Resistance Exercise Training Alters Mitochondrial Function in Human Skeletal Muscle

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ABSTRACT
PORTER C., P. T. REIDY, N. BHATTARAI, L. S. SIDOSSIS, and B. B. RASMUSSEN. Resistance Exercise Training Alters Mitochondrial Function in Human Skeletal Muscle. Med. Sci. Sports Exerc., Vol. 47, No. 9, pp. 1922–1931, 2015. Introduction: Loss of mitochondrial competency is associated with several chronic illnesses. Therefore, strategies that maintain or increase mitochondrial function will likely be of benefit in numerous clinical settings. Endurance exercise has long been known to increase mitochondrial function in the skeletal muscle. Comparatively little is known regarding the effect of resistance exercise training (RET) on skeletal muscle mitochondrial respiratory function. Purpose: The purpose of the current study was to determine the effect of chronic resistance training on skeletal muscle mitochondrial respiratory capacity and function. Methods: Here, we studied the effect of a 12-wk RET program on skeletal muscle mitochondrial function in 11 young healthy men. Muscle biopsies were collected before and after the 12-wk training program, and mitochondrial respiratory capacity was determined in permeabilized myofibers by high-resolution respirometry. Results: RET increased lean body mass and quadriiceps muscle strength by 4% and 15%, respectively (P < 0.001). Coupled mitochondrial respiration supported by complex I, and complex I and II substrates increased by 2- and 1.4-fold, respectively (P < 0.01). The ratio of coupled complex I-supported respiration to maximal respiration increased with RET (P < 0.05), as did complex I protein abundance (P < 0.05), whereas the substrate control ratio for succinate was reduced after RET (P < 0.001). Transcripts responsible for proteins critical to electron transfer and NAD+ production increased with training (P < 0.05), whereas transcripts involved in mitochondrial biogenesis were unaltered. Conclusion: Collectively, 12 wk of RET resulted in qualitative and quantitative changes in skeletal muscle mitochondrial respiration. This adaptation was accompanied by modest changes in mitochondrial proteins and transcript expression. RET seems to be a means to augment the respiratory capacity and intrinsic function of skeletal muscle mitochondria. Key Words: RESISTANCE TRAINING, SKELETAL MUSCLE, MITOCHONDRIA, BIOENERGETICS

Mitochondria are the cellular organelles responsible for aerobic adenosine triphosphate (ATP) production; thus, mitochondrial "health" is of importance to overall cellular function. The abundance and functional characteristics of mitochondria within an organ influence its physiological function (17,23,24,27). For example, aging is synonymous with reduced skeletal muscle mitochondrial density and function (29), which is associated with reduced exercise capacity. Conversely, the number and functionality of skeletal muscle mitochondria positively correlate with exercise capacity in healthy humans (15,25,38). Furthermore, aerobic exercise is known to be a potent regulator of skeletal muscle oxidative capacity (12,36), where mitochondrial density and function are elevated in both animals and humans acclimated to endurance exercise (13,15,25).

Resistance exercise has emerged as an efficacious intervention, which augments muscle mass and function, particularly in populations with diminished lean body mass. However, the effect of resistance exercise on skeletal muscle bioenergetics remains poorly understood. Early reports suggested that skeletal muscle mitochondrial volume (22) and oxidative capacity (5) were reduced by chronic resistance exercise training (RET). More recently, others have shown that resistance exercise increases the activity of oxidative enzymes in tissue homogenates (33) and respiration in skinned muscle fibers (25). However, many other studies have also shown that mitochondrial density (21) and the activity of oxidative enzymes thought to reflect mitochondrial abundance and/or function, such as citrate synthase (CS), β-hydroxyl-acyl-CoA dehydrogenase, and succinate dehydrogenase, are largely unaltered by chronic RET (1,10,11,26,34,35,37). The discordance in these results are
likely attributable to numerous factors, such as the training regimen used, participants’ preexisting training status, the timing of biopsy collection after exercise (19), and the numerous analytical techniques used to quantify the abundance and activity of mitochondrial enzymes (18).

Most studies to date concerning the effect of RET on skeletal muscle bioenergetics have determined surrogate measures of oxidative capacity (5,10,11,22,26,34,35,37), which are more likely to reflect the abundance of mitochondrial proteins rather than mitochondrial function. Surprisingly, only a small number of studies have attempted to determine the effect of RET on skeletal muscle mitochondrial function. Salvadego et al. (28) measured skeletal muscle mitochondrial respiration in a cross-section of resistance-trained athletes and untrained controls. These researchers found that coupled (ATP producing) mitochondrial respiration per milligram of muscle was greater in resistance-trained athletes, as was the respiratory control ratio (RCR) for adenosine diphosphate (ADP) (28), suggesting that muscle ATP producing capacity is greater in resistance-trained individuals.

