

The impact of aging on mitochondrial function and biogenesis pathways in skeletal muscle of sedentary high- and low-functioning elderly individuals

Anna-Maria Joseph,¹ Peter J. Adhihetty,² Thomas W. Buford,¹ Stephanie E. Wohlgenuth,¹ Hazel A. Lees,¹ Linda M.-D. Nguyen,² Juan M. Aranda,³ Bhanu D. Sandesara,¹ Marco Pahor,¹ Todd M. Manini,¹ Emanuele Marzetti^{1,4} and Christiaan Leeuwenburgh¹

¹Departments of Aging and Geriatric Research, University of Florida, Gainesville, FL 32610, USA

²Applied Physiology and Kinesiology, University of Florida, Gainesville, FL 32611, USA

³Division of Cardiovascular Medicine, University of Florida, Gainesville, FL 32610, USA

⁴Department of Orthopedics and Traumatology, Catholic University of the Sacred Heart, Rome 00168, Italy

Summary

Age-related loss of muscle mass and strength (sarcopenia) leads to a decline in physical function and frailty in the elderly. Among the many proposed underlying causes of sarcopenia, mitochondrial dysfunction is inherent in a variety of aged tissues. The intent of this study was to examine the effect of aging on key groups of regulatory proteins involved in mitochondrial biogenesis and how this relates to physical performance in two groups of sedentary elderly participants, classified as high- and low-functioning based on the Short Physical Performance Battery test. Muscle mass was decreased by 38% and 30% in low-functioning elderly (LFE) participants when compared to young and high-functioning elderly participants, respectively, and positively correlated to physical performance. Mitochondrial respiration in permeabilized muscle fibers was reduced (41%) in the LFE group when compared to the young, and this was associated with a 30% decline in cytochrome c oxidase activity. Levels of key metabolic regulators, SIRT3 and PGC-1 α , were significantly reduced (50%) in both groups of elderly participants when compared to young. Similarly, the fusion protein OPA1 was lower in muscle from elderly subjects; however, no changes were detected in Mfn2, Drp1 or Fis1 among the groups. In contrast, protein import machinery components Tom22 and cHsp70 were increased in the LFE group when compared to the young. This study suggests that aging in skeletal muscle is associated with impaired mitochondrial function and altered biogenesis pathways and that this may contribute to muscle atrophy and the decline in muscle performance observed in the elderly population.

Key words: aging; mitochondria; PGC-1 α ; sarcopenia; skeletal muscle.

Correspondence

Christiaan Leeuwenburgh, University of Florida, Institute on Aging, 1600 SW Archer Road, PO Box 100143, Gainesville, FL 32610, USA. Tel.: 1 352 273 6796; fax: 1 352 294 5058; e-mail: cleeuwen@ufl.edu

Accepted for publication 29 May 2012

Introduction

The aging process is associated with a progressive decline in muscle mass and is referred to as sarcopenia. The sarcopenic phenotype is characterized by lower muscle strength, increased muscle fatigability, and reduced endurance capacity that may contribute to increased frailty and a greater risk of falls and other disabilities (Hurley, 1995). While a number of factors have been associated with age-related sarcopenia, the primary mechanisms contributing to this process are still uncertain.

A decline in mitochondrial function is a common phenomenon that has been readily observed in several different types of aged tissues and is particularly prominent in highly oxidative tissues such as skeletal muscle, in both animals and humans (Lanza & Nair, 2010). The mitochondrial theory of aging is based on the premise that cumulative damage caused by the production of free radicals can alter mitochondrial DNA (mtDNA; e.g., point mutations and deletions; Hiona & Leeuwenburgh, 2008). Defects in mtDNA can lead to a decline in mtDNA abundance and a reduced number of genes encoding mitochondrial proteins. This results in the improper assembly of the electron transport chain (ETC), as well as components of oxidative phosphorylation (Short *et al.*, 2005). Lower mitochondrial protein synthesis rates, disturbances in mitochondrial enzyme activities, and lower oxidative capacity and ATP synthesis have all been reported in aged tissue (Rooyackers *et al.*, 1996; Short *et al.*, 2005). Furthermore, damage to mtDNA, lipids, and proteins by free radicals can activate cell death pathways, inducing the release of mitochondrial proteins such as cytochrome c into the cytosol, and the fragmentation of DNA that has been linked to muscle fiber atrophy and reduced physical function with aging (Marzetti *et al.*, 2011). A role for mitochondrial dysfunction in aging is evidenced by mtDNA mutator mice that express a proofreading deficient mitochondrial DNA polymerase gamma (Polg; Kujoth *et al.*, 2005). These mutant mice have increased mtDNA mutations and display age-related phenotypes, including severe sarcopenia which is likely due to defects in the assembly of functional ETC holoenzyme complexes (Hiona *et al.*, 2010). Similar findings of increased mtDNA mutagenesis and reduced mitochondrial enzyme activities have been reported in human skeletal muscle with age (Hiona & Leeuwenburgh, 2008). Although the impact of these somatic mutations and deletions on organismal survival has not been completely established, these studies implicate a role for mitochondria in the aging process.

Mitochondrial biogenesis is an intricate process tightly controlled by key mitochondrial regulators. The proper assembly and functioning of mitochondria is achieved largely by nuclear-encoded proteins. Thus, a coordinated effort by both the nuclear and mitochondrial genomes is required to maintain mitochondrial integrity. In recent years, the importance of coactivators and transcription factors has been highlighted. In particular, the peroxisome proliferator-activated receptor γ coactivator α (PGC-1 α) has gained wide acceptance as being the major regulator of mitochondrial content and oxidative metabolism in several tissues, including skeletal muscle. Overexpression of PGC-1 α in skeletal muscle induces a fast-to-slow-fiber-type transition and increases mitochondrial content and oxidative metabolism through its activation of target genes involved in substrate metabolism (Handschin & Spiegelman, 2006). Furthermore,



PGC-1 α is upregulated in both animals and humans during acute and chronic contractile activity (Handschin & Spiegelman, 2006). Previous studies have reported reduced levels of PGC-1 α in aged muscle (Baker *et al.*, 2006; Safdar *et al.*, 2010), as well as in conditions of muscle wasting (Sandri *et al.*, 2006; Satchell *et al.*, 2007). Interestingly, overexpression of PGC-1 α preserves mitochondrial function and muscle integrity in these conditions, partly through the stimulation of mitochondrial biogenesis and the attenuation of cell death pathways (Sandri *et al.*, 2006; Wenz *et al.*, 2009).

In addition to mitochondrial biogenesis regulatory proteins, the significance of mitochondrial fusion and fission proteins has recently been highlighted in aging. These proteins regulate mitochondrial morphology and function and protect the integrity of the organelle by facilitating the mixing of intracellular metabolites and mtDNA. Fusion and fission proteins also segregate damaged and energy-deficient organelles through autophagy (Seo *et al.*, 2010). Defects in mitochondrial fusion and fission proteins have dramatic consequences on mitochondrial function. For example, mutations in the fusion proteins Mfn2 and OPA1 cause two neurodegenerative diseases, Charcot-Marie-Tooth type 2A, and dominant optic atrophy, respectively (Seo *et al.*, 2010). Muscle-specific Mfn1 and Mfn2 knockouts result in mtDNA depletion and the accumulation of organelles with a high level of mutant mtDNA (Chen *et al.*, 2010). Consequently, these animals have severe mitochondrial dysfunction and muscle atrophy. In human skeletal muscle, the mitochondrial fusion protein Mfn2, a downstream target of PGC-1 α and estrogen-related receptor alpha (ERR α), is upregulated with acute exercise and downregulated with obesity, insulin resistance, and aging (Seo *et al.*, 2010). Furthermore, mitochondrial morphology factors have also been linked to another group of proteins known as the mitochondrial protein import machinery (PIM; Seo *et al.*, 2010). Collectively, the primary task of this machinery is the import and targeting of all nuclear-encoded mitochondrial proteins to subcompartments of the organelle where they are assembled into a functional reticulum. Similar to the aforementioned groups of proteins, the PIM are also sensitive to changes in muscular activity, altering the import rate of proteins into mitochondrial subcompartments and contributing to mitochondrial biogenesis (Takahashi *et al.*, 1998). While the effect of aging on the protein import pathway was recently demonstrated in animals (Joseph *et al.*, 2010), not much is known regarding this translocation system in humans.

