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Regulation of apoptosis and autophagy in mouse and human skeletal muscle with aging and lifelong exercise training

Maja Munk Dethlefsen[a], Jens Frey Halling[a], Henrik D. Møller[a], Peter Plomgaard[b], Birgitte Regenberg[a], Stine Ringholm[a], Henriette Pilegaard[a,*]

[a] Section for Cell Biology and Physiology, Department of Biology, University of Copenhagen, Denmark
[b] Department of Clinical Biochemistry, Rigshospitalet and The Centre of Inflammation and Metabolism and Centre for Physical Activity Research, Rigshospitalet, University of Copenhagen, Denmark

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ABSTRACT

Exercise training has been reported to prevent the age-induced decline in muscle mass and fragmentation of mitochondria, as well as to affect autophagy and mitophagy. The interaction between these pathways during aging as well as the similarity between such changes in human and mouse skeletal muscle is however not fully understood. Therefore the aim of the present study was to test the hypothesis that cellular degradation pathways, including apoptosis, autophagy and mitophagy are coordinately regulated in mouse and human skeletal muscle during aging and lifelong exercise training through a PGC-1α-p53 axis. Muscle samples were obtained from young untrained, aged untrained and aged lifelong exercise trained men, and from whole-body PGC-1α knockout mice and their littermate controls that were either lifelong exercise trained or sedentary young and aged. Lifelong exercise training prevented the aging-induced reduction in PGC-1α, p53 and p21 mRNA as well as the increase in LC3II and BNIP3 protein in mouse skeletal muscle, while aging decreased the BAX/Bcl-2 ratio, LC3I and BAX protein in mouse skeletal muscle without effects of lifelong exercise training. In humans, aging was associated with reduced PGC-1α mRNA as well as decreased p62 and p21 protein in skeletal muscle, while lifelong exercise training increased BNIP3 protein and decreased p53 mRNA. In conclusion, there was a divergent regulation of autophagy and apoptosis in mouse muscle with aging and lifelong exercise training, whereas healthy aged human skeletal muscle seemed rather robust to changes in apoptosis, autophagy and mitophagy markers compared with mouse muscle at the investigated age.

1. Introduction

Age-related changes have been shown to include decreased muscle mass (Novak, 1972) and reduced content of oxidative proteins in both human and mouse skeletal muscle (Leick et al., 2010; Conley et al., 2000; Essen-Gustavsson and Borges, 1986; Hollmann et al., 2007) as well as fragmented mitochondria in mouse skeletal muscle (Halling et al., 2017). In addition, numerous studies have provided evidence that maintenance of efficient degradation and turnover of proteins and organelles like mitochondria is important for muscle function during aging (Lira et al., 2013; Wohlgemuth et al., 2010; Masiero et al., 2009; Carnio et al., 2014).

Apoptosis-mediated degradation has been suggested to be involved in the age-related muscle mass decline (Dupont-Versteegden, 2006). The pro-apoptotic Bcl-2-like protein 4 (BAX) to anti-apoptotic BAX/B-cell lymphoma 2 (Bcl-2) protein ratio is often used as a marker of apoptotic signaling (Motyl, 1999; Oltvai et al., 1993) and the BAX/Bcl-2 ratio has been shown to increase in rat skeletal muscle with aging (Ziaaldini et al., 2015; Song et al., 2006). On the other hand, one mouse study demonstrated no change in BAX and Bcl-2 protein (Leick et al., 2010), while another reported increased Bcl-2 and unchanged BAX protein levels (Sin et al., 2015) with aging in mice. Autophagy also contributes in maintaining skeletal muscle homeostasis through the removal of damaged cellular components (Klionsky et al., 2016). Lipidated microtubule-associated proteins 1A/1B light chain 3B (LC3)II is often used as a marker of the number of autophagosomes, and elevated LC3II/LC3I protein ratio in combination with decreased cargo protein Sequestosome-1 (p62) protein levels as increased autophagy (Mizushima and Yoshimori, 2007; Klionsky et al., 2016). In addition, mitophagy is the selective removal of mitochondria by autophagy, where damaged mitochondria are marked for degradation after being removed from the mitochondrial network. Thus fission regulated by

* Corresponding author at: Universitetsparken 13, 2100 Copenhagen Ø, Denmark.
E-mail address: hpilegaard@bio.ku.dk (H. Pilegaard).

