Experimental Gerontology 58 (2014) 269–278

Contents lists available at ScienceDirect

Experimental Gerontology

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

Two weeks of one-leg immobilization decreases skeletal muscle respiratory capacity equally in young and elderly men

Martin Gram [a,*], Andreas Vigelsø [a], Takashi Yokota [a,b], Christina Neigaard Hansen [a], Jørn Wulff Helge [a], Martin Hey-Mogensen [a], Flemming Dela [a]

[a] Xlab, Center for Healthy Aging, Department of Biomedical Sciences, Faculty of Health Sciences, University of Copenhagen, DK-2200 Copenhagen, Denmark
[b] Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan

ARTICLE INFO

Article history:
Received 8 June 2014
Received in revised form 18 August 2014
Accepted 26 August 2014
Available online 2 September 2014

Section Editor: Christiaan Leeuwenburgh

Keywords:
Aging
Disuse
Muscle oxidative capacity
Immobilization
Mitochondrial biogenesis

ABSTRACT

Physical inactivity affects human skeletal muscle mitochondrial oxidative capacity but the influence of aging combined with physical inactivity is not known. This study investigates the effect of two weeks of immobilization followed by six weeks of supervised cycle training on muscle oxidative capacity in 17 young (23 ± 1 years) and 15 elderly (68 ± 1 years) healthy men. We applied high-resolution respirometry in permeabilized fibers from muscle biopsies at inclusion after immobilization and training. Furthermore, protein content of mitochondrial complexes I–V, mitochondrial heat shock protein 70 (mtHSP70) and voltage dependent anion channel (VDAC) were measured in skeletal muscle by Western blotting. The elderly men had lower content of complexes I–V and mtHSP70 but similar respiratory capacity and content of VDAC compared to the young. In both groups the respiratory capacity and protein content of VDAC, mtHSP70 and complexes I, II, IV and V decreased with immobilization and increased with retraining. Moreover, there was no overall difference in the response between the groups. When the intrinsic mitochondrial capacity was evaluated by normalizing respiration to citrate synthase activity, the respiratory differences with immobilization and training disappeared. In conclusion, aging is not associated with a decrease in muscle respiratory capacity in spite of lower complexes I–V and mtHSP70 protein content. Furthermore, immobilization decreased and aerobic training increased the respiratory capacity and protein contents of complexes I–V, mtHSP70 and VDAC similarly in the two groups. This suggests that inactivity and training alter mitochondrial biogenesis equally in young and elderly men.

1. Introduction

Aging is a complex process involving a number of physiological alterations including loss of muscle mass, strength and whole body aerobic capacity, which are associated with increased mortality and morbidity. In addition, physical inactivity is a major public concern and has the potential to adversely impact the effects of aging. On a global scale WHO has estimated that 28 and 34% of adult men and women, respectively, are insufficiently physically active (WHO, 2012). Physical inactivity is associated with a higher risk of dependence and mortality among elderly people with impaired mobility (Hirvensalo et al., 2000). Also, adopting a physically active lifestyle decreased all-cause mortality in a large cohort of elderly subjects (Paffenbarger et al., 1994) and attenuated the decline in skeletal muscle mitochondrial function in active elderly compared to sedentary elderly (Safdar et al., 2010). Thus, muscle mass, strength and endurance diminish with age, partly due to a decrease in daily physical activity. However, the loss of skeletal muscle performance with age may also occur as a consequence of dysfunctional skeletal muscle mitochondria. This view is supported by data showing accumulating damage to lipids, proteins and mtDNA as well as decreased mitochondrial protein synthesis rates in aged humans (Fano et al., 2001; Gianni et al., 2004; Rooyackers et al., 1996; Short et al., 2005; Zhang et al., 1998). These factors may contribute to age-related mitochondrial dysfunction and reduced energy producing capacity in skeletal muscle, independent of decreased daily physical activity level.

