Effects of age and unaccustomed resistance exercise on mitochondrial transcript and protein abundance in skeletal muscle of men

Daniel I. Ogborn,1 Bryon R. McKay,2 Justin D. Crane,2 Adeel Safdar,5 Mahmood Akhtar,3 Gianni Parise,2 and Mark A. Tarnopolsky3,4

1Departments of Medical Sciences, 2Kinesiology, 3Pediatrics and 4Medicine, McMaster University, Hamilton, ON, Canada; and 5Cardiovascular Institute, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts

Submitted 6 January 2014; accepted in final form 13 February 2015

Ogborn DI, McKay BR, Crane JD, Safdar A, Akhtar M, Parise G, Tarnopolsky MA. Effects of age and unaccustomed resistance exercise on mitochondrial transcript and protein abundance in skeletal muscle of men. Am J Physiol Regul Integr Comp Physiol 308: R734–R741, 2015. First published February 18, 2015; doi:10.1152/ajpregu.00005.2014.—Mitochondrial dysfunction may contribute to age-associated muscle atrophy. Previous data has shown that resistance exercise (RE) increases mitochondrial gene expression and enzyme activity in older adults; however, the acute response to RE has not been well characterized. To characterize the acute mitochondrial response to unaccustomed RE, healthy young (21 ± 3 yr) and older (70 ± 4 yr) men performed a unilateral RE bout for the knee extensors. Muscle biopsies were taken at rest and 3, 24, and 48 h following leg press and knee extension exercise. The expression of the mitochondrial transcriptional regulator proliferator-activated receptor γ coactivator 1-α (PGC-1α) mRNA was increased at 3 h postexercise; however, all other mitochondrial variables decreased over the postexercise period, irrespective of age. ND1, ND4, and citrate synthase (CS) mRNA were all lower at 48 h postexercise, along with specific protein subunits of complexes II, III, IV, and ATP synthase. Mitochondrial DNA (mtDNA) copy number decreased by 48 h postexercise, and mtDNA deletions were higher in the older adults and remained unaffected by acute exercise. Elevated mtDNA copy number could not explain the reduction in mitochondrial proteins and DNA, because there was no increase in ubiquitinated voltage-dependent anion channel (VDAC) or its association with PTEN-induced putative kinase 1 (PINK1) or Parkin, and elevated p62 content indicated an impairment or reduction in autophagic flux. In conclusion, age did not influence the response of specific mitochondrial transcripts, proteins, and DNA to a bout of RE.

AGING IS CHARACTERIZED by a progressive muscular atrophy (sarcopenia) that results in significant functional impairments and reductions in quality of life for the elderly (30). Sarcopenia has a complex etiology thought to involve multiple processes including the loss of motor units and denervation of muscle (12), impaired protein synthesis and turnover (3), altered systemic inflammation (36), oxidative stress (17), and mitochondrial dysfunction (47). Of those, mitochondrial dysfunction has been suggested as a primary contributor to the aged and sarcopenic phenotype of skeletal muscle (44) and may play a role in the additional causative factors of aging including telomere shortening and DNA damage (41). Free radical production over time results in the accumulation of damage to the mitochondria and its associated components (DNA) that ultimately exacerbates electron transport chain function, resulting in additional free radical production and oxidative stress, creating a vicious cycle of oxidative damage (20). This theory is supported by the accumulation of oxidative damage in aged muscle (14, 17) and the gradual attenuation of mitochondrial function (3), protein synthesis (38), and transcript abundance (29) with increasing age. The occurrence of ragged red fibers, cytochrome-c oxidase deficiency, and those with elevated succinate dehydrogenase activity increase with age (8), in association with mitochondrial DNA (mtDNA) deletions in aged muscle (7, 9). Relevant to the concept of sarcopenia, these regions of mitochondrial abnormalities are associated with atrophic regions of the myofiber (8, 47), although no definitive, mechanistic link has been made between mitochondrial dysfunction and muscle atrophy.

