Progressive resistance training reduces myosin heavy chain coexpression in single muscle fibers from older men

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Williamson, David L., Michael P. Godard, David A. Porter, David L. Costill, and Scott W. Trappe. Progressive resistance training reduces myosin heavy chain coexpression in single muscle fibers from older men. J. Appl. Physiol. 88: 627–633, 2000.—The purpose of this study was to examine myosin heavy chain (MHC) and myosin light chain (MLC) isoforms following 12 wk of progressive resistance training (PRT). A needle biopsy was taken from the vastus lateralis to determine fiber-type expression [ATPase (pH 4.54) and MHC/MLC] in seven healthy men (age = 74.0 ± 1.8 yr). Subjects were also tested for 1-repetition maximum (1-RM), pre- and posttraining. The progressive knee extensor protocol consisted of three sets at 80% of 1-RM 3 days/wk for 12 wk. Freeze-dried, single muscle fibers were dissected for MHC and MLC analysis and then subjected to SDS-PAGE and silver staining, pre- and posttraining. MHC expression increased in the I (10.4%; P < 0.05) and decreased in I/IIa (9.0%; P < 0.05), IIa/IIx (0.9%; P < 0.05), and IIa/IIx (8.9% P < 0.05) isoforms, with no change in the IIa and IIx isoforms, pre- vs. posttraining. The MLC3f-to-MLC2 ratio did not change with the PRT in either the MHC I or MHC IIa isoforms (total fibers = 902), pre- to posttraining. ATPase fiber distribution did not significantly differ following training (I: 50.4 ± 6.7 vs. 51.9 ± 7.9, IIa: 36.8 ± 5.3 vs. 41.1 ± 7.0, IIb: 12.8 ± 5.6 vs. 7.0 ± 4.0%; pre- vs. posttraining, respectively). 1-RM increased (51.9%; P < 0.05) from pre- to posttraining. The PRT provide a stimulus for alterations in MHC isoforms, which demonstrated a decrease in all hybrid isoforms and an increase in MHC I expression (not found in the ATPase results), unlike the MLC ratio (3:2), which was not altered with training. No apparent alteration in fiber-type distribution was observed post PRT.

METHODS

Subjects

Seven healthy older men (age = 74.0 ± 1.8 yr, weight = 74.0 ± 5.1 kg, height = 176.5 ± 3.2 cm) were recruited from the community to serve as subjects in this investigation. The criteria for volunteers included nonexercising (aerobic, resistance, or other), nonobese, nonsmoking, normotensive, healthy men as judged by a physician. All potential subjects underwent a telephone interview, followed by a visit to the laboratory. This visit included a medical history and an interview with a physician, a complete physical examination, and a graded stress test (12-lead electrocardiogram) performed on a treadmill. Before they were screened, all potential subjects were informed of all procedures and risks associated with the screening, testing, and training. On entry into the study, the subject’s activity level outside of the laboratory was monitored. Informed consent was obtained from each volunteer, which was approved by the Internal Review Board of Ball State University and Ball Memorial Hospital.

SKELETAL MUSCLE FUNCTION AND morphology have been shown to deteriorate with increasing age (10, 20, 21, 23, 25). These changes have been demonstrated in whole muscle strength [e.g., 1-repetition maximum (1-RM)] and size (measured by computed tomography), as well as single fiber size and type (via NADH and ATPase histochemistry, respectively). Resistance training of older individuals has been used as a countermeasure for skeletal muscle atrophy and strength (14, 15, 17),
Muscle Biopsy

Needle biopsies (~100–200 mg) were taken from the vastus lateralis muscle with the aid of suction (6, 13). Before the biopsy, the area was cleaned with betadine and then anesthetized with 1% Xylocaine (~1 ml). Approximately 5 min after the injection of Xylocaine, an incision was made through the skin and subcutaneous fat to accommodate the biopsy needle (5 mm). The needle was inserted into the muscle belly, and a sample was drawn into the needle port by aid of suction from a 140-ml syringe. The muscle was immediately divided into longitudinal sections. One muscle section was frozen in liquid nitrogen for MHC and MLC analysis and then freeze dried at −35°C for 4 days (Bio-Dry Trivac, FTS Systems, Stone Ridge, NY). Another section was vertically mounted in tragacanth gum (Sigma-G-1128, St. Louis, MO) and frozen in isopentane for ATPase analysis.

