Aerobic exercise training improves whole muscle and single myofiber size and function in older women

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Harber MP, Konopka AR, Douglass MD, Minchev K, Kaminsky LA, Trappe TA, Trappe S. Aerobic exercise training improves whole muscle and single myofiber size and function in older women. Am J Physiol Regul Integr Comp Physiol 297: R1452–R1459, 2009. First published August 19, 2009; doi:10.1152/ajpregu.00354.2009.—To comprehensively assess the influence of aerobic training on muscle size and function, we examined seven older women (71 ± 2 yr) before and after 12 wk of cycle ergometer training. The training program increased (P < 0.05) aerobic capacity by 30 ± 6%. Quadriceps muscle volume, determined by magnetic resonance imaging (MRI), was 12 ± 2% greater (P < 0.05) after training and knee extensor power increased 55 ± 7% (P < 0.05). Muscle biopsies were obtained from the vastus lateralis to determine size and contractile properties of individual slow (MHC I) and fast (MHC IIa) myofibers, myosin light chain (MLC) composition, and muscle protein concentration. Aerobic training increased (P < 0.05) MHC I fiber size 16 ± 5%, while MHC IIa fiber size was unchanged. MHC I peak power was elevated 21 ± 8% (P < 0.05) after training, while MHC IIa peak power was unaltered. Peak force (Po) was unchanged in both fiber types, while normalized force (Po/cross-sectional area) was 10% lower (P < 0.05) for both MHC I and MHC IIa fibers after training. The decrease in normalized force was likely related to a reduction (P < 0.05) in myofibrillar protein concentration after training. In the absence of an increase in Po, the increase in MHC I peak power was mediated through an increased (P < 0.05) maximum contraction velocity (Vo) of MHC I fibers only. The relative proportion of MLC1s (Pre: 0.62 ± 0.01; Post: 0.58 ± 0.01) was lower (P < 0.05) in MHC I myofibers after training, while no differences were present for MLC2s and MLC3s isoforms. These data indicate that aerobic exercise training improves muscle function through remodeling the contractile properties at the myofiber level, in addition to pronounced muscle hypertrophy. Progressive aerobic exercise training should be considered a viable exercise modality to combat sarcopenia in the elderly population.

AERobic exercise training is widely recommended as part of the exercise prescription for older adults because of its positive benefits on cardiovascular health, glucose metabolism, and body composition (31). Surprisingly, the influence of aerobic exercise on muscle size and function in older adults has not been well characterized. There is evidence that aerobic exercise can improve muscle function (13, 53), although many investigations have reported that aerobic training does not alter whole muscle size in the elderly population (11, 13, 23, 38, 39, 53, 54). However, most of these studies permitted weight loss during the exercise intervention and/or examined subject populations with conditions, such as type 2 diabetes or heart failure, which may be confounding factors for muscular growth and adaptation.

Historically, it is known that aerobically trained athletes display larger muscle fibers compared to controls (9, 10, 18, 19); however, limited data are available to describe the influence of aerobic training on myofiber size in older adults. A cross-sectional analysis of older runners (63 yr) reported larger type I (slow) myofibers than younger subjects (8). These findings were later verified by the same group, who reported significant myofiber hypertrophy of both slow and fast myofibers after 9–12 mo of a walking/jogging program in older (64 yr) men and women (7). More recently, it has been shown that aerobic exercise acutely (37) and chronically (38) stimulates protein synthesis in old individuals, suggesting that aerobic exercise alters protein metabolism in a manner that may be conducive for skeletal muscle growth. To date, no study has examined both whole muscle and individual myofiber size in response to aerobic training in older adults to verify whether the training induced myofiber hypertrophy was associated with hypertrophy at the whole muscle level.

There is a growing body of evidence that aerobic exercise alters the intrinsic contractile properties at the cellular level in younger adults. We recently reported that slow and fast myofibers from collegiate runners have a higher contraction velocity and generate greater peak power compared to recreationally active runners (19). Additionally, 13 wk of aerobic training increased normalized force, contraction velocity, and peak power production at the cellular level, independent of changes in muscle fiber size (47). The enhanced contraction velocity that we have reported with aerobic training is supported by data in run-trained rats (36) and chronically trained distance runners (57). Thus, it appears that aerobic training alters muscle function through remodeling the contractile characteristics at the cellular level, independent of changes in muscle size.

