

Effect of resistance training on single muscle fiber contractile function in older men

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Trappe, Scott, David Williamson, Michael Godard, David Porter, Greg Rowden, and David Costill. Effect of resistance training on single muscle fiber contractile function in older men. *J Appl Physiol* 89: 143–152, 2000.—The purpose of this study was to examine single cell contractile mechanics of skeletal muscle before and after 12 wk of progressive resistance training (PRT) in older men ($n = 7$; age = 74 ± 2 yr and weight = 75 ± 5 kg). Knee extensor PRT was performed 3 days/wk at 80% of one-repetition maximum. Muscle biopsy samples were obtained from the vastus lateralis before and after PRT (pre- and post-PRT, respectively). For analysis, chemically skinned single muscle fibers were studied at 15°C for peak tension [the maximal isometric force (P_o)], unloaded shortening velocity (V_o), and force-velocity parameters. In this study, a total of 199 (89 pre- and 110 post-PRT) myosin heavy chain (MHC) I and 99 (55 pre- and 44 post-PRT) MHC IIa fibers were reported. Because of the minimal number of hybrid fibers identified post-PRT, direct comparisons were limited to MHC I and IIa fibers. Muscle fiber diameter increased 20% (83 ± 1 to 100 ± 1 μm) and 13% (86 ± 1 to 97 ± 2 μm) in MHC I and IIa fibers, respectively ($P < 0.05$). P_o was higher ($P < 0.05$) in MHC I (0.58 ± 0.02 to 0.90 ± 0.02 mN) and IIa (0.68 ± 0.02 to 0.85 ± 0.03 mN) fibers. Muscle fiber V_o was elevated 75% (MHC I) and 45% (MHC IIa) after PRT ($P < 0.05$). MHC I and IIa fiber power increased ($P < 0.05$) from 7.7 ± 0.5 to 17.6 ± 0.9 $\mu\text{N} \cdot \text{fiber lengths} \cdot \text{s}^{-1}$ and from 25.5 to 41.1 $\mu\text{N} \cdot \text{fiber lengths} \cdot \text{s}^{-1}$, respectively. These data indicate that PRT in elderly men increases muscle cell size, strength, contractile velocity, and power in both slow- and fast-twitch muscle fibers. However, it appears that these changes are more pronounced in the MHC I muscle fibers.

skeletal muscle; myosin heavy chain; peak tension; shortening velocity

DECREASES IN WHOLE SKELETAL muscle strength and size are features commonly associated with old age (13, 21, 24). In addition, it has been shown that the single muscle cell contractile velocity can decrease by as much as 40% in skeletal muscles of older animals (32) and humans (22) compared with that in young animals and humans. These impairments have been shown to contribute to the difficulty in locomotion and the increased risk for falls and subsequent fractures often observed in the elderly. These sarcopenia-related re-

ductions in skeletal muscle function have profound social and economic impacts for older adults and the health care system. It is estimated that 30 billion dollars of health care costs are directly related to sarcopenia (30).

More recently, resistance training performed by older adults has proven effective for increasing whole muscle strength and size (6, 14). In fact, humans older than 90 yr have shown positive adaptations to a resistance training stimulus (10), demonstrating that skeletal muscle maintains a degree of adaptability even in very old people. Although the resistance exercise-induced changes in whole muscle have been well documented, alterations in the contractile properties at the cell level of elderly human skeletal muscle are not known. Furthermore, it is also unclear how the contractile function of different muscle fiber types respond to a typical resistance training program in older humans.

Our goal was to determine whether the contractile function at the single fiber level is altered with resistance training in older adults. To accomplish this goal, we strength trained seven older men for 12 wk using a high-intensity progressive resistance training (PRT) program, which has been used in several previous studies (7, 10, 14). We hypothesized that 1) the PRT will cause an increase in single muscle fiber diameter, maximum shortening velocity (V_o), and power of the slow myosin heavy chain (MHC) I and fast MHC IIa muscle fiber; 2) the MHC IIa muscle fibers will be more affected than the MHC I muscle fibers in response to the PRT program; and 3) alterations in structural proteins [MHC and myosin light chain (MLC) expression] after PRT will, in part, account for the improvement in single cell contractile function in these older men.

METHODS

Subjects

Seven older male subjects participated in this investigation. Their age, height, and mass were 74.0 ± 1.8 yr, 176.5 ± 3.2 cm, and 74.6 ± 5.1 kg, respectively. Each participant

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underwent a thorough physical examination, which included a medical history evaluation, resting and exercise electrocardiograms, blood pressure measurement, and orthopedic evaluation, before the PRT program was initiated. These volunteers were not obese (less than 28 kg/m²) and were normotensive, nonsmokers, nonmedicated, and healthy, as judged by a physical examination. The volunteers were sedentary and had not performed resistance training for at least 1 yr before this study. The experimental protocol was approved by the Human Research Committees of Ball State University and Ball Memorial Hospital before data collection.

Resistance Training Program

All subjects performed a 12-wk PRT program designed to strengthen the vastus lateralis (6, 10, 14). This PRT program consisted of bilateral isotonic leg extension at 80% of their concentric one-repetition maximum (1 RM). Subjects raised the weight using a 2- to 3-s interval and used this same interval for lowering the weight. The 1 RM was reevaluated every 2 wk, and the weight was adjusted accordingly to ensure that intensity was maintained at 80%. Subjects performed the PRT three times per week (for a total of 36 training sessions) with at least 24–48 h between training sessions. Each training session consisted of two sets of 10 repetitions and a third set to failure. There was a 2-min rest period between each set. Each PRT session was preceded by a warm-up and cooldown period of 10 min of stationary cycling at low resistance (50 W) and slow speed, as well as stretching of the muscle groups involved in the strength measurements.

Muscle Biopsy

A percutaneous muscle biopsy sample (~100 mg) was obtained from the vastus lateralis 1 wk before initiation of the PRT program (2). A second muscle biopsy was obtained after 12 wk of PRT from the vastus lateralis 3 days after the last resistance training session. Each muscle sample was sectioned longitudinally into several portions and placed in cold skinning solution and stored at –20°C for later analysis of permeabilized single muscle fiber physiology and myosin stoichiometry as described below. After each muscle biopsy sample, all single fiber contractile measurements were completed on fresh tissue over a 30-day period.