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DRP1 has been shown to precede mitophagy (Twig and Shirihai, 2011). Autophagy and mitophagy have been suggested to be altered in aging skeletal muscle (Halling et al., 2017; Sebastian et al., 2016; Carnio et al., 2014; Wohlgemuth et al., 2010; Russ et al., 2012), but this remains controversial as studies have reported both decreased (Russ et al., 2012; Carnio et al., 2014) increased (Halling et al., 2017) and unchanged (O'Leary et al., 2013; Sakuma et al., 2016; Fritzen et al., 2016) LC3II/LC3I protein ratios in skeletal muscle of various species with aging. Taken together, the impact of aging on skeletal muscle apoptosis and autophagy, as well as the molecular association between apoptosis, autophagy and mitophagy in mice and human skeletal muscle with aging, remain to be fully elucidated.

Exercise training has been shown to enhance the content of skeletal muscle mitochondrial oxidative proteins (Hollmann et al., 2007; Leick et al., 2010). Moreover, lifelong endurance exercise training has been shown to prevent age-induced fragmentation of mitochondria in mouse skeletal muscle (Halling et al., 2017) as well as increase citrate synthase (CS) activity and maximal oxygen consumption (VO_2max) in aged mice and humans (Kohrt et al., 1991; Leick et al., 2010; Harber et al., 2009; Bori et al., 2012). In addition, endurance exercise training at old age has been reported to increase the LC3II/LC3I ratio in skeletal muscle of aged rats (Luo et al., 2013; Carnio et al., 2014), while lifelong endurance exercise training was shown to prevent an age related change in the LC3II/LC3I ratio in triceps muscle of aged mice (Halling et al., 2017) and vastus lateralis of humans (Carnio et al., 2014). Together this suggests that exercise training-induced metabolic adaptations in aging skeletal muscle include regulation of autophagy. However, a simultaneous investigation of the impact of lifelong exercise training on apoptosis, mitophagy and autophagy in human and mouse skeletal muscle remains to be examined.

Factors previously suggested to mediate exercise training-induced prevention of age-associated metabolic changes in skeletal muscle include the transcriptional coactivator peroxisome proliferator activated receptor-γ coactivator (PGC)-1α (Leick et al., 2010; Ringholm et al., 2013; Halling et al., 2017; Vina et al., 2009; Safdar et al., 2011; Conley et al., 2007), known as a master regulator of mitochondrial biogenesis. Thus, PGC-1α has been shown to be required for lifelong exercise training-induced prevention of an age-associated decline in the content of metabolic proteins and mitochondrial fragmentation in mouse skeletal muscle (Halling et al., 2017; Leick et al., 2010). Skeletal muscle PGC-1α mRNA has been reported to decline with aging and increase with exercise training in a mouse model of premature aging (Safdar et al., 2011). In addition, PGC-1α overexpression has been reported to protect against mitochondria-mediated apoptosis (Bianchi et al., 2006) and it has been suggested that absence of PGC-1α leads to skeletal muscle atrophy (Sandri et al., 2006; Adhihetty et al., 2009). Moreover, PGC-1α has been shown to be required for basal and acute exercise-induced autophagy in mouse skeletal muscle (Vainshtein et al., 2015; Halling et al., 2016). Although PGC-1α was not required for maintaining skeletal muscle LC3I, LC3II or p62 protein levels during lifelong exercise training in mice (Halling et al., 2017), the role of PGC-1α in the concerted regulation of apoptosis, autophagy and mitophagy in skeletal muscle with aging and lifelong exercise training remains to be determined.

