Skeletal Muscle Mitochondria in the Elderly: Effects of Physical Fitness and Exercise Training

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Context: Sarcopenia is thought to be associated with mitochondrial (Mito) loss. It is unclear whether the decrease in Mito content is consequent to aging per se or to decreased physical activity.

Objectives: The objective of the study was to examine the influence of fitness on Mito content and function and to assess whether exercise could improve Mito function in older adults.

Design and subjects: Three distinct studies were conducted: 1) a cross-sectional observation comparing Mito content and fitness in a large heterogeneous cohort of older adults; 2) a case-control study comparing chronically endurance-trained older adults and sedentary (S) subjects matched for age and gender; and 3) a 4-month exercise intervention in S.

Setting: The study was conducted at a university-based clinical research center.

Outcomes: Mito volume density (MitoVd) was assessed by electron microscopy from vastus lateralis biopsies, electron transport chain proteins by Western blotting, mRNAs for transcription factors involved in M biogenesis by quantitative RT-PCR, and in vivo oxidative capacity (ATPmax) by ^{31}P-magnetice resonance spectroscopy. Peak oxygen uptake was measured by graded exercise test.

Results: Peak oxygen uptake was strongly correlated with MitoVd in 80 60- to 80-year-old adults. Comparison of chronically endurance-trained older adults vs S revealed differences in MitoVd, ATPmax, and some electron transport chain protein complexes. Finally, exercise intervention confirmed that S subjects are able to recover MitoVd, ATPmax, and specific transcription factors.

Conclusions: These data suggest the following: 1) aging per se is not the primary culprit leading to Mito dysfunction; 2) an aerobic exercise program, even at an older age, can ameliorate the loss in skeletal muscle Mito content and may prevent aging muscle comorbidities; and 3) the improvement of Mito function is all about content. (*J Clin Endocrinol Metab* 99: 1852–1861, 2014)

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Abbreviations: ATPmax, maximal adenosine-tri-phosphate regeneration capacity; BMI, body mass index; Ct, cycle threshold; ETC, electron transport chain; FM, fat mass; HR, heart rate; k, PCr recovery rate constant; LBM, lean body mass; MitoVd, mitochondrial volume density; NRF, nuclear respiratory factor; PCr, phosphocreatine; PGC-1, peroxisome proliferator-activated receptor-γ coactivator-1; TFAM, mitochondrial transcription factor A; VO$_2$peak, peak oxygen uptake.

Mitochondrial dysfunction and reduced oxidative capacity in skeletal muscle have been linked to the pathogenesis of sarcopenia, aging disabilities, and frailty (1). Sedentary lifestyle, an escalating epidemic in Western societies, is associated with loss of mitochondrial content and function (2, 3). Increased mitochondrial content in response to exercise training was first reported by Holloszy in 1967 (4). Since then, exercise training has been shown to be an effective strategy to improve muscle oxidative capacity (5, 6).

Aerobic exercise training up-regulates mitochondrial genes (7). Adaptations of skeletal muscle to exercise (8) include the up-regulation of the master regulator of mitochondrial biogenesis, the peroxisome proliferator-activated receptor-γ coactivator-1α (*PGC-1α*) (9). *PGC-1α* is a transcriptional regulator that induces mitochondrial biogenesis by coactivating a large spectrum of transcription factors, including the nuclear respiratory factors 1 and 2 (*NRF-1*, *NRF-2*) (10, 11). In turn, *NRF-1* and *NRF-2* control the expression of a significant number of the proteins that make up the five respiratory complexes (12, 13) and modulate the expression of the mitochondrial transcription factor A (*TFAM*), which regulates mitochondrial DNA replication (13, 14). Several studies to date indicate that, in addition to *PGC-1α*, aerobic exercise also up-regulates *TFAM* and *NRF-1* in humans (2, 15, 16).

Aging is associated with a loss of mitochondrial content (17, 18) and function (18–20) in muscle. However, studies comparing younger with older subjects are conflicting (21, 22). Indeed, it is not clear whether the decrease in mitochondrial content is associated with aging per se or with the decreased physical activity that comes with aging. Given the important role of aerobic exercise in up-regulating genes and transcription factors controlling mitochondrial content and function, it remains to be seen whether aerobic exercise training could play a protective role for mitochondria in aging and whether training can help older individuals recover mitochondrial content. Therefore, this study had two main objectives: first, to examine the relationship between mitochondrial content and physical fitness in older men and women focusing on external validity with a broad population in terms of physical fitness and body composition; and second, focusing on internal validity using a comprehensive picture of mitochondrial biology from the molecular level (mRNA transcripts, protein expression), the organelle level (mitochondrial density), the whole muscle level (in vivo organelle capacity), and whole-body level [peak oxygen uptake (VO$_2$peak)], combining invasive and noninvasive techniques, to assess whether exercise could improve mitochondrial content and function in older adults through the up-regulation of mitochondrial master regulators.