Experimental Setup

The cellular studies on the individual muscle fibers were carried out on a single fiber physiology station. A 2- to 3-mm muscle fiber was isolated from a muscle bundle and transferred to a small experimental chamber filled with relaxing solution where the ends were securely fastened between a force transducer (model 400A, Cambridge Technology, Woburn, MA) and a direct-current torque motor (model 308B, Cambridge Technology) as described by Moss (26). The instrumentation was arranged so that the muscle fiber could be rapidly transferred back and forth between experimental chambers filled with relaxing or activating solutions. The apparatus was mounted on a microscope (Olympus BH-2) so that the fiber could be viewed ($\times 800$) during an experiment. Unamplified force and length signals were fed to a digital oscilloscope (Nicolet 310, Madison, WI), enabling fiber performance to be monitored throughout data collection. Analog force and position signals were amplified (dual differential amplifier, model 300-DIF2, Positron Development, Inglewood, CA), converted to digital signals (National Instruments), and transferred to a computer [Millennia (pentium

processor), Micron Electronics, Nampa, ID] for analysis using customized software. Servo-motor arm and isotonic force clamps were controlled with a computer-interfaced force-position controller (force controller, model 300-FC1, Positron Development).

The sarcomere length for each muscle fiber was adjusted to 2.5 μ m using an eyepiece micrometer. The eyepiece micrometer had a total length of 250 μ m with 100 divisions, thus making each division equivalent to 2.5 μ m. To set the sarcomere length at 2.5 μ m, each division mark from the eyepiece micrometer was lined up with a sarcomere along the fiber segment at a magnification of $\times 800$. A video camera (Sony CCD-IRIS, DXC-107A) fitted to the camera tube of the microscope and connected to a computer allowed viewing on a computer monitor and storage of the digitized images of the muscle specimens during the experiment. Fiber diameter was determined from a captured computer image taken with the fiber briefly suspended in air (4, 11, 33). Fiber width was determined at three points along the length of the captured computer image using public domain software (National Institutes of Health Image software, version 1.60), and fiber cross-sectional area was calculated from the mean width, with the assumption that the fiber forms a cylindrical cross section when suspended in air (<5 s).

All experiments were performed at 15°C. The temperature of the experimental chambers was constantly monitored (copper-constantan thermocouple, PP-T-24S, Omega Engineering, Stamford, CT) during the experiments. To cool the relaxing and activating solutions in the experimental chambers, a bath (RTE 111, Neslab, Portsmouth, NH) constantly circulated water through a milled aluminum plate. A thermoelectric pump peltier junction current source, with a feedback circuit temperature controller (E5AX-VAA, sensitivity of $\pm 0.3\%$, Omron), lowered the temperature of the experimental chambers to 15°C and maintained this temperature throughout the experiment. For all muscle fiber experiments, a fiber with a compliance [calculated as fiber length (FL) divided by y -intercept] $> 10\%$ and/or a decrease in peak tension [the maximal isometric force (P_0)] of $> 10\%$ was discarded and not used for analysis. The average series compliance for the fibers studied in the present investigation was 4%.

Experimental Solutions

The skinning solution contained (in mM) 125 potassium propionate, 2.0 EGTA, 4.0 ATP, 1.0 MgCl₂, and 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 (15, 20).

The compositions of the relaxing and activating solutions were calculated by using an interactive computer program (9). These solutions were adjusted for temperature, pH, and ionic strength with the use of stability constants in the calculations (17). Each solution contained (in mM) 7.0 EGTA, 20.0 imidazole, 14.5 creatine phosphate, 1.0 free Mg²⁺, 4 free MgATP, and KCl and KOH to produce an ionic strength of 180 mM and a pH of 7.0. The relaxing and activating solutions had a free Ca²⁺ concentration of pCa 9.0 and pCa 4.5, respectively (where pCa = $-\log$ Ca²⁺ concentration).

Experimental Procedures

The individual muscle fibers were analyzed for 1) P_0 , 2) V_0 , and 3) force-velocity-power parameters.

Force determination. The output of the force and position transducers were amplified and sent to a microcomputer via a Lab-PC+ 12-bit data acquisition board (National Instru-

ments). Resting tension was monitored first, and then the fiber was maximally activated in pCa 4.5 solution. Active P_o was determined in each fiber by computer subtraction of the force baseline from the P_o in the pCa 4.5 solution.

Determination of V_o . Fiber V_o was measured by the slack test technique (see Fig. 1) as described previously (8). The fiber was fully activated in pCa 4.5 and then rapidly released to a shorter length, such that tension fell to baseline. The fiber shortened, taking up the slack, after which tension