It is generally thought that resistance exercise (RE) does not benefit mitochondrial function in young skeletal muscle (2, 45); however, mitochondrial protein synthesis and enzyme activities increase acutely following RE (42, 49), and peroxisome proliferator-activated receptor γ coactivator 1-α (PGC-1α) expression, a master regulator of mitochondrial biogenesis (52), is elevated following both acute (10) and chronic strength training (39). It has been proposed that mitochondrial adaptations to RE may be heightened in aged muscle (44). At the functional level, RE can increase maximal oxygen uptake (VO2max) (46) in the elderly and result in improved endurance capacity (1), adaptations typically associated with endurance exercise. Melov et al. (29) demonstrated that the aged transcriptome has a high number of differentially expressed mitochondrial and metabolic-related genes; however, after 6 mo of RE the aged transcriptome more closely resembled that of young skeletal muscle. Perhaps more reflective of actual mitochondrial function/capacity, mitochondrial creatine kinase protein content, and complex IV enzyme activity are increased with RE (34) in addition to reduced oxidative stress and increased antioxidant enzyme activity (35). Collectively these data indicate that mitochondria are sensitive to RE in older adults; however, the extent to which RE can promote favorable mitochondrial signaling in aged and young skeletal muscle is yet to be clearly defined.

Strength training is a safe and effective therapeutic intervention to maintain or increase muscle mass and strength in the elderly (15); however, the role of mitochondrial adaptations in the preservation of function is not clear. Direct comparisons of the acute mitochondrial response to RE in young and aged skeletal muscle are lacking and require further clarification. We hypothesized that older adults would have reduced basal mitochondrial content and therefore have an elevated mitochondrial response to an equivalent training stimulus compared with the young.
MATERIALS AND METHODS

Subjects. Eighteen community-dwelling younger (Y; n = 9, 21 ± 3 yr) and older men (O; n = 9, 70 ± 4 yr) were recruited to complete a single RE bout. Participants underwent a routine screening before the study and were required to complete a health questionnaire and to have not been involved in a lower-body RE program for at least 6 mo before the study. Exclusion criteria were as previously described (28): evidence of heart disease, respiratory disease, uncontrolled hypertension, renal disease, diabetes, orthopedic disabilities involving the lower limb, the use of NSAIDs or statin-related drugs and smoking. Participants were instructed to refrain from physical activity during the study period and to abstain from the consumption of alcohol and nonsteroidal anti-inflammatories. All participants gave written, informed consent before participation. This study was approved by the Hamilton Health Sciences Human Research Ethics Board and conformed to all declarations on the use of human subjects as research participants.

Acute exercise protocol. One week before the acute exercise session, participants completed a baseline blood collection and a body composition assessment with dual-energy X-ray absorptiometry (DEXA, GE, Canada) and were familiarized with the strength training exercises and equipment. Participants arrived at the clinic at 0600 h and performed a unilateral RE program for the knee extensors, as previously described (27, 28). Initially each subject completed an incremental, unilateral one repetition maximum (IRM) protocol for the leg press and knee extension exercises. After determination of the IRM, subjects completed four sets of 10 repetitions of each exercise at 75%-1RM with 2 min rest between each set. The order of exercise was alternated such that half of the participants commenced the exercise bout with the leg press, the other half with the leg extension exercise.

Muscle biopsies. Three hours after the exercise protocol, a muscle biopsy was obtained from the unexercised and exercised leg, followed by subsequent biopsies of the exercised leg only at 24 and 48 h after the exercise bout. A percutaneous needle with manual suction was used to biopsy the vastus lateralis, and each biopsy was spaced with ~3 cm between adjacent biopsy sites (43), with the use of each site distributed across participants such that each site was equally sampled across time points (distal, middle, proximal). Each biopsy was sectioned into smaller portions and then immediately frozen in liquid nitrogen for storage at −80°C for future use.

RNA analysis. RNA was extracted from 30 mg of quadriceps tissue in 1 ml of TRIzol Reagent (Invitrogen, Burlington, ON, Canada). After electric homogenization 0.2 ml of chloroform was added per sample and the resultant clear aqueous phase was transferred to RNeasy spin columns (Qiagen, Germantown, MD). After these steps were completed, RNA was isolated as per the manufacturer’s recommendations. To prevent contamination with genomic DNA, all RNA samples were treated with RNaseA (Qiagen) while on the isolation columns. Concentration of the RNA was spectrophotometrically determined by measuring the absorbance of the solution at 260 nm (ND-1000, Nanodrop, Willmington, DE).