Research Design and Methods

Study design

Three distinct studies were conducted. Study 1 is a cross-sectional study comparing the baseline levels of mitochondrial content and physical fitness in a heterogeneous cohort of older adults across a spectrum of fitness levels. Study 2 is a case-control study comparing chronically endurance trained older adults and sedentary subjects matched for age and gender. Study 3 is an interventional study comprising a 4-month exercise intervention in sedentary older adults.

Study 1 was partially conducted at the University of Pittsburgh and finished at the University of Lausanne because the last author was in the process of changing institutions. All tests were conducted under the exactly same conditions, and the analyses were conducted in exactly the same way. Studies 2 and 3 were conducted at the University of Lausanne. The institutional review boards of both sites approved all the studies, and all the subjects provided written informed consent.

Subjects

Volunteers between 60 and 80 years of age in good general health and stable weight were recruited for the studies. Active smokers and participants with abnormal thyroid, liver, or kidney function, anemia, and participants taking anticoagulation agents or medication known to affect skeletal muscle homeostasis (such as glucocorticoids or insulin sensitizers) were excluded. All subjects underwent a standard 75-g oral glucose tolerance test to rule out diabetes. For study 2 (case-control) and study 3 (interventional), volunteers were considered physically active or sedentary based on their self-declared levels of physical activity. Physically active volunteers (named here as active) were engaging in three or more structured aerobic exercise sessions per week for more than 1 year. Sedentary individuals were defined as those participating in a structured exercise session no more than 1 day per week.

Exercise intervention (study 3)

The exercise training was a 16-week, supervised, moderate-intensity aerobic protocol. Sedentary subjects were asked to engage in at least three supervised sessions in the gym. Each session was progressively increased from 30 to 60 minutes. Moderate intensity was defined as 75% of the subjects' heart rate (HR). Exercise prescription was individualized based on the subject's peak HR achieved during the baseline VO$_2$peak test and adapted at the midpoint of the intervention with a submaximal ergometer test as described in details by Dube et al (23). HR monitors (Polar Electro Oy) and exercise logs were used to monitor intensity. Subjects could bike, walk, run, or row within their

HR target range with at least 80% of the training as walking or biking. Frequency, duration, and volume of exercise were recorded and computed as described elsewhere (23). During the training regimen, subjects were instructed to follow their typical food intake and not to undertake dietary changes while engaged in the study.

Clinical outcome measures

Height was measured using a wall-mounted stadiometer and weight using a calibrated medical digital scale (Seca). Lean body mass (LBM) was determined by dual-energy X-ray absorptiometry (DiscoveryA; Hologic Inc). Physical fitness was determined by VO_2peak using a graded exercise test on an electronically braked cycle ergometer (Lode B.V.). HR, blood pressure, and electrocardiogram were recorded before, during, and after the exercise test. Oxygen consumption was computed via indirect calorimetry (Metalyzer3B; Cortex GmbH). The protocol was adapted from previously used protocols well suited for older volunteers of various degrees of fitness or fatness (24). Briefly, after an initial warm-up consisting of 2 minutes of no-load pedaling, the graded exercise test began at 25 W for women or 50 W for men for the first 2 minutes and was then increased 25–50 W thereafter until volitional exhaustion or if one of the American College of Sports Medicine established criteria for maximal testing had been reached.

Ex vivo skeletal muscle outcome measures

Percutaneous muscle biopsies were obtained in the fasted state from the vastus lateralis under local anesthesia (buffered lidocaine) as previously described (24). Controlled conditions included no exercise for 48 hours, a standardized dinner followed by an overnight fast prior to the biopsy. After trimming of visible adipose tissue with a dissecting microscope (MZ6; Leica Microsystems), one portion of the specimen (~5 mg) was fixed for transmission electron microscopy and two portions (~30 mg each) were flash frozen in liquid nitrogen and stored at −80°C for Western blotting and RT-PCR. Analyses were performed in a blind manner. Transmission electron microscopy (studies 1, 2, and 3) was used to measure mitochondrial volume density (MitoVd) as a marker of mitochondrial content. A recent validation and detailed description of this stereological method has been described elsewhere (25). For protein expression (studies 2 and 3), frozen tissue was homogenized in 200 μL of ice-cold lysis buffer containing 50 mm Tris-HCl (pH 7.5), 150 mm NaCl, 1% (vol/vol) Nonidet P-40, 1 mm EDTA, and freshly added protease inhibitor cocktail tablet (Roche Diagnostics International), using a motor-driven Eppendorf homogenizer. Homogenates were then rotated for 30 min-

utes at 4°C before centrifugation at 15 000 rpm for 10 minutes at 4°C. The pellet was discarded, and the supernatant was collected and stored at −80°C until used. Protein was measured by the BCA method (Pierce, Thermo-Fisher Scientific Inc). Western blotting was performed as previously described (26). Protein band intensity was measured by ImageJ (National Institutes of Health), and the target protein levels were normalized over the corresponding α-tubulin loading controls for each subject. All antibodies for mitochondrial complex subunits have been purchased from Mitosciences (Abcam). The list of antibodies can be found in Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org. Gene expression analysis (studies 2 and 3), in which the total mRNA preparations, cDNA synthesis, and quantitative RT-PCR were performed as described previously (26). Primers are described in Supplemental Table 2. Target mRNA levels were normalized over the geometric mean of β-actin and cyclophilin B, which were selected as housekeeping genes after having checked their expression stability (27). Relative mRNA expression levels were calculated with the ΔΔ cycle threshold (Ct) method, by which we used the mean of the ΔCts from five sedentary subjects as ΔCt calibrator.