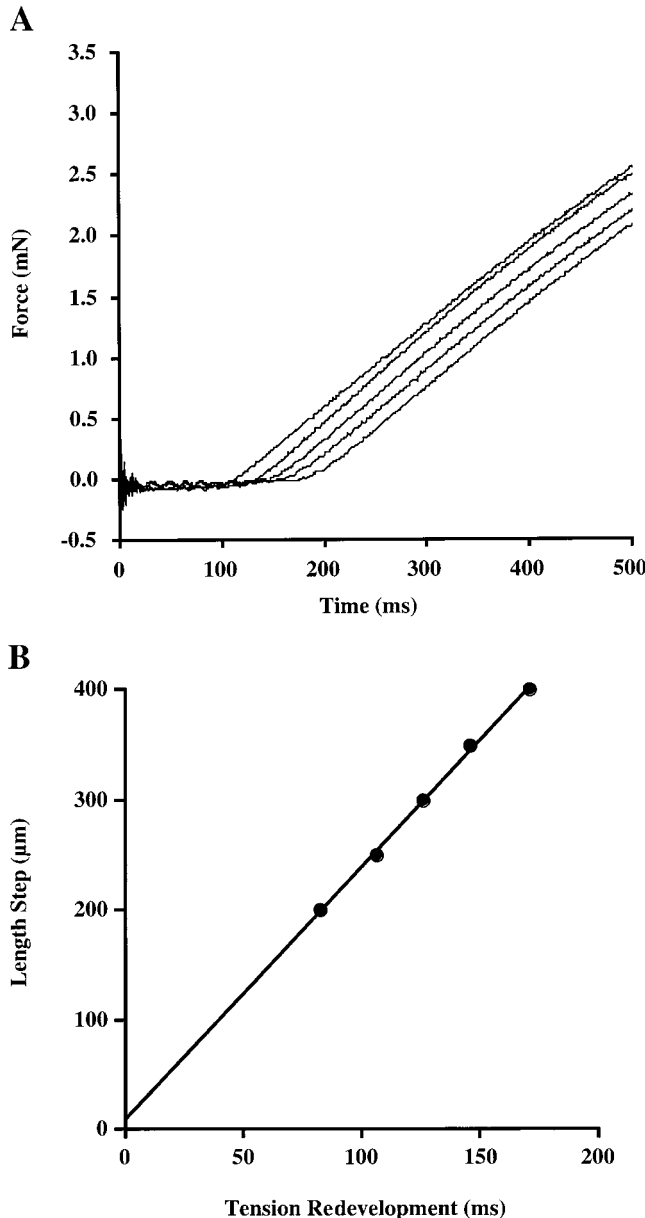


Fig. 1. Representative force records of a maximally Ca^{2+} -activated muscle fiber from the vastus lateralis of a 75-yr-old man. A: original force recordings following slack steps of 200, 250, 300, 350, and 400 μ m. Records have been superimposed to illustrate the increasing duration (time) to redevelop force with increasing slack distance. B: time required to redevelop force plotted vs. imposed slack distance. Slope of this line defines fiber unloaded shortening velocity (V_o). In this example, V_o of the fiber was 1.41 fiber lengths (FL)/s, with a y-intercept of 16.4. Fiber segment studied was 2.10 mm, thus giving a compliance of 0.8%; r^2 was 0.99. Gel electrophoresis indicated that this fiber expressed only myosin heavy chain (MHC) I.

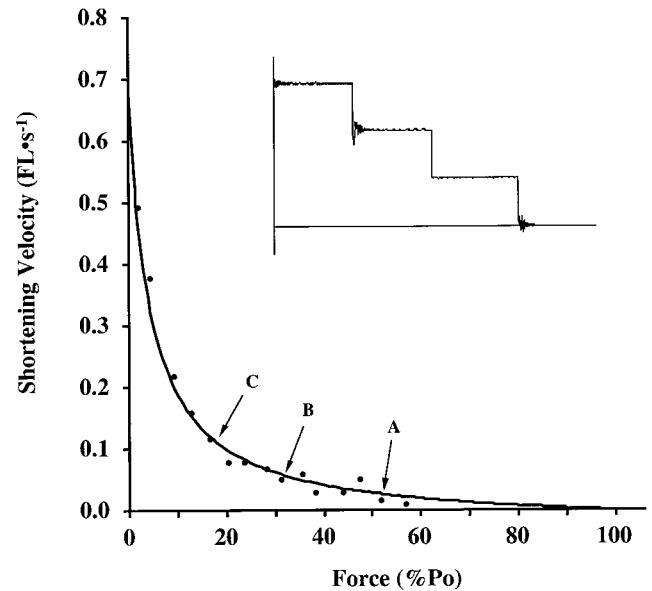


Fig. 2. Representative force records and resulting force-velocity relationship of a human vastus lateralis fiber from a 75-yr-old man. Inset: series of 3 submaximal isotonic load clamps, each step lasting 100 ms. Force and shortening velocity were determined over the final one-third of each isotonic load clamp. Data were fit using the Hill equation, $(P + a)(V + b) = (P_o + a)b$, where P is force, V is velocity, P_o is peak isometric force, and a and b are constants of force and velocity, respectively. A total of 15 points were used to construct the force-velocity curve. For this fiber, the V_{max} was 0.68 FL/s, with an a/P_o of 0.029 and an r^2 of 0.99. Gel electrophoresis indicated that this fiber expressed only MHC I.

began to redevelop. The fiber was then placed in relaxing solution and returned to its original length. The duration of unloaded shortening, or time between onset of slack and redevelopment of tension, was determined by computer analysis. Six different activation and length steps (each $\leq 20\%$ of FL) were used for each fiber, with the slack distance plotted as a function of the duration of unloaded shortening. Fiber V_o (FL/s) was calculated by dividing the slope of the fitted line by the segment length; the data were normalized to a sarcomere length of 2.50 μ m.

Force-velocity and power. Isotonic load clamps were performed on each fiber for determination of force-velocity parameters (see Fig. 2). Each fiber segment was fully activated in pCa 4.5 solution and then subjected to three isotonic load steps. After the third isotonic step, the position motor imposed a slack length step of $<20\%$ of the original FL and the fiber was transferred back into relaxing solution, where it was reextended to its original FL. The entire procedure was performed five to six times at various loads so that each fiber was subjected to a total of 15–18 isotonic contractions.

Shortening velocity and relative fiber force (% P_o) were determined over the final one-third of each isotonic contraction by computer analysis of the slopes of the position and force recordings, respectively. During the isotonic force clamps, the initial phase of each contraction elicited small variations in force and velocity. This small variation lasted approximately one-third (~ 30 ms) of each isotonic load clamp, which was stabilized for the final two-thirds of each contraction. Thus the final one-third of each isotonic load clamp was used for analysis.

All shortening velocities were normalized to a unit of FL and expressed as FL per second (FL/s). Because the length of each fiber segment studied varied slightly, it was necessary

to normalize all velocity measurements to FL to account for the variation in the number of sarcomeres in series. To construct the force-velocity relationships, load was expressed as P/P_o , where P is the force during load clamping and P_o is the peak isometric force developed before the submaximal load clamps. The Hill equation (see Ref. 19), $(P + a)(V + b) = (P_o + a)b$, where P is force, V is velocity, and a and b are constants of force and velocity, respectively, was used to fit the data obtained for an individual fiber by an iterative nonlinear curve-fitting procedure and to determine V_{max} . Only individual experiments in which r^2 was greater than or equal to 0.98 were included for analysis.

Fiber power was calculated from the fitted force-velocity parameters and the maximum isometric force observed during the experiment. Absolute power ($\mu\text{N} \cdot \text{FL} \cdot \text{s}^{-1}$) was defined as the product of force (in μN) and shortening velocity (in FL/s). Normalized power ($\text{kN} \cdot \text{m}^{-2} \cdot \text{FL} \cdot \text{s}^{-1}$) was defined as the product of normalized force, i.e., fiber tension or force/fiber cross-sectional area.

Myosin Stoichiometry

After single muscle fiber physiology measurements were made, each fiber was solubilized in 20 μl of 1% SDS sample buffer and stored at -80°C until assayed (16). To determine the MHC and MLC composition, fibers were run on a Hoefer SE 600 gel electrophoresis system that consisted of a 3.5% (wt/vol) acrylamide stacking gel with 5% (MHC) or 12% (MLC) separating gel at 4°C . After gel electrophoresis, the gels were silver stained as described by Giulian et al. (16). Representative 5 and 12% gels are presented in Fig. 3. A computer-based image analysis system and software (Alpha Innotech, ChemImager 4000, San Leandro, CA) were used to quantify the relative density of each MLC band.