Recently, Pesta et al. (25) showed that endurance training and strength training performed under hypoxia resulted in similar qualitative and quantitative changes in skeletal muscle mitochondrial respiration. Unfortunately, while Pesta et al. (25) attempted to study the effect of strength training under normoxic conditions, only three participants completed the intervention, meaning statistical power was not achieved. As such, a significant knowledge gap remains concerning the effect of RET on skeletal muscle respiratory capacity and function. To address this, we set out to determine the effect of chronic resistance exercise on skeletal muscle mitochondrial respiratory function. To do this, skeletal muscle biopsies were collected from young, healthy, untrained men before and after a 12-wk RET program and mitochondria function measurements were performed on fresh muscle biopsy samples. We hypothesized that RET would augment skeletal muscle mitochondrial respiratory capacity.

**METHODOLOGY**

**Human subjects and screening.** Eleven young healthy volunteers were recruited for the current study. Participants were young, recreationally active males and free of any physical impairment that would prevent participation in an RET program. Participants, although physically active, were not engaged in any structured resistance or endurance training programs at the time of or 6 months before participation. As such, all participants can be considered healthy but untrained. The participants were recruited through locally posted flyers, newspaper advertisements, and by word of mouth. Screening of participants was performed in the morning after an overnight fast at the Institute for Translational Sciences—Clinical Research Center (ITS-CRC) at the University of Texas Medical Branch in the afternoon after an overnight fast. All participants provided informed written consent before enrolment in the study. The study was approved by the institutional review board of the University of Texas Medical Branch and was performed in compliance with the Declaration of Helsinki as revised in 1983.

**Experimental design.** After enrolment, participants completed a 10- to 14-d pretraining run-in period that consisted of the pretraining study day at the ITS-CRC, and then three nonconsecutive days of exercise familiarization and baseline 1-repetition maximum (1RM) strength testing at the University of Texas Medical Branch Alumni Field House. The pretraining study day included assessment of body composition, muscle biopsy collection, and isokinetic and isometric strength testing. Two to three days later, the participants reported to the University of Texas Medical Branch Alumni Field House for familiarization/testing before beginning the 12 wk of training. After 12 wk of training, participants were retested exactly 3 d after the final exercise session of the training program (see following section). Participants reported to the ITS-CRC at the same time in the morning as the pretraining study day to repeat the same laboratory tests and sample collection.

**Pre- and posttesting study day.** Participants reported to the ITS-CRC at the University of Texas Medical Branch in the morning after an overnight fast. After voiding, participants lay supine for 30 min before assessment of body composition by dual-energy x-ray absorptiometry scan (ADR 4500W; Hologic, Bedford, MA).

To maintain a supine position, participants were transported to and from the CRC in a stretcher. After a dual-energy x-ray absorptiometry scan, ultrasound (Phillips HDI 5000) of the vastus lateralis (VL) was conducted while the participant lay in bed. Ultrasound assessment of VL muscle thickness was assessed as others have previously described (32) with some minor modifications. Briefly, several B-mode real-time images of the VL were taken in the midsagittal position at 50% and 75% of the femur length (from the anterior superior iliac spine). The ultrasound head position, before and after training, was placed relative to specific measured landmarks. The image that offered the sharpest contrast with the femur was chosen to ensure perpendicular placement of the scan head. VL muscle thickness was assessed as the average distance from the superficial aponeurosis to the deep aponeurosis at these two locations. Preliminary testing, on the same individuals, revealed that the coefficients of variation for measurements taken on the day of or several weeks apart were 1.42% ± 0.20% and 1.84% ± 0.40%, respectively.

A percutaneous biopsy sample of the VL muscle was collected from a randomly selected leg under local lidocaine anesthesia using a suction-adapted 6-mm Bergström needle (3). One portion of muscle (approximately 20–30mg) was immediately submerged in an ice-cold, pH-adjusted (7.1) relaxation solution (BIOPS buffer) containing 10-mM CaK$_2$-EGTA, 7.23-mM K$_2$-EGTA, 20-mM imidazole, 20-mM
taurine, 50-mM K-MES, 0.5-mM dithiothreitol, 6.56-MgCl₂, 5.77-mM ATP, and 15-mM creatine phosphate. The remainder of the sample was snap-frozen in liquid nitrogen and stored at −80°C for future enzyme activity, protein content analysis, lysate separation, and RNA isolation.

Peak torque of the quadriceps and biceps femoris muscles of the nonbiopsied leg was subsequently determined by dynamometry (Biodex, Shirley, NY). Participants were previously familiarized to the test at the screening. Briefly, subjects were restrained in the dynamometer, with the anatomical access of the knee joint of their dominant leg aligned with the mechanical axis of the dynamometer. After demonstration of proper technique and an explanation of the strength test protocol, subjects performed practice contractions to refamiliarize themselves with the dynamometer and to warm up. Thereafter, isometric peak torque (extension and flexion) was determined at a 60° angle. Then, isometric peak torque (extension and flexion) was determined at an angular velocity of 120° per second.