Reverse transcription was performed on 100 ng total RNA with random hexamers as per the manufacturer’s instructions (Applied Biosystems, Foster City, CA). Real-time PCR was performed with gene-specific primers on the 7300 real-time PCR System (Applied Biosystems) using SYBR Green chemistry (PerfeCTa SYBR Green Supermix with ROX, Quanta Biosciences, Gaithersburg, MD). The primers used were as follows: β2-microglobulin forward: 5′-ctggagaatggacacat; PGC-1β forward: 5′-ctgtttagctgctctcaca; reverse: 5′-ggtgaagctggtgcttgac; PGC-1-related coactivator (PRC) forward: 5′-cttcaacagtgtgaggttcggtgcag; reverse: 5′-ggttcatcctggaatgtgggtggattcc; mitochondrial transcription factor a (Tfm) forward: 5′-ttgacacgccatttc; reverse: 5′-ttggaactctggcagatg; nuclear respiratory factor 1 (Nrf1) forward: 5′-gagtgtcgtgcaacagaaac; reverse: 5′-ttatatgaggtttttacatgtgctg; Nrf2 forward: 5′-ttgctgcacagccagactt; reverse: 5′-ccgaaatttgaggttggtgxx; Nix forward: 5′-ttggaactcataactcctcttg; reverse: 5′-ttgaaactgctcagtcctacat; Beclin 1 forward: 5′-agccttaacctgatcctacat; reverse: 5′-tgctctcttctggcttgg; p62 forward: 5′-ggagaagtagattgggttgataac; reverse: 5′-tttagagctgaggtctcgtg; light Chain 3 (LC3) forward: 5′-ccagcttgctggctttcggat; reverse: 5′-gcctgattaattgcagttga; tubulin alpha-related protein 7 (ATG7) forward: 5′-gttgtgagaggtgctctgct; reverse: 5′-acctccactcttctgtaac; and BCL2 adenosine E1B 19 kDa protein-interacting protein 3 (BNIP3) forward: 5′-ggtagctgctgctgagggattggagtt; reverse: 5′-ttcgaagacctgtttggtacat. All data are expressed relative to β2-microglobulin, which did not alter expression in response to the exercise bout (data not shown) (27).

Protein analysis. Approximately 30 mg of each muscle biopsy was homogenized with an electric tissue micer (Pro Scientific, Oxford, CT) and processed into nuclear and cytosolic fractions as per the manufacturer’s recommendations (Pierce, Rockford, IL). Protein concentration was determined using the bichinconic acid method as per the manufacturer’s recommendations (Pierce) with a spectrophotometer (Benchmark Plus, Bio-Rad, Hercules, CA). Proteins were probed using various conditions depending on the protein using the following antibodies: OXPHOS Cocktail (Abcam ab11041, Toronto, ON, Canada), ATG7 (Cell Signaling Technology no. 8558, Boston, MA), LC3b (Sigma L7543, St. Louis, MO), p62 (Cell Signaling no. 5114), voltage-dependent anion channel (VDAC; Abcam ab15895), Ubiquitin (Santa Cruz sc-8017, Dallas, TX), PTEN-induced putative kinase 1 (Pink1) (Cell Signaling no. 6946), and Parkin (Cell Signaling no. 4211). All proteins were probed under similar conditions with primary antibodies (overnight incubation at 4°C) followed by a 1-h incubation at room temperature with anti-rabbit or anti-mouse secondary antibodies (1:10,000, GE Healthcare). All blots were developed with ECL plus (GE Healthcare) and exposed to X-ray film (GE Healthcare). All films were digitized and band density was determined with ImageJ (NIH, Bethesda, MD). Protein data are expressed relative to lactate dehydrogenase A (LDHA; Cell Signaling no. 2012), voltage-dependent anion channel (VDAC; Abcam ab158792) protein content, or Ponceau S staining (mitochondrial blot) all of which were stable with both age and exercise.