Numerous studies have linked mitochondrial dysfunction with reduced skeletal muscle function during aging (Hiona & Leeuwenburgh, 2008). The extent of this association, however, remains under debate because some studies have revealed no effect of age on muscle mitochondrial properties (Barrientos *et al.*, 1996; Rasmussen *et al.*, 2003). In part, some of the divergent results may be due to differences in physical activity status that may influence mitochondrial enzyme activity levels and the extent of muscle mass loss that is experienced with age (Barrientos *et al.*, 1996; Safdar *et al.*, 2010). Recently, Safdar *et al.* (2010) reported that mitochondrial function was preserved in active older individuals compared to age-matched sedentary individuals and that this was partly due to differences in antioxidant capacity, inflammatory markers, and mitochondrial oxidative capacity between the two groups. Hence, these data suggest the potential of a physically active lifestyle in attenuating the decline in mitochondrial function previously reported with age and highlight the role of physical activity in human aging research.

The primary purpose of this study was to investigate skeletal muscle changes in cellular and molecular mechanisms involved in mitochondrial biogenesis in two distinct groups of sedentary elderly subjects, classified as high and low functioning on the basis of physical performance measures. While these subjects differed in their overall functionality, their

physical activity levels were similar. Thus, we specifically sought to assess whether high and low functionality in the elderly was associated with distinct mitochondrial protein expression profiles in skeletal muscle irrespective of physical activity status. We hypothesized that the extent of mitochondrial decline with aging would be correlated with physical function parameters, with the highest mitochondrial decay being observed in low-functioning elderly (LFE) individuals when compared to high-functioning elderly (HFE) individuals. Firstly, our findings provide support for mitochondrial dysfunction contributing to functional decline in muscle with age. Additionally, our data suggest that the extent of mitochondrial impairment correlates to physical function in the elderly.

Results

Participant characteristics

The main participant characteristics are provided in Table 1. A total number of 35 individuals participated in the study, including 23 older and 12 young men and women. No significant differences were observed in gender, height, weight, and body mass index (BMI) among the three groups. However, elderly participants had higher amounts of intermuscular adipose tissue (IMAT) compared to young participants ($P < 0.05$). Older participants in the LFE group were on average 6 years older than in the HFE group ($P < 0.05$), and appropriate statistical analyses (ANCOVA) were used to account for this difference. The thigh muscle mass was 38% and 30% lower ($P < 0.05$) in the LFE group when compared to the young and HFE group, respectively. The greater atrophy observed in muscle from the LFE group was not attributable to age or gender differences between the two elderly populations. Additionally, muscle size was positively correlated with the SPPB score in HFE and LFE individuals ($r = 0.48$, $P < 0.05$; data not shown). The SPPB test has been extensively validated and is predictive of future mobility disability risk and mortality in the elderly (Guralnik *et al.*, 1994). The two elderly groups did not significantly differ in terms of the number of conditions they exhibited (Buford *et al.*, 2012).

Muscle fiber cross-sectional area and fiber type differences

Type II fibers in LFE subjects displayed a reduced cross-sectional area (CSA) when compared to young participants (Table 2; $P < 0.05$). No

Table 1 Participant characteristics

Characteristic	Y	HFE	LFE	<i>P</i> -value for group difference
Sample size (<i>n</i>)	12	12	11	
Age (years)	23 ± 1	75 ± 1*	81 ± 1*†	< 0.05
Females, % (<i>n</i>)	33 (4)	33 (4)	45 (5)	0.789
Weight (kg)	73 ± 3	82 ± 5	78 ± 4	0.299
Height (cm)	173 ± 3	170 ± 3	167 ± 3	0.211
BMI (kg m ⁻²)	24 ± 1	28 ± 1	28 ± 1	0.09
Muscle size (cm ³)	527 ± 29	466 ± 39	325 ± 34*†	< 0.05
Subcutaneous fat (cm ³)	361.6 ± 62	291.8 ± 34	348.9 ± 61	0.6
IMAT (cm ³)	88.57 ± 5	119.3 ± 11*	121.4 ± 8*	< 0.05
SPPB total score	N/A	11.42 ± 0.2	6.091 ± 0.5†	< 0.001

BMI, body mass index; IMAT, intermuscular adipose tissue; N/A, not applicable; SPPB, short physical performance battery; Y, young; HFE, high-functioning elderly; LFE, low-functioning elderly.

Values are reported as means ± SE. Y, HFE, and LFE. HFE determined by a score of ≥ 11 and LFE ≤ 7 on the SPPB test. Young participants did not perform the SPPB.

* $P < 0.05$ vs. young; † $P < 0.05$ LFE vs. HFE.

Table 2 Fiber type composition and area in vastus lateralis from Y and HFE and LFE participants

Variable	Y	HFE	LFE
Type I (%)	55 ± 3	45 ± 7	56 ± 6
Type II (%)	45 ± 3	55 ± 7	44 ± 6
Type I area (μm ²)	6759 ± 843	8626 ± 1153	6713 ± 961.6
Type II area (μm ²)	6578 ± 777	7614 ± 1662	4335 ± 592.3*

Y, young; HFE, high-functioning elderly; LFE, low-functioning elderly.

Values are reported as means ± SE.

* $P < 0.05$ vs. young.

difference in type II fiber CSA was detected between HFE and young subjects. In addition, neither the number nor the CSA of type I fibers differed among the groups (Table 2).

Effect of age on mitochondrial function in skeletal muscle

To assess mitochondrial bioenergetics and function, oxygen (O₂) consumption and cytochrome c oxidase (COX) activity were measured in permeabilized fibers. Basal rates of pyruvate-malate-induced state 4 respiration were reduced in the elderly groups when compared to young. Moreover, LFE individuals displayed significantly lower state 4 respiration relative to the HFE group (Fig. 1A; $P < 0.05$), a difference that could not be attributed to age or gender. ADP-stimulated respiration (state 3) was 41% lower in the LFE subjects compared to young participants ($P < 0.05$). Although not statistically significant, there was a strong trend for a decrease in state 3 respiration between HFE subjects and young individuals ($P = 0.06$). COX activity in whole muscle homogenates, used as an indicator of mitochondrial function (Adihetty *et al.*, 2009; Safdar *et al.*, 2010), was reduced by 30% in LFE subjects relative to the young group (Fig. 1B; $P < 0.05$). No difference in COX activity was observed between HFE and young participants. The decrease in mitochondrial content does not entirely account for the decline in state 3 respiration rates observed in the LFE group suggesting that mitochondrial bioenergetics is compromised in skeletal muscle from LFE individuals compared to young and that there is a trend for mitochondrial function decline in the HFE group.

Age-related changes in AMPK α and p38 signaling pathways

To determine whether upstream mitochondrial signaling pathways were altered in young and elderly participants, levels of two key signaling molecules, 5' AMP-activated protein kinase (AMPK α) and p38 mitogen-activated protein kinase (MAPK) were assessed. No significant difference in either parameter was detected between groups (Fig. 2).

Age-related changes in transcriptional and metabolic regulators in aging muscle

The induction of mitochondrial gene expression and biogenesis is largely controlled by the coordinated actions of various transcriptional and metabolic regulators such as PGC-1 α and Sirtuin 3 (SIRT3). Levels of both these proteins were reduced by ~50% in elderly participants compared to young (Fig. 3A; $P < 0.05$). Furthermore, PGC-1 α protein levels were positively correlated with COX activity in all groups (Fig. 3B; $r = 0.48$, $P < 0.05$). In contrast, levels of the cofactor receptor-interacting proteins 140 (RIP140), a repressor of PGC-1 α target genes (Powelka *et al.*, 2006), were not different with age or between LFE and HFE participants.