After the strength test, participants were fed a meal before leaving the unit. All testing was repeated on the posttesting day in the same order.

**RET.** After familiarization and 1RM strength testing, participants began a 12-wk whole-body progressive RET program. All exercise training sessions were performed on the same equipment at the Alumni Field House at the University of Texas Medical Branch. Exercise sessions were performed on nonconsecutive days, three times weekly, with four rest days per week, under supervision of qualified personal trainers. RET was performed at an intensity of 60%–80% of 1RM and consisted of 3–4 sets of 8–10 repetitions performed to failure for each exercise. In weeks 1–8, two sessions per week were performed at an intensity of 70% 1RM, where three sets of 10 repetitions were performed to momentary muscular failure. Each session consisted of whole-body resistance exercise that lasted approximately 60–70 min. The remaining session was performed at an intensity of 60% 1RM with the goal of not reaching momentary muscular failure on this day. In weeks 9–12, two sessions per week were performed an intensity of 80% 1RM, where four sets of eight repetitions were performed to momentary muscular failure. The third session was performed at an intensity of 60% 1RM as before. Each session consisted of whole-body resistance exercise that lasted approximately 70–90 min. Resistance exercises included flat and incline chest press; leg press, curl, and extension; seated pull-downs and rows; calf raises; and abdominal exercises. Participants rested for 1–2 min between exercises and individuals sets. Strength was retested at 3, 6, and 9 wk, so as the participants’ strength increased, absolute training loads could be adjusted to maintain a relative training intensity between 60% and 80% 1RM. A 1RM strength test was performed again upon completion of the training program as the final exercise session. To allow for unforeseen life events, participants were given 13 wk after the familiarization period to complete 36 exercise sessions. This allowed for 100% attendance. Throughout the RET intervention, subjects were asked to maintain their normal physical activity and dietary habits.

**Preparation of permeabilized muscle fibers.** After collection from participants, muscle samples preserved in BIOPS buffer were immediately transferred to the laboratory to be prepared for high-resolution respirometry (HRR). Briefly, in a six-well plate, myofiber bundles were manually separated using sharp forces in ice-cold BIOPS buffer where all visible connective tissues were removed. Chemical permeabilization of the sarcolemmal membrane was achieved by transferring myofiber bundles to 2 mL of pH-adjusted (7.1) MIR05 buffer (0.5-mM EGTA, 3-mM MgCl₂, 60-mM K-lactobionate, 20-mM taurine, 10-mM KH₂PO₄, 20-mM HEPES, 110-mM sucrose, and 1 mg/mL−1 of essential fatty acid free bovine serum albumin) containing 50μg/mL−1 saponin. Samples were agitated in MIR05 buffer containing saponin for 30 min at 4°C. Myofiber bundles were then transferred to 2 mL of MIR05 buffer alone and agitated for 15 min to ensure that any residual saponin was rinsed away. Approximately 1–3 mg of permeabilized myofiber bundles were then weighed on a precision microbalance (Mettler-Toledo, Zaventem, Belgium) and immediately transferred to an Oxygraph-2k (O2K) respirometer (Oroboros Instruments, Innsbruck, Austria).

**HRR.** Before each experiment, the polygraphic oxygen sensors of the O2K were calibrated in MIR05 buffer at air saturation. HRR measurements were performed in 2 mL of MIR05 buffer. Temperature was maintained at 37°C ± 0.01°C throughout each experiment by an electronic Peltier. Oxygen concentration within the MIR05 was determined at 2-s intervals and used to compute oxygen flux per milligram of tissue by DatLab software (Oroboros Instruments, Innsbruck, Austria). Once myofiber bundles had been introduced into the O2K chamber, an air phase was established by slightly opening the chamber. Approximately 2–3 mL of 99% oxygen was injected into each chamber to increase the oxygen concentration of the MIR05 buffer. Typically, oxygen concentration was increased to approximately 450 μM before each experiment. All experiments were performed with in a range of 200–450 μM of oxygen to ensure that oxygen diffusion would not be limiting to respiration.

Mitochondrial respiratory function was determined by the sequential addition of substrates and uncouplers. State 2 leak respiration supported primarily by electron flow through complex I of the respiratory chain (L₁) was achieved by the titration of 1.5-mM octanoyl-L-carnitine, 5-mM pyruvate, 2-mM malate, and 10-mM glutamate into the O2K chamber. Electron transfer was then coupled to phosphorylation by the addition of 5-mM ADP, accessing coupled state 3 respiration with electron transfer supported by complex I (P₁). Succinate 10 mM was added to the O2K chamber to induce maximal state 3 (oxphos) respiration with parallel electron input from complex I and II (P₁II). Cytochrome C (cyt C) 10 μM was added to access the competence of the outer mitochondrial membrane. The absence of a significant increase in respiratory flux after the addition of cyt C indicates that the outer mitochondrial membranes are intact. Finally, oxidative phosphorylation was uncoupled by the titration of carbonyl cyanide m-chlorophenylhydrazone (CCCP) to a.
final concentration of 5 μM to access maximal electron transfer capacity (E).