Statistical Analysis

Data were expressed as means and SE. For a given fiber type (MHC) and variable (diameter, P_o , V_o , power, and so forth), data were averaged and pooled by subject. Statistical significance of the subject means for a given variable was then assessed with the use of a two-tailed Student's t -test. A probability of less than 5% ($P < 0.05$) was considered to be significant.

Given the small number of hybrid (I/IIa and IIa/IIa) and IIx fibers studied in these experiments (Table 1), they were not included in the analysis. Therefore, the pretraining-to-posttraining comparisons of single myofiber function are limited to the MHC I and IIa fiber types.

RESULTS

Whole Muscle Strength

Knee extensor strength, as assessed by 1 RM, improved $50 \pm 6\%$, from 52.9 ± 5.3 to 79.1 ± 7.0 kg, as a result of the 12-wk PRT program.

MHC and MLC Isoform Composition

Contractile properties were determined for a total of 344 (175 pre-PRT and 169 post-PRT) fibers randomly isolated from the vastus lateralis biopsies (Table 1). This represents ~ 25 fibers that were studied from each subject before and after the PRT program. All fibers reported were subjected to the slack test technique for the determination of V_o and to load-clamp maneuvers to obtain the force-velocity curves. As can be seen in

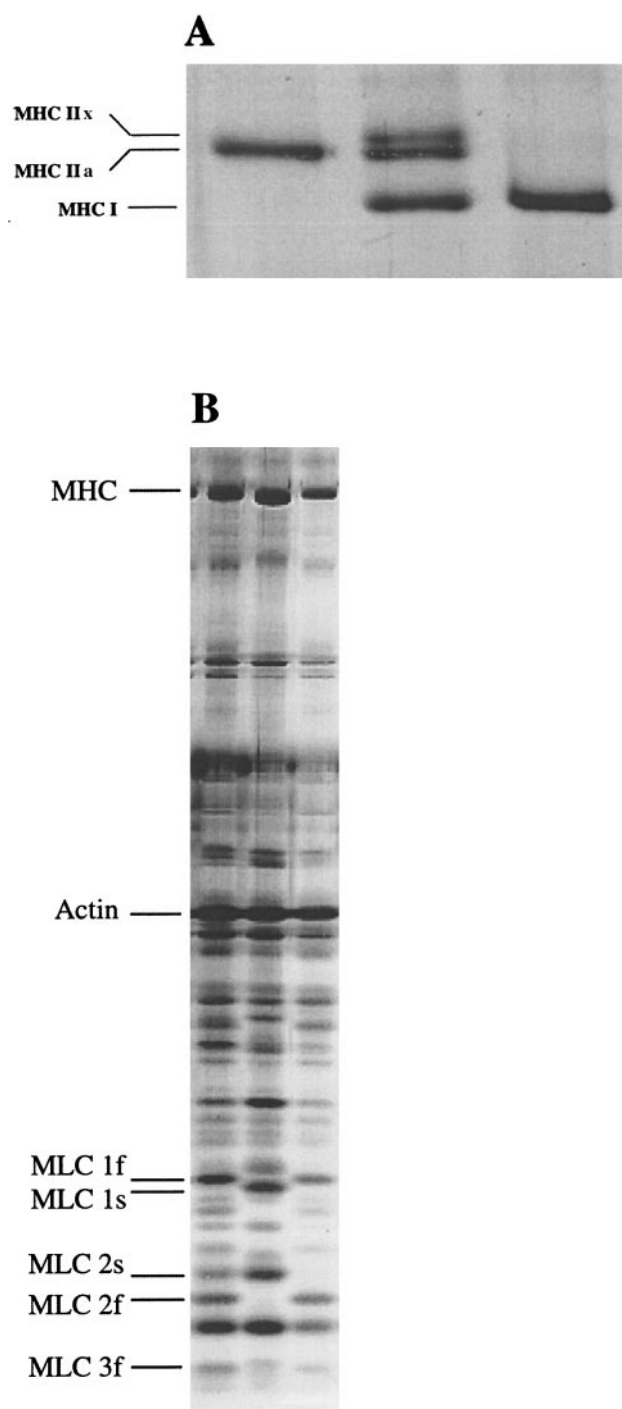


Fig. 3. Representative 5% (A) and 12% (B) PAGE demonstrating identification of single human muscle fibers for MHC and myosin light chain (MLC). Each lane (1–3) represents a single fiber. A: single muscle fibers (MHC) from the vastus lateralis of a 75-yr-old man. B: single muscle fibers (MLC) from the vastus lateralis the same individual.

Fig. 3, all three MHC bands (I, IIa, IIx) could be separated and identified.

The number of hybrid and the number of IIx fibers studied pre- and posttraining were 31 (18%) and 15 (9%), respectively. This represented a 9% decrease ($P < 0.05$) in this fiber population after PRT. Conversely,

Table 1. *Myosin heavy chain composition of vastus lateralis fibers before and after resistance training*

	Pre		Post	
	<i>n</i>	Percent	<i>n</i>	Percent
MHC Isoform				
I	89	51	110	65*
I/IIa	15	9	7	4
IIa	55	31	44	26
IIa/IIx	13	7	8	5
IIx	3	2	0	0
No. of hybrid + IIx fibers	31		15*	
Total no. of fibers	175		169	

n = No. of fibers. Hybrid fiber defined as I/IIa or IIa/IIx. MHC, myosin heavy chain; Pre, before training; Post, after training. **P* < 0.05 from pre- vs. postresistance training.

there was a 14% increase (*P* < 0.05) in the number of MHC I fibers with no change in the MHC IIa fibers after PRT.

A complete analysis of the MLC profile is presented in Table 2. There were no differences in relative content of the MLC isoforms (MLC_{1f}, MLC_{1s}, MLC_{2s}, MLC_{2f}, MLC_{3f}). In addition, the pure MHC I and IIa isoforms did not coexpress the fast (MLC_{1f} and MLC_{2f}) or slow (MLC_{1s} and MLC_{2s}) isoforms, respectively. However, both MHC I and IIa fibers expressed the MLC_{3f} isoform before and after PRT.

There was no significant difference in the relative ratio of alkali MLC_{3f} to the dithionitrobenzoic acid MLC_{2f} (MLC_{3f}/MLC_{2f}) as a result of the 12-wk PRT program. The MLC_{3f}/MLC_{2f} of the MHC I fibers was 0.221 ± 0.023 before the training and 0.197 ± 0.015 after the training (*P* > 0.05). Similarly, the MLC_{3f}/MLC_{2f} of the MHC IIa fibers was 0.371 ± 0.033 and 0.302 ± 0.025 before and after the training, respectively. Although there was no observed differences within the specific fiber-type populations, the MLC_{3f}/MLC_{2f} was significantly (*P* < 0.05) lower in the MHC I compared with that in the MHC IIa fibers.