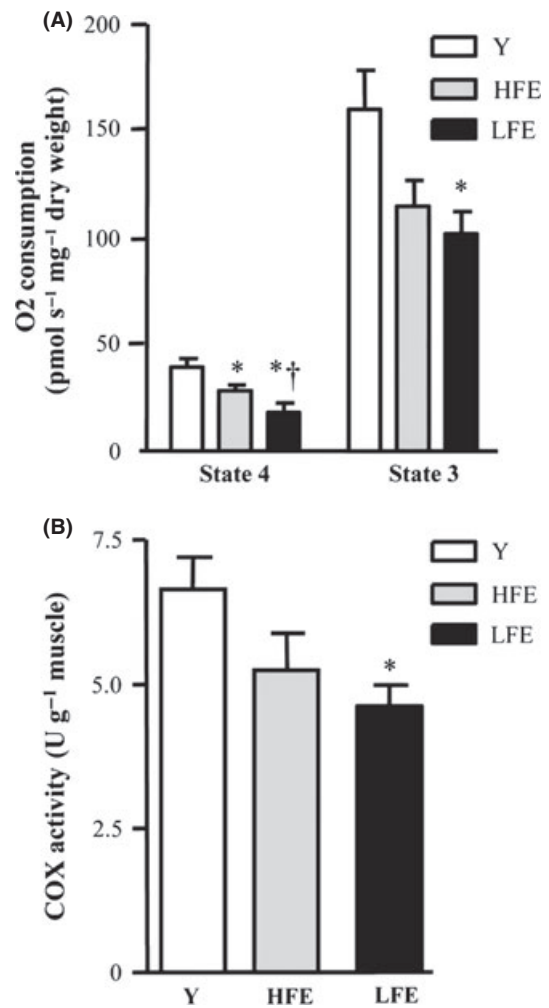


Fig. 1 Mitochondrial bioenergetics in muscle from young and functionally distinct elderly individuals. (A) Respiration in permeabilized muscle fibers from young (Y), high-functioning elderly (HFE), and low-functioning elderly (LFE) subjects was measured with pyruvate/malate (5 mM/2 mM) in basal respiratory state 4 or ADP-stimulated (2 mM) respiratory state 3 and expressed as pmol s⁻¹ per mg of dry weight. Values are means ± SE; $n = 5-9$ per group. * $P < 0.05$ vs. young; † $P < 0.05$ vs. HFE (B) Cytochrome c oxidase activity, expressed as unit per gram of tissue. Values are means ± SE; $n = 5-9$ per group. * $P < 0.05$ vs. young.

Similarly, levels of the PGC-1 α targeted proteins, nuclear respiratory factor 1 (NRF-1) and mitochondrial transcription factor A (Tfam) did not differ across groups. However, abundance of the mtDNA-encoded subunit COX I was lower with aging ($P < 0.05$). Finally, no significant changes in the nuclear-encoded COX IV protein were observed. Interestingly, decreased mitochondrial function and content in the LFE subjects were associated with reduced functional capacity as indicated by gait speed on the 4-m walk test (Fig. 4A; $P < 0.05$). Furthermore, a positive correlation was determined between PGC-1 α and the 4-m walk test (Fig. 4B; $r = 0.42$, $P < 0.05$).

Effect of age on mitochondrial fusion and fission pathways

While no changes were observed in Mfn2 protein levels among the groups, a significant decline in the fusion protein OPA1 was detected in elderly participants compared to young (Fig. 5; $P < 0.05$). Finally, no

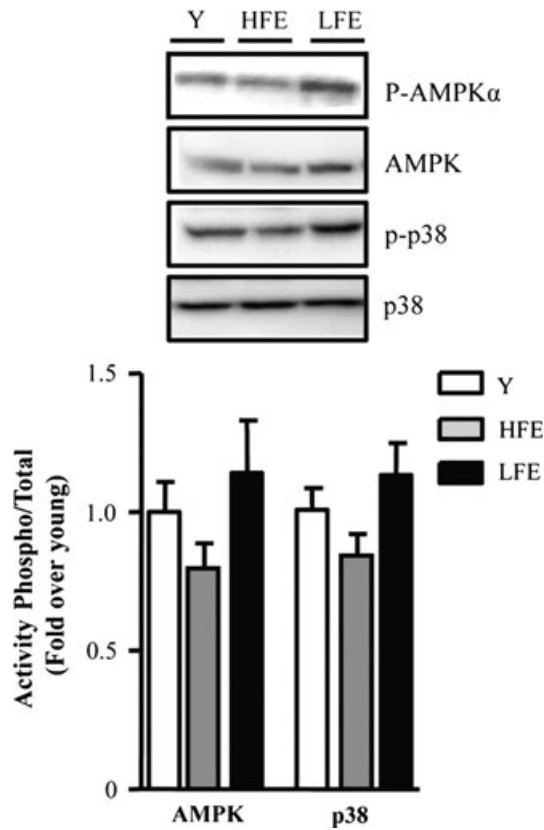


Fig. 2 Effect of age on cellular signaling molecules. Representative Western blots of phosphorylated 5' AMP-activated protein kinase (P-AMPK α), total AMPK (AMPK), phosphorylated p38 mitogen-activated protein kinase (p-p38), and total p38 (p38). Summary of experiments of AMPK and p38 activation are shown below as the phosphorylated form of the kinase over the total content and expressed as a fold of young. Values are means \pm SE; $n = 11$ – 12 per group.

differences in the levels of the mitochondrial fission proteins Drp1 and Fis1 were observed (Fig. 5).

Effect of age on mitochondrial protein import components

The PIM consists of two primary assembly complexes known as the translocases of the outer membrane (TOM complex) and the translocases of the inner membrane (TIM complex). TOM22, as well as cytosolic heat shock protein 70 (cHSP70) were upregulated in skeletal muscle from LFE participants compared to young (Fig. 6; $P < 0.05$). cHsp70 was also higher in the LFE group when compared to the HFE group ($P < 0.05$) and independent of age and gender. In contrast, levels of mitochondrial Hsp70 (mtHsp70) were reduced with aging ($P < 0.05$) in the LFE compared to the young group. A trend toward an age-related decrease in Hsp60 ($P = 0.06$) protein levels was also evident.

Discussion

Aerobic capacity declines with age because of a loss in skeletal muscle mass and a lower muscle oxidative capacity (Rooyackers *et al.*, 1996; Short *et al.*, 2005). The molecular and cellular disturbances responsible for the decline in oxidative capacity with progressive age are currently unknown. While mitochondria have been implicated, the extent of mitochondrial decline with aging and how this relates to poorer muscle quality