CS activity. Approximately 20 mg of wet muscle tissue was lyophilized for 48 h. Freeze-dried muscle tissue was dispersed free of visible blood and connective tissue. One to two milligrams of freeze-dried tissue was homogenized in a glass tissue grinder in an ice-cold 175-mM KCl buffer containing 2-mM EDTA and 1% Triton. Muscle homogenates were then centrifuged at 2000 rpm at 4°C. Supernatants were stored at −80°C until analysis for CS activity and protein content.

CS activity was determined spectrophotometrically by a modified version of the method originally devised by Srere (30). Briefly, muscle lysates were diluted one in 10 in a 100-mM Tris-HCl buffer (pH, 8.1) containing 300-mM (30). Briefly, muscle lysates were diluted one in 10 in a 100-mM Tris-HCl buffer (pH, 8.1) containing 300-μM acetyl-CoA and 100 μM of 5,5′-dithiobis-(2-nitrobenzoic acid). Thereafter, oxaloacetate was added to each well at a final concentration of 500 μM to initiate the CS reaction. Light absorbance at 412 nM was then recorded every 30 s for 10 min at 37°C (BioTek Eon™, Winooski, VT). The change in light absorbance is proportional to the reaction of 5,5′-dithiobis-(2-nitrobenzoic acid) with free thiols (Coenzyme A production after the condensation of oxaloacetate and acetyl-CoA). CS activity (μmol·g−1·min−1) was calculated from the linear change in absorbance over time.

CS activity was normalized to the protein content of the muscle lysates. Lysate protein concentration was determined using a modified Bradford assay. Samples and bovine serum albumin standards were incubated at room temperature in protein quantification dye (BIO-RAD, Hercules, CA) for 30 min. Light absorbance was read in a 96-well plate at 595 nM (BioTek® Eon™, Winooski, VT) in a 200-μL aliquot of sample mixed with protein quantification dye. Lysate protein concentrations were calculated from the extinction coefficient of the 1 mg·mL−1 bovine serum albumin standard.

Protein expression. Homogenates were prepared from 40–50 mg of muscle tissue for Western blotting analysis as previously described (7). Mitochondrial respiratory complexes I–V protein abundance was quantified using a Mitosciences® OXPHOS human antibody cocktail ab110411 (Abcam, Eugene, Oregon). The β-mercaptethanol was omitted from the sample buffer to ensure nonreducing denaturing conditions for SDS-PAGE, as per manufacturer’s instructions. Immunoblot data were normalized to the manufacturer’s internal loading control, isolated human heart mitochondria, which was loaded on all gels for comparison across blots. Data were further normalized to Ponceau S to account for differences in loading.

RNA extraction and semiquantitative real-time PCR. RNA isolation, complementary DNA (cDNA) synthesis, and real-time quantitative PCR were performed as we have previously described (8). Total RNA was isolated by homogenizing 10- to 20-mg of tissue with a handheld homogenizing dispenser (T10 Basic Ultra Turrax; IKA, Wilmington, NC) in 1 mL of TRI reagent. The RNA was separated into an aqueous phase using 0.2 mL of chloroform and subsequently precipitated from the aqueous phase using 0.5 mL of isopropanol. RNA was washed twice with 1 mL of 75% ethanol, air-dried, and suspended in a known amount of nuclease-free water. RNA concentration was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE), and RNA was DNase-treated using a commercially available kit (DNA-free; Ambion, Austin, TX). A total of 2 μg of RNA was reverse-transcribed into cDNA according to the directions provided by the manufacturer (iScript; BioRad, Hercules, CA). Real-time quantitative PCR was carried out using the PrimePCR system with a CFX Connect PCR cycler (BioRad). cDNA was analyzed with SYBR green fluorescence (Qiagen Green mix; BioRad). Validated gene targets and unique Bio-Rad assay ID are as follows: cyt C oxidase subunit 4 (COX4I1), qHsaCID0006289; cyt C oxidase assembly factor (COX18), qHsaCID0017694; mitofusin 1 (MFN1), qHsaCED0046771; nicotinamide phosphoribosyltransferase (NAMPT), qHsaCED0043104; N-myc downstream-regulated gene 2 (NDRG2), qHsaCED0056853; PPARC1A, qHsaCID0006418; and mitochondrial transfer factor A (TFAM), qHsaCED0037846. The geometric mean of three targets (ACTB, qHsaCED0036269; B2M, qHsaCID0015347; and RPL13A, qHsaCED0020417) was selected as normalization/housekeeping gene. B2M was stable before to after training for all but one subject; however, ACTB and RPL13A were stable for all subjects. Thus, the geometric mean of two targets (ACTB and RPL13A) was used as normalization/housekeeping gene. The target stability values for these normalization genes were exceptional as demonstrated by a coefficient of variation of 0.027 and M value of 0.078. Relative fold changes were determined from the Ct values using the 2−ΔΔCT method (20) calculated via Bio-Rad’s CFX Manager software.