In vivo skeletal muscle outcome measures (studies 2 and 3)

The rate of postexercise phosphocreatine (PCr) recovery reflects oxidative ATP synthesis rate and was shown to be correlated with in vitro measurements of oxidative capacity (28). PCr recovery experiments were performed on a 3T MR system (VERIO; Siemens) in the supine position. A double-tuned 31P/1H surface coil (RAPID Biomedical) was placed on the center of the quadriceps muscle, and spectra were collected with an adiabatic excitation pulse. One fully relaxed spectrum was obtained on resting muscle with a repetition time of 20 seconds and four averages. For the PCr recovery spectra, the repetition time was 2 seconds with two scans per spectrum, resulting in a time resolution of 4 seconds before, during, and for 9 minutes after dynamic knee extensions against a rubber band. Contraction frequency was one extension per second (acoustic cues). The resistance of the rubber band was adapted to each subject's strength, which was determined beforehand by maximal isokinetic torque of the knee extensors. Default exercise duration was 28 seconds. If the relative decrease of PCr was outside the target of 20%–40%, exercise duration was changed to 22 seconds, 36 seconds, or 44 seconds; otherwise, it was unchanged for a second repetition. Because pH did not decrease below 6.8 in any experiment and recovery rates of experiments 1 and 2 were not significantly different from each other (P =

.92), results are shown as average of the two experiments. For the postprocessing, spectra were analyzed with jMRUI (29) using AMARES for quantitation. The recovery of PCr was fitted to the following formula: PCr (t) = PCr_0 + ΔPCr $(1 - e^{-k \cdot \tau})$, with PCr_0 as the PCr intensity at the beginning of recovery and ΔPCr as the exercise-induced decrease of the PCr signal. The pH was calculated from the chemical shift between inorganic phosphate and PCr. The oxidative phosphorylation capacity (ATPmax) was computed, as previously suggested (20), as the product of the recovery rate, PCr recovery rate constant (k) and the resting PCr content obtained from the resting spectrum and assuming a constant ATP concentration of 8.2 mM.

Statistical procedures

Data are presented as means ± SEM. For study 1 (cross-sectional), the relationship between variables was explored using linear regression. For studies 2 (case-control) and 3 (interventional), data were first explored using nonparametric statistical tests appropriate for small sample sizes including the Wilcoxon rank-sum test (between group comparison study 2) and the Wilcoxon signed rank test (before and after comparison, study 3). After assessing normality, parametric tests were performed. These included independent *t* tests for study 2 and paired *t* tests for study 3. *P* values reported in *Results* are two tailed and from parametric tests unless otherwise specified. Correlations were performed with Spearman correlation coefficient. Significance level was set at .05. The statistical analyses were performed using JMP version 9 (SAS Institute), SPSS version 20 (IBM), and Prism version 6c (GaphPad Inc) for Macintosh.

Results

Mitochondria content correlates with exercise capacity in older adults (study 1: cross-sectional study)

A total of 80 subjects, 33 men and 47 women, were included in this study. The cohort was heterogeneous with wide ranges of MitoVd, VO_2peak, body mass index (BMI), and body fatness (Table 1). A strong relationship was observed between MitoVd and VO_2peak (Figure 1). This relationship was similar when VO_2peak was normalized by LBM or body weight (BW). These data show that skeletal muscle mitochondrial content is positively associated with peak oxygen uptake in the elderly.

Case-control comparison between age-matched sedentary and chronically trained older volunteers (study 2)

In an attempt to evaluate the effects of chronic exercise on mitochondrial content and function, we compared 60-
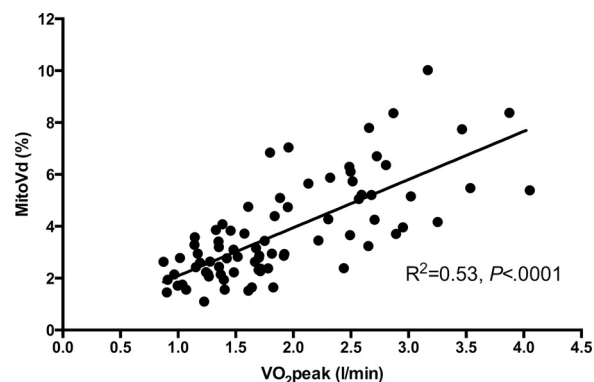
Table 1. Study 1: Subjects' Characteristics