Training Program

All training was performed under supervision at the Human Performance Laboratory. Before the training bout, the subject warmed up on a cycle ergometer (Met 100, Cybex, NY, NY) for ~5 min at a low level of intensity (25–50 W). After the warm-up, the subject was seated on the bilateral isotonic knee extensor device (Cybex Eagle). The training involved a concentric and eccentric component, in which the subject was required to lift the weight to full knee extension (concentric) and lower the weight to the starting position (eccentric). Each component (concentric and eccentric) was ~2–3 s in duration. The subject was asked to perform three sets with 2–3 min of rest in between, the first two sets to 10 repetitions and the last set to volitional exhaustion. All leg exercise sessions were performed at 80% of 1-RM and assessed every 2 wk to ensure that the intensity was being maintained. 1-RM was determined by increasing the weight one-half (2.88 kg) or one full plate (5.75 kg) (Cybex Eagle) with each successful bilateral knee extension. The test continued until the subject was not able to maintain proper form and/or fully extend at the given weight. The training was conducted three times per week, with at least 24 h between sessions (i.e., no back-to-back sessions), lasting 12 wk in duration.

ATPase Histochemistry

Several transverse sections (10 µm) for histochemical analysis were cut at −20°C in a cryostat (Tissue-Tek II, Miles Laboratory, Elkhart, IN). Fiber-type analysis was performed using the ATPase method, preincubated at pH 4.54. Fibers were counted and then classified as types I, IIA, and IIX (8).

Myosin Analysis

Fiber-type expression was determined by teasing out individual freeze-dried fibers under a microscope and then subjecting them to SDS-PAGE for analysis of MHC and MLC expression. For this analysis, we dissected 200 fibers from the pre- and the postraining muscle samples. After dissection, the fibers were solubilized in 50 µl of 1% SDS sample buffer [1% SDS, 6 mg/ml EDTA, 0.06 M Tris (pH 6.8), 2 mg/ml bromophenol blue, 15% glycerol, and 5% β-mercaptoethanol] and stored at −80°C until assayed. The SDS-PAGE allowed us to evaluate the MHC (I, IIA, IIX) and the MLC (1f, 1s, 2f, 2s, 3f) on the same single fiber by using a loading and a separating gel. The MHC were run on a 3.5% loading gel and a 5% separating gel, and the MLC were analyzed using a 3.5% loading gel and a 12% separating gel at 4°C (Hoefer SE 600 series, San Francisco, CA). Silver staining revealed the distribution of fiber-type expression, which corresponded to known molecular weights (Sigma) and standards (16).

The inter- and intra-assay variability for the SDS-PAGE and silver-staining technique in our laboratory was 0.0%. This was based on 100 fibers run twice in the same gel run (28 well combs, 8 gel slabs per run) (interassay), and the same 100 fibers were run on separate days and months for determination of intra-assay variability. The coefficient of determination (r2) comparing MHC expression in 100 vs. another 100 single fibers from the same biopsy sample (1,600 fibers; n = 4 pre- and postraining) was 0.960 and 0.997, pre- and postraining, respectively. The coefficient of variation for the SDS-PAGE + silver-staining technique was 2.1% and 2.6%, pre- and postraining, respectively.