Statistical analysis. Data are presented as group means ± SE unless stated otherwise. Group means were analyzed for significance by a two-tailed paired t-test using GraphPad Prism version 6 (GraphPad, La Jolla, CA). Significance was accepted when P ≤ 0.05.

RESULTS

Subject characteristics. Eleven young (26 ± 5 yr) healthy men participated in the current study. Basic subject characteristics before and after RET are presented in Table 1.

### Table 1. Subject characteristic before and after RET.

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>Before RET</th>
<th>After RET</th>
<th>Δ</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>26 ± 5</td>
<td>26 ± 5</td>
<td></td>
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<tr>
<td>Height (cm)</td>
<td>180 ± 9</td>
<td>180 ± 9</td>
<td></td>
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<tr>
<td>Weight (kg)</td>
<td>86 ± 14.4</td>
<td>88 ± 13.5</td>
<td>1.87 ± 2.0</td>
<td>0.010</td>
</tr>
<tr>
<td>Body mass index (kg·m−2)</td>
<td>26.5 ± 3.1</td>
<td>27.1 ± 2.7</td>
<td>0.57 ± 0.58</td>
<td>0.009</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>23.1 ± 8.2</td>
<td>22.7 ± 7.8</td>
<td>0.45 ± 1.45</td>
<td>0.332</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>62.7 ± 8.1</td>
<td>65.2 ± 7.9</td>
<td>2.54 ± 1.41</td>
<td>0.000</td>
</tr>
<tr>
<td>BMI (kg·m−2)</td>
<td>3.3 ± 0.5</td>
<td>3.3 ± 0.4</td>
<td>0.01 ± 0.03</td>
<td>0.459</td>
</tr>
<tr>
<td>% fat mass</td>
<td>27.4 ± 6.4</td>
<td>26.3 ± 6.2</td>
<td>1.1 ± 1.4</td>
<td>0.023</td>
</tr>
<tr>
<td>% fat-free mass</td>
<td>72.6 ± 6.4</td>
<td>73.7 ± 6.2</td>
<td>1.1 ± 1.4</td>
<td>0.023</td>
</tr>
<tr>
<td>VL thickness (cm)</td>
<td>2.5 ± 0.4</td>
<td>2.8 ± 0.3</td>
<td>0.3 ± 0.2</td>
<td>0.000</td>
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</table>

*Values are presented as group means ± SD.

*Bone mineral content.

*VL muscle thickness.
Total body mass increased by 1.9 ± 2 kg after 12 wk of RET (P < 0.01). Body mass index also increased significantly with RET (P < 0.01). Subjects gained a significant amount (2.5 ± 1.4 kg) of fat-free mass with RET (P < 0.001), whereas absolute fat mass was not significantly different before and after RET. Subsequently, there was a significant increase in the relative proportion of fat-free mass (P < 0.05) and a significant decrease in the relative proportion of fat mass (P < 0.05) after RET. Muscle thickness data before and after RET are presented in Table 1. RET significantly increased muscle thickness of the VL by 12% (P < 0.001). Although habitual physical activity and dietary habits were not determined in the current study, it is likely that participants increased their dietary caloric and nitrogen intake, as indicated by the increase in body mass and fat-free mass after RET.

**Muscle strength.** Muscle strength data before and after RET are presented in Table 2. RET significantly increased the isometric peak torque of both the quadriceps muscles also increased with RET (P < 0.001). The increase in isokinetic peak torque of the biceps femoris muscle group after RET did not reach statistical significance.

**Skeletal muscle mitochondrial respiration.** Mitochondrial respiratory capacity per milligram wet weight of muscle tissue was increased by RET (Fig. 1). State 2 leak respiration (L) was increased twofold by RET (10.7 ± 1.5 vs 21.8 ± 3.2 pmol s⁻¹ mg⁻¹; P < 0.05). Similarly, coupled state 3 respiration with electron input from complex I (P₁) increased twofold after RET (30.2 ± 2.8 vs 58.9 ± 3.5 pmol s⁻¹ mg⁻¹; P < 0.001). Maximal coupled (oxphos) respiration with electron input from both complex I and II of the electron transport chain (P₁,II) increased 1.4-fold after RET (54.5 ± 6.5 vs 75.6 ± 5.8 pmol s⁻¹ mg⁻¹; P < 0.05). The addition of cyt C had little effect on respiratory flux, suggesting that the muscle biopsy procedure and the preparation of permeabilized myofiber bundles did no damage to the outer mitochondrial membrane. Addition of 10 μM of cyt C increased respiration by 2.1% ± 1.5% on average before RET and 1.6% ± 1.0% on average after RET. Maximal uncoupled mitochondrial respiration, a marker of mitochondrial respiratory capacity, was achieved by titration of the ionophore CCCP. RET significantly increased electron transfer system capacity (E) by 65% (64.0 ± 5.1 vs 104.4 ± 9.8 pmol s⁻¹ mg⁻¹; P < 0.001).