	Mean ± SEM	Minimum	Maximum
Age, y	66.6 ± 0.5	60	79
BW, kg	79.3 ± 1.5	55.4	106.6
BMI, kg/m²	28.1 ± 0.5	19.9	37.3
Body fat, %	35.0 ± 1.4	7.7	51.8
LBM, kg	47.9 ± 1.01	31.3	71.0
VO_2peak, L/min	1.91 ± 0.08	0.87	4.05
VO_2peak/BW, mL/min/kg	25.2 ± 1.3	11.0	59.1
VO_2peak/LBM, mL/min/kg	39.2 ± 1.2	21.9	66.4
MitoVd, %	3.78 ± 0.21	1.09	10.02

to 80-year-old active with age- and gender-matched sedentary adults. Subjects' characteristics are presented in Table 2.

The active subjects exhibited significantly higher MitoVd (+48.9%) compared with sedentary peers (Figure 2A). At the protein level, differences between groups could be observed for the electron transport chain (ETC) complexes I, IV, and V, which were significantly higher in the active subjects (Figure 2, B and C). Complexes IV and V were positively correlated with MitoVd (rho = 0.52 and 0.70, respectively; $P < .05$). Complexes I, IV, and V were positively correlated with VO_2peak/LBM (rho = 0.56, 0.76, and 0.55, respectively; $P \leq .03$). Complexes IV and V were negatively correlated with fat mass (rho ≤ −0.59, $P \leq .01$) and percentage body fat (rho ≤ −0.52, $P \leq .05$). No significant differences were detected in the expression levels of genes involved in mitochondrial biogenesis (ie, *PGC-1α*, *PGC-1β*, *NRF-1*, *NRF-2*, and *TFAM*; Figure 2D) despite a clear tendency for *PGC-1α* to have a higher expression in the active group.

In vivo oxidative phosphorylation capacity (reflected in k and ATPmax) was greater in the active than in the sedentary volunteers (+22.0% for k and +21.2% for ATPmax, Table 2). MitoVd and ATPmax were positively correlated (rho = 0.74, $P < .0001$); the same was observed for MitoVd and k (rho = 0.61, P = .002). When taking the

Figure 1. Study 1 (n = 80): linear relationship between physical fitness (VO_2peak) and mitochondrial volume density (MitoVd).

Table 2. Study 2: Subjects' Characteristics and in Vivo Skeletal Muscle Oxidative Capacity (PCr Recovery)

	Active	Sedentary	P Value[a]
Subjects Characteristics			
n	14	14	
Gender, M/F	7/7	8/6	
Age, y	67.4 ± 1.2	65.6 ± 0.7	.21
BW, kg	59.6 ± 2.2	83.9 ± 4.7	*<.0001*
BMI, kg/m^2	21.5 ± 0.5	27.8 ± 1.3	*<.0001*
LBM, kg	45.7 ± 2.2	54.7 ± 3.1	*.03*
Fat mass, kg	12.0 ± 0.7	27.9 ± 3.0	*<.0001*
Body fat, %	20.2 ± 1.2	32.3 ± 2.5	*.0003*
VO$_2$ peak, L/min	2.16 ± 0.15	2.06 ± 0.14	.64
VO$_2$ peak/LBM, mL/min/kg	46.1 ± 2.02	37.7 ± 1.8	*.005*
PCr recovery			
n	12	14	
k, L/min	2.33 ± 0.11	1.91 ± 0.11	*.009*
ATPmax, mmol/L/sec	1.37 ± 0.07	1.13 ± 0.05	*.01*
pH end exercise	7.11 ± 0.01	7.12 ± 0.01	.39
pH, min	6.94 ± 0.01	6.96 ± 0.01	.16
Decrease in PCr, %	28.3 ± 1.9	32.1 ± 1.5	.13
k/MitoVd, L/min/%	0.35 ± 0.03	0.41 ± 0.03	.16
ATPmax/MitoVd	0.21 ± 0.04	0.24 ± 0.01	.13

Data are means ± SEM. Significant *P* values are in italics.

[a] Two-tailed independent *t* test.

ratio rate constant k to MitoVd or ATPmax to MitoVd as a marker of mitochondrial function per volume, there was no difference between groups (Table 2). This suggests that the increase in ATPmax is due to a higher mitochondrial number or content but not to intrinsic changes per mitochondria.

Exercise intervention in previously sedentary older subjects (study 3)

To investigate the capacity of skeletal muscle from untrained elderly individuals to respond to aerobic training, the sedentary subjects followed a 16-week training program (endurance exercise intervention) with a postintervention evaluation. Two subjects were excluded from the final data analyses: one man initiated a calorie-restriction diet during intervention and had substantial weight loss; the second was a woman who received steroid treatment for acute rheumatoid disease during intervention. Of the 12 finishers, muscle specimen data were obtained in 10 subjects (six males, four females).