The purpose of this investigation was to comprehensively assess the influence of aerobic training on whole muscle and single myofiber size and function in older women. We hypothesized that muscle function would be enhanced in the absence of muscle hypertrophy after aerobic training. Further, we hypothesized that a remodeling of the contractile properties at the cellular level would reflect changes at the whole muscle level.

MATERIALS AND METHODS

Subjects

Seven older women (aged 71 ± 2 yr) volunteered to participate in this investigation (Table 1). Each subject underwent a thorough physical examination, which included a detailed medical history, blood chemistry profile, pulmonary function test, and resting and peak fitness testing. The participants were re-assessed in the laboratory following a 2-wk familiarization to the cycle ergometer. Subjects were asked to maintain their normal diet, but were instructed to avoid vigorous physical activity 3 days before each testing session. The study was conducted in accordance with the human research ethics regulations of Ball State University and with all procedures conducted in accordance with the Declaration of Helsinki. Each participant provided written informed consent. After the first meeting, the subjects were then randomly assigned to either a control group (n = 4) or an aerobic training group (n = 3). The control group was instructed to maintain their normal diet and lifestyle and to refrain from all forms of exercise during the study period. The aerobic training group was instructed to perform lower extremity endurance training, three times per week, on a cycle ergometer. Each training session consisted of 60 min of continuous cycling at 60% of the peak heart rate achieved during the familiarization test. The training program lasted 12 wk and included progression of training load.

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exercise electrocardiograms (ECG). Subjects were excluded on the basis of the following criteria: 1) body mass index \(\geq 28 \text{ kg/m}^2\); 2) type 1 or type 2 diabetes; 3) uncontrolled hypertension; 4) active cancer, cancer in remission, or having received treatment for any form of cancer in the previous 5 yr; 5) coronary artery disease; 6) cardiovascular disease (e.g., peripheral arterial disease and peripheral vascular disease); 7) abnormal thyroid function; 8) physically active defined as one who has completed regular aerobic or resistance exercise more than once per wk for 20 min or longer during the previous year; 9) chronic and/or regular NSAID consumption; and 10) any condition that presents a limitation to exercise training (e.g., severe arthritis, chronic obstructive pulmonary disease, neuromuscular disorder, moderate or severe cognitive impairment, Alzheimer's disease, vertigo, dizziness). All subjects were cleared to participate by the study physician. Written informed consent was obtained from each subject for all procedures following approval by the Institutional Review Boards of Ball State University and Ball Memorial Hospital.

General Study Design

Eligible volunteers underwent a series of baseline measurements for the determination of aerobic capacity using a graded exercise test on a cycle ergometer, body composition assessment via dual energy X-ray absorptiometry, whole muscle volume assessed with magnetic resonance imaging (MRI), whole muscle function (i.e., power), of the knee extensor muscle group, and a muscle biopsy procedure for the assessment of single muscle fiber size, contractile mechanics, and protein morphology. Upon completion of the baseline measurements, subjects performed 12-wk of progressive aerobic exercise training. Following the training intervention, all subjects repeated the testing procedures that occurred at baseline.

Experimental Procedures

Aerobic capacity/graded exercise test. Subjects performed a physician-supervised graded exercise test for the assessment of aerobic power (\(\text{VO}_2\text{max}\)) before and after the 12-wk aerobic training intervention. The test was performed on an electronically braked cycle ergometer (SensorMedics Ergometrics 800) beginning at a very low workload (\(\approx 10\) W). After a brief warm-up at the initial workload, the workload was progressively increased in 1-min stages until exhaustion with a total test time of 10–12 min. During the test, the subject's heart rate, blood pressure, rating of perceived exertion, and electrocardiograms (ECG) were monitored. Subjects were excluded on the basis of any condition that presents a limitation to exercise training (e.g., severe arthritis, chronic obstructive pulmonary disease, neuromuscular disorder, moderate or severe cognitive impairment, Alzheimer's disease, vertigo, dizziness). All subjects were cleared to participate by the study physician. Written informed consent was obtained from each subject for all procedures following approval by the Institutional Review Boards of Ball State University and Ball Memorial Hospital.

