA in the skeletal muscle and phenotypic pattern in a large panel of isogenic strains of BXD mice.

Key findings: While acute aerobic exercise increased Sesn1 accumulation and induced a discrete augment of Sesn2 protein content and AMPK threonine phosphorylation, chronic exercise reduced the basal levels of Sesn1 and Sesn2 as well as of AMPK threonine phosphorylation in the quadriceps muscles of C57BL/6 J mice. In accordance with these experimental approaches, transcriptomic analysis revealed that *Sesn1* and *Sesn2* mRNA levels in the skeletal muscle were inversely correlated with the locomotor activity in several strains of BXD mice.

Significance: Our data suggest that physical exercise has role on Sestrin1 and Sestrin2 expression on skeletal muscle, providing new insights into the mechanism by which physical exercise affects stress-related proteins in skeletal muscles.

1. Introduction

Sestrins (SESNs) are a class of proteins that are induced by stress [1,2]. Currently, three isoforms of the SESNs family are known: Sestrin 1, Sestrin 2 and Sestrin 3. Studies have shown that these proteins are important for the maintenance of metabolic homeostasis, for the protection of cells against age-related physiological damage and, mainly, for the control of Adenosine Monophosphate Kinase (AMPK)/Mammalian Target of Rapamycin (mTOR) signaling [3]. According to Budanov and colleagues [4], this regulation occurs through the encoding of antioxidant proteins. Sestrins lead to AMPK action and Mammalian Target of Rapamycin Complex 1 (mTORC1) activity suppression [5]. The deficiency of this class of proteins results in organic dysfunctions as muscle degeneration, fat accumulation, mitochondrial dysfunction and insulin resistance [6].

Different conditions induce specific SESNs expression. For instance, hypoxia induces Sesn1 and Sesn2 accumulation in human cancer cell lines [1]. Also, several factors that decrease cellular ATP concentration induced, by unknown mechanisms, the Sesn2 expression [7]. Sesn1 and Sesn2 are highly expressed in skeletal muscle [2]. When SESNs are inactivated, the reactive oxygen species (ROS) are accumulated, which promotes oxidative stress [4].

On the other hand, physical exercise is considered an important non-pharmacologic strategy to prevent and treat several metabolic

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https://doi.org/10.1016/j.lfs.2017.12.023
Received 26 June 2017; Received in revised form 21 November 2017; Accepted 18 December 2017
Available online 19 December 2017
0024-3205/

dysfunctions related to obesity and aging. Aerobic exercise promotes, by cellular stress, morphological and metabolic consistent responses in several organic tissues, including skeletal muscle, liver, adipose tissue and hypothalamus [8–12]. It has been proposed that, at least in part, these adaptations are due to AMPK activation [13]. The physical exercise promotes the intracellular ATP depletion and the increase of the AMP content, triggering the AMPK activation. Another classic effect of physical exercise is the increase of antioxidant activity. Several studies have demonstrated that physical exercise is capable to induce the expression of antioxidant enzymes including superoxide dismutase, catalase and glutathione peroxidase in mice skeletal muscle and cardiac tissue [14,15]. Altogether, these data suggest that the physical exercise-induced stress leads to organic adaptations. In this scenario, Sesns expression may play an important role, since they can connect the stress promoted by the physical exercise to AMPK activation and to the antioxidant response in multiple tissues. Thus, the aim of this study was to evaluate the effects of chronic and acute physical exercise protocols on the Sesn1 and Sesn2 accumulation in the skeletal muscle of mice.

2. Methods

2.1. Antibodies

Anti-SESN1(AB134091) and anti-SESN2 (AB198717) antibodies were from Abcam, anti-GAPDH (SC-25778), anti-Phospho-AMPKα 1/2 (Thr172) (SC-33524), anti-AMPKα 1/2 (SC-25792) and anti-SOD2 (SC-30080) antibodies were from Santa Cruz and Ponceau was from Sigma-Aldrich.