Subjects' characteristics and effect of the intervention on clinical outcomes are presented in Table 3. On average, subjects exercised 3.1 ± 0.1 sessions/wk, with an average of 55 ± 1.9 minutes per session. Based on their recorded HR, exercise intensity was of 8.5 ± 0.6 kcal/min, thus achieving the goal of a moderate endurance exercise program corresponding to an average of 5.2 ± 0.4 kcal/kg of BW expanded per session. The exercise intervention promoted modest, but significant, changes in BW and BMI (both −2.2%). Body composition changed with improve-

ments in LBM (+1.5%), and marked decrease in fat mass (FM; −6.6%) and percent body fat (−5.9%). Overall fitness was remarkably improved by the exercise program, with a change of +13.9% in absolute VO$_2$peak, corresponding to +12.5% relative VO$_2$peak/LBM.

MitoVd increased by 50.7% with training (Figure 3A). Furthermore, the levels of complexes III, IV, and V were significantly increased postintervention, accompanied by a strong tendency for complex I toward up-regulation (+29.1%) (Figure 3, B and C). In line with previous reports (30), we also observed a significant increase in *PGC1α* and *TFAM* expression levels after the 4 months of exercise intervention (Figure 3D). The changes in the expression levels of *PGC1α* and *NRF-2* were significantly correlated to the increase in *TFAM* expression (rho = 0.86 and rho = 0.76, respectively, $P \leq .03$). The above observations indicate that exercise training increases MitoVd and VO$_2$peak in older sedentary subjects, probably by up-regulating the key orchestrators of the mitochondrial biogenesis program.

ATPmax improved by 22.5% (Table 3). ATPmax/MitoVd was not significantly changed with intervention (Table 3). This again highlights that the increase in ATPmax is due to enhanced mitochondrial content, not to intrinsic changes in mitochondrial function.

Discussion

It is well established that mitochondrial dysfunction and reduced oxidative capacity are associated with insulin re-
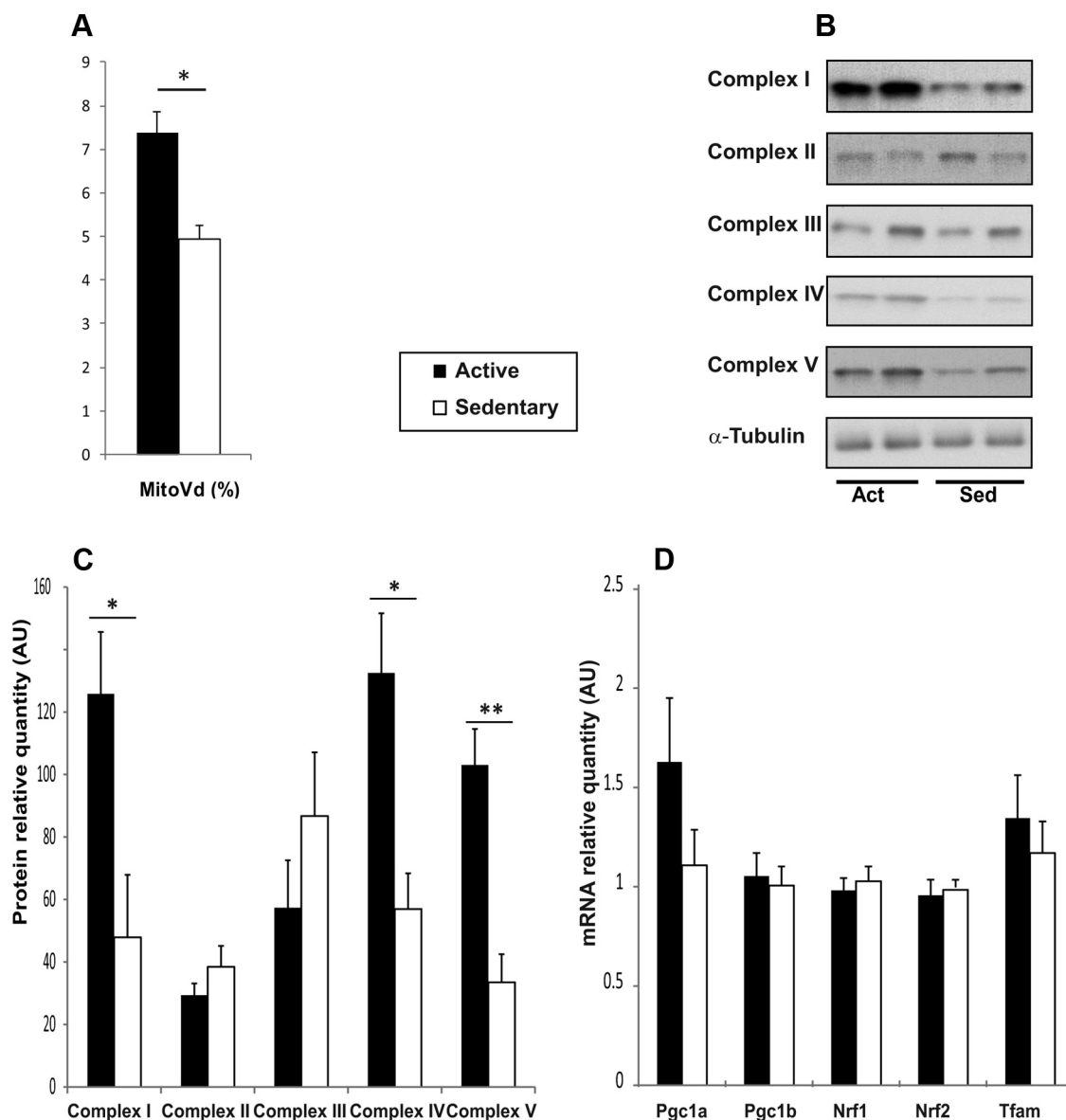
Figure 2. Study 2: skeletal muscle comparison between age-matched older active and sedentary subjects. A, MitoVd (active, n = 13; sedentary, n = 12). *, $P = .0003$. B, Western blots from representative subjects belonging either to the active (Act) or sedentary (Sed) group. C, Electron transport chain complex relative abundance (active, n = 7; sedentary, n = 8; the values normalized over the corresponding α-tubulin levels are shown). *, $P \leq .02$; **, $P = .0001$. D, Relative mRNA abundance (active, n = 7; sedentary, n = 9). For all panels, error bar, SEM; black bar, active; white bar, sedentary.