Whole muscle volume/magnetic resonance imaging. Proton MR images of the thigh were measured before and after the 12-wk aerobic training intervention using a General Electric Signa 1.5 Tesla imaging system at standard settings (TR/TE = 2,000/9 ms), as we have previously described (52). Bilateral scans were obtained after 1 h of supine rest to avoid the influence of potential fluid shifts (4). Subjects were positioned with an adjustable foot restraint for fixation of joint angles and thus, muscle lengths. A standard of 1% CuSO\(_4\) was placed along the length of the leg, such that it appears in the field of view of all images to eliminate bias in viewing images, resulting from day to day variations in the magnetic field and thus, pixel density. The pixel density of each scan was normalized to this standard for the determination of the relative muscle density. Contiguous, 1-cm interleaved serial scans were obtained from the femoral artery to the arterial surface of the femur. MR images were electronically transferred to a personal computer (Macintosh Power PC) and analyzed with National Institutes of Health (NIH) Image software (ver. 1.60) using manual planimetry. Average muscle cross-sectional area for all analyzed images was taken as the average of each slice from the first distal image containing the vastii (vastus lateralis, vastus medialis, vastus intermedius) and rectus femoris and summed for total quadriiceps femoris. Muscle volume (cm\(^3\)) was calculated by multiplying the CSA by the slice thickness (1 cm) for all analyzed images. All measurements were made by the same investigator in a blinded fashion.

Skeletal muscle function. Peak power and peak isometric force of the knee extensor muscle group were assessed before and after the 12-wk aerobic training intervention using an inertial ergometer (Inertial Technology) connected to a strain gauge load cell and potentiometer interfaced with a personal computer (Gateway E-4200) (64, 71). Following multiple orientation sessions to become familiar with the knee extensor device, subjects performed three identical sessions separated by at least 2 days. All tests were bilateral. Prior to any testing, subjects performed a 10-min warm-up on a stationary bicycle at a self-selected intensity, as well as with stretching exercises of the lower limbs followed by small loads on the resistance apparatus. Peak isotonic force was assessed at a fixed knee joint angle of 120°. For both peak power and peak force, subjects completed three submaximal repetitions followed by three maximal attempts with 3-min rest between sets. The concentric power output normalized throughout the full range of motion was used. Knee extensor power and force were normalized to quadriceps muscle CSA for an index of normalized power (Watts/cm\(^2\)) and normalized force (Nm/cm\(^2\)) at the whole muscle level.

Muscle biopsy procedure. Percutaneous needle biopsies were obtained from the vastus lateralis muscle, according to the methods of Bergstrom (5) before and after the 12-wk aerobic training intervention. The muscle specimen was immediately divided into longitudinal sections, dissected free of adipose and connective tissue, frozen in liquid nitrogen, or processed for single muscle fiber physiology, as described below. Posttraining muscle biopsies were obtained 48–72 h after the last exercise session.

Single fiber physiology studies. A bundle of muscle fibers was placed in cold solution for the determination of contractile properties. The solution contained (in mM): 125 K propionate, 2.0 EGTA, 4.0 ATP, 1.0 MgCl\(_2\), 20.0 imidazole (pH 7.0), and 50% (vol/vol) glycerol. Fibers were kept in this solution for a minimum of 1 day but not longer than 4 wk. Individual muscle fibers were analyzed for diameter, peak force (\(P_{\text{max}}\)), shortening velocity (\(V_s\)), and power characteristics. Detailed descriptions and illustrations of these procedures have been presented in our laboratory's previous work (45, 46).

Single fiber size. A video camera (Sony CCD-IRIS, DXC-107A, Japan) connected to the microscope and interfaced to a computer allowed viewing on a computer monitor and storage of the digitized images of the muscle fibers during the experiment. Fiber diameter was determined from a captured computer image taken with the fiber briefly suspended in air (<5 s). Fiber width (diameter) was determined at three points along the length of the captured computer image using public domain software (NIH Image v1.61) and averaged to provide a mean diameter measurement. Fiber CSA was determined from the fiber diameter with the assumption that the fiber forms a circular shape while suspended in air, as we have previously done (34, 43, 45, 46, 50).

Force determination. Resting force was monitored with the fiber in pCa 9.0 solution and then the fiber was maximally activated in pCa 4.5 solution. Force determination (\(P_{\text{max}}\)) was determined in each fiber by computer subtraction of the force baseline from the peak in the pCa 4.5 solution. Normalized force (\(P_{\text{max}}/\text{CSA}\)) was determined from the relationship of peak force and fiber CSA.

Unloaded contraction velocity determination. Fiber velocity determination (\(V_s\)) was measured by the slack test technique, as described by Edman (12). Briefly, the fiber was brought to peak tension and then rapidly shortened, so that tension returned to baseline. The time between the onset of slack and redevelopment of tension (i.e., the period of unloaded shortening) was measured by computer analyses. Four different slack distances (each <15% of fiber length) were used...
for each fiber and the slack length was plotted as a function of the duration of unloaded shortening. Velocity (FL·s⁻¹) was calculated by dividing the slope of the fitted line by the fiber segment length, and the data were normalized to a sarcomere length of 2.50 μm.