Immunoprecipitation. For immunoprecipitation, subject samples were combined to create three pools of 125 µg for both young and old subjects at each time point (24 total samples) ensuring that equal proteins amounts were used from each subject. Cytosolic protein lysates were initially solubilized with 1% n-dodecyl maltoside in equivalent volumes for 30 min on ice and then spun in a centrifuge at 16,000 g at 4°C. Concurrently, antibody to bead coupling was performed by adding 20 µl of protein A/G plus agarose beads to spin columns (Pierce), washing twice with PBS, then additionally washing twice with 200 µl of wash buffer (PBS/0.05% n-dodecyl maltoside with protease inhibitors (Roche)). The columns were then spun to remove residual wash solution and incubated with 2 µg of anti-VDAC antibody (Abcam, ab14734) in PBS for 2 h at 4°C to conjugate the antibody to the beads. After two additional washes in wash buffer, the solubilized cytosolic lysate was added to the columns and allowed to rotate end-over-end overnight at 4°C with the beads. The following morning, the columns were spun to collect the proteins not bound to the beads (IP supernatant). After the supernatant was collected, the beads were washed twice with 200 µl of wash buffer and incubated with 50 µl of 1% SDS and spun to elute any immunoprecipitated proteins. All proteins were then spun at 3,000 rpm for 1 min at 4°C. The immunoprecipitates were run on gels using SDS-PAGE and transferred to nitrocellulose membranes. Membranes were probed using anti-Ubiquitin (Santa Cruz, sc-8017), anti-Parkin (Cell Signaling no. 4211), and anti-Pink1 (Cell Signaling no. 6946) to detect the amount
of Parkin and Pink1 associated with VDAC and the amount of ubiquitinated VDAC according to the SDS-PAGE methods. No signal was present when using an IgG control antibody for immunoprecipitation.

Mitochondrial DNA. Total genomic DNA was isolated from ~20 mg of skeletal muscle as per the manufacturer’s specifications (DNeasy, Qiagen, Toronto, ON, Canada). The concentration and purity of the DNA were assessed using a spectrophotometer (Nanodrop-1000, Thermo Scientific, Wilmington, DE). MiDNA copy number was assessed with primers designed for ND1 (forward: 5'-aagtcaccctagccatcattctac, reverse: 5'-aagtcaccctagccatcattctac) in the mitochondrial genome and considered relative to the nuclear gene =ber was assessed with primers designed for ND1 (forward: 5'-gacagatc-3'-globin (forward: 5'-gggcagagccatctattgctt, reverse: 5'-gggcagagccatctattgctt) in the mitochondrial genome and considered relative to the nuclear gene (beta-globin) for normalization.

Table 1. Subject characteristics and resting age differences

<table>
<thead>
<tr>
<th></th>
<th>Young Adults</th>
<th>Older Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>21 ± 3</td>
<td>70 ± 4*</td>
</tr>
<tr>
<td>Height, cm</td>
<td>179.6 ± 8.2</td>
<td>176.0 ± 4.9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>91.7 ± 21.9</td>
<td>87.6 ± 6.4</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>65.9 ± 10.8</td>
<td>62.9 ± 6.4</td>
</tr>
<tr>
<td>Leg press 1RM, lbs</td>
<td>228.0 ± 93.8</td>
<td>160.8 ± 50.1*</td>
</tr>
<tr>
<td>Leg press extension 1RM, lbs</td>
<td>117.6 ± 47.4</td>
<td>81.9 ± 22.8*</td>
</tr>
<tr>
<td>Isometric knee extension, N-m</td>
<td>324.6 ± 85.4</td>
<td>228.3 ± 45.1*</td>
</tr>
<tr>
<td>NRF1 mRNA, au</td>
<td>0.015 ± 0.01</td>
<td>0.023 ± 0.01*</td>
</tr>
<tr>
<td>ND1 mRNA, au</td>
<td>59.4 ± 32.2</td>
<td>50.0 ± 18.0</td>
</tr>
<tr>
<td>ND4 mRNA, au</td>
<td>101.6 ± 27.6</td>
<td>86.1 ± 20.0*</td>
</tr>
<tr>
<td>mtDNA Deletions, au</td>
<td>0.01 0.02</td>
<td>0.93 ± 0.10*</td>
</tr>
<tr>
<td>mtDNA copy number, au</td>
<td>2,481 ± 1,392</td>
<td>2,953 ± 1,659</td>
</tr>
<tr>
<td>Complex II subunit protein, au</td>
<td>0.22 ± 0.03</td>
<td>0.2 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SD. 1RM, one-repetition maximum; NRF1, nuclear respiratory factor 1; mtDNA, mitochondrial DNA. *P < 0.05.