sistance and type 2 diabetes. Aging is similarly associated with a loss of mitochondrial content and function (17–20), which might contribute to the development of age-related insulin resistance and physiological decline. Although the positive relationship between mitochondrial content and physical fitness has been acknowledged in younger populations (31, 32), the relationship in older populations has yet to be recognized. Furthermore, it is not clear whether the mitochondrial function decline during aging is a direct consequence of the aging process per se or secondary to the sedentary lifestyle that is more prevalent in the aging population (33). Finally, it is also not clear whether the possible mitochondrial defects in the aged population are due

to a defective ability to trigger mitochondrial biogenesis programs.

Herein we demonstrate that physical fitness is exquisitely correlated with mitochondrial density in skeletal muscle in older adults (60–80 y old). Similarly, using a comprehensive picture of mitochondrial biology from the molecular level (mRNA transcripts, protein expression), the organelle level (mitochondrial density), the whole muscle level (in vivo organelle capacity), and whole-body level (VO_2peak), we demonstrate that the mitochondrial content and function of aged individuals can be largely enhanced by an endurance exercise program. As a whole, our results indicate that aging per se does not impede mi-

Table 3.   Study 3: Subjects' Characteristics and in Vivo Skeletal Muscle Oxidative Capacity (PCr Recovery) Before and After a 4-Month Endurance Training Intervention

	Sedentary Before	Sedentary After	P Value[a]
Subject Characteristics			
n	12	12	
Gender, M/F	7/5		
BW, kg	83.3 ± 5.4	81.5 ± 5.1	.04
BMI, kg/m^2	27.5 ± 1.3	26.9 ± 1.3	.04
LBM, kg	54.6 ± 3.7	55.4 ± 3.5	.04
FM, kg	27.4 ± 3.0	25.6 ± 2.9	.0008
Body fat, %	32.0 ± 2.6	30.1 ± 2.6	.0005
VO$_2$peak, L/min	2.01 ± 0.16	2.29 ± 0.17	.006
VO$_2$peak/LBM, mL/min/kg	36.97 ± 1.92	41.60 ± 2.03	.004
PCr recovery			
n	12	12	
k, L/min	1.88 ± 0.12	2.41 ± 0.13	.0009
ATPmax, mmol/L/sec	1.11 ± 0.06	1.36 ± 0.06	.006
pH end exercise	7.13 ± 0.01	7.12 ± 0.01	.30
pH min	6.96 ± 0.01	6.96 ± 0.02	.35
Decrease in PCr, %	31.4 ± 1.4	30.5 ± 2.0	.72
k/MitoVd, L/min/% (n = 10)	0.42 ± 0.04	0.35 ± 0.04	.22
ATPmax/MitoVd, (n = 10)	0.24 ± 0.02	0.20 ± 0.02	.13

Data are means ± SEM. Significant P values are in italics.

[a] Two-tailed paired t test.

tochondrial biogenesis in response to exercise and that the decreases in mitochondrial function observed in elder adults are likely due to decreased physical activity.

To our knowledge, this is the largest cohort used to date to evaluate this relationship with a direct measure of skeletal muscle mitochondrial content. Thus, although the overall mitochondrial content is known to decrease with age, its positive relationship with whole body oxygen uptake persists. Further confirming this, in the case-control comparison between older sedentary and age-matched active adults, the active exhibited higher levels of fitness with greater mitochondrial volume density. Although our study uses direct measures of mitochondrial content and objective measures of physical fitness, our results are consistent with previous reports in smaller cohorts of both young and old individuals or using indirect markers of mitochondrial content (24, 34, 35).