**Power determination.** Submaximal isotonic load clamps were performed on each fiber for determination of force-power parameters. Each fiber segment was fully activated and subjected to a series of three isotonic load steps, resulting in a total of 15–18 isotonic contractions. Po and Vo data points derived from the isotonic contractions were fit using the Hill equation (22). Only individual experiments with r² greater than or equal to 0.98 were included for analysis. Fiber power was calculated from the fitted force-velocity parameters (P₀, Vₘₐₓ, and αP₀, where α is a force constant). Absolute peak power (μN·FL⁻¹·s⁻¹) was defined as the product of force (in micronewtons) and shortening velocity (Vₘₐₓ in FL·s⁻¹), while normalized power (W·l⁻¹) was defined as the product of normalized force, (i.e., force/fiber CSA) and shortening velocity (Vₘₐₓ in FL·s⁻¹).

**Myosin heavy chain and myosin light chain isoform composition.** Following physiological measurements of single muscle fibers, each fiber was solubilized in 80 μl of 10% SDS sample buffer and stored at −20°C until assayed. In order to determine the myosin heavy chain (MHC) and myosin light chain (MLC) composition, fibers were run on a Hoefer SE 600 gel electrophoresis system that consisted of a PAG gel slab in a horizontal tank, 20 mM imidazole, and 5 mM EDTA; pH 6.8. Following gel electrophoresis, gels were silver-stained as described by Giulian et al. (17). MHC and MLC isoforms were identified according to final relative migration position from the SDS-PAGE/silver staining. The MHC were categorized as MHC I, Iia, Ix, I/IIa, I/IIa/Ix, I/IIx, and IIa/Ix.

**Water content and protein concentration.** The wet weight of a muscle sample (~10 mg) was determined on a precision microbalance at −35°C, and the sample was then freeze-dried for 72 h. The dry weight of each muscle sample was then determined at −35°C. Muscle water content was determined from the difference in dry and wet weight for each muscle sample and expressed as a percentage of initial wet weight. Each muscle sample was then homogenized in 40 volumes of cold buffer (250 mM sucrose, 100 mM potassium chloride, 20 mM imidazole, and 5 mM EDTA; pH 6.8) in a ground glass homogenizer. Samples were then centrifuged at 21,000 g for 30 min at 4°C. The supernatant was taken as the sarcoplasmic protein fraction, and the pellet was resuspended in 40 volumes of buffer and taken as the myofibrillar protein fraction (40). Aliquots of the homogenate (total protein), sarcoplasmic, and myofibrillar protein fractions were measured for protein concentration using the bicinchoninic acid assay (Thermo Scientific) with bovine serum albumin used as the protein standard, as we have previously performed (45). The amount of protein in each of the three fractions was normalized to the wet weight of each muscle sample.

**Aerobic exercise training protocol.** Subjects performed 12 wk of aerobic exercise training on an electronically braked cycle ergometer (Stairmaster Stratus 3300 CE). Exercise intensity was based on the resting heart rate, determined from resting ECG, and maximum heart rate achieved during the graded exercise test [i.e., heart rate reserve (HRR)]. Exercise intensity, as %HRR, was based on the difference between maximum heart rate and resting heart rate. The ergometer workload (Watts) was adjusted in order for subjects to maintain target heart rate, and subjects were exercised at a pedaling frequency of 70–90 rpm. A total of 42 exercise sessions were performed. Duration (20–45 min), intensity (60–80% HRR), and frequency (3 or 4 sessions/wk) of exercise were progressively increased throughout the 12 wk to optimize the training response. The last 5 wk of the exercise program consisted of 45 min at 80% intensity per week. Each exercise session was monitored in its entirety by a member of the investigative team to ensure that the prescribed exercise intensity and duration were obtained. Additionally, the subject’s body weight was measured and recorded prior to each exercise session (3 or 4 times/wk) and subjects were counseled, if necessary, to make modifications in dietary intake in order to maintain body weight.

**Statistical analysis.** Data are expressed as means ± SE. Statistical significance for group means for Pre and Post time points for all variables was assessed with the use of a paired two-tailed Student’s t-test. Single muscle fiber physiology variables were averaged in a fiber-type specific manner and pooled by subject. The average of the subject means was used for statistical analysis. A P value of less than 5% (P < 0.05) was considered statistically significant.