**Skeletal muscle oxidative capacity.** Skeletal muscle CS activity, a marker of oxidative capacity, is presented in Figure 2A. RET tended to increase CS activity in the skeletal muscle (56.1 ± 4.5 vs 65.8 ± 5.4 (μmol·g⁻¹·min⁻¹)/ (mg protein)). However, this 17% increase was not statistically significant. CS activity was not correlated with maximal coupled mitochondrial respiration (P) before (Fig. 2B) or after (Fig. 2C) RET. Maximal respiratory capacity (E) correlated with maximal coupled respiration (P) both before (R = 0.96, P < 0.001) (Fig. 2D) and after (R = 0.92, P < 0.001) (Fig. 2E) RET.

**Skeletal muscle mitochondrial function.** Skeletal muscle mitochondrial respiration values were normalized to maximal uncoupled respiration (E) to control for mitochondrial respiratory capacity (Fig. 3A). Leak respiration with complex I substrates (L) normalized to E was not altered by RET (0.17 ± 0.02 vs 0.22 ± 0.03). Coupled respiration supported by complex I (P₁) was significantly increased by RET when normalized to E (0.48 ± 0.04 vs 0.60 ± 0.04, P < 0.05). Coupled respiration supported by both complex I and II was not altered by RET when normalized to E (0.83 ± 0.05 vs 0.73 ± 0.03). The RCR for ADP was not different after RET (3.2 ± 0.4 vs 3.5 ± 0.6) (Fig. 3B). The substrate control ratio (SCR) for succinate was significantly reduced after RET (1.8 ± 0.1 vs 1.3 ± 0.1; P < 0.001) (Fig. 3C).

**Electron transport chain protein expression.** The protein abundances of complex I to IV of the electron transport chain and ATP synthase (complex V) are presented in Figure 4. Complex II, III, and IV protein expression was not different after RET. Similarly, complex V protein expression was unaltered by RET. The protein expression of complex I of the electron transport chain (NADH oxidase) increased by 11% with RET (P = 0.054).

**Select mitochondrial mRNA expression.** The mRNA expression of COX18, MNF1, NDRG2, peroxisome proliferator-activated receptor gamma coactivator (PGC1α), and TFAM was unaltered by RET, whereas the mRNA

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**TABLE 2**. Muscle strength before and after 12 wk of RET.

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<th>Before</th>
<th>After</th>
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<th>P</th>
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<tbody>
<tr>
<td></td>
<td></td>
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<tr>
<td>Leg isometric peak torque (N m⁻¹)</td>
<td>282 ± 18</td>
<td>324 ± 19</td>
<td>15</td>
<td>0.0001</td>
</tr>
<tr>
<td>Flexion</td>
<td>153 ± 10</td>
<td>170 ± 11</td>
<td>11</td>
<td>0.0008</td>
</tr>
<tr>
<td>Extension</td>
<td>195 ± 9</td>
<td>212 ± 11</td>
<td>9</td>
<td>0.0008</td>
</tr>
<tr>
<td>Leg isokinetic peak torque (N m⁻¹)</td>
<td>121 ± 6</td>
<td>126 ± 8</td>
<td>5</td>
<td>0.2104</td>
</tr>
<tr>
<td>Flexion</td>
<td>121 ± 6</td>
<td>126 ± 8</td>
<td>5</td>
<td>0.2104</td>
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Values are presented as group means ± SE.
expression of COX4I1 and NAMPT was significantly increased after RET ($P < 0.05$) (Fig. 4C).

**DISCUSSION**

The number and intrinsic function of mitochondria within skeletal muscle influence substrate metabolism and exercise capacity. Skeletal muscle mitochondria exhibit marked plasticity in response to numerous physiological stimuli and pathophysiological states (4,17,23,24,27), making them a target for interventions aimed at improving metabolic health and physical function. Aerobic exercise has long been known to be a potent regulator of skeletal muscle oxidative capacity (12,13,36), as chronic endurance exercise increases mitochondrial number/density and intrinsic mitochondrial function (15). In contrast, surprisingly little is known regarding the effect of resistance exercise on skeletal muscle mitochondrial function.

We determined the effects of chronic RET on skeletal muscle mitochondrial function. We show that RET elicits both quantitative and qualitative adaptations in skeletal muscle mitochondrial respiration. This data suggest that RET is a means of augmenting mitochondrial respiratory capacity and function in skeletal muscle.