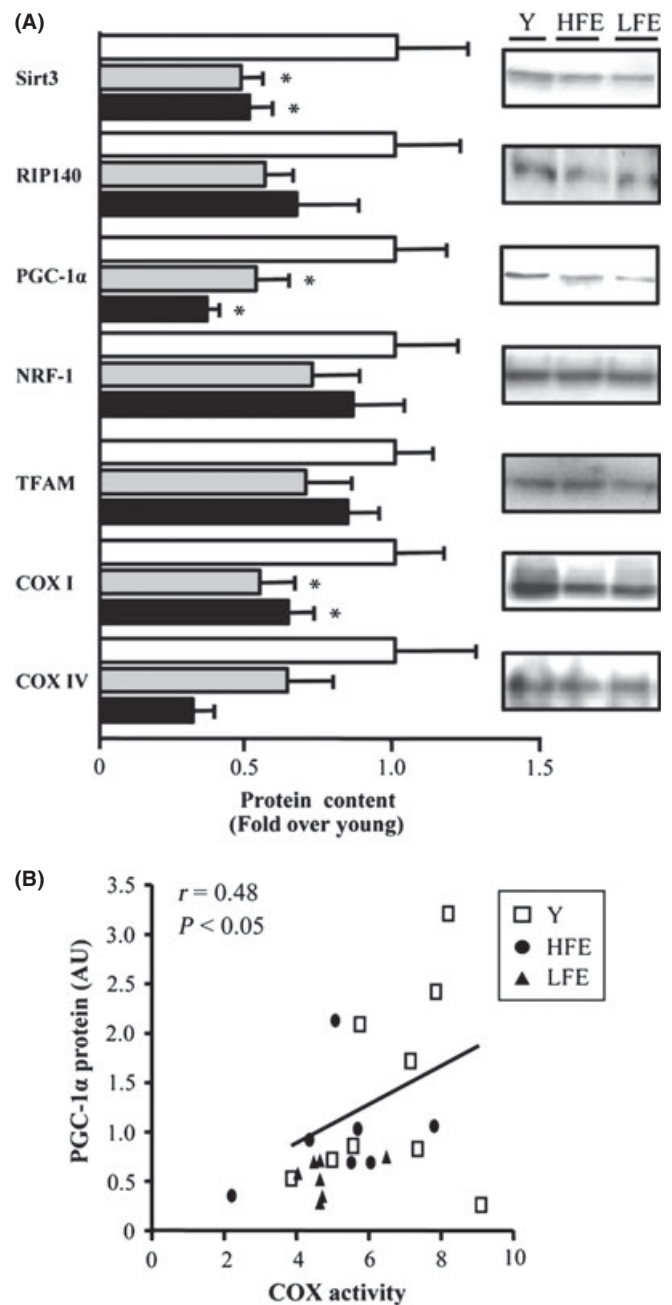


Fig. 3 Age-related changes in key metabolic and transcriptional regulators. (A) Western blot analysis of SIRT3 = sirtuin 3; RIP140 (nrip1) = nuclear receptor-interacting protein 140; PGC-1 α = peroxisome proliferator-activated receptor gamma (PPAR γ) coactivator 1-alpha; NRF-1 = nuclear respiratory factor 1; Tfam = mitochondrial transcription factor A; cytochrome c oxidase (COX) I and IV = COX subunit I and IV. A summary of repeated experiments (*left*) along with the corresponding protein blot (*right*). All values were normalized to actin and expressed as a fold of young. Values are means \pm SE; $n = 11$ – 12 per group. * $P < 0.05$ vs. young. (B) Relationship between COX activity and PGC-1 α in young and elderly individuals. $n = 7$ – 9 per group. r = Pearson correlation coefficient.

and function remain controversial. Thus, the present study was designed to investigate age-related changes in skeletal muscle mitochondrial function and to determine whether mitochondrial properties differ in elderly sedentary individuals with low physical performance relative to high physical performance.

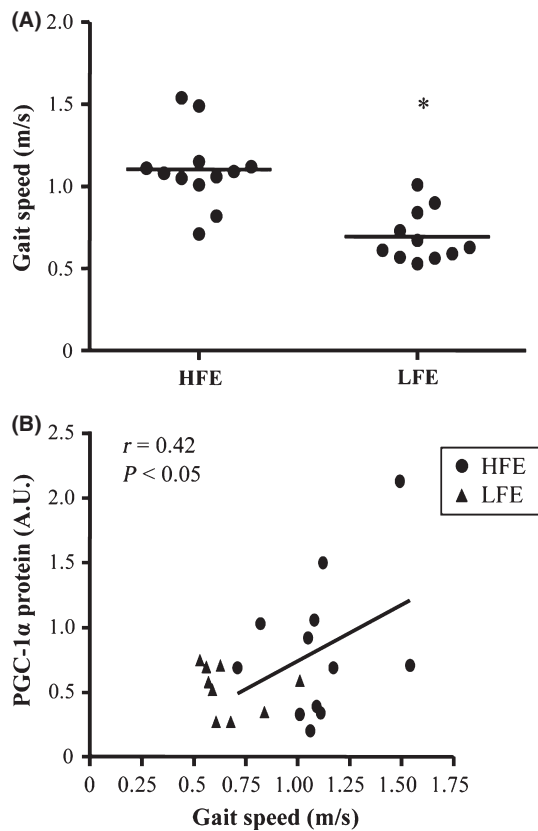


Fig. 4 Association between PGC-1 α and physical function in elderly subjects. (A) Gait speed in high-functioning elderly (HFE) and low-functioning elderly (LFE) individuals with each point representing an individual. $n = 11$ –12 per group. * $P < 0.05$ vs. HFE. (B) Relationship between gait speed and PGC-1 α protein in HFE and LFE subjects. $n = 9$ –12 per group. $r =$ Pearson correlation coefficient.

Consistent with previous studies, our results show that muscle mass declines with age and that this is likely attributed to fiber atrophy that preferentially occurs in type 2 fibers (Lexell & Taylor, 1991). There are also reports that aging leads to a greater proportion of hybrid fibers that cannot strictly be classified as type I or type IIA but rather co-express both MHC I and IIA isoforms (referred to as type IIC fibers). While the mechanisms responsible for these hybrid fibers are not well understood, a dysregulation in the expression of MHC isoforms by nuclei within individual fibers has been postulated (Andersen, 2003). These hybrid fibers are most accurately assessed using electrophoresis of MHC isoforms in single fibers that allow examination of a longer section of the muscle fiber. Therefore, the number of fibers detected co-expressing MHC I and MHC IIA is drastically reduced by histological methods where analysis is limited to a small CSA of the fiber. Thus, although we were not able to accurately estimate the percentage of these hybrid fibers with the methods used in this study, we intend to include this unique fiber population in future studies with similar subject populations.

Reductions in the quantity of muscle in elderly individuals were also associated with mitochondrial decay as demonstrated by reduced bioenergetics and the content of mitochondria in muscle. Interestingly, these changes were most prominent in LFE individuals. Previous reports have shown that skeletal muscle from elderly individuals and particularly those with poor physical performance are more prone to oxidative damage when compared to elderly individuals with higher physical performance. This is evidenced by higher levels of 8-hydroxy 2-deoxyguanosine (8-OHdG) and

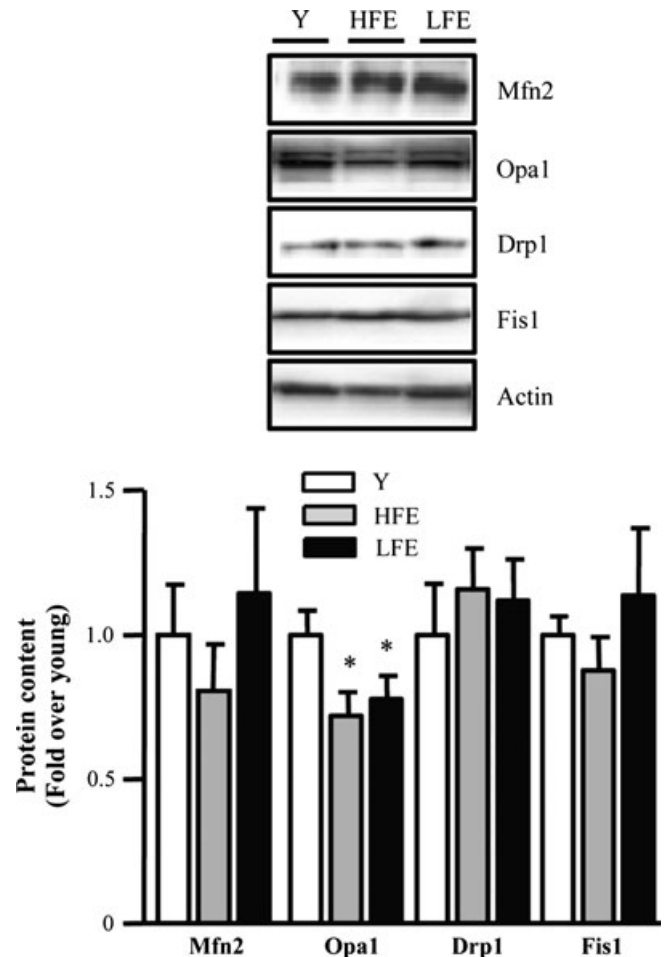


Fig. 5 Mitochondrial fusion and fission proteins in young and elderly subjects. Western blots of fusion Mfn2 (mitofusin 2) and Opa1 (optic Atrophy Type 1), and fission proteins Drp1 (dynamin-related protein 1) and Fis1 (fission 1). Two bands were detected for Opa1 representing the long and short isoforms. Both bands were quantified for total Opa1 protein. Summary of repeated experiments is shown below. All values were normalized to actin and expressed as a fold of young. Values are means \pm SE; $n = 11$ –12 per group. * $P < 0.05$ vs. young.