**RESULTS**

**Subject Characteristics and Exercise Compliance**

Each subject completed all 42-exercise sessions for an exercise compliance of 100%. The cumulative exercise time for the training program was 28 h and each thigh muscle group performed >120,000 muscle contractions over the 12-wk program (assuming an average of at least 70 rpm).

The progressive aerobic training program improved (P < 0.05) absolute and relative aerobic capacity by 29 ± 6% and 30 ± 6%, respectively. Body weight remained stable (P > 0.05) during the training intervention, while there were trends (P ≤ 0.10) for decreasing absolute fat mass and body fat percentage and increasing absolute fat-free mass as a result of the training (Table 1).

**Whole Muscle Volume and Function**

Whole muscle volume of the quadriceps femoris muscle group, determined with MRI, increased (P < 0.05) 12 ± 2% with aerobic training. Whole muscle knee extensor function, determined as peak power during knee extension exercise, was 55 ± 7% higher (see Table 1; P < 0.05) after the aerobic training program. Peak power normalized to muscle size was increased (P < 0.05) 14 ± 4% after aerobic training. Peak isometric force production was 35 ± 7% higher (P < 0.05) and normalized force was 22 ± 7% higher (P < 0.05) after training. Additionally, peak work output during the graded cycle exercise test was 40 ± 5% higher (P < 0.05) after training.

**Table 1. Aerobic capacity, body composition, muscle size, and muscle function parameters before (Pre) and after (Post) 12 wk of aerobic training in older (71 ± 2 yr) women (n = 7)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre</th>
<th>Post</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic capacity, L·min⁻¹</td>
<td>11.1±0.1</td>
<td>14.1±0.1*</td>
<td>29±6</td>
</tr>
<tr>
<td>ml·kg⁻¹·min⁻¹</td>
<td>15.9±1.0</td>
<td>20.5±0.9*</td>
<td>30±6</td>
</tr>
<tr>
<td>Maximum heart rate, bpm</td>
<td>154±7</td>
<td>157±6</td>
<td>N/A</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>68.0±4.8</td>
<td>67.2±4.5</td>
<td>N/A</td>
</tr>
<tr>
<td>BMI, kg·m⁻²</td>
<td>25.2±1.8</td>
<td>25.1±1.8</td>
<td>N/A</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>40.7±3.4</td>
<td>39.8±3.5*</td>
<td>&lt;2.4±0.0</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>27.6±4.3</td>
<td>26.4±4.1*</td>
<td>&lt;3.9±1.6</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>39.2±1.4</td>
<td>39.6±1.4*</td>
<td>0.9±0.4</td>
</tr>
<tr>
<td>Quadriceps muscle volume, cm³</td>
<td>587±55</td>
<td>654±60*</td>
<td>12±2</td>
</tr>
<tr>
<td>Knee extensor power, W</td>
<td>241±42</td>
<td>296±45*</td>
<td>55±7</td>
</tr>
<tr>
<td>Normalized power, W·cm⁻²</td>
<td>5.8±0.8</td>
<td>6.5±0.8*</td>
<td>14±4</td>
</tr>
<tr>
<td>Knee extensor peak isometric force, Nm</td>
<td>199±25</td>
<td>261±27*</td>
<td>35±7</td>
</tr>
<tr>
<td>Normalized force, Nm·cm⁻²</td>
<td>4.9±0.4</td>
<td>5.9±0.5*</td>
<td>22±7</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. *P < 0.05 compared to Pre. †P < 0.10 compared to Pre. N/A, not applicable.
Single Muscle Fiber Physiology

A total of 336 individual myofibers, 168 pretraining and 168 posttraining, were isolated and examined for size and contractile properties. Pretraining analysis consisted of 83 MHC I myofibers, 77 MHC IIa, four MHC I/IIa, three MHC IIa/IIx, and one pure MHC 2x. Posttraining analysis consisted of 82 MHC I myofibers, 79 MHC IIa, and seven MHC I/IIa. Because of the low amount of hybrid fiber studies, presentation and discussion of results are restricted to MHC I (slow) and MHC IIa (fast) myofibers.

Myofiber size. CSA of MHC I fibers was 16 ± 5% greater (P < 0.05) after training, while changes in the CSA of MHC IIa fibers were not statistically significant (Fig. 1). A histogram of fiber-type-specific CSAs for individual myofibers is presented in Fig. 2.