RESULTS

Subject characteristics. Subject characteristics have been previously published for the groups used in the present study (28). While there was no difference in lean body mass between the groups (O: 62.9 ± 6.4 kg, Y: 65.9 ± 10.8 kg), the older men (age 70 ± 4 yr) were significantly weaker, scoring lower on the leg press (O: 160.8 ± 50.1 lbs, Y: 228 ± 93.8 lbs; P < 0.05) and knee extension one-repetition maximum (1RM; O: 81.9 ± 22.8 lbs, Y: 117.6 ± 47.4 lbs.; P < 0.05) than the younger participants (age 21 ± 3 yr). Group characteristics and resting differences between young and old participants are presented in Table 1.

Mitochondrial response to RE. Exercise (f = 38.68, P < 0.001; Fig. 1), but not age (f = 0.05, P = 0.82), altered total PGC-1α expression. PGC-1α mRNA was increased 3 h postexercise (407% of baseline, P < 0.001) but returned to basal levels by 24 h. Two alternative isoforms of PGC-1α were differentially affected by RE (Fig. 1). There was no effect of age on either transcript; however, exercise decreased PGC-1α1 (f = 5.82, P = 0.002) and increased PGC-1α4 (f = 25.76, P < 0.001; Fig. 1). PGC-1α1 was progressively decreased to 20% of resting expression at 48 h postexercise, whereas PGC-1α4 was increased at 3 h (P < 0.01).

Exercise but not age increased Tfam mRNA (f = 7.98, P = 0.003 Fig. 2A). Tfam mRNA increased to 124% of resting levels at 24 h postexercise (P < 0.05). There was an effect of age and a trend for exercise on NRF1 (age: f = 5.97, P = 0.0296; exercise: f = 2.26, P = 0.096) and only exercise for NRF2 mRNA (f = 3.96, P = 0.0137) mRNA (Fig. 2A). NRF1 mRNA was 54% higher in the older men at baseline (P < 0.05, Table 1), and NRF2 mRNA was reduced at 48 h postexercise (70% P < 0.01, respectively).

Based on the differential regulation of the mRNA for mitochondrial transcription factors, we tested to see if there was also discrepancy in abundance of either mitochondrial or nuclear encoded mitochondrial-related transcripts (Fig. 2B). Exercise (CS: f = 6.12, P = 0.0014) but not age influenced CS mRNA (nuclear encoded), with a trend for decreased CS mRNA at 48 h (68% of rest, P = 0.08). ND1 and ND4 mRNA were lower with age (ND1: f = 5.07, P = 0.04; ND4: f = 5.92, P = 0.02) as the transcripts were 19% and 18% higher in the young at baseline (P < 0.05, Table 1). Exercise decreased ND1 and ND4 mRNA (ND1: f = 3, P = 0.04; ND4: f = 13.8, P < 0.001), such that both were lower relative to the resting values by 48 h postexercise (P < 0.05 and P < 0.001, respectively).

Consistent with the reduced mRNA abundance, the 30-kDa subunit of complex II (f = 16.3, P < 0.001), complex III subunit core 2 (f = 21.5, P < 0.001), and the α subunit of ATP synthase (f = 31.7, P < 0.001) were reduced 48 h postexercise (80%, P < 0.001; 78%, P < 0.001; and 80% of rest, P < 0.001, respectively; Fig. 3), with no effect of age or exercise on complex I content (data not shown) and a trend for exercise on complex IV subunit II (f = 4.67, P < 0.01, 76% of rest, P = 0.05).

Fig. 1. Expression data indicates that resistance exercise (RE) had a stimulatory effect on total proliferator-activated receptor γ coactivator 1-α (PGC-1α) mRNA 3 h after RE. Expression of the PGC-1α1 isoform was decreased by 48 h, whereas PGC-1α4 mRNA was increased at 3 h postexercise. Age groups have been collapsed to demonstrate the exercise effect over time. †P < 0.01 and ‡P < 0.001.
Mitochondrial DNA deletions were higher in the older adults at baseline (15% higher, P = 0.01; Fig. 4A, Table 1); however, this ratio was unaffected by exercise (Fig. 4B). Consistent with the mRNA and protein results, mtDNA copy number decreased over the postexercise period (f = 3.25, P = 0.032; Fig. 4C) to 68% of resting levels by 48 h (P = 0.03), irrespective of age.