Several studies have investigated how aging influences the oxidative capacity in human skeletal muscle, but there is no consensus in the

Abbreviations: mtHSP70, mitochondrial heat shock protein 70 (also known as Grp75 or mortalin); GM3, glutamate and malate supported state 3 respiration; GMS3, glutamate, malate and succinate supported state 3 respiration; State 4o, glutamate, malate and succinate supported respiration in the presence of oligomycin; Complexes I–V, mitochondrial oxidative complex I–V; VDAC, voltage dependent anion channel (also known as mitochondrial porin); VO_2max, maximal whole body oxygen uptake; DXA, dual-energy X-ray absorptiometry; ECG, electrocardiography; CS activity, citrate synthase activity; HbA1c, glycated hemoglobin; LBM, lean body mass; BMI, body mass index.

* Corresponding author at: Xlab, Center for Healthy Aging, Department of Biomedical Sciences, Faculty of Health Sciences, University of Copenhagen, Blegdamsvej 3B, DK-2200 Copenhagen, Denmark.
E-mail addresses: mgram@sund.ku.dk (M. Gram), avhansen@sund.ku.dk (A. Vigelsø), t-yokota@med.hokudai.ac.jp (T. Yokota), cnh@sund.ku.dk (C.N. Hansen), jhelge@sund.ku.dk (J.W. Helge), martin.hey.mogensen@gmail.com (M. Hey-Mogensen), fdela@sund.ku.dk (F. Dela).

http://dx.doi.org/10.1016/j.exger.2014.08.013
0531-5565/

literature. Lower oxidative capacity with aging is found in some (Conley et al., 2000; McCully et al., 1991, 1993; Petersen et al., 2003; Short et al., 2005; Taylor et al., 1997; Tonkonogi et al., 2003) but not all studies (Barrientos et al., 1996; Brierly et al., 1997; Chretien et al., 1998; Hutter et al., 2007; Kent-Braun and Ng, 2000; Lanza et al., 2008; Rasmussen et al., 2003). However, the level of physical activity is a major confounding factor in studies examining skeletal muscle mitochondrial oxidative capacity. A likely reason for the lack of consensus is that many studies have not controlled for differences in levels of physical activity and/or age-associated diseases in the design of the studies. Thus, in studies in young and elderly athletes (Brierly et al., 1997; Lanza et al., 2008; Rimbert et al., 2004) or between groups objectively matched for physical activity levels (Kent-Braun and Ng, 2000) skeletal muscle mitochondrial oxidative capacity was not different.

The potential difference in adaptation to aerobic training between young and elderly subjects in muscle oxidative capacity has been investigated previously. The available evidence suggests that the response to training on muscle oxidative capacity is similar with age even in spite of large baseline differences in VO_2max (Konopka et al., 2014; Short et al., 2003, 2004). Likewise, two cross sectional studies including trained and untrained young and elderly subjects revealed only very few interactions between age and training status for the majority of variables (Lanza et al., 2008; Rimbert et al., 2004). However, it is not known whether physical inactivity exerts an effect that is opposite to the effect of aerobic training. In addition, there is a void of studies on the effect of physical inactivity in non-athletes, which is unfortunate because such a study design better reflects real life course trajectories.

The impairment in mitochondrial function with age or physical inactivity might manifest itself in a decrease in mitochondrial content and/or in an altered intrinsic mitochondrial function. To this end, the permeabilized fiber technique allows a measure of the respiration per wet weight indicating a change in mitochondrial content, and respiration normalized to a mitochondrial marker indicating the intrinsic mitochondrial function. A reduced respiration per wet weight or normalized to citrate synthase activity (CS activity) is therefore interpreted as a mitochondrial dysfunction. To further evaluate the impact of age on the adaptation of mitochondrial biogenesis to profound changes in physical activity level, we analyzed the protein content of markers of mitochondrial protein import (mtHSP70) and mitochondrial mass (VDAC1 and complexes I–V). We tested the hypothesis that aging per se is not a factor in the adaptation of muscle oxidative capacity to profound changes in the physical activity level.

2. Materials and Methods

2.1. Ethical Approval

The young and elderly men were informed orally and in writing about the study, potential risks and experimental procedures before written consent to participate were obtained. The study was approved by the Ethics Committee of Copenhagen (h-4-2010-85) and was performed according to the Declaration of Helsinki. The young and elderly men received remuneration for participation and were reimbursed for transportation expenses during the immobilization period and to/from meetings at the department.