Similar results describing single muscle cell protein alterations (MHC and MLC) following PRT have been reported by our laboratory from these same older men (34). The protein isoform data presented here are from a different set of single muscle fibers that were subjected to physiological studies to examine contractile behavior before and after PRT. The novelty of the MHC and MLC data presented in the present investigation is the relationship of the structural and functional data from the same individual fibers.

Fiber Diameter and Peak Force

Table 3 shows the increase in fiber diameter that occurred with the 12-wk PRT program. On average, the diameter of the fibers expressing MHC I increased 20%, whereas the MHC IIa fibers increased 13%. This corresponded to a 45 and 28% increase in fiber cross-sectional area (calculated from fiber diameter) of the MHC I and IIa fibers, respectively. Thus the *P*₀ of the MHC I fibers increased out of proportion to the increase in fiber per cross-sectional area.

The overall average *P*₀ increased (*P* < 0.05) by 55% in the MHC I fibers and 25% in the MHC IIa fibers (Table 3). Before the PRT, the absolute *P*₀ of the MHC IIa was significantly (*P* < 0.05) greater than the MHC I fibers. However, the magnitude of change in the MHC I fiber population was greater than that in the MHC IIa fibers, resulting in similar absolute *P*₀ between the fiber types. In fact, the MHC I fibers were stronger (nonsignificant) than the MHC IIa fibers after the 12-wk PRT program. When corrected for fiber per cross-sectional area, the MHC I fibers increased ~7% (nonsignificant); however, there was no change in the specific tension of the MHC IIa fibers as a result of the PRT. Interestingly, there was no difference in fiber diameter between the MHC I and IIa fiber types before or after the PRT.

Fiber *V*₀

The *V*₀ results for both MHC I and IIa fiber types were significantly (*P* < 0.05) elevated after the 12-wk PRT program (Table 4). The *V*₀ of MHC I fibers was elevated by 0.64 FL/s, which corresponded to a 75% increase. The MHC IIa fiber *V*₀ was elevated, on average by 0.98 FL/s, equivalent to an increase of 45%. This is further illustrated in Fig. 4. The *V*₀ results for both MHC I and IIa fibers were shifted to the right, indicating an overall faster shortening velocity after the training. Of the MHC I fibers, 71% (*n* = 63) had *V*₀ values <1.0 FL/s before the training, whereas only 12% (*n* = 13) had *V*₀ values of <1.0 FL/s after the training. Similarly, of the MHC IIa fibers, 55% (*n* = 30) had *V*₀ values <2.0 FL/s before the training, whereas only 18% (*n* = 8) had *V*₀ values <2.0 FL/s after the training.

Isotonic Contractile Properties

All three parameters describing the force-velocity relationship (*V*_{max}, *P*₀, and *a/P*₀) were significantly (*P* < 0.05) altered in both MHC I and MHC IIa fiber types after the

Table 2. *Myosin light chain composition of vastus lateralis fibers before and after resistance training*

MLC Isoform	Pre	Post
<i>MHC I fibers</i>		
MLC _{1f}	NE	NE
MLC _{1s}	0.52 ± 0.01	0.51 ± 0.02
MLC _{2s}	0.37 ± 0.01	0.39 ± 0.01
MLC _{2f}	NE	NE
MLC _{3f}	0.11 ± 0.01	0.10 ± 0.01
<i>MHC IIa fibers</i>		
MLC _{1f}	$0.44 \pm 0.01^*$	$0.43 \pm 0.02^*$
MLC _{1s}	NE	NE
MLC _{2s}	NE	NE
MLC _{2f}	$0.40 \pm 0.00^*$	0.40 ± 0.01
MLC _{3f}	$0.16 \pm 0.01^*$	$0.17 \pm 0.02^*$

Values are means ± SE. The relative contribution of each myosin light chain (MLC) band is represented as a fraction of the entire MLC band region (total = 1.00) for that particular fiber type (as identified by the MHC band). NE = not expressed; for the "pure" MHC I and IIa isoforms, these specific MLC isoforms were not expressed. **P* < 0.05 between MHC I vs. IIa fibers.

Table 3. *Fiber diameter, peak isometric force, and peak isometric force per fiber cross-sectional area of vastus lateralis MHC I and IIa muscle fibers before and after resistance training*

Fiber Type	Diameter, μm		P_o , mN		P_o/CSA , kN/m^2	
	Pre	Post	Pre	Post	Pre	Post
MHC I	83 ± 1	$100 \pm 2^*$	0.58 ± 0.01	$0.90 \pm 0.02^*$	109 ± 2	117 ± 3
MHC IIa	86 ± 1	$97 \pm 2^*$	$0.68 \pm 0.02^\dagger$	$0.85 \pm 0.03^*$	118 ± 4	118 ± 7

Values are means $\pm$ SE. P_o , peak isometric force; P_o/CSA , P_o per fiber cross-sectional area (CSA). $^*P < 0.05$ from pre- vs. postresistance training; $^\dagger P < 0.05$ from MHC I vs. MHC IIa.

PRT program (Tables 3 and 5). Fiber $V_{\max}$ was elevated by 61 and 54% in the MHC I and IIa fibers, respectively. Although the $V_{\max}$ values were slightly lower than the V_o values from the same fibers in this study, the relative change in both of these measurements of shortening velocity was of similar magnitude.

Composite force-velocity curves of the pre- and post-PRT MHC I and IIa fibers are shown in Fig. 5. The mean a/P_o for the MHC I and IIa fibers increased significantly ($P < 0.05$) (therefore less curvature) as a result of the strength training. The absolute and normalized composite power curves from MHC I and IIa fibers are illustrated in Fig. 6. Absolute peak power increased ($P < 0.05$) by 128% in the MHC I fibers and by 61% in the MHC IIa fibers. When normalized to fiber per cross-sectional area, power was still elevated by 57 and 29% in the MHC I and IIa fibers, respectively. Peak power of the MHC I fibers occurred at 14.9% of P_o before the training and 15.2% of P_o after the training. Similarly, peak power of the MHC IIa fibers occurred at 14.9 and 15.7% of P_o before and after PRT, respectively.