Interestingly, sedentary older individuals submitted to an exercise intervention displayed large improvements in MitoVd. Actually, postintervention MitoVd values were similar to levels observed in the active group (sedentary post intervention vs active P > .05). This again clearly indicates that aged individuals do not have any acquired problem to enhance mitochondrial biogenesis. Although one of the limitations of our study is the lack of comparison with a younger cohort and the fact that other authors suggested that chronic exercise is not able to completely restore mitochondrial content in older subjects (2), it is important to note that postintervention MitoVd are in the

range of younger cohorts (36) or previously published chronically trained older subjects (24).

To further solidify the information from MitoVd, we also evaluated mitochondrial function in our patients by means of in vivo oxidative capacity. Our endurance trained active subjects displayed greater in vivo oxidative capacity, as determined by the rate of PCr recovery and ATPmax, compared with age-matched sedentary subjects. However, sedentary subjects improved their in vivo oxidative capacity by ~22% after training. Importantly, neither active nor sedentary subjects before or after intervention displayed changes in the ratio of ATPmax to MitoVd. This suggests that the increase in the ability to replenish ATP is not primarily due to mitochondrial intrinsic changes in oxidative function but rather to the higher mitochondrial content. Remarkably, a recent paper by Conley et al (37)[based on a previous study from the same group (20, 38)] showed an increase in the ratio of ATPmax (23%) but no significant increase in mitochondrial volume (8.8%) after 6 months of endurance training in older men and women. Thus, their reported ratio of ATPmax to MitoVd, which the authors termed energy coupling, was increased. However, a large difference in comparing our study is that their intervention [one legged press exercise described by Jubrias et al (38)] improved VO$_2$max by only approximately 5%. Further initiatives will be required to evaluate how different exercise protocols mitigate the enhancement of ATP synthesis by increasing the intrinsic respiratory coupling or by inducing mitochondrial bio-
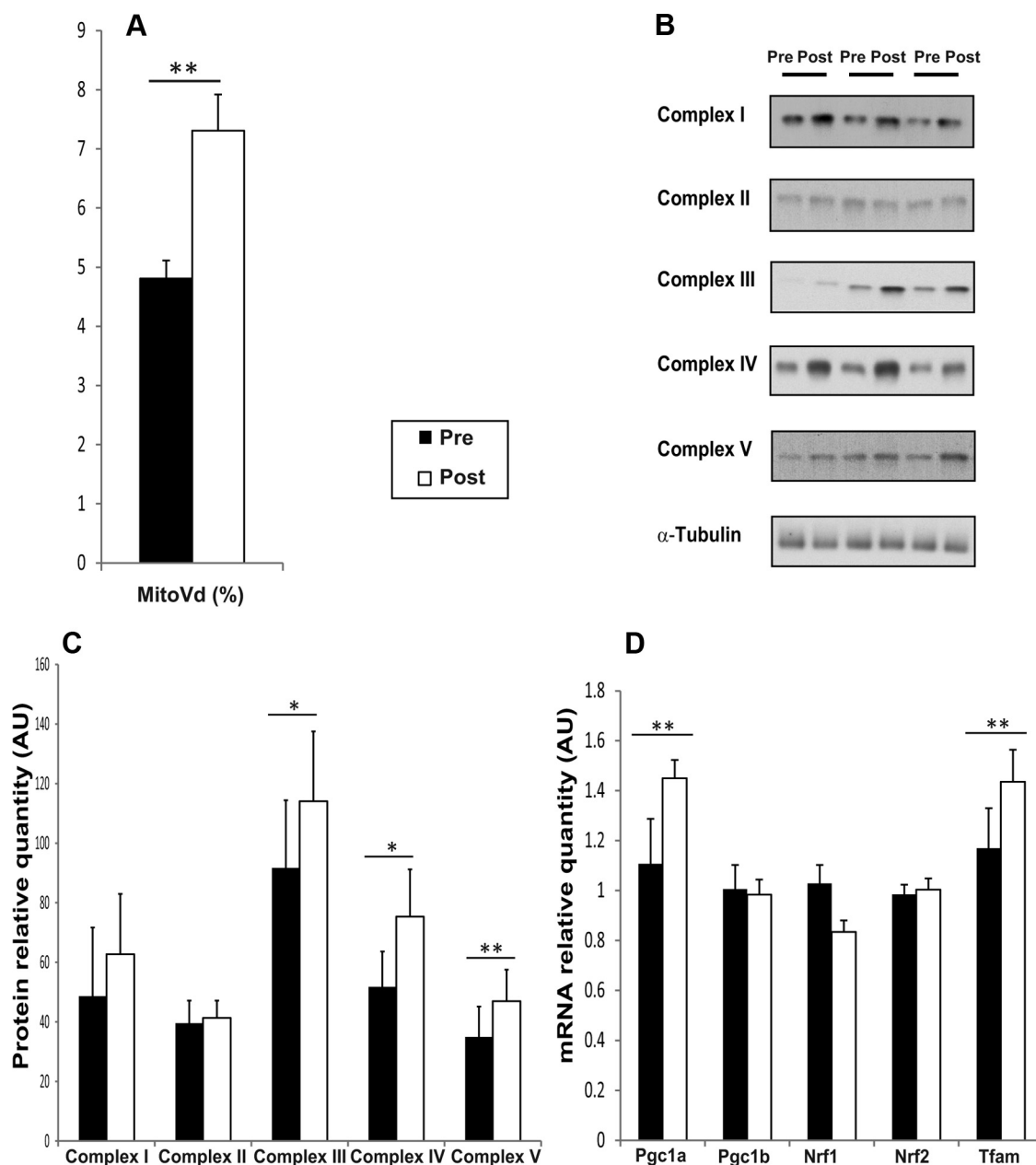
Figure 3. Study 3: skeletal muscle of older sedentary adults before and after 4-month endurance training intervention. A, MitoVd (n = 10). B, Paired Western blots on ETC complexes from representative subjects before and after intervention. C, Electron transport chain complex relative abundance (n = 7; the values normalized over the corresponding α-tubulin levels are shown). D, Gene expression profiles (n = 8). For all panels, error bar, SEM; black bar, before intervention; white bar, after intervention. *, $P = .03$ (one tail); **, $P < .05$.