RET has received significant interest recently as a relatively straightforward strategy for increasing muscle mass and strength. In the current study, a 12-wk progressive RET program resulted in a 1.8-kg increase in body mass. This was attributable to increased fat-free mass. Indeed, the relative contribution of fat and fat-free mass to whole-body mass significantly changed over the study period, with participants becoming leaner. Thus, RET was effective in increasing body mass, and in particular, fat-free mass (i.e., skeletal muscle), as evidenced by a 12% increase in VL thickness. Twelve weeks of RET also significantly increased strength. Specifically,
A novel finding of the current study was that RET significantly increased skeletal muscle mitochondrial respiration. To our knowledge, this is the first prospective study to show that chronic RET augments skeletal muscle mitochondrial respiration. Specifically, we show a marked increase in mitochondrial respiration coupled to ATP production (P) after RET, where the increase in P when complex I substrates were provided (P<sub>1</sub>) was particularly striking. These data show that the respiratory capacity and perhaps more importantly, the ATP-producing capacity of skeletal muscle mitochondria significantly increased after RET. These results are in good agreement with those of Salvadego et al. (28), who showed that coupled skeletal muscle mitochondrial respiration was significantly greater in a cross-section of resistance-trained athletes when compared with that in untrained individuals. Furthermore, the current data are similar to those showing that aerobic training increases skeletal muscle mitochondrial function (15,25).

Mitochondrial respiratory capacity (E) was assessed in uncoupled organelles using the proton ionophore CCCP. CCCP dissipates mitochondrial membrane potential and increases respiration above that of maximal coupled respiration because the inhibitory effect of proton accumulation on electron transfer is removed (9). RET significantly increased mitochondrial respiratory capacity (E) (Fig. 1). These findings are similar to those showing that mitochondrial respiratory capacity is greater in endurance-trained individuals compared with that in untrained individuals (15). Furthermore, mitochondrial respiratory capacity (E) was correlated with maximal coupled mitochondrial respiration (P) before (R = 0.96) (Fig. 3E) and after (R = 0.92) (Fig. 3F) RET. Collectively, this suggests that like aerobic exercise training (8,13), RET increases coupled mitochondrial respiration and mitochondrial respiratory capacity.

A benefit of determining mitochondrial function by HRR is that numerous respiratory states can be measured sequentially within the same sample. In the current study, leak (L), phosphorylating (P), and maximal (E) mitochondrial respiration were determined in each myofiber preparation within a single experiment. Critically, this means that respiratory fluxes were determined within the same pool of mitochondria. An advantage of this approach is that sensitivity of a sample to a particular substrate or uncoupler reflects the intrinsic functional characteristics of the mitochondrial pool within that sample and is independent of mitochondrial density. In the current study, normalizing leak (L) and phosphorylating (P)

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**FIGURE 3**—Specific mitochondrial respiration and coupling control. Skeletal muscle mitochondrial respiration normalized to respiratory capacity (E) before and after RET (A). B, The RCR for ADP calculated as P<sub>1</sub>/L<sub>1</sub>. C, The SCR for succinate calculated as P<sub>1</sub>−P<sub>1</sub>/P<sub>1</sub>. *P < 0.05, ***P < 0.001.
respiratory states to respiratory capacity (E) provides a means of normalizing absolute respiratory rates to an internal control and thus accounts for any change in respiratory capacity/mitochondrial density after RET.

Our present data show that the ratio of leak mitochondrial respiration to mitochondrial respiratory capacity (L/E) was unchanged by 12 wk of RET (Fig. 3A). Interestingly, the ratio of coupled respiration supported by complex I substrates to respiratory capacity (PI/E) was significantly increased by RET (Fig. 3A), suggesting that in addition to quantitative alterations in respiratory capacity, chronic RET brings about qualitative changes in skeletal muscle mitochondria. However, when coupled respiration was supported by both complex I and II substrates, the ratio of P/E was not different, again suggesting that increases in maximal coupled respiration after RET (P_{I+II}) are largely supported by increased respiratory capacity.

The RCR for ADP, an indicator of how coupled mitochondria are, was calculated as the ratio of P/I to L/I respiration. The RCR for ADP was not altered by chronic RET (Fig. 3B). These current data are in contrast to those of Salvadego et al. (28), who demonstrated that skeletal muscle mitochondrial RCR for ADP was greater in a cohort of resistance-trained and untrained individuals. Furthermore, Salvadego et al. (28) used a slightly different substrate combination than the one used in the current study. With that said, our finding that chronic resistance exercise does not alter the RCR for ADP (Fig. 3B) is supported by our flux control ratio data (Fig. 3A), where L/E and P_{I+II}/E were not different after RET, suggesting that the proportions of respiratory capacity accounted for by leak and coupled respiration were similar before and after RET.