5% gel for MHC. The 5% separating gel (acrylamide/bis, 19.1%) was poured into a 140 × 160 × 0.75-mm gel cast 4 cm from the top of the glass plate. The gel reagents consisted of 3.75 ml of 1.5 M Tris buffer (18.17 g Tris base and 4 ml of 10% SDS in 100 ml, pH 8.8) for the separating gel, 8.75 ml of 51.4% glycerol, 0.045 ml of 10% ammonium persulfate (APS), and 0.015 ml of N,N,N′,N′-tetramethylethlenediamine (TEMED). 12% gel for MLC. The 12% separating gel (acrylamide/bis, 200:1) was poured into a 140 × 160 × 0.75-mm gel cast 4 cm from the top of the glass plate. The gel reagents consisted of 4.24 ml of 3.0 M Tris·HCl buffer for the separating gel (5.4 g of Tris·HCl and 32.2 g of Tris base in 100 ml, pH 9.3), 5.5 ml of 51.4% glycerol, 0.045 ml of 10% APS, and 0.026 ml of TEMED.

Stacking gel. Once the separating gel polymerized, the stacking gel was poured on top; then, the 28-well combs were placed between the glass plates. The stacking gel for the 5% gel consisted of 3.25 ml of distilled water, 0.5 ml of acrylamide/bis (19:1), 1.25 ml of 0.05 M Tris buffer (6.06 g Tris base and 4.0 ml of 10% SDS in 100 ml, pH 6.8), 0.02 ml of 10% APS, and 0.01 ml of TEMED. The 12% separating gel consisted of 3.17 ml of distilled water, 0.5 ml of acrylamide/bis (20:1), 1.25 ml of 0.05 M Tris·HCl buffer for the stacking gel (7.7 g of Tris·HCl and 1.5 g of Tris base in 100 ml, pH 6.8), 0.05 ml of 10% SDS, 0.018 ml of 10% APS, and 0.014 ml of TEMED.

Silver stain. Once the bromphenol blue had run off the gel slab (~13 h for MHC, ~5 h for MLC), the electric current was discontinued. After the loading gel was discarded and a small portion of the right corner was cut off the separating gel for orientation purposes, the slab was placed into 250 ml of solution A (alcohol-acid fixing solution) (500 ml ethanol, 100 ml glacial acetic acid, 400 ml distilled water) for a minimum of 30 min. The gel was then transferred into 250 ml of solution B (gluteraldehyde cross-linking solution) (200 ml of 50% gluteraldehyde, 800 ml distilled water) for no more than 24 h. One hour before the gel was developed, a series of rinses with distilled water (one every 20 min) was performed until the yellow color was no longer detectable in the gel. Typically, the gel was agitated (Thermolyne Roto Mix, Barnstead, Newington, NH) in a covered Pyrex glass dish to allow for proper rinsing and to prevent gel adhesion to the glassware.

The staining began by transferring the gel into solution C (ammonial silver stain solution) (6 ml of 1.14 M AgNO3, 31.5 ml of 90 mM NaOH, 2.1 ml of 14.8 M NH4OH in 110 ml of distilled water at 4°C) for 6 min while it was agitated. Once finished, the gel was transferred into distilled water for a series of 2-min rinses (distilled water) for 6 min. The gel was then placed into solution D (citric acid-formaldehyde developer) (2.5 ml of 47.6 mM citric acid, 250 µl of 37% formaldehyde in 10% methanol solution in 500 ml of distilled water) for developing. When the stain had reached a desired intensity, the gel was transferred into a series of 1-min distilled water rinses for 5–6 min and then agitated in distilled water for at least 3–5 h. At this point, the gels were ready for...
preservation. A gel dryer (Easy Breeze, Hoefer, San Francisco, CA) was employed, and two sheets of cellophane (Hoefer) were used to sandwich the gel, accompanied by a light coat of glycerol on each side to prevent cracking. This would allow the gels to retain staining intensity and color (16).

MHC protein expression distribution was identified according to migration rates and compared with molecular weight standards of each single fiber. The MHC and MLC were categorized as MHC I, IIa, IIx, I/IIa, I/IIa/IIx, IIa/IIx and as 1s, 1f, 2s, 2f, 3f, respectively. The classification of the MHC isoforms in the single fibers were based on their protein expression (i.e., MHC I, I/IIa, I/IIa/IIx, IIa/IIx), as determined by migration rates from the SDS-PAGE plus silver staining and known standards. A subject's MHC fiber-type distribution was determined from the 200 fibers per biopsy pretraining and the 200 fibers posttraining. From these fibers, the percentage of the total (200) for each MHC isoform was represented. This distribution of 200 fibers acts as one data set for an individual subject. This was done for each subject's fibers, pre- and posttraining. Our mean data were derived from the individual subject data: the individual subject distributions were added to determine a mean for the respective time point (i.e., pre- and posttraining separately).