Myofiber force production. Peak isometric force (P0) was unaltered with aerobic training in both MHC I and MHC IIa fiber types (see Table 2). However, normalized force production (P0/CSA) was 8 ± 2% and 10 ± 3 lower (P < 0.05) for MHC I and MHC IIa fibers, respectively (Fig. 3).

Myofiber contraction velocity. Maximum unloaded shortening velocity (V0) was 16 ± 4% higher (P < 0.05) posttraining for MHC I fibers, while V0 of MHC IIa was unaltered by the training program (see Table 2). Vmax was not changed in either fiber type with aerobic training.

Myofiber power. Absolute peak power production of the MHC I fibers was 21 ± 8% higher (P < 0.05) after aerobic training, while no differences were observed for MHC IIa fibers (Table 2). Peak power normalized to cell volume was unaltered by the training program.

Muscle Water Content and Protein Concentration

Muscle water content increased (P < 0.05) from 71.9 ± 1.0 pre-training to 74.7 ± 0.9% post-training. In support of this finding, mean grayscale pixel value determined from the MRI scans of the quadriceps muscle was greater (P < 0.05) post-training, suggesting a higher water content in the muscle tissue. Conversely, total protein concentration trended to be lower (P < 0.06) after aerobic training and this was associated with a reduction (P < 0.05) in myofibrillar protein concentration (Fig. 4). Sarcoplasmic protein concentration was unaltered by the training program.

MLC composition. A relatively lower (P < 0.05) proportion of MLC1s was apparent in MHC I myofibers after aerobic training, while no differences were detected for MLC2s and MLC3f isoforms (see Table 3). Further, no differences were observed for the MLC3f to MLC2s ratio after training.

DISCUSSION

The goal of this investigation was to comprehensively assess the influence of aerobic training on skeletal muscle size and

<table>
<thead>
<tr>
<th></th>
<th>MHC I</th>
<th>MHC IIa</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Po, mN</td>
<td>0.81±0.06</td>
<td>0.86±0.08</td>
</tr>
<tr>
<td>V0, FL/s</td>
<td>1.02±0.05</td>
<td>1.18±0.03*</td>
</tr>
<tr>
<td>Vmax, FL/s</td>
<td>0.82±0.05</td>
<td>0.90±0.01</td>
</tr>
<tr>
<td>Absolute power,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>μN·FL·s⁻¹·s⁻¹</td>
<td>13.6±1.1</td>
<td>16.4±1.7*</td>
</tr>
<tr>
<td>Normalized power,</td>
<td></td>
<td></td>
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<tr>
<td>W/l</td>
<td>2.50±0.12</td>
<td>2.59±0.08</td>
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</table>

Data are expressed as means ± SE. *P < 0.05 compared to Pre. FL, fiber length; MHC, myosin heavy chain.

Fig. 1. Cross-sectional area (CSA) of slow [myosin heavy chain I (MHC I)] and fast (MHC IIa) myofibers before (PRE) and after (POST) 12-wk of progressive aerobic exercise training. Data are expressed as means ± SE. *P < 0.05 compared to PRE.

Fig. 2. Frequency histograms of individual slow (MHC I) (top) and fast (MHC IIa) (bottom) myofiber CSA before (PRE) and after (POST) 12-wk of progressive aerobic exercise training. Data are reported as a percentage of myofibers examined for a given CSA.
function in older women (71 ± 2 yr). The primary findings from this study were that 12 wk of progressive aerobic exercise training resulted in significant muscle hypertrophy and increased power production at the whole muscle and single muscle fiber levels. Additionally, in conjunction with improvements at the whole muscle level, normalized force production of both slow (MHC I) and fast (MHC IIa) myofibers was reduced after training, and this was associated with a lower concentration of myofibrillar protein. These results provide unique insights in the regulation of myofiber structure and function by physical activity in older individuals. Further, these data suggest that if performed with vigorous intensity in the absence of weight loss, aerobic exercise training can be an effective modality for improving muscle mass and function for older adults.