protein carbonylation, as well as altered redox homeostasis and lower antioxidant capacity (Parise *et al.*, 2005; Safdar *et al.*, 2010). Additionally, poor physical performance in the elderly is associated with elevated levels of chronic inflammatory markers including interleukin 6 (IL-6) and nuclear factor kappa B (NF- κ B; Buford *et al.*, 2010; Cesari *et al.*, 2004). This hypothesis is supported by the greater decline observed in mitochondrial respiration, as well as COX activity in the LFE individuals that may contribute to higher rates of free radical formation and muscle damage. Moreover, recent data obtained from our group in the same population of elderly subjects demonstrate a strong correlation between apoptotic signaling proteins and reduced muscle mass and function further implicating increased oxidative stress as a possible contributor of muscle aging (Marzetti *et al.*, 2012). Collectively these reports point to an association between oxidative damage, mitochondrial function, and muscle mass loss in aging skeletal muscle, particularly in individuals with poor physical function. The specific molecular details, however, regarding this relationship are still unclear and require further investigation.

It is well established that PGC-1 α is an important regulator of mitochondrial mass and function in skeletal muscle (Handschin & Spiegelman,

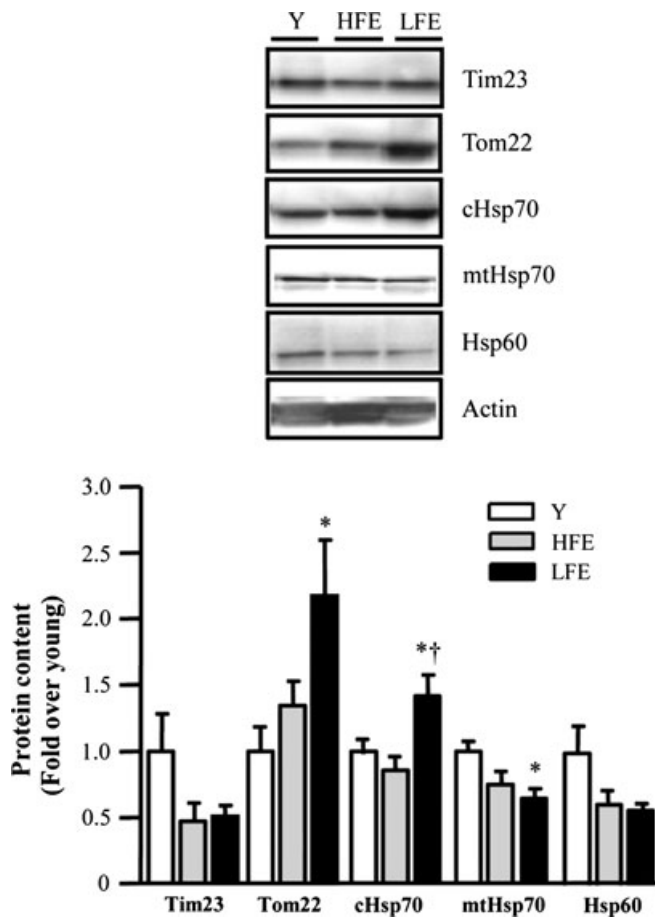


Fig. 6 Alterations in mitochondrial protein import machinery (PIM) components. Western blot analysis of PIM with a graphical representation depicted below. Tim23 = translocase of the inner membrane protein 23; Tom22 = translocase of the outer membrane protein 22; cHsp70 = cytosolic heat shock protein 70; mtHsp70 = mitochondrial heat shock protein 70; Hsp60 = heat shock protein 60. All values were normalized to actin and expressed as a fold of young. Values are means \pm SE; $n = 11$ – 12 per group. * $P < 0.05$ vs. young; † $P < 0.05$ vs. high-functioning elderly.

2006). Furthermore, declines in PGC-1 α levels precede the loss of muscle mass in aging (Baker *et al.*, 2006), as well as in a variety of muscle wasting conditions including fasting and denervation (Sandri *et al.*, 2006; Sackey *et al.*, 2007). In these models of atrophy, muscle wasting was prevented by the ectopic expression of PGC-1 α (Sandri *et al.*, 2006; Sackey *et al.*, 2007). In aged mice, PGC-1 α overexpression was also associated with improved whole body health compared with age-matched wild-type animals (Wenz *et al.*, 2009). In our study, levels of PGC-1 α were reduced in both high- and LFE individuals. In addition, PGC-1 α levels were positively correlated with COX activity, as well as gait speed in elderly participants suggesting that this protein may be an important predictor in the degree of physical function decline linked to mitochondrial dysfunction. This is supported by previous data illustrating a strong relationship between COX activity and muscle strength in the elderly (Safdar *et al.*, 2010). Such an assumption is also in line with evidence showing that PGC-1 α mRNA levels are significantly correlated with oxidative capacity and training status in healthy subjects (Garnier *et al.*, 2005). As discussed above, PGC-1 α levels increase in response to physical activity and can be used as a marker of mitochondrial biogenesis in muscle. This is also evidenced in active

elderly individuals where levels of this protein were preserved and similar to young individuals when compared to sedentary individuals (Safdar *et al.*, 2010). The finding that this protein was equally reduced in both HFE and LFE individuals when compared to young subjects would suggest that both elderly groups had similar physical activity lifestyles.

Our study also demonstrates that sirtuin 3 (SIRT3) levels are affected with aging. Lanza *et al.* (2008) reported similar findings in human skeletal muscle from individuals with a sedentary proteomic profile. Human SIRT3 is a mitochondrial NAD⁺-dependent deacetylase that has been associated with longevity in humans (Rose *et al.*, 2003). Perhaps a clue linking SIRT3 to aging is supported by recent studies demonstrating that SIRT3 in muscle promotes the expression of PGC-1 α (Palacios *et al.*, 2009) and vice versa (Kong *et al.*, 2010). In addition, overexpression of SIRT3 or PGC-1 α in cultured myotubes suppresses levels of ROS (Kong *et al.*, 2010). These data are in agreement with the finding that SIRT3 similar to PGC-1 α has the ability to reduce oxidative damage by influencing key antioxidant defense systems (Someya *et al.*, 2010). Intriguingly, a PGC-1 α /Tfam complex was recently identified in association with mtDNA nucleotides suggesting that PGC-1 α translocates to mitochondria where it may play a role in retrograde signaling between the nucleus and mitochondria (Aquilano *et al.*, 2010). Although the exact molecular understanding has yet to be fully elucidated, it appears that age-related disturbances in PGC-1 α and SIRT3 levels may increase oxidative damage and mutations to DNA, compromising mitochondrial function and contributing to muscle atrophy observed with aging.

