Experimental Gerontology 102 (2018) 19–27

Contents lists available at ScienceDirect

Experimental Gerontology

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

Skeletal muscle from aged American Quarter Horses shows impairments in mitochondrial biogenesis and expression of autophagy markers

Chengcheng Li, Sarah H. White, Lori K. Warren, Stephanie E. Wohlgemuth*

Department of Animal Sciences, University of Florida, Gainesville, FL, USA

ARTICLE INFO

Keywords:
Aging
Skeletal muscle
Mitochondrial content
mtDNA copy number
Autophagy

ABSTRACT

Aging is associated with decreased mitochondrial content and function in skeletal muscle, possibly due to compromised biogenesis and autophagic removal of dysfunctional mitochondria. The aim of this study was to compare markers of mitochondrial content and biogenesis and of autophagy between skeletal muscle from young and aged American Quarter Horses. Citrate synthase protein and mtDNA copy number were decreased in *triceps brachii* (TB) muscle ($P < 0.05$) from aged horses, suggesting an age-related decline in mitochondrial content. Concomitantly, mRNA expression of *PGC-1α* and *TFAM*, regulators of mitochondrial biogenesis, was lower in aged compared to young TB ($P < 0.05$). Expression of autophagy markers suggested an age-associated decline of autophagy. The autophagosomal cargo protein SQSTM/p62 accumulated with age in both muscles ($P < 0.05$). Expression of Autophagy-related protein Atg5 ($P < 0.05$) and the autophagosome-bound form of Microtubule-associated protein light chain 3 (LC3-II; $P < 0.05$) were lower in aged compared to young TB. While LC3 transcript level was elevated in aged compared to young GM ($P < 0.001$), protein expression of LC3-II was unaffected. Gene expression of Lysosomal Membrane-Associated Protein 2 (LAMP2) was not affected by age in either muscle. However, LAMP2 protein expression declined with age ($P < 0.05$), suggesting a decline in autophagosome-lysosome fusion. Taken together, our data indicate that equine skeletal muscle mitochondrial content and biogenesis were impaired with age. Further, autophagosome formation and lysosomal degradation were negatively affected in aged TB and GM, respectively. Future research needs to explore whether interventions targeting these cellular processes can prolong health and performance of aging American Quarter Horses.

1. Introduction

Mitochondria serve as the combustion engines of life, providing the ATP necessary for skeletal muscle contraction. They do so through oxidative phosphorylation (OXPHOS), in which ATP and CO_2 are produced at the expense of nutrient substrates and molecular O_2. Given the mitochondria's role in energetic support of locomotion, proper maintenance of a healthy mitochondrial population is critical to ensure efficient energy supply. In human and rodent models, extensive investigation has revealed changes in mitochondrial content and function in a variety of disease models. For example, data collected in old individuals suggest that reduced skeletal muscle mitochondrial content and quality is associated with aging (Hepple, 2014; Picard et al., 2010; Short et al., 2005).

The total mitochondrial content of a cell is tightly regulated by two opposing cellular processes, mitochondrial biogenesis and mitochondria-selective autophagy (mitophagy) (Mishra and Chan, 2016; Wai and Langer, 2016). Mitochondrial biogenesis, the creation of new mitochondria, is responsible for replenishment of new functional mitochondria. On the other hand, mitophagy, one of the key mitochondrial quality control mechanisms, sequesters and degrades dysfunctional or aged mitochondria to maintain a healthy mitochondrial population. Thus, the balance between mitochondrial renewal and elimination of damaged mitochondria is essential for maintaining a certain level of healthy mitochondria to meet energy demands (reviewed by Palikaras et al., 2015). It is not surprising that an impaired balance between these opposing processes can result in numerous pathological conditions, as well as aging, in diverse organisms ranging from yeast to mammals (Artal-Sanz and Tavernarakis, 2009; Kaeberlein, 2010; Preston et al., 2008). Mitochondrial biogenesis is a complex process, which requires coordinated synthesis and assembly of thousands of proteins encoded by both the nuclear and mitochondrial genomes (Scarpulla, 2008b). In addition, mitochondrial DNA (mtDNA) replication must be coordinated to meet the requirements of the new mitochondrial generation. Growing evidence demonstrates that mitochondrial content in human skeletal muscle declines gradually with