Autophagocytic response to RE. We found no effect of age or exercise on the expression of ATG7, Beclin1, or BNIP3 while LC3b mRNA was increased (f = 3.95, P = 0.014 Fig. 5A) at 3 h postexercise (134% of rest, P = 0.012), with a trend toward increased expression at 24 h (127% of rest, P = 0.057). In addition, p62 mRNA was increased (f = 12.21, P < 0.001) at 3 h postexercise (133% of rest, P = 0.003). Beclin1, BNIP3, and VPS34 mRNA did not differ with either age or exercise (data not shown). ATG7 protein content did not change with age or exercise (Fig. 5B). Total LC3b protein (f = 8.53, P < 0.001) trended to increase 24 h postexercise (123% of rest, P = 0.076) and was significantly increased at 48 h (144% of rest, P < 0.001) independent of age (Fig. 5B). P62 protein did not differ by age but was affected by exercise (f = 7.856, P < 0.001), increasing to 136% (P < 0.05) and 178% (P < 0.01) by 24 and 48 h, respectively (Fig. 5B).

Mitochondria are targeted for autophagy through the ubiquitination of VDAC via Pink1 and Parkin and subsequent binding to LC3 via p62 or through the direct interaction of the outer mitochondrial membrane protein Nix with LC3 (23, 31, 48). Pink1 and Parkin protein content did not differ postexercise in cytosolic fractions (data not shown), and Nix1 mRNA was not different from rest at any point postexercise (f = 4.2, P < 0.01); however, the mRNA content at 24 and 48 h was greater than at 3 h (data not shown). No significant effect of age or exercise was detected for the ubiquitination of VDAC or its association with Pink1 and Parkin (Fig. 6).

DISCUSSION

Despite the positive mitochondrial and functional adaptations with long-term strength training (44) and the acute stimulation of mitochondrial protein synthesis (49) and enzyme activity (42), we found a distinct reduction in mitochondrial transcripts (ND1, ND4, Nrf2), proteins (Complex II 30kDa subunit, ATP synthase subunit alpha, Complex III subunit Core 2), and mtDNA 48 h after RE. While chronic RE is associated with the cumulative reversal of the age-related mitochondrial and metabolic transcriptional phenotype (29), our data suggests the acute mitochondrial-related transcriptional response to RE is one of reduced abundance, irrespective of age. In agreement with our data, Gordon et al. (19) found an overall reduction in mitochondrial-related genes acutely following RE; however, the suppression of oxidative genes was attenuated after 12 wk of RE training. Collectively, these data indicate that the beneficial effects of RE on the mitochondria may require cumulative bouts of RE, and that the early response to an acute bout of exercise may not be representative or predictive of the ultimate training outcome or chronic steady-state abundance of either mRNA or protein.

Despite the fact that the elderly participants had equivalent lean body mass to the younger participants, there was evidence

**Fig. 2.** A: expression of mitochondrial transcription varied over the postexercise period. Mitochondrial transcription factor a (Tfam) mRNA was elevated at 24 h postexercise, whereas Nuclear Respiratory Factor 2 (Nrf-2) was significantly reduced by 48 h and Nrf-1 did not change (P = 0.096). B: both mitochondrial and nuclear encoded mitochondrial-related transcripts were reduced after exercise. Expression of ND1 and ND4 (mitochondrial encoded) were reduced 48 h postexercise, whereas citrate synthase (CS) approached significance (P = 0.08). Age groups have been collapsed to demonstrate the exercise effect over time.

*P < 0.05, †P < 0.01, and ‡P < 0.001.

**Fig. 3.** RE reduced the abundance of specific mitochondrial protein subunits (Complex II 30kDa subunit, ATP synthase subunit alpha, Complex III subunit Core 2) 48 h after the exercise bout. Age groups have been collapsed to demonstrate the exercise effect over time. *P < 0.05, †P < 0.01, and ‡P < 0.001.
of reduced mitochondrial content and muscle dysfunction. We have previously characterized that the elderly individuals had reduced strength across both training exercises and maximal isometric knee extension torque (27, 28). In addition, the area of type II fibers was reduced relative to the young, despite preservation of type I fiber area. While many markers of mitochondrial content were similar across age groups, we did find reduced abundance of ND1 and ND4 mRNA and elevated NRF1 mRNA and mtDNA deletions. Reduced mitochondrial content and increased mtDNA deletions are often considered
hallmarks of the aging process in skeletal muscle (21), and compensatory increases in NRF1 have been previously characterized with aging (6). While we cannot causally associate the age-related mitochondrial alterations in these individuals with type II fiber atrophy with age, evidence does suggest mitochondrial dysfunction in aging may be more prevalent in type II fibers (47). Nevertheless, the alterations in mitochondrial content with age, whether associated with muscle atrophy or not, did not appear to influence the acute mitochondrial response to RE in the elderly. These results are limited to the population sampled, which given the comparable muscle mass or not, did not appear to influence the acute mitochondrial response to RE in the elderly. These results are limited to the population sampled, which given the comparable muscle mass to the young represent healthy, nonsarcopenic individuals. Further research is required to determine whether a similar response would occur in a sarcopenic elderly population.