Age-related sarcopenia has also been associated with altered mitochondrial morphology, as illustrated by the appearance of enlarged and depolarized mitochondria with aberrant mitochondrial cristae formation (Navratil *et al.*, 2008). OPA1 is present in the mitochondrial inner membrane and is important for mitochondrial fusion and cristae morphology (Arnoult *et al.*, 2005). Interestingly, giant mitochondria in cultured myoblasts display reduced levels of OPA1 and are associated with impaired fusion capacity (Navratil *et al.*, 2008). These findings imply that aberrant mitochondria may not restore their function by fusing and exchanging their contents with functional organelles, leading to compromised bioenergetics. Noteworthy, our results indicate that OPA1 levels but not Mfn2 are reduced in aged human muscle, suggesting perhaps an additional pathway leading to mitochondrial dysfunction in late life. Moreover, repression of OPA1 can also result in mitochondrial fragmentation through greater fission. These alterations are accompanied by decreased cell growth and oxygen consumption, as well as the induction of apoptosis (Arnoult *et al.*, 2005; Chen *et al.*, 2005). The latter occurs primarily through the remodeling of cristae junctions that control the release of cytochrome c. Interestingly, mitochondrial morphology proteins interact with members of the Bcl-2 family of cell death proteins (Brooks *et al.*, 2007); however, the significance of such interactions is yet unclear. Thus, dysregulation in fusion and/or fission events resulting in improper mixing of intracellular metabolites and mtDNA may compromise the integrity of the mitochondrial genome by favoring the accumulation of mtDNA mutations. This leads to enhanced oxidative damage, triggering the release of pro-apoptotic proteins, and irreversible cell death pathways. Altogether, these data suggest the importance of maintaining proper mitochondrial dynamics through fusion and fission proteins and the consequences of aberrant mitochondrial morphology for cellular health.

With regard to the PIM, results from the present study are consistent with previous findings in animals (Joseph *et al.*, 2010). Indeed, in muscle from aged rats, levels of the outer membrane receptor protein Tom22 are increased relative to their younger counterparts. In the same animals, these changes resulted in higher rates of precursor protein import and assembly into mitochondrial complexes (Joseph *et al.*, 2010). Tom22 is

crucial for the import and assembly of Tom40, a protein that forms the TOM complex, the main entry gate into mitochondria (Humphries *et al.*, 2005). The upregulation of Tom22 in aged muscle is likely a compensatory response by the cell to increase the import and assembly of nuclear-encoded proteins into mitochondria and maintain oxidative capacity in the face of impaired synthesis of mtDNA-encoded components. This phenomenon has also been observed in other conditions of compromised bioenergetics including mtDNA depletion and mitochondrial disease (Joseph *et al.*, 2004). A direct interaction between components of the PIM and mitochondrial morphology, as well as apoptosis proteins, has also been reported (Seo *et al.*, 2010), implying that mitochondrial regulatory pathways might not be functionally independent and that their roles in mitochondrial biogenesis are multifaceted. This study is the first to show the adaptability of the PIM components in muscle from elderly individuals and highlights the potential of this previously unexplored area of mitochondrial biology in aging research.

In summary, results from the present study indicate that mitochondrial regulation and function are reduced in aged human skeletal muscle, and this appears to be related to the level of physical performance in the elderly. Despite correcting for age and gender, LFE individuals displayed greater declines in mitochondrial bioenergetics and muscle mass loss than HFE individuals. This finding supports our hypothesis of an association between the extent of skeletal muscle mitochondrial decline and physical function status with aging. However, it is important to note that mitochondrial dysfunction was also observed in HFE participants, which implicates aging as a primary contributor to this process. Moreover, the finding that key regulatory proteins including Sirt3, PGC-1 α , and COX I are reduced to the same extent in both elderly groups would suggest that non-mitochondrial factors may be involved in declines in muscle function and potentially muscle mass losses with age. Additionally, while we wanted to control for differences in physical activity levels, the possibility of variations in recreational activity levels between the groups cannot be completely ruled out. Despite these limitations, this study highlights the importance of mitochondria in aging skeletal muscle and the complex interplay among various regulatory pathways such as mitochondrial dynamics and mitochondrial protein import in muscle. The data from this study suggest that mitochondrial dysregulation may contribute toward skeletal muscle decline with age and that functionality in the elderly is correlated with mitochondrial parameters irrespective of physical activity. Collectively, these data provide insight for the therapeutic potential of mitochondrially targeted interventions/strategies in the treatment of sarcopenia and the improvement of physical function in the elderly population.

Experimental procedures

Participants

A total of 35 participants including 12 young (eight male and four female) and 23 older subjects (14 men and nine women) were included in this study. These participants were part of a subset from a larger study protocol assessing sarcopenia in aging. Elderly participants were further divided into high functioning (HF) and low functioning (LF) based on their performance on the Short Physical Performance Battery (SPPB) test that includes a standing balance, a 4-m walk course, and a chair stand test (Guralnik *et al.*, 1994). Participants with a SPPB score of ≥ 11 were classified as HF, while participants with a score of ≤ 7 were classified as LF. Participants in all groups were sedentary and exercised < 20 min per week in the 2 months prior to the study. Participants were informed of the proce-

dures involved prior to enrollment in the study and gave their written informed consent, as approved by the University of Florida Institutional Review Board. Additional exclusion criteria were as follows; history of smoking in the prior 12 months, congestive heart failure NYHA Class III or IV, previous stroke with upper and/or lower extremity involvement within prior 6 months, peripheral vascular disease Fontaine Class III/IV, history of life-threatening cardiac arrhythmias, active treatment for cancer or history of cancer in prior 3 years, severe neurological disorders including Parkinson's disease, renal disease requiring dialysis, lung disease requiring steroids, lower extremity amputation, severe osteoarthritis that interferes with physical function, complicated diabetes, inflammatory diseases (e.g., active rheumatoid arthritis, vasculitis, autoimmune disorders, and inflammatory bowel disease), life-threatening illnesses with an estimated life expectancy of < 1 year.

Muscle biopsy

Skeletal muscle samples were obtained under local anesthesia from the vastus lateralis muscle using a percutaneous needle biopsy (Buford *et al.*, 2010). Muscles were immediately processed for respiration measurements and histochemistry. Remaining tissue was immediately frozen and stored at -80 °C.

Preparation of permeabilized muscle fibers

Permeabilized fibers were prepared as previously described (Kuznetsov *et al.*, 2008). Briefly, muscle samples were placed in ice-cold *Buffer X* containing 60 mM K-MES, 35 mM KCl, 7.23 mM K₂EGTA, 2.77 CaK₂EGTA, 20 mM imidazole, 0.5 mM DTT, 20 mM taurine, 5.7 mM ATP, 15 mM PCr, and 6.56 mM MgCl₂·6 H₂O (pH, 7.1, 295 mosmol/kgH₂O) and connective tissue removed. Muscle pieces were cut into small bundles (~ 8 –10 mg) and gently separated into single fibers. Myofibers were permeabilized in *Buffer X* with saponin (50 μ g mL⁻¹) and incubated on a rotator for 30 min at 4 °C. Permeabilized fibers were washed in ice-cold *Buffer Z* containing 110 mM K-MES, 35 mM KCl, 1 mM EGTA, 5 mM K₂HPO₄, and 3 mM MgCl₂·6 H₂O, 0.05 mM pyruvate, and 0.02 mM malate with 0.5 mg mL⁻¹ BSA (pH, 7.1, 295 mosmol/kgH₂O) on a rotator at 4 °C for 45 min. Fiber bundles were washed in *Buffer Z* containing 5 mM pyrophosphate to deplete fibers of endogenous nucleotides.

Mitochondrial respiration in permeabilized muscle fibers

Respiration was measured using an Oroboros O2K Oxygraph (Innsbruck, Austria) according to methods previously described (Kuznetsov *et al.*, 2008). Once the O₂ flux rate was stable, permeabilized muscle fibers were weighed and added to the chamber consisting of *Buffer Z* containing 20 mM creatine. The O₂ flux rate was measured following the addition of a substrate and inhibitor protocol, which consisted of 5 mM pyruvate and 2 mM malate, 2 mM ADP, 10 μ M cytochrome *c*, 10 mM succinate, 2 μ g mL⁻¹ oligomycin, and 0.5 μ M carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone. Respiratory control ratios calculated as state 3/state 4 indicated well-coupled mitochondria in all groups measured (mean, RCR = 5.0 ± 0.48). At the end of the experiment, fiber bundles were removed and dried overnight.