DISCUSSION

The aim of the present investigation was to examine how skeletal muscle adapts to a resistance training stimulus in older men. To accomplish this goal, we utilized the skinned muscle fiber preparation. This preparation disrupts the sarcolemma, allowing for direct chemical stimulation of the contractile machinery. Thus alterations in cross-bridge mechanics can be examined. To our knowledge, this is the first investigation to characterize single muscle fiber contractile structure and function in response to resistance training in older adults. The primary findings from this research were that MHC I and IIa fibers from the vastus lateralis were larger, produced greater P_o , con-

tracted faster (V_o), and were more powerful subsequent to the 12-wk PRT program. However, the MHC I fibers of these older men demonstrated a greater degree of adaptation in response to the PRT program compared with that shown in the MHC IIa fibers.

Effect of Resistance Training on Fiber Diameter and Force

In the present study, the PRT program induced a significant increase in single muscle cell diameter of 20 and 13% in the fibers expressing the MHC I and IIa isoform, respectively. Previous studies examining fiber hypertrophy (using histochemical methods) of the vastus lateralis following resistance training in older men and women have reported increases ranging from 7 to 52% (27), with the majority of studies reporting a greater increase in type II fiber size. Interestingly, the MHC I fibers were $\sim 4\%$ smaller before the training and $\sim 3\%$ larger after the training compared with that shown in the MHC IIa fibers. Thus these data show that fibers expressing the MHC I and IIa isoforms before the training are larger than fibers expressing the MHC I and IIa isoform after a PRT program in older adults. Furthermore, it appears that muscle cells with the MHC I protein increase in size as much, if not more, than the muscle cells with the MHC IIa protein.

As a result of the PRT, P_o was significantly increased in both fiber types. Overall, P_o was increased by 55 and 25% in the MHC I and IIa fibers, respectively. Before the PRT program, the MHC IIa fibers produced significantly more force ($\sim 17\%$) than the MHC I fibers. However, after 12 wk of PRT, there was no difference in the P_o of the MHC I and IIa fibers. In fact, the MHC I fibers developed, on average, $\sim 6\%$ more force than the MHC IIa fibers as a result of the PRT. When absolute P_o was corrected for fiber size ($P_o/\text{cross-sectional area}$), there were no apparent differences in specific force. Although there was a 7% increase in specific force of the MHC I fibers, this was not statistically significant. This suggests that the increased absolute P_o after the PRT could be explained by the observed single cell hypertrophy. This is an important finding and suggests that other mechanisms intrinsic to the single muscle cells must be responsible for the increased contractile velocity (V_o) and power observed in these older men.

It is interesting that the PRT program employed in these older men resulted in a greater improvement in absolute force production such that the MHC I fibers

Table 4. *Unloaded shortening velocity of vastus lateralis muscle fibers before and after resistance training*

Fiber Type	Fiber V_o , FL/s		%Change
	Pre	Post	
MHC I	0.85 ± 0.03	$1.49 \pm 0.04^*$	75
MHC IIa	$2.20 \pm 0.10^\dagger$	$3.18 \pm 0.16^*^\dagger$	45

Values are means $\pm$ SE. V_o , unloaded shortening velocity; FL, fiber length. $^*P < 0.05$ from pre- vs. postresistance training; $^\dagger P < 0.05$ from MHC I vs. MHC IIa.

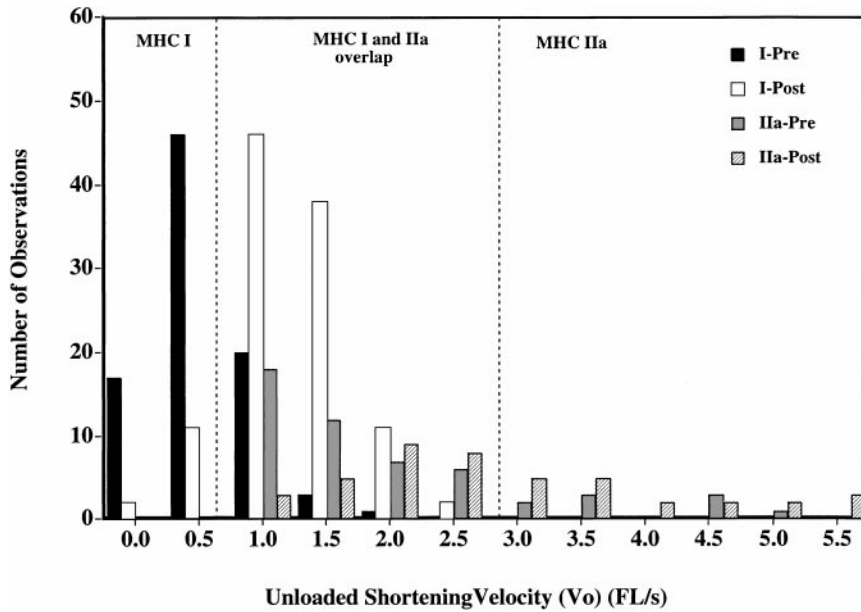


Fig. 4. Frequency distribution of MHC I and IIa fiber V_o values before (pre) and after (post) the progressive resistance training program. The x-axis distribution is in 0.5 FL/s increments. For example, the 1.0 FL/s increment represents all fibers that had a V_o ranging from 1.0 to 1.49 FL/s.

were slightly stronger after the intervention. The mechanisms for this result cannot be explained by the present data. To date, there is limited information on single muscle fiber function in humans that may help explain the present results. Two recent reports by Botinelli et al. (4, 5) and one by Larsson and Moss (23) clearly showed that fast-twitch fibers (MHC IIa) develop more force compared with slow-twitch fibers (MHC I). This is in agreement with the present results on pretraining fiber P_o . However, this relationship among the fiber types and force development did not hold true in these older men after 12 wk of training.

Effect of Resistance Training on Fiber V_o

The V_o of skeletal muscle is highly correlated to the MHC isoform composition (1) and is thought to be the main determinant of V_o in human muscle fibers (29). Furthermore, V_o is generally believed to reflect the maximum speed at which the actin and myosin filaments are able to interact. In the present study, V_o increased substantially in MHC I and IIa fibers as a result of PRT. Thus the relationship between fiber V_o and MHC isoform was altered or uncoupled with the resistance training. In fact, the resistance training stimulus used in this study was sufficient to elevate fiber V_o by 75 and 45% in fibers expressing solely MHC I and IIa isoforms, respectively. On average, the majority (70%) of the pre-PRT fibers displayed V_o between 0.3 and 1.3 FL/s, whereas the majority (73%) of the post-PRT fibers fell between 1.0 and 2.0 FL/s.