### Results

#### Whole Muscle Strength

The 12-wk PRT program significantly increased knee extensor strength by 51.9 ± 6.5% (52.9 ± 5.3 kg during pretraining, 79.1 ± 7.0 kg during posttraining) in the older men (P < 0.05).

#### ATPase Analysis for Fiber Type

Muscle fiber-type distributions, as determined by ATPase histochemistry (pH 4.54), were performed pre- and posttraining and are shown in Table 1. A representative histochemical stain is displayed in Fig. 1. The total amount of fibers analyzed was 3,002 pre- and 3,198 posttraining. On average, 500 ± 68 fibers per subject were used for pretraining analysis, and 533 ± 94 fibers were used for posttraining analysis (n = 6, pre- and posttraining). Because of an insufficient tissue sample available for analysis, one subject (subject A) was not included in the histochemical data analysis. Although there were modest increases in type IIa fibers (+4.3%) and slight decreases in type IIb fibers (−5.8%) following PRT, these differences were not significant (Table 1; P > 0.05). Type I fibers showed no difference following the PRT.

#### MHC

SDS-PAGE analyses of MHC isoforms (total = 3,059 single muscle fibers) were studied (1,568 fibers pre- and 1,491 fibers posttraining). Representative polyacrylamide gels for MHC and MLC are shown in Fig. 2. MHC analysis revealed a significant decrease (P < 0.05) in all of the hybrid isoforms (i.e., I/IIa, I/IIa/IIx, IIa/IIx, I/IIa/IIx and as 1s, 1f, 2s, 2f, 3f, respectively).
IIa/Ix) (Table 2). The MHC I/IIa, IIa/Ix, and I/IIa/Ix isoforms decreased in distribution by 9.0%, 8.9%, and 1.0% pre- vs. posttraining, respectively.

The total number of hybrid fibers pretraining was 478 of 1,568 single fibers analyzed or 35.0% of the total fibers. This number was significantly reduced (P < 0.05) following the resistance training, with hybrid fibers accounting for only 12.6% (168 hybrids per 1,491 single muscle fibers analyzed). The MHC I fibers significantly increased by 10.4% (P < 0.05) following the PRT. Despite an increase in MHC IIa distribution of 8.7%, the values were not significantly different. The MHC IIx fibers remained unchanged after the training (Table 2 and Fig. 3).

When the pretraining measurements of ATPase histochemistry (type I, IIa, IIb) were compared with MHC (MHC I, IIa, IIx), a significant correlation of \( r = 0.80 \) (P < 0.05) was found. After PRT, the ATPase histochemistry...
training (MHC IIa fibers) was significantly lower pre- and post-training compared to MHC IIx isoforms (36 of 3,059 single muscle fibers; 1.2%), these fibers were not used in the MLC_3/MLC_2 calculation in the analysis.

DISCUSSION

The purpose of this study was to examine the morphological changes in single muscle fibers following 12 wk of resistance training in seven older men. Along with the single fiber analysis for MHC and MLC distributions, ATPase histochemistry was analyzed pre- and posttraining. The single muscle fiber adaptations to the PRT stimulus were further supported by whole muscle measurements of strength. The major findings of this study were a significant decrease in all of the “hybrid” myosin isoforms (i.e., MHC I/IIa, I/IIa/IIx, IIa/IIx), along with a significant increase in MHC I distribution. However, there was no change in fiber-type distribution using the ATPase histochemical technique. In addition, there was no change in the MLC_3-to-MLC_2 ratio pre- vs. posttraining in MHC I or MHC Ila single fibers.