genesis. Another important difference with the work of Conley et al (37) is that their sedentary subjects were less fit than ours to start with (average VO_2peak 1.7 vs 2.0 l/min with equivalent BW); this could mean that a certain minimal activity is needed to keep up the energy coupling, but this again would point to the effect of exercise and lifestyle and not to aging per se.

This higher mitochondrial content in chronically trained individuals was concurrent to an increase in electron transport chain complexes content, particularly in complexes I, IV, and V. Similar differences in complex IV levels were observed between sedentary knee osteoar-

thritic older patients and age-matched active controls (39). Interestingly, in our cohort, sedentary older adults have lower MitoVd than those who are physically active yet exhibit no differences in complexes II and III concentrations. Similarly, a recent study (40) of young healthy volunteers showed no relationship between MitoVd and the content of complexes I and IV but a strong correlation with complexes II, III, and V. In light of these cumulative data, we propose that MitoVd appears to provide a better representation of mitochondrial content than individual or relative abundance of ETC complexes. It must be kept in mind that analyzing mitochondrial content or function

through the evaluation of mitochondrial complex subunit abundance or by in vitro single complex activities may be misleading. This overlooks possible additional layers of regulation such as supercomplex assemblies or posttranslational modifications, which can heavily affect ETC complex function without necessarily changing their global content.

Consistent with the increase in mitochondrial content induced by our exercise intervention, transcriptional regulators of mitochondrial biogenesis were markedly upregulated. We observed significant increases in the gene expression of both *PGC-1α* and *TFAM* after the 16-week training but not in *NRF-1* and *NRF-2*. Prior reports demonstrate that protein expression levels of *PGC1α*, *TFAM*, and *NRF-1* are increased after 10 weeks of endurance training (2). For *PGC1α*, the magnitude observed in our study (∼50%) was similar to the one observed in a 16-week intervention in both younger and older subjects (30) as well as the one observed for *PGC1α* protein content in a recent 12-week intervention (41). Therefore, exercise can stimulate mitochondrial biogenesis in aged populations and increase this global respiratory capacity.

This work is not without limitations. First, the common thread between the three parts of this work was the relationship between physical fitness and mitochondrial content/function in older adults. Further studies are needed to address other controversial debates, such as the relationship between mitochondrial function and insulin sensitivity or with the genesis and development of sarcopenia in aged patients. Second, we did not explore gender differences, which are thought to influence mitochondrial ATP production (42). Indeed, our measurements of in vivo mitochondrial function (studies 2 and 3) were performed in a relative small number of volunteers not permitting further stratifications. Lastly, we did not compare our older adults cohorts with a control group of young individuals. Thus, we cannot rule out that exercise training in a younger population would have enhanced effects in mitochondrial content and function compared with the changes we observed in our 60- to 80-year-old population of interest.

In summary, our work, using in vivo and ex vivo methodologies, thus allowing a comprehensive model of mitochondrial biology, demonstrates the following: 1) that physical fitness is tightly linked to mitochondrial content in a broad and heterogeneous population of older individuals, 2) that aging per se is not the primary culprit leading to mitochondrial dysfunction, as 3) aged individuals largely enhance mitochondrial function in response to exercise training. Therefore, the lower oxidative capacity observed in old individuals is likely due to a higher tendency toward a sedentary lifestyle and lower energy demand because mitochondrial biogenesis programs can be efficiently activated upon stimulation. Accordingly, commencing an aerobic exercise program, even at an older age, can help ameliorate the loss in skeletal muscle mitochondrial content and may prevent muscle aging comorbidities such as sarcopenia and insulin resistance.

Acknowledgments

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