Recent research by Lowey et al. (25) suggested that the MLC isoforms play a key role in regulating speeds of muscle shortening. In addition, the MLC isoforms appear to modulate muscle performance to a greater degree in the faster contracting fibers (3, 18, 31). It is believed that changes in MLC content, especially MLC_{3f} content, are important in determining the shortening velocity of a muscle fiber. In the present

study, we found no alterations in MLC composition after the PRT program that could help explain why these fibers could contract at much faster speeds. However, the MHC IIa fibers were found to have a MLC_{3f}/MLC_{2f} that was significantly higher than that in the MHC I fibers, which is in agreement with the findings of others (3). However, the fact that the MLCs were not altered from pre- to post-PRT in the MHC I and IIa muscle fibers indicates that the observed changes in fiber V_o cannot be explained by alterations in the MLC isoforms.

An age-related slowing of fiber V_o (~40%) has also been shown in both human and rat skeletal muscles (22, 32). Given the increase in V_o observed in the present study, resistance training appears to offer an effective countermeasure to attenuate or reverse the decline in fiber V_o that has been observed with old age. Thus the present results provide evidence of qualitative changes in the muscle cell, which most likely play an important role in preserving muscle function in older adults.

Table 5. Isotonic contractile properties of vastus lateralis muscle fibers before and after resistance training

Fiber Type	V_{max} , FL/s	a/P_o	Absolute Peak Power, $\mu N \cdot FL \cdot s^{-1}$
MHC I			
Pretraining	0.65 ± 0.05	0.032 ± 0.004	7.73 ± 0.53
Posttraining	$1.05 \pm 0.05^*$	$0.044 \pm 0.004^*$	$17.62 \pm 0.94^*$
MHC IIa			
Pretraining	$2.06 \pm 0.11^\dagger$	0.035 ± 0.009	$25.50 \pm 1.03^\dagger$
Posttraining	$3.18 \pm 0.16^{*\dagger}$	$0.053 \pm 0.009^{*\dagger}$	$41.09 \pm 2.38^{*\dagger}$

Values are means $\pm$ SE. a , Force constant; V_{max} , shortening velocity. $^*P < 0.05$ from pre- vs. postresistance training; $^\dagger P < 0.05$ from MHC I vs. MHC IIa.

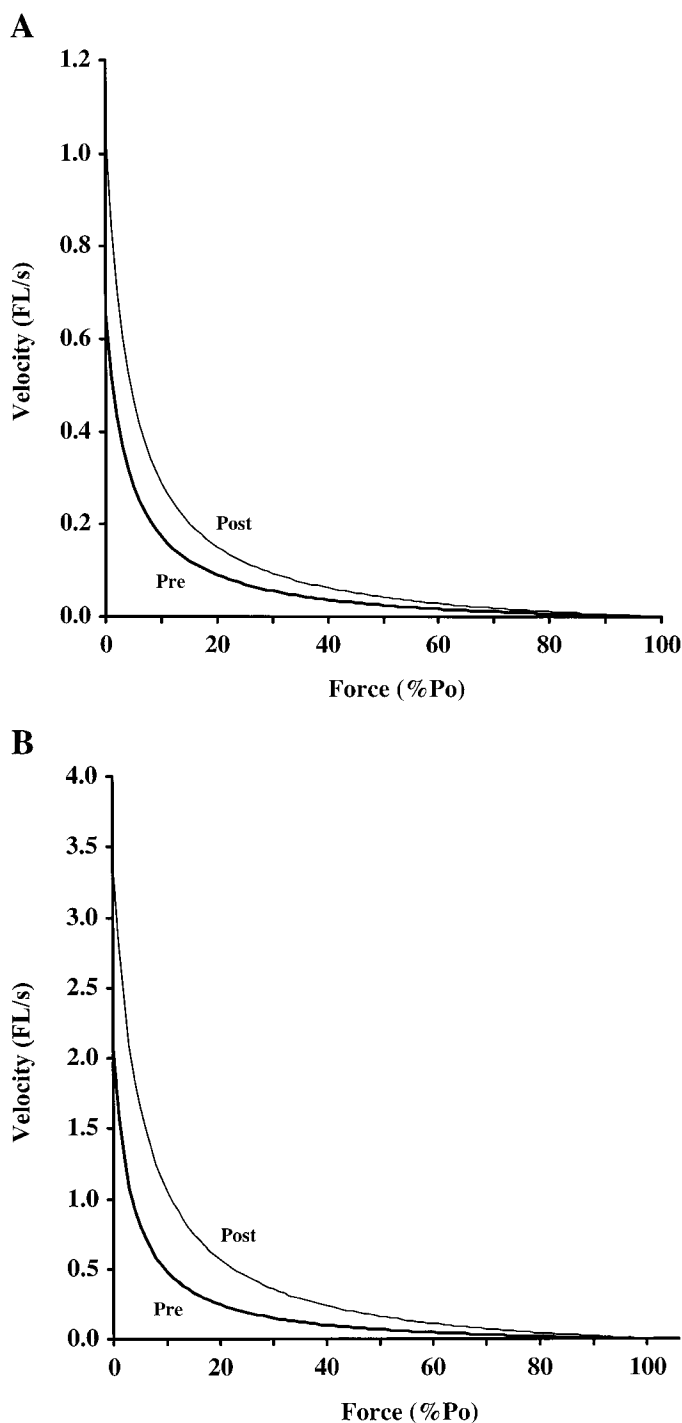


Fig. 5. Composite force-velocity curves for MHC I (A) and MHC IIA (B) vastus lateralis muscle fibers before and after the 12-wk progressive resistance training program. Measurements were made with isotonic load clamps (force-velocity relationships) while maintained at 15°C.