* Corresponding author at: Department of Animal Sciences, University of Florida, 2250 Shealy Drive, Gainesville, FL 32611, USA.
E-mail address: steffiw@ufl.edu (S.E. Wohlgemuth).

https://doi.org/10.1016/j.exger.2017.11.022
Received 31 July 2017; Received in revised form 27 October 2017; Accepted 30 November 2017
Available online 02 December 2017

advancing age (Crane et al., 2010). Concomitantly, an age-associated impairment of mitochondrial biogenesis capacity has been reported in animal models (Fannin et al., 1999; Sugiyama et al., 1993).

Mitophagy is a specific form of autophagy that selectively degrades dysfunctional mitochondria. The mitochondria are sequestered by a unique double-membrane organelle, called autophagosome, and targeted to be degraded in the lysosomes (Ding and Yin, 2012). Accumulating evidence shows that a decline in autophagy is associated with age, and that increased autophagy delays aging in lab animals (Schiavi et al., 1810; Wu et al., 2013). In humans, an age-related decline in mitophagy was observed (Cavallini et al., 2007) and associated with an accumulation of damaged mitochondria (Masiero and Sandri, 2010). Given that mitochondria are increasingly damaged during the aging process (reviewed by Shigenaga et al., 1994), decreased mitophagic activity might further exacerbate dysfunction of the mitochondrial population as a whole in older individuals. Hence, decreased mitophagy likely contributes to the decline in mitochondrial quality and function that contributes to the aging phenotype.

Previously we have shown that mitochondrial content and function decreased gradually with advanced age in skeletal muscle from American Quarter Horses (Li et al., 2016). However, it is not completely understood what regulates mitochondrial content and function in the equine. One underlying cause for the age-associated decrement of mitochondrial density and function could be an impaired balance between mitochondrial biogenesis and autophagy in muscle from aged horses. Therefore, the purpose of the current study was to explore potential mechanisms that contribute to the age-associated decline in mitochondrial content and quality in equine skeletal muscle by examining the factors involved in mitochondrial biogenesis and autophagy. Because of their distinct locomotor functions and metabolic properties, we compared *gluteus medius* (GM) and *triceps brachii* (TB) muscles from young and aged American Quarter Horses (Li et al., 2016). We hypothesized that different muscles respond differently to aging, and that the distinct response is due to differences in the activation of mitochondrial content control mechanisms. A better understanding of the cellular and molecular mechanisms responsible for the maintenance of a healthy mitochondrial population in equine skeletal muscle is a prerequisite to design interventions to prolong health and performance of aging horses.

2. Materials and methods

2.1. Animals

Healthy young (1.8 ± 0.1 years old, 14 fillies and 10 geldings) and aged (17–25 years old, 11 mares and 1 gelding) American Quarter Horses owned by the University of Florida were enrolled in this study. None of the horses had received forced exercise 6 months prior to the study. The Henneke body condition score (BCS) system (Henneke et al., 1983) with a scale ranging from 1 (emaciated) to 9 (extremely obese) was used to estimate the horse body condition score of the horses enrolled in the study. The BCS was 5.00 for the young group and 5.80 ± 0.25 for the aged group ($P < 0.001$), respectively. This study was approved by the University of Florida Institute of Food and Agricultural Sciences Animal Research Committee.