2.2. Animals and housing

Male C57BL/6 J mice, 2 months old, were used from the University of Campinas animal facility. The animals were placed five animals per cages, exposed to 12 h light and 12 h dark cycle, temperature between 20 °C and 22 °C, and received water and standard rodent chow (brand Nuvilab®) ad libitum. The animals were distributed in control group, acute exercise group and chronic exercise group. The experiments were approved by the ethics committee of UNICAMP, protocol number 3830–1 and followed the Guide for the Care and Use of Laboratory Animals, published by the U.S. National Institutes of Health (NIH publication no. 85–23 revised 1996).

The number of animals used in each experiment is specified in the figure legends.

2.3. Incremental load test

Firstly, mice from acute exercise and chronic exercise groups were adapted to the exercise on a motor treadmill during 5 days at 3 m/min during 10 min. After 2 days, the animals were submitted to an incremental load test. The initial intensity was 6 m/min at 0% grade, with increments of 3 m/min every 3 min until exhaustion. The exhaustion occurred after the animals reached the end of the treadmill five times in 1 min gap. The peak workload was measured at the exhaustion point of the test and 60% of the peak workload was determined as the exercise training intensity [16].

2.4. Exercise protocol

2.4.1. Acute protocol

The animals from the acute exercise group, were submitted to a single 60 min aerobic exercise session, on a treadmill, at 60% of the peak workload, defined in an incremental test performed previously. Two and four hours after the exercise session, the quadriceps muscle was extracted.

2.4.2. Chronic protocol

The animals from chronic exercise group performed during 4 weeks, 5 days/week, sessions of 60 min of aerobic exercise on a treadmill, with intensity corresponding to 60% of the peak workload. Extraction of the quadriceps muscle occurred 24 h after the last exercise session.

2.5. Tissue sampling

The mice were anesthetized with an intraperitoneal injection of chlorohydrate of ketamine (50 mg/kg, ketamine, Parke-Davis, Ann Arbor, MI) and xylazine (20 mg/kg, Rompun, Bayer, Leverkusen). The tissue was homogenized in extraction buffer and the samples were stored as previously described [12].

2.6. Western blotting

The Western blotting was performed as previously described [17]. The Western blotting results were normalized by the respective loading control and the final values were given in percentage of respective control group. The entire membranes and the statistical analyses of the Western blots are shown in the supplementary Fig. 1 (Fig. S1).

2.7. Analysis of mRNA content

Total RNA was isolated using the TRIzol reagent (Invitrogen, Grand Island, NY, USA). A 2 μg quantity of total RNA was used as template for the synthesis of cDNA using SuperScript® III First-Strand Synthesis System (Invitrogen). Real-time PCR reactions were performed using 150 ng cDNA, 0.6 μM primers (5′ -GGCTCAATGGTTTAGAGAATGC-3′ (forward), 5′ - AGGAGGCAAGAGAGTGGTAGTG -3′ (reverse) synthetized by Merck, Darmstadt, Germany for SESN1 and Exxtend, Paulinia, Brazil for SESN2 as described before by Lenhare and collegues [18]), and HOT FIREPol® EvaGreen® qPCR Mix Plus (Solis BioDyne, Tartu, Estonia). The cycling parameters were: 10 min at 95 °C followed by 40 cycles of 15 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C. Relative content of mRNAs was determined after normalization with β2M using the ΔΔCt method. Each set of primers was designed to recognize unique regions of gene sequences.

2.8. Bioinformatics analysis

Bioinformatics analysis was performed using a dataset from skeletal muscles of genetically-diverse BXD mice (EPFL/LISP BXD CD Muscle Affy Mouse Gene 1.0 ST (Nov12) RMA Exon Level (Log2)) [19] and the locomotor activity values were obtained using a dataset as previously published [20]. These data sets are accessible on Genetwork (http://www.genenetwork.org). The Pearson's correlation graphs were built using Prism GraphPad 6.1® and the heat map graph was obtained using the Gene-E® software. All individual values used in the bioinformatics analysis are described in the supplementary table (Table S1).

3. Statistics

All results were expressed as mean ± standard error from the mean (SEM). The data were analyzed by "*t*-Student" test when two groups were compared and analysis of variance (ANOVA) followed by the Bonferroni multiple mean test when more than two groups were compared. The statistical significance used was $p < 0.05$. The Pearson's and Spearman's correlations were performed using Prism GraphPad 6.1® and positive or negative correlation were considered when $r > 0.5$ or $r < -0.5$, respectively and $p < 0.05$.