Cytochrome *c* oxidase activity

Enzyme activity was measured spectrophotometrically in whole muscle homogenates by measuring the maximal rate of oxidation of fully reduced cytochrome *c* (Adhietty *et al.*, 2009).

Immunohistochemistry for fiber typing and CSA

Serial sections from samples were prepared as previously described (McClung *et al.*, 2007). Briefly, sections were exposed to primary antibodies specific to dystrophin (Thermo Scientific, Rockford, IL, USA), MHC type I (Development Studies Hybridoma Bank, Iowa City, IA, USA), and MHC type IIa (Developmental Studies Hybridoma Bank). Sections were incubated with rhodamine red anti-rabbit secondary antibody, Alexa Fluor 350 goat anti-mouse secondary antibody, and Alexa Fluor 488 goat anti-mouse secondary antibody (Molecular Probes, Eugene, OR, USA). Sections were washed, mounted, and sealed with a cover slip and viewed under an inverted fluorescence microscope (Carl Zeiss Axiovert 200). Images were acquired at 10X and analyzed for myofiber CSA (μm^2) using SCION IMAGE software (Scion Technologies, Frederick, MD, USA).

Immunoblotting

Whole muscle homogenates were prepared as previously described (Adhihetty *et al.*, 2009). Proteins were separated on 8–15% polyacrylamide gels and transferred to nitrocellulose membranes. Blots were blocked for 1 h in StartingBlock T20 (Thermo Scientific) and probed with the appropriate primary antibody (1:200 for Tom40; 1:500 for pAMPK α , T-AMPK α , p-p38, T-p38, Tim23, PGC-1 α , NRF-1, SIRT3, RIP140, Mfn2, Opa1, Drp1; 1:1000 for Tfam, mtHsp70, cHsp70, Hsp60, Tom22, Fis1; 1:5000 for actin). Following, blots were incubated with anti-rabbit secondary antibodies at a dilution of 1:1000 (PGC-1 α , Mfn2, Fis1), 1:1500 (p-AMPK, T-AMPK, p-p38, T-p38, SIRT3) or 1:2500 (RIP140, Tfam, NRF-1), anti-mouse secondary at a dilution of 1:1000 (Opa1, Drp1, Tim23, Tom22, mtHsp70, cHsp70, Hsp60) or 1:10 000 (actin). Blots were washed and exposed to the DuoLux chemiluminescence kit, and proteins visualized using a ChemiDoc XRS imager from Bio-Rad (Hercules, CA, USA). A description of antibodies used in this study can be found in Table S1 (Supporting information). Immunoblotting of actin or Ponceau S staining was used to normalize for the amount of protein loaded.

Data were initially analyzed for normal distribution and homogeneity of variance. For continuous variables, descriptive statistics were evaluated using a one-way analysis of variance (ANOVA; Table 1). The Tukey's post hoc test was applied to identify individual group differences. The chi-square test was used to evaluate categorical descriptive variables. Primary outcomes were evaluated using an ANOVA with Tukey's follow-up. Where differences were observed between HF and LF groups (muscle size, state 4 respiration, cHsp70 protein), a follow-up one-way ANCOVA was performed to adjust for potential confounders, for example, age and gender. Measures of functional status obtained from elderly subjects were analyzed using an independent sample *t*-test. Pearson's correlation coefficient (*r*) was performed to assess associations between PGC-1 α and mitochondrial content, as well as gait speed. Differences were considered statistically significant if $P < 0.05$. Data are presented as mean \pm standard error (SE).

Acknowledgments

This study was supported by the University of Florida (UF), Claude D. Pepper Older Americans Independence Center (OAIC). The OAIC is funded by a grant from the NIH/NIA (1P30AG028740). We are grateful to the OAIC Recruitment Core at UF, as well as the technical assistance of L.A. Gilbert and L. J. Myers. We would like to thank all subjects for their participation in this study.

Author's contribution

A.-M.J. and C.L. were responsible for all aspects of the study. P.J.A. performed experiments and assisted with the manuscript write-up. J.M.A. and B.D.S. performed muscle biopsies. T.W.B., L.M.N., H.A.L., S.E.W., T.M.M., E.M., and M.P. contributed to the design of the original study, sample preparation, and the manuscript.

References

- Adhihetty PJ, Uguccioni G, Leick L, Hidalgo J, Pilegaard H, Hood DA (2009) The role of PGC-1 α on mitochondrial function and apoptotic susceptibility in muscle. *Am. J. Physiol. Cell Physiol.* **297**, C217–C225.
- Andersen JL (2003) Muscle fibre type adaptation in the elderly human muscle. *Scand. J. Med. Sci. Sports* **13**, 40–47.
- Aquilano K, Vigilanza P, Baldelli S, Pagliei B, Rotilio G, Ciriolo MR (2010) Peroxisome proliferator-activated receptor gamma co-activator 1 α (PGC-1 α) and sirtuin 1 (SIRT1) reside in mitochondria: possible direct function in mitochondrial biogenesis. *J. Biol. Chem.* **285**, 21590–21599.
- Arnout D, Grodet A, Lee YJ, Estaquier J, Blackstone C (2005) Release of OPA1 during apoptosis participates in the rapid and complete release of cytochrome c and subsequent mitochondrial fragmentation. *J. Biol. Chem.* **280**, 35742–35750.
- Baker DJ, Betik AC, Krause DJ, Hepple RT (2006) No decline in skeletal muscle oxidative capacity with aging in long-term calorically restricted rats: effects are independent of mitochondrial DNA integrity. *J. Gerontol. A Biol. Sci. Med. Sci.* **61**, 675–684.
- Barrientos A, Casademont J, Rotig A, Miro O, Urbano-Marquez A, Rustin P, Cardellach F (1996) Absence of relationship between the level of electron transport chain activities and aging in human skeletal muscle. *Biochem. Biophys. Res. Commun.* **229**, 536–539.
- Brooks C, Wei Q, Feng L, Dong G, Tao Y, Mei L, Xie ZJ, Dong Z (2007) Bak regulates mitochondrial morphology and pathology during apoptosis by interacting with mitofusins. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 11649–11654.
- Buford TW, Cooke MB, Manini TM, Leeuwenburgh C, Willoughby DS (2010) Effects of age and sedentary lifestyle on skeletal muscle NF- κ B signaling in men. *J. Gerontol. A Biol. Sci. Med. Sci.* **65**, 532–537.
- Buford TW, Lott DJ, Marzetti E, Wohlgemuth SE, Vandenberg K, Pahor M, Leeuwenburgh C, Manini TM (2012) Age-related differences in lower extremity tissue compartments and associations with physical function in older adults. *Exp. Gerontol.* **47**, 38–44.
- Cesari M, Pahor M, Bartali B, Cherubini A, Penninx BW, Williams GR, Atkinson H, Martin A, Guralnik JM, Ferrucci L (2004) Antioxidants and physical performance in elderly persons: the Invecchiare in Chianti (InCHIANTI) study. *Am. J. Clin. Nutr.* **79**, 289–294.
- Chen H, Chomyn A, Chan DC (2005) Disruption of fusion results in mitochondrial heterogeneity and dysfunction. *J. Biol. Chem.* **280**, 26185–26192.
- Chen H, Vermulst M, Wang YE, Chomyn A, Prolla TA, McCaffery JM, Chan DC (2010) Mitochondrial fusion is required for mtDNA stability in skeletal muscle and tolerance of mtDNA mutations. *Cell* **141**, 280–289.
- Garnier A, Fortin D, Zoll J, N'Guessan B, Mettauer B, Lampert E, Veksler V, Ventura-Clapier R (2005) Coordinated changes in mitochondrial function and biogenesis in healthy and diseased human skeletal muscle. *FASEB J.* **19**, 43–52.
- Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, Scherr PA, Wallace RB (1994) A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *J. Gerontol.* **49**, M85–M94.
- Handschin C, Spiegelman BM (2006) Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. *Endocr. Rev.* **27**, 728–735.
- Hiona A, Leeuwenburgh C (2008) The role of mitochondrial DNA mutations in aging and sarcopenia: implications for the mitochondrial vicious cycle theory of aging. *Exp. Gerontol.* **43**, 24–33.
- Hiona A, Sanz A, Kujoth GC, Pamplona R, Seo AY, Hofer T, Someya S, Miyakawa T, Nakayama C, Samhan-Arias AK, Servais S, Barger JL, Portero-Otin M, Tanokura M, Prolla TA, Leeuwenburgh C (2010) Mitochondrial DNA mutations induce mitochondrial dysfunction, apoptosis and sarcopenia in skeletal muscle of mitochondrial DNA mutator mice. *PLoS ONE* **5**, e11468.