To our knowledge, this is the first investigation to report whole muscle hypertrophy following aerobic exercise training in older adults. The large improvement in muscle mass was unexpected and is similar to results from our laboratory (46, 49) and others (2, 6, 15) with regards to resistance exercise in similar subject populations. It has been suggested that aerobic exercise may help preserve muscle mass with advancing age (33, 41); however, studies that have examined whole muscle size before and after training interventions have concluded that aerobic training does not alter size at the whole muscle level (11, 13, 23, 38, 39, 53, 54). A major confounding factor in many of those studies is that subjects experienced weight loss during the exercise intervention, which may limit the capacity for muscular growth (11, 13, 38, 39, 54). Additionally, subject populations in many of these studies were overweight, obese, diabetic, or heart failure patients, introducing additional confounding variables (11, 13, 21, 54). Our investigation was unique in that we examined healthy older subjects and ensured that body weight was maintained during the training intervention. Although our subjects did not experience weight loss, favorable improvements in body composition were suggested by trends (P < 0.10) for lower fat mass and higher fat-free mass after training.

The concept of aerobic exercise as an anabolic stimulus is not intuitive; however, there are several precedents in the literature to support our findings. Endurance-trained athletes have been shown to display larger muscle fibers compared to sedentary and moderately active individuals (8, 10, 18, 19). Furthermore, the addition of aerobic exercise to the exercise

Table 3. Single myofiber myosin light chain composition of MHC I fibers before (Pre) and after (Post) 12 wk of aerobic training

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLC1s</td>
<td>0.62±0.01</td>
<td>0.58±0.01*</td>
</tr>
<tr>
<td>MLC2s</td>
<td>0.34±0.01</td>
<td>0.37±0.01</td>
</tr>
<tr>
<td>MLC3f</td>
<td>0.036±0.004</td>
<td>0.048±0.006*</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. *P < 0.05 compared to Pre. Data are presented as the average relative proportion of each MLC isoform for each myofiber. MLC, myosin light chain.
countermeasure during chronic unloading prevents skeletal muscle atrophy more effectively than resistance exercise alone (48, 51). The hypertrophy of the slow myofibers observed after the aerobic training intervention is consistent with a previous CSA of older distance runners (63 yr) (8). Additionally, aerobic exercise has been shown to acutely stimulate muscle protein synthesis (37) and can elevate basal muscle protein synthesis in older adults when performed chronically (38). These aerobic exercise-induced alterations in protein metabolism in older individuals are similar to what is observed following resistance exercise, and these responses produce an intramuscular environment that is favorable for muscle growth. The additional muscle mass that we observed after aerobic training does not appear to be solely comprising new protein accretion, as muscle water content was elevated after training. Additionally, a reduction in myofibrillar protein concentration, relative to muscle wet weight, suggests that proteins other than the primary contractile proteins, such as mitochondrial proteins, were increased with aerobic training. Furthermore, it cannot be delineated whether the muscle hypertrophy we observed was specifically due to the direct training stimulus on the muscle or a greater sensitivity to feeding in the postexercise period. Fujita et al. (16) reported that a single session of aerobic exercise enhanced the anabolic response to insulin (i.e., feeding) by stimulating muscle protein synthesis and creating a positive protein balance. Therefore, it is possible that this enhanced sensitivity to feeding in response to each exercise session contributed to the accretion of protein over the course of the training intervention.

Importantly, the muscle hypertrophy was accompanied by improvements in whole muscle power and force production. While not unequivocal, it has been reported that aerobic training can improve muscle function in the elderly in the absence of muscle hypertrophy (1, 13, 21, 53). Accordingly, we originally hypothesized that aerobic training would improve muscle function independent of changes in muscle mass by remodeling the contractile characteristics at the cellular level, which has been demonstrated in young competitive endurance athletes (19) and with endurance training in young subjects (36, 47). At the cellular level, we observed that peak power of the slow myofibers was elevated with aerobic training, which was associated with a faster contraction velocity (i.e., $V_o$), as peak force was unaltered. A similar influence of aerobic training on myofiber contraction velocity has been consistently shown in human (14, 19, 47, 58) and rodent models (36). The mechanism by which aerobic training increases contraction velocity is unclear, but may be related to MLC morphology (36, 57), although this relationship is tenuous in human skeletal muscle (26, 44, 46, 50). Interestingly, we observed a reduction in a decrease in MLC $\alpha$ with a nonsignificant ($P > 0.05$) increase in MLC$\beta$, which may partially explain the elevation in contraction velocity after training. In addition to subtle changes in MLC composition, we also observed an increase in skeletal muscle water content and a reduction in myofibrillar protein concentration that may have contributed to the elevation in contraction velocity, as both these factors influence contraction velocity by altering myofilament spacing (30, 35, 55). The higher water content of the muscle posttraining may be secondary to greater muscle glycogen stores after training, as muscle glycogen content can exert a measurable impact on muscle water content (32), and aerobic training has been shown to improve muscle glycogen levels in older subjects (29, 42). Regardless of the potential mechanisms, increasing contraction velocity at the cellular level appears to be a strategic adaptation to enhance muscle power in response to aerobic training.