In the current study, P_{I}/E increased after RET. This alteration in intrinsic mitochondrial function is indicative of skeletal muscle mitochondria becoming more sensitive to the complex I substrate NADH. In support of this, the SCR for succinate (P_{I+II}/P_i) was significantly reduced after RET. The conversion of succinate to fumarate in the Krebs cycle produces FADH₂, from which electrons are transferred to ubiquinone via the inner mitochondrial membrane-bound enzyme succinate dehydrogenase (complex II of the electron transport chain). This suggests that the contribution of complex I and II to total electron transfer and thus mitochondrial respiration is altered.

FIGURE 4—Altered mitochondrial gene expression and protein abundance after RET. A, Mitochondrial respiratory chain protein levels determined in cytoplasmic skeletal muscle lysates before (black bars) and after (gray bars) 12 wk of RET. I, II, III, IV, and V represent complex I (NADH oxidase), complex II (succinate dehydrogenase), complex III (cytochrome oxidoreductase), complex IV (cytochrome C oxidase), and complex V (ATP synthase) of the respiratory chain. B, Representative blot images of the human heart mitochondria isolate internal control and a pre- and postsample (loaded in duplicate). C, Changes in RNA levels of COX18, COX4I1, MFN1, NAMPT, NDRG2, PGC1α, and TFAM before and after RET. *P < 0.05.
after RET, where complex I electron transfer to ubiquinone increases, while complex II electron transfer to ubiquinone decreases after RET.

Blunted mitochondrial sensitivity to succinate after RET suggests reduced electron flow from complex II. However, because electron flow from complex I to ubiquinone increased after RET, as did both coupled (P) and uncoupled (E) respiration, it would seem that there is a shift in the relative contribution of complex I and II to maximal electron transfer, as opposed to diminished complex II function. This is an important point, as previous studies have reported reduced complex II activity after chronic RET (2,5), concluding that RET may reduce skeletal muscle oxidative capacity. Furthermore, although we show that the contribution of complex II to total respiratory capacity is diminished, mitochondrial respiratory capacity increases with RET. Such a finding underscores the fact that caution should be exercised when attempting to infer deficits in mitochondrial function from single measurements of mitochondrial enzyme activity.

In accordance with functional data showing significant increase in coupled respiration supported by electron input from complex I of the electron transport chain, we found that the complex I protein abundance was also significantly elevated after 12 wk of RET (Fig. 4). This adds credence to our supposition that RET results in qualitative changes in mitochondrial respiratory function, where complex I function is augmented. Interestingly, although the protein abundance of the other four components of the electron transport chain was unaltered by chronic RET, the fact that complex II protein levels did not change (Fig. 4) further suggests that reduced succinate sensitivity after RET is likely due to increased complex I function rather than deficit in complex II abundance or function.

To further examine the mechanisms underpinning improvements in mitochondrial function after RET, we examined the expression of several mRNA transcripts before and after training. It has been postulated that increased content of mitochondrial proteins is driven by increased transcriptional regulation of mitochondrial biogenesis (14). Similar to previous reports (1), we did not see a pronounced increase in the expression of mRNA involved in mitochondrial biogenesis (COX18, MFN1, NDRG2, PPARC1A (PGC1α), and TFAM) assessed 3 d after the last day of RET. It should be noted that marked improvements in aerobic capacity can be seen without chronic increases (31) or even a decrease (16) in markers of mitochondrial biogenesis. It seems that PGC1α, because of its short half-life, may be more reflective of a transcriptional regulation of the acute response, and thus, the timing of biopsy 3 d after exercise in the case of the current study) may explain the absence of any transcriptional response. However, mRNA expression of COX4I1 and NAMPT significantly increased after RET. Increased COX4I1 expression after RET has been reported previously (1) and unlike PGC1α may reflect chronic transcriptional regulation of mitochondria not necessarily due to an acute training response (31). Given that complex IV (COX) is the location of electron transfer to molecular oxygen within mitochondria lends some mechanistic insight into the increased respiratory capacity seen after RET. NAMPT controls conversion of nicotinamide to nicotinamide adenine dinucleotide (NAD+), the principle electron carrier within the mitochondrion. Cross-sectional and longitudinal studies demonstrate that NAMPT mRNA and protein are increased with aerobic-like exercise and that the NAMPT protein content is correlated with mitochondrial content (6). This current finding may be linked to increased complex I function because complex I (NADH oxidase) respiratory flux after RET may require greater mitochondrial NAD+.

To the best of our knowledge, this is the first study to comprehensively determine the effect of chronic RET on mitochondrial respiratory capacity within the skeletal muscle. Our novel findings demonstrate that a 12-wk RET program results in both qualitative and qualitative adaptations in skeletal muscle mitochondria of young healthy adults. These changes seem to occur with modest changes in mitochondrial proteins and mRNA. Increased capacity for coupled respiration particularly supported by complex I activity was accompanied by increased mitochondrial respiratory capacity. Collectively, these findings demonstrate that chronic RET improves mitochondrial respiratory function within the skeletal muscle.

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