Historically, resistance training studies in older populations have used ATPase histochemistry to determine fiber-type distribution and composition (14, 15). They have consistently reported that aging muscle fiber-type distributions were not altered with resistance training, although increases in fiber area (hypertrophy) in both type I and II fibers were shown. This study has confirmed previous findings that resistance training cannot alter fiber-type distribution when using the ATPase method of staining. This technique is limited to three main fiber types (type I, Ila, and Iib) (8) and generally is not able to accurately detect hybrid fibers. A series of stains at various pH levels can indicate potential hybrid fibers, but staining intensity is highly pH dependent and interpretation is subjective.

SDS-PAGE in combination with silver staining has been used to identify components of the contractile machinery (1, 3, 7, 9, 17, 18, 24) in human, rabbit, and rat skeletal muscles. Only a few studies (17, 18, 23, 29, 31) have used this technique to assess aging human muscle. Klitgaard et al. (18) profiled the vastus lateralis of five older men (69 yr) using both the ATPase histochemical staining and the SDS-PAGE + silver-staining methods. Fiber-type compositions from the ATPase data of Klitgaard et al. (18) (48% type I, 37% type Ila, 15% type Iib) were similar to the findings from this investigation (50.4% type I, 36.8% type Ila, and 12.8% type Iib for pretraining). With the use of the SDS-PAGE and silver-stain method for single fiber analysis of MHC composition, Klitgaard et al. (18) detected hybrids (33% MHC Ila/Iix and 20% MHC I/Ila) in the aging single muscle fibers. The results from the present investigation support Klitgaard et al. (18); we report hybrids pre- (35%) and posttraining (12.6%) using the silver-stain method. It appears that the ATPase method was not sensitive enough to detect these hybrid fibers, unlike the silver-staining technique, and may not be the proper technique to accurately determine fiber-type distributions in an older population (trained or untrained). When comparing the ATPase

Table 3. Effect of training on myosin light chain 3-to-myosin light chain 2 ratio in single fibers

<table>
<thead>
<tr>
<th>MHC</th>
<th>Pretraining</th>
<th>Posttraining</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.151±0.02*</td>
<td>0.137±0.03*</td>
</tr>
<tr>
<td>Ila</td>
<td>0.215±0.02</td>
<td>0.214±0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as a ratio of myosin light chain 3 (MLC_3) to total MLC_2. Number of fibers used were as follows: 401 pretraining (MHC I: 25±7 and 32±8 fibers/subject, pre- and posttraining, respectively) and 501 posttraining (MHC Ila: 32±5 and 40±8 fibers/subject, pre- and posttraining, respectively) (n = 7).

*Significant difference (P < 0.05) between MHC I and Ila.
results to the single fiber isoforms, we found a significant correlation ($r = 0.80$ for pretrained and 0.95 for posttrained; $P < 0.01$) between the two methods, but this did not include the hybrid fibers. The stronger posttraining correlation may be due to the decrease in hybrid isoforms.

Hybrid fibers may be in a state of transition toward an isoform that is stimulus dependent or in a state of stability. More recent work addressing the issue of isoform stability examined the protein expression and mRNA of the same fiber following bed rest (2). Andersen et al. (2) suggested that the muscle was in a period of transition, demonstrated by an increase in fibers expressing one isoform at the protein level and another isoform at the mRNA level (e.g., a fiber expressing MHC IIx at the mRNA level but expressing MHC IIa at the protein level). This type of fiber was labeled “mismatched,” suggesting an incomplete transition. These results did not concur with what was shown at the protein level, which may be due to the limitations of ATPase histochemical staining for fiber typing used by Andersen et al. (2). SDS-PAGE + silver staining may have been a more appropriate technique in determining fiber type.

The hypothesis of the present investigation was that MLC3 would increase in proportion to MLC2, thus translating into changes at the single fiber level and in the whole muscle (i.e., strength). Results from this study did not demonstrate an increase in the MLC3-to-MLC2 ratio; rather, a slight decrease in the MHC I fibers pre- vs. posttraining was shown. Light chain modulation of shortening velocity ($V_o$) has been an area of intense study since the findings of Lowey et al. (26). A variation in $V_o$ of single fibers, expressing similar MHC, has been correlated to the amount of MLC3 (24, 31); the more MLC3 content, the higher the $V_o$.