An interesting finding from the present study was a reduction in normalized force (i.e., peak force normalized to cell size) at the cellular level in both slow and fast myofibers. Notably, normalized force was the only variable to be altered after aerobic training in the fast myofiber population. The decrease in normalized force resulted from an increase in cell size with no change in peak force; therefore, it is important to note that aerobic training did not compromise absolute peak force. This finding is surprising, as peak force at the cellular level is typically proportional to cell size (14, 43, 50, 56). Further, to our knowledge, this is the only exercise intervention that has observed a decrease in normalized force. The reduction in myofibrillar protein concentration that we observed in response to training likely contributed to the decrease in normalized force. These data suggest that in light of the substantial muscle hypertrophy, the myofibrillar proteins, namely actin and myosin, were not upregulated concomitantly with total muscle protein, which may be related to an age-related reduction in basal MHC synthesis rates (3). This finding is in contrast to recent reports from our laboratory that the myofibrillar protein fraction and the concentration of the main contractile proteins, myosin and actin, remain consistent during conditions of atrophy and while increasing or maintaining muscle mass with exercise countermeasures during unloading (20, 27). Additionally, the concentration of the myofibrillar protein fraction appears to increase in proportion with the total protein concentration during resistance training-induced hypertrophy over the same training duration in older subjects (Trappe T, unpublished observations). These findings highlight differential adaptations at the cellular level between aerobic and resistance exercise training in older adults. These exercise mode-specific responses are supported by a recent investigation reporting that a single session of aerobic exercise stimulates protein synthesis of mitochondrial proteins but not myofibrillar proteins, while a single session of resistance exercise stimulated the synthesis of both mitochondrial and myofibrillar protein fractions in young subjects (59). While these alterations in normalized force are intriguing and provide novel insights into muscle adaptation, the most important aspect is that these changes did not negatively impact muscle function (i.e., peak power) at the whole muscle and single muscle fiber levels.

At the myocellular level, age-related atrophy and loss of function are more pronounced in the fast myofibers (25, 28, 45), which is supported by the pretraining profile in the current set of older women (Fig. 2). This phenomenon is of high clinical relevance as the fast myofibers exhibit dramatically higher functional properties, namely, 5- to 6-fold greater power production compared to the slow myofibers. For this reason, it is important to develop interventions that target the fast-myofiber population in aging individuals. The majority of adaptations at the cellular level after aerobic training occurred in the slow-myofiber population, which is consistent with what we have reported previously in response to resistance training in similar subjects (46). Taken together, these findings suggest that the capacity for muscle hypertrophy and adaptation may be restricted to the slow-myofiber population in older women.
However, a long-term (9–12 mo) aerobic exercise program elicited hypertrophy of both slow and fast myofibers in a cohort of older (63 yr) subjects, suggesting that aerobic exercise possesses the potential to target the fast myofibers in older subjects (7). In the current study, although not significant, the fast myofibers were 20% larger on average after aerobic training. As shown in Fig. 2, there is a clear shift toward larger myofibers, for both slow and fast fibers, after aerobic training. The lack of statistical significance represents the heterogeneity in fast-myofiber response to aerobic training (Fig. 5). It is possible that a longer duration exercise intervention is necessary to completely reach the fast myofibers in the elderly population. Obviously, more work is needed to clearly elucidate the potential of aerobic training to positively influence the fast myofibers.

**Perspectives and Significance**

The benefits of aerobic exercise on cardiovascular and metabolic health in older adults have been well established (31). Our findings extend these benefits and provide evidence, for the first time, that progressive aerobic exercise training can elicit robust improvements in muscle size and performance in healthy older women. Although adaptations at the whole muscle level (size and function) were similar to resistance training-induced adaptations in this subject population, it appears that aerobic training results in qualitative changes in muscle composition, namely, increases in water content and a reduction in myofibrillar protein concentration that are not typically observed with resistance training. Additionally, aerobic training appears to remodel the contractile characteristics at the cellular level (i.e., increased contraction velocity and reduced normalized force) in conjunction with myofiber hypertrophy, mostly of the slow myofibers, to achieve an enhanced power production. These data provide novel insights into the regulation of the slow myofibers, to achieve an enhanced power production.

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**REFERENCES**

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