- Humphries AD, Streimann IC, Stojanovski D, Johnston AJ, Yano M, Hoogenraad NJ, Ryan MT (2005) Dissection of the mitochondrial import and assembly pathway for human Tom40. *J. Biol. Chem.* **280**, 11535–11543.
- Hurley BF (1995) Age, gender, and muscular strength. *J. Gerontol. A Biol. Sci. Med. Sci.* **50**, 41–44.
- Joseph AM, Rungi AA, Robinson BH, Hood DA (2004) Compensatory responses of protein import and transcription factor expression in mitochondrial DNA defects. *Am. J. Physiol. Cell Physiol.* **286**, C867–C875.
- Joseph AM, Ljubovic V, Adihetty PJ, Hood DA (2010) Biogenesis of the mitochondrial Tom40 channel in skeletal muscle from aged animals and its adaptability to chronic contractile activity. *Am. J. Physiol. Cell Physiol.* **298**, C1308–C1314.
- Kong X, Wang R, Xue Y, Liu X, Zhang H, Chen Y, Fang F, Chang Y (2010) Sirtuin 3, a new target of PGC-1 α , plays an important role in the suppression of ROS and mitochondrial biogenesis. *PLoS ONE* **5**, e11707.
- Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, Hofer T, Seo AY, Sullivan R, Jobling WA, Morrow JD, Van RH, Sedivy JM, Yamasoba T, Tanokura M, Weindruch R, Leeuwenburgh C, Prolla TA (2005) Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science* **309**, 481–484.
- Kuznetsov AV, Veksler V, Gellerich FN, Saks V, Margreiter R, Kunz WS (2008) Analysis of mitochondrial function in situ in permeabilized muscle fibers, tissues and cells. *Nat. Protoc.* **3**, 965–976.
- Lanza IR, Nair KS (2010) Mitochondrial function as a determinant of life span. *Pflügers Arch.* **459**, 277–289.
- Lanza IR, Short DK, Short KR, Raghavakaimal S, Basu R, Joyner MJ, McConnell JP, Nair KS (2008) Endurance exercise as a countermeasure for aging. *Diabetes* **57**, 2933–2942.
- Lexell J, Taylor CC (1991) Variability in muscle fibre areas in whole human quadriceps muscle: effects of increasing age. *J. Anat.* **174**, 239–249.
- Marzetti E, Calvani R, Bernabei R, Leeuwenburgh C (2011) Apoptosis in skeletal myocytes: a potential target for interventions against sarcopenia and physical frailty – a mini-review. *Gerontology* **58**, 99–106.
- Marzetti E, Lees HA, Manini TM, Buford TW, Aranda Jr JM, Calvani R, Capani G, Marsiske M, Lott DJ, Vandenborne K, Bernabei R, Pahor M, Leeuwenburgh C, Wohlgemuth SE (2012) Skeletal muscle apoptotic signaling predicts thigh muscle volume and gait speed in community-dwelling older persons: an exploratory study. *PLoS ONE* **7**, e32829.
- McClung JM, Kavazis AN, Whidden MA, DeRuisseau KC, Falk DJ, Criswell DS, Powers SK (2007) Antioxidant administration attenuates mechanical ventilation-induced rat diaphragm muscle atrophy independent of protein kinase B (PKB Akt) signalling. *J. Physiol.* **585**, 203–215.
- Navratil M, Terman A, Arriaga EA (2008) Giant mitochondria do not fuse and exchange their contents with normal mitochondria. *Exp. Cell Res.* **314**, 164–172.
- Palacios OM, Carmona JJ, Michan S, Chen KY, Manabe Y, Ward III JL, Goodyear LJ, Tong Q (2009) Diet and exercise signals regulate SIRT3 and activate AMPK and PGC-1 α in skeletal muscle. *Aging (Albany, NY)* **1**, 771–783.
- Parise G, Brose AN, Tarnopolsky MA (2005) Resistance exercise training decreases oxidative damage to DNA and increases cytochrome oxidase activity in older adults. *Exp. Gerontol.* **40**, 173–180.
- Powelka AM, Seth A, Virbasius JV, Kiskinis E, Nicoloso SM, Guilherme A, Tang X, Straubhaar J, Cherniack AD, Parker MG, Czech MP (2006) Suppression of oxidative metabolism and mitochondrial biogenesis by the transcriptional corepressor RIP140 in mouse adipocytes. *J. Clin. Invest.* **116**, 125–136.
- Rasmussen UF, Krstrup P, Kjaer M, Rasmussen HN (2003) Human skeletal muscle mitochondrial metabolism in youth and senescence: no signs of functional changes in ATP formation and mitochondrial oxidative capacity. *Pflügers Arch.* **446**, 270–278.
- Rooyackers OE, Adey DB, Ades PA, Nair KS (1996) Effect of age on in vivo rates of mitochondrial protein synthesis in human skeletal muscle. *Proc. Natl. Acad. Sci. U. S. A.* **93**, 15364–15369.
- Rose G, Dato S, Altomare K, Bellizzi D, Garasto S, Greco V, Passarino G, Feraco E, Mari V, Barbi C, BonaFe M, Franceschi C, Tan Q, Boiko S, Yashin AI, De BG (2003) Variability of the SIRT3 gene, human silent information regulator Sir2 homologue, and survivorship in the elderly. *Exp. Gerontol.* **38**, 1065–1070.
- Sacheck JM, Hyatt JP, Raffaello A, Jagoe RT, Roy RR, Edgerton VR, Lecker SH, Goldberg AL (2007) Rapid disuse and denervation atrophy involve transcriptional changes similar to those of muscle wasting during systemic diseases. *FASEB J.* **21**, 140–155.
- Safdar A, Hamadeh MJ, Kaczor JJ, Raha S, deBeer J, Tarnopolsky MA (2010) Aberrant mitochondrial homeostasis in the skeletal muscle of sedentary older adults. *PLoS ONE* **5**, e10778.
- Sandri M, Lin J, Handschin C, Yang W, Arany ZP, Lecker SH, Goldberg AL, Spiegelman BM (2006) PGC-1 α protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 16260–16265.
- Seo AY, Joseph AM, Dutta D, Hwang JC, Aris JP, Leeuwenburgh C (2010) New insights into the role of mitochondria in aging: mitochondrial dynamics and more. *J. Cell Sci.* **123**, 2533–2542.
- Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, Nair KS (2005) Decline in skeletal muscle mitochondrial function with aging in humans. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 5618–5623.
- Someya S, Yu W, Hallows WC, Xu J, Vann JM, Leeuwenburgh C, Tanokura M, Denu JM, Prolla TA (2010) Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. *Cell* **143**, 802–812.
- Takahashi M, Chesley A, Freyssenet D, Hood DA (1998) Contractile activity-induced adaptations in the mitochondrial protein import system. *Am. J. Physiol.* **274**, C1380–C1387.
- Wenz T, Rossi SG, Rotundo RL, Spiegelman BM, Moraes CT (2009) Increased muscle PGC-1 α expression protects from sarcopenia and metabolic disease during aging. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 20405–20410.

Supporting information

Additional supporting information may be found in the online version of this article:

Table S1 Description of commercially available antibodies used in the study.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.