The large increase in single cell V_0 in fibers expressing the MHC I and IIA isoforms after the PRT was probably the most notable finding from this investigation. The observed increase in fiber V_0 could not be explained by the structural data (MHC and MLC) from these same muscle fibers. Thus other aspects of con-

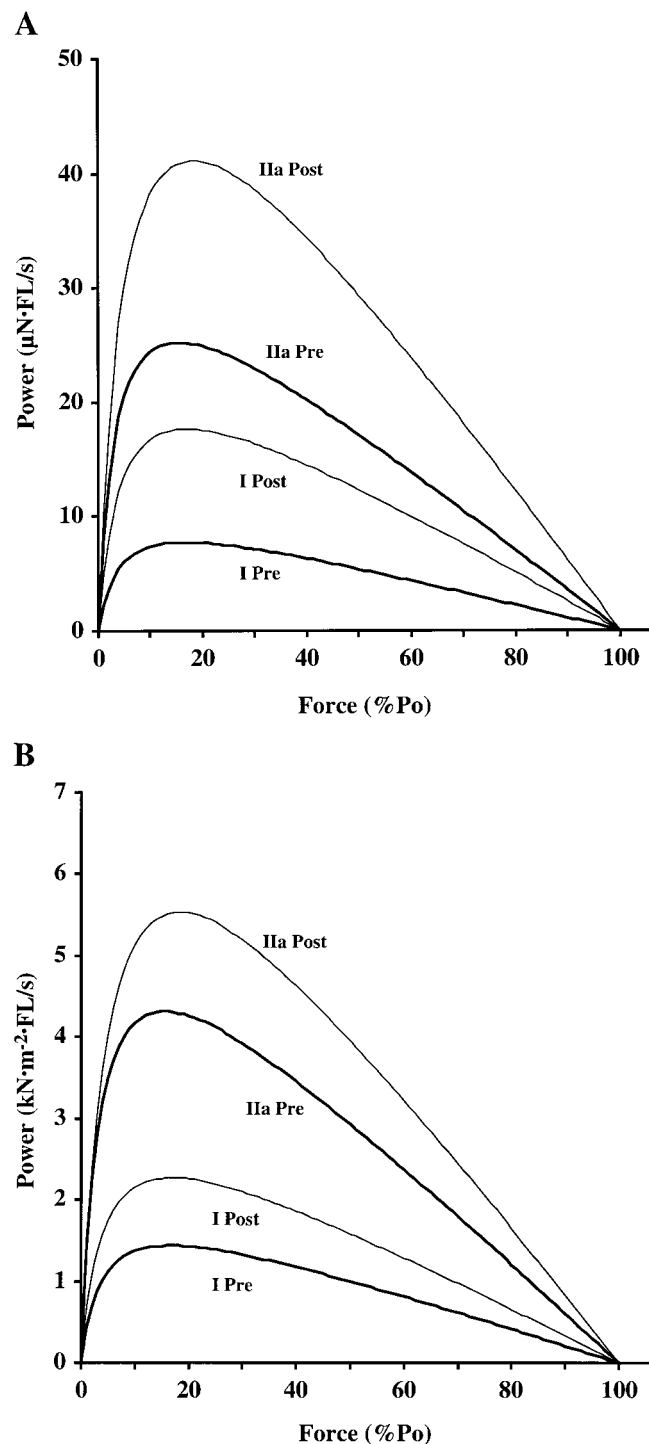


Fig. 6. Composite force-power curves for MHC I and IIA vastus lateralis muscle fibers before and after the progressive resistance training program. A: absolute power curves (in $\mu\text{N} \cdot \text{FL} \cdot \text{s}^{-1}$). B: normalized (to cell size) power curves (in $\text{kN} \cdot \text{m}^{-2} \cdot \text{FL} \cdot \text{s}^{-1}$). Measurements were made with isotonic load clamps (force-velocity-power relationships) while maintained at 15°C.

tractile behavior (e.g., calcium kinetics, number of cross bridges acting in parallel, and/or other contractile protein alterations that may influence V_0) may have occurred during the muscle remodeling process caused by the resistance training that are modulating contrac-

tile velocity. Although the increase in fiber V_o cannot be explained with the present data, it points out that our current understanding in the regulation of the speed of shortening is incomplete.

Effect of Resistance Training on Fiber Power

The present investigation is the first to demonstrate that single muscle cell power is significantly increased with resistance training in older men. Interestingly, fiber power was improved to a larger extent in the MHC I fiber compared with that in the MHC IIa fibers. This is in contrast to previous reports that indicated that the muscle mechanics of type II (MHC II) fibers appear to be more affected by resistance training than the MHC I fibers (12). However, before this investigation, functional information describing alterations in the contractile properties of single muscle cells after resistance training was unavailable. Thus previous thoughts about fiber behavior in response to resistance training were only speculation.

Peak power output is a function of P_o , V_{max} , and the curvature of the force-velocity relationship. All three of these variables were improved with the PRT. The curvature of the force-velocity curve (a/P_o) increased by ~30% in both the MHC I and IIa fiber types. Thus, for any given load (P/P_o), the muscles contracted at a greater velocity. Rome et al. (28) demonstrated that muscle fibers shorten within a range of physiological speeds, which will optimize mechanical power output and efficiency. This typically occurs at ~20% of P_o to achieve maximum power development. In the present study, we observed that both MHC I and IIa fibers produced peak power at ~15% of their peak load, which was unaltered with the PRT. In addition, Rome et al. (28) also demonstrated that carp muscle fibers are selectively recruited to perform work such that power and efficiency are optimized. This may provide insight as to why the MHC I fibers were "more responsive" to the PRT program employed in this study. Because the human fibers from this study had a large range of shortening velocities (0.32–5.62 FL/s) (Fig. 4; Tables 3 and 4), it is conceivable that the in vivo speed of movement with the resistance training was better suited for fibers with slow-to-moderate shortening velocities and the MHC I fibers were selectively recruited to perform the work. In support of this theory, we found little change in shortening velocity of fibers with a $V_o > 3.0$ FL/s. Furthermore, it appears that the largest shift in shortening velocity was in the 0.5 to 1.5 FL/s range, which is the range that MHC I fibers appear to functionally dominate.

Practical Implications

The results from this investigation are the first to show that the contractile function of individual slow- and fast-twitch muscle fibers from older men is improved with resistance training. This has important implications because whole muscle performance and single muscle cell performance have been shown to be drastically reduced with aging (14, 21, 22, 32). Of

particular interest was the large increase in contractile velocity and peak power in the MHC I and IIa fibers as a result of the training. This suggests that muscle performance may not solely rely on cell size for functional gains because specific tension was not altered with the training. Increases in power output, which is critical for human movement, appear to rely on the ability to improve the contractile velocity of the fibers rather than hypertrophy alone. Thus, although retaining muscle mass remains an important objective for the elderly, an increase in contractile velocity also appears to be important for preserving muscle function in older adults.

In conclusion, the present results provide evidence that the cross-bridge mechanics involving force production and shortening velocity in single human muscle cells expressing MHC I and IIa isoforms are altered after 12 wk of high-intensity resistance training in older men. More specifically, we observed an increase in single cell diameter, P_o , V_o , and power of MHC I and IIa fibers. Because increases in muscle cell size could account for the increased force production, the most notable findings were the large increases in fiber V_o and power, which could not be explained by protein alterations (MHC and MLC) or increases in cell size.

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