An increase in PGC-1α mRNA is often used as an indicator or predictor of consequent mitochondrial and metabolic adaptations to exercise (52); however, it appears that this is not the case for RE. Our data are in agreement with others by showing that, similar to endurance exercise, RE can also increase total PGC-1α mRNA (10). We cannot exclude the possibility that while PGC-1α mRNA is elevated, any related PGC-1α activity/function may serve to modulate other cellular processes such as the unfolded protein response (UPR) (50) and not mitochondrial biogenesis. Given the stimulatory effects of RE on skeletal muscle protein synthesis (13), it is possible that increased postexercise protein abundance may stimulate the UPR (11), a process modulated by the interaction of PGC-1α with the UPR-transcription factor ATF6 (50). In addition, recent work has detected alternate PGC-1α isoforms that are differentially expressed dependent on the exercise modality (39), although the specificity of such a response (53) and the role of PGC-1α in compensatory hypertrophy has recently come into question (37). In a series of experiments Ruas et al. (39) demonstrated that the PGC-1α isoform is specifically increased following either chronic resistance or mixed mode resistance/endurance exercise and correlates with the percentage change in the performance of the leg press exercise. This specific PGC-1α isoform does not regulate the commonly reported mitochondrial targets but rather regulates insulin-like growth factor 1 (IGF-1) and ultimately represses myostatin to have a favorable affect on muscle mass. In agreement, we have established that the preferential expression of PGC-1α occurs with acute RE as well, and that PGC-1α is decreased following acute RE. In addition, we have previously shown a reduction in myostatin mRNA (28) in the same participants used in the present study. Ultimately, the fact that we found increased PGC-1α mRNA coincident with reduced mitochondrial transcripts, protein and DNA content further suggests that differing PGC-1α isoforms are induced dependent on the mode of exercise or that PGC-1α coactivates differing transcription factors following resistance or endurance exercise [ATF6 (39)] as opposed to NRF1 and NRF2 (51). Such a relationship may be graded in nature or temporally staggered, as we did find an elevation in Tfam mRNA 24 h postexercise, indicative of NRF1 coactivation by PGC-1α (51).

Along with the downregulation of mitochondrial transcripts (19), it is possible that the reduction in mitochondrial protein and DNA content was modulated by an alternate cellular process. Autophagocytic removal of mitochondria from the cell can occur through the bulk removal of organelles via macroautophagy but also through a targeted mechanism known as mitophagy (48). While our understanding of selective autophagy is emerging, targeting of the mitochondria to the lysosome can occur through two mechanisms (23). In the first, the mitochondrial outer membrane protein VDAC is ubiquitinated by E3-ubquitin ligase Parkin, which is targeted to dysfunctional mitochondria by the accumulation of Pink1 on the outer mitochondrial membrane (31). The polyubiquitin binding protein p62 then binds to ubiquitinated VDAC on the mitochondrial outer membrane and links the mitochondria to the lysosome through an interaction with LC3 (5). Alternatively, the mitochondrial outer membrane protein Nix is able to interact with LC3 directly to target mitochondria to the lysosomal membrane (23). Our immunoprecipitation data found no increase in the ubiquitination of VDAC postexercise or its association with Pink1 and Parkin, suggesting that this pathway is not stimulated by RE, or that this occurs at a different postexercise time point than those studied in the current study. In addition, we failed to detect any change in Nix mRNA indicating mitophagy is not responsible for the reduction in

---

Fig. 6. A: immunoprecipitation of voltage-dependent anion-selective channel (VDAC) found there was no change in ubiquitinated VDAC or its association with PTEN-induced putative kinase 1 (Pink1) or Parkin followed the RE bout. Age groups have been collapsed to demonstrate the exercise effect over time. B: representative Western blotting of a pooled sample for each postexercise time point.
mitochondrial protein and DNA abundance postexercise, although we cannot rule out a posttranscriptional role of Nix in the regulation of mitochondria following RE. Future studies will require the use of electron microscopy (the gold standard) to conclusively rule out a role for macroautophagy as an explanation for the acute reduction in mitochondrial protein abundance and mtDNA content following RE.