2.2. Muscle tissue sampling

Skeletal muscle microbiopsies were obtained from the *gluteus medius* (young: n = 24; aged: n = 12) and *triceps brachii* of a subset of young horses (n = 12) and of all aged horses (n = 12) under local anesthesia following the procedure described previously (Li et al., 2016). Muscle samples were collected at a sampling depth of 5 cm, using a 14-gauge SuperCore ™ Biopsy needle (Angiotech, Gainesville, FL, USA), immediately snap-frozen in liquid nitrogen and transported to the laboratory in a dry shipper (MVE SC4/2V, CHART, Inc., Ball Ground, GA, USA), where samples were transferred to a −80 °C freezer.

2.3. Analysis of mtDNA copy number

To evaluate mitochondrial content in horse skeletal muscle, the relative amount of mtDNA to nuclear DNA (nDNA) was determined using a CFX Connect real-time PCR detection system (Bio-Rad, Laboratories, Inc., Hercules, CA). Total DNA was extracted from muscle samples using Wizard® Genomic DNA purification kit (Promega Corporation, Madison, WI), according to the manufacturer's instructions. One primer pair specific for the mtDNA (NADH dehydrogenase 1, *ND1*) and another specific for the nuclear DNA (β-actin, *ACTB*), were designed using PrimerQuest (Integrated DNA Technologies, Coralville, IA). The primer sequences for the *ND1* gene were: 5′-GGA TGG GCC TCA AAC TCA A-3′ (forward) and 5′-GGA GGA CTG AGA GTA GGA TGA T-3′ (reverse). The primer sequences used to amplify *ACTB* gene were: 5′-CTC CAT TCT GGC CTC ATT GT-3′ (forward) and 5′-AGA AGC ATT TGC GGT GGA-3′ (reverse). Another primer pair designed within the *COX1* (*cytochrome c oxidase subunit 1*) region of the mitochondrial genome was also tested to confirm the result assessed with *ND1*.The primer sequences used for *COX1* gene were: 5′-CAG ACC GTA ACC TGA ACA CTA C-3′ (forward) and 5′-GGG TGT CCG AAG AAT CAG AAT AG-3′ (reverse). The relative amount of *ND1*, *COX1* and *ACTB* was determined for each sample from a standard curve prepared from a serial dilution of a pool of all the samples. Relative mtDNA copy number was calculated from the ratio *ND1/ACTB* and *COX1/ACTB*, which were subsequently averaged.

2.4. RNA isolation

Total RNA was isolated from ~30 mg of snap-frozen muscle using RNeasy® Plus Universal Mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. With the DNA elimination solution included in the Kit, genomic DNA was removed from the samples. Immediately following extraction, the RNA concentration and purity were determined using a UV spectrophotometer (Synergy HT, BioTek Instruments, Winooski, VT) by measuring the absorbance at 260 (OD260) and 280 (OD280) nm. All measurements were performed in duplicate.

2.5. Analysis of mRNA expression

First-strand cDNA was synthesized with random primers using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems Inc., Foster City, CA) according to the manufacturer's directions. Quantitative real-time PCR (qRT-PCR) analysis was performed using the CFX Connect real-time PCR detection system with a 20 μL reaction volume containing cDNA, primers, and iTaq™ Universal SYBR® Green Supermix (Bio-Rad). All samples were analyzed in duplicate simultaneously with a negative control that contained no cDNA. The data were normalized to GAPDH mRNA in each reaction, and results were expressed as a fold change in mRNA compared with expression in GM from the young horse group. Forward and reverse primer sequences are listed in Table 1. Melting point dissociation curves generated by the instrument were used to confirm the specificity of the amplified product. The relative quantification was done using the relative standard curve method.

2.6. Analysis of protein expression by Western blot

Frozen muscle samples were cryopulverized using a BioPulverizer (BioSpec Products, Inc., Bartlesville, OK, USA) prior to protein extraction. The cryopulverized tissues were immersed 1:50 (w/v) in lysis buffer (25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.1% SDS, 10% glycerol, 1 mM DTT, 0.5% sodium deoxycholic acid (Wu et al., 2009)) supplemented with 1% Halt™ protease-