Several studies encompass the tumor suppressor protein 53 (p53) as a regulator of metabolic processes in addition to the extensive role of p53 in regulating senescence and apoptosis (Saleem et al., 2009; Zhu and Prives, 2009; Kim et al., 2017). p53 and PGC-1α have been reported to interact and the presence of PGC-1α is thought to determine the impact of p53 (Sen et al., 2011; Safdar et al., 2016a), as well as regulate the transcriptional activity of p53 (Sen et al., 2011; Kim et al., 2017). p53 has been reported to have proapoptotic function (ie. BAX binding) when PGC-1α is lacking (Sen et al., 2011), while moderate stress has been reported to induce PGC-1α-p53 interaction (Safdar et al., 2016a; Sen et al., 2011). The general understanding is that p53 protein content is increased in aged rodent skeletal muscle (Sin et al., 2015; Ziaaldini et al., 2015; Kim et al., 2017). However, the impact of lifelong exercise training on p53 mRNA in human and mouse skeletal muscle as well as the requirement of PGC-1α for age and lifelong exercise training-mediated regulation of p53 mRNA remains to be determined.

Therefore, the aim of the present study was to test the hypothesis that the cellular degradation pathways, apoptosis, autophagy and mitophagy are coordinately regulated in mouse and human skeletal muscle through a PGC-1α-p53 axis during aging and lifelong exercise training. To address this, analyses were performed on muscle biopsies from young untrained, aged untrained and aged lifelong exercise trained men. In addition, muscle samples were analyzed from whole-body PGC-1α knockout mice and their littermate controls that were either lifelong exercise trained or sedentary young and aged mice.

2. Materials & methods

2.1. Human subjects

The present human study included 9 healthy untrained young (Young UT), 8 untrained aged (Aged UT) and 8 lifelong exercise trained aged (Aged ExT) men. Activity level was determined by self-reported questionnaire. The young untrained group (age 23.5 ± 2.4 years, bodyweight 84.3 ± 14 kg) (mean ± SD) was physically active < 1 h per week. The aged untrained group (age 62.8 ± 1.3 years, bodyweight 82.9 ± 14 kg) had a lifelong sedentary lifestyle with physical activity once per week at the most, throughout their life. The aged exercise trained group (age 62.1 ± 1.4 years, bodyweight 82.3 ± 12 kg) had exercised more than three times per week throughout their life. The reported physical activity differed over the years for several of the participants and included soccer, bicycling, hiking, running, gymnastics, handball, badminton, military training, fitness, swimming, tennis, boxing, track and field and ice hockey. Moreover, the lifelong exercise trained subjects trained at least 5 h/week at the time of the experiment. After being informed verbally and in writing of the experimental procedures and associated risks, all participants gave their written consent to take part in the study. The experimental procedure was approved by the Ethics Committee of Copenhagen and Frederiksberg communities (H-1-2012-108 and H-7-2014-001) and was conducted in accordance with guidelines of The Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects. Analyses on tissue samples and descriptive characteristics have previously been presented for the young subjects (Olesen et al., 2015; Bienso et al., 2015) and the aged subjects (Møller et al., 2018; Sailani et al., 2018).

2.2. Experimental setup

2.2.1. Pretesting

Young untrained subjects: At least 7 days prior to the experimental day, the physical fitness (training state) was confirmed by determining VO_2max (< 45 ml $O_2 \cdot min^{-1} \cdot kg^{-1}$ body weight), in an incremental bicycle ergometer test, as previously described (Olesen et al., 2015). The subjects were instructed to refrain from physical activity 24 h prior to the experimental day, and to ingest a light meal 2 h prior to sampling.

Aged untrained and aged exercise trained subjects: At least 7 days prior to the experimental day, the physical fitness of the aged subjects was confirmed by an endurance exercise test. Thus, an incremental ergometer cycling challenge was performed consisting of cycling at 120 watts for 5 min, increasing by 20 watts every other minute to perceived exertion of 18 on the Borg scale, after which participants continued until exhaustion, as previously described (Møller et al., 2018). The cycling test showed longer exercise duration in the trained than in the untrained subjects as anticipated. The subjects were instructed to refrain from physical activity 48 h prior to the experimental day, but the time of the last meal was not controlled.