2.2. Subject Characteristics

17 young and 15 elderly healthy untrained men were included. Age inclusion criteria were 20–27 and 60–75 years, respectively. The present report is part of a large study investigating the effect of one-leg immobilization on muscle metabolism. Thus, data from the same subjects have been published previously but in a different context (Reihmane et al., 2013). To investigate the effect of aging per se the young and elderly men were chosen to have a body mass index (BMI), fat percent and whole body maximal oxygen uptake (VO_2max) in a similar percentile based on the Danish Health Examination Survey (Eriksen et al., 2011). Also, an effort was made to include young and elderly men with the same daily physical activity level. Potential differences between groups in the investigated variables are therefore interpreted as an age effect. Both the young and elderly men were screened prior to inclusion to exclude individuals with diabetes (measured by glycated hemoglobin (HbA1c)), musculoskeletal disease, cardiovascular disease (resting ECG in the elderly) or any known predisposition to deep venous thrombosis. None of the young men took medication, but some of the elderly men were in medical treatment for hypertension (n = 2; thiazide diuretic + angiotensin II inhibitor; angiotensin II receptor antagonist), prostate enlargement (n = 2; α-blocker), mild asthma (n = 1; anticholinergica pro re nata), mild depression (n = 1; selective serotonin reuptake inhibitor) and attention deficit hyperactive disorder (n = 1; modafinil). None of the young and elderly men were smokers.

2.3. Experimental Design

The immobilization and training protocol as well as standardization procedures have previously been described (Reihmane et al., 2013). In brief, one of the legs was randomly immobilized with a Donjoy knee brace (DJO Nordic, Malmö, Sweden) locked at 60° for 14 days. The subjects were given a pair of crutches and were repeatedly instructed not to engage in any weight-bearing activity with the immobilized leg but they ambulated freely in the entire period. Three days after the immobilization period ended, the subjects engaged in a structured training program. The training period consisted of 20 sessions of supervised aerobic cycle ergometer training (~48–58 min effective exercise per session), divided into 12 sessions of continuous exercise (at 84 ± 1% and 85 ± 1% of maximal heart rate) and 8 sessions of interval exercise (at 89 ± 1% and 90 ± 1% of maximal heart rate), in young and older subjects, respectively. The training intensity was modified after two and four weeks of training according to a VO_2max test (data not shown). One elderly and two young men failed to complete the training period. In total the subjects reported to the laboratory ~30 times during the intervention (Fig. 1). To assess the daily physical activity level a combined tri-axial accelerometer and heart rate sensor (Actitrainer, Actigraph, Pensacola, United States) was used for three consecutive days before, during immobilization, and during the training period, while continuing normal daily activities (30 Hz, 1°S epoch). The main outcome variable, the vector magnitude, was calculated as the square root of the sum squared of activity counts for each vector (axis 1, axis 2 and axis 3) at every epoch and then averaged over the registration period. This was then averaged in each group at each time point (Fig. 1). To take the altered activity level of the one leg immobilization into account, the data was post processed using a low frequency extension algorithm. Wear time validation was performed to infer real heart rate from noise by removing data in the lower range defined as four consecutive epochs with a heart rate value of zero and in the higher range defined as 11 consecutive epochs with a heart rate value of four and/or more. If less than 60% of the recorded data remained after the wear time validation the dataset was removed (MATLAB 8.0, The MathWorks, Inc., Natick, Massachusetts, United States). As a result subjects were excluded entirely from the analysis during the screening in: young = 2; elderly = 1, during immobilization in young = 2; elderly = 2 and during the training period in young = 1; elderly = 1. Accelerometer data were accepted whenever heart rate wear time was present. This resulted for the remaining subjects in an average acceptance rate of 56 h and 20 min for the young subjects and 55 h and 54 min for the elderly subjects.

2.4. Skeletal Muscle Biopsies

Subjects arrived to the laboratory in the morning after an overnight fast. As seen in Fig. 1, we obtained biopsies from vastus lateralis at inclusion in the control leg (T1CON) and the leg-to-be-immobilized (T1IM) (week zero); immediately after the Donjoy was removed (week two)