Few studies have investigated the effects on autophagy or mitophagy specifically, and previous data are equivocal on the induction of autophagy with either acute (16, 18) and chronic RE (26) or exercise in general (24, 33). At the induction of autophagy, LC3b-I is conjugated by a complex of ATG proteins to the lipophilic phosphatidylethanolamine to generate LC3-II that can readily integrate into autophagosome membranes and therefore the ratio of LC3b-II to I is a marker of the stimulation of autophagy (4). Both Fry and Glynn et al. (16, 18) have previously demonstrated either no change or a decrease in the LC3b-II-to-LC3b-I ratio following RE, suggesting either an inability of RE to activate or that RE inhibits autophagy. Conversely Luo et al. (26) demonstrated increased ATG7, ATG5, and Beclin 1 protein with reduced p62 and a lower LC3b-II-to-I ratio after 9 wk of RE. These discrepant findings may be explained as differences between the acute (16, 18) and chronic response to RE (26) or the use of young adults or aged rodents.

Similar results have been obtained using acute treadmill exercise in murine and rodent skeletal muscle. Ogura et al. (33) found a biphasic response of LC3b-II in cardiac muscle and found a strong negative correlation ($r = 0.79$) between phosphorylation of serine-2448 of mammalian target of rapamycin (mTOR) and LC3b-II postexercise. As mTOR is a potent inhibitor of autophagy (22), and RE increases phosphorylation of serine-2448 of mTOR (13), it is possible that the acute postexercise protein synthetic demand outweighs any stimulus for autophagy, and this relationship may shift during training. The control of mTOR may be central to the divergent acute and chronic autophagic responses, as an attenuated response of mTOR or its downstream targets has been observed with training (32), which could create a permissive autophagic environment in contrast to that of acute exercise. In addition, Kim et al. (24) demonstrated a simultaneous reduction in multiple markers (LC3b-II, LC3b-I, ATG7, Beclin1, LAMP2a) of autophagy over the initial 12 h after a single bout of treadmill exercise. Our data are in agreement with previous work (16, 18) and indicates that acute RE either fails to activate or actively suppresses autophagy, such that accumulations in total LC3b and p62 are detected 48 h after RE. The results of Luo et al. (26) indicate that, as suggested for the mitochondrial response, the acute autophagic response to RE may not be equivalent to the cumulative effect of training, and further studies are required to clarify the response of autophagy, mitophagy, and the mitochondria to chronic RE.

**Perspectives and Significance**

This work demonstrates that, despite older adults having greater mtDNA deletions, mitochondrial transcripts (ND1, ND4, CS, Nrf2), proteins (Complex II 30kDa subunit, ATP synthase subunit alpha, Complex III subunit Core 2), and mtDNA copy number decline following unaccustomed, acute RE irrespective of age. Furthermore, while long-term strength training may promote favorable mitochondrial adaptations in aged skeletal muscle (44), the immediate postexercise response indicates reduced mitochondrial protein, transcript, and mtDNA abundance, which may be related to preferential induction of specific PGC-1α isoforms (39). This suggests that the favorable long-term mitochondrial response is dependent on cumulative bouts of RE, and that the response to an isolated bout is not indicative of the ultimate adaptation to training. Further work is required in the immediate hours after RE to determine whether reductions in mitochondrial content are the result of RE-stimulated macro or mitophagy, and how PGC-1α is differentially regulated to produce the divergent adaptations associated with either resistance or endurance exercise. In addition, further research is required to clarify the role of the alternate PGC-1α isoforms in the adaptation to RE (25, 37, 39, 53).

**GRANTS**

This work was supported by a CIHR Pilot Grant 112223 held by G. Parise.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**

ACUTE MITOCHONDRIAL RESPONSE TO RESISTANCE EXERCISE