In aging muscle, two studies (23, 31) have reported decreased MLC3 expression in sedentary vs. physically active individuals. Mechanical unloading has been associated with an increase in MLC3 and an increase in $V_o$ (29, 30). Likewise, resistance training in rats has demonstrated an increase in single fiber $V_o$, and the combination of the two (unloading and resistance training) results in increases in $V_o$ vs. control rats (weight bearing) (29). The two mechanisms may be under differing controls for increasing skeletal muscle $V_o$. The MLC3 expression may be enhanced in aging muscle due to a decreased activity. Similarly, the resistance exercise stimulus would increase MLC3 expression but without measurable difference compared with pretraining in the older men ("pseudounloaded" state).

Aging skeletal muscle and mechanical unloading both have demonstrated denervation/reinnervation, leading to fiber-type grouping (25). The difference between the two lies within the fiber-type alterations, in which unloading has been shown to preferentially affect the slow-twitch fibers and aging seems to affect the fast-twitch fibers. Unloading protocols are normally no more than 12 wk, so these divergent lines of data may be due to the length of exposure; i.e., aging occurs over a period of several decades. The changes we see with unloading may represent the short-term changes occurring in the aging process. Possibly, there is an initial shift toward a faster fiber with aging (40–50 yr) as with unloading; then, as age further increases and activity decreases, denervation/reinnervation occurs. Degradation of the higher threshold motor units (type II motor units) (10) causes a decrease in the type II fiber population, with little or no change in the fiber-type distribution (25). With aging, there is a decrease in the distribution of type II fibers (20, 21, 25, 28), possibly due to a decrease in physical activity and an increase denervation/reinnervation of the corresponding motor units (10). The slower type fiber (MHC I), which predominates in aging muscle, may have to maintain force production; that is, MHC I isoforms display a greater magnitude of change than the MHC IIa fibers. If this is the case, the results from this study support this notion. The MHC I fiber distribution significantly increased by 10.4% after PRT, whereas the MHC IIa and IIx remained unchanged. Other studies (1, 3, 11, 12) have demonstrated increases in the MHC IIa fiber distribution using similar or higher intensity protocols in younger individuals. We hypothesized a similar MHC IIa increase expression following the training in the older men, and these findings may be age related or stimulus dependent. There was a 8.7% increase in MHC IIa in this study, although not significant. The inability to significantly increase MHC IIa distribution may be due to the above-mentioned denervation/reinnervation of type II fibers (10). The 12-wk resistance training protocol may have not been long enough to strengthen the denervated/reinnervated fibers; thus type II fibers would have adapted at a slower rate than the type I fibers.

Lastly, it should be mentioned that the muscle biopsy procedure represents a possible confounding variable in this investigation. The posttraining biopsy was performed proximal to the pretraining biopsy (same leg), so as to avoid possible scar tissue or damage from the previous biopsy site, and was taken at approximately the same depth each time. Lexell et al. (25) have reported the predominance of type II fibers superficially and type I fibers in deep regions of the whole muscle cross section (vastus lateralis) in younger age groups. Conversely, Lexell et al. (25) reported that two older age groups did not show a similar pattern (occurrence of the two fiber types varied only slightly between the deep and superficial regions). In addition, all subjects in the investigation did display the same trends in MHC alterations (i.e., MHC hybrid decrease, MHC I increase, and little change in the MHC IIa and IIx).

In conclusion, this is the first investigation, to our knowledge, to report fiber-type alterations with PRT in an older population. Previous studies of this nature have reported no change in fiber-type distribution using ATPase histochemistry, along with significant increases in strength, as was shown in this investigation. Furthermore, all myosin hybrid isoforms significantly decreased and MHC I expression significantly increased, with no change in MHC IIa expression. Moreover, this investigation found no change in the
MLC3/MLC2 expression ratio after 12 wk of PRT. This investigation found the use of ATPase histochemistry less reliable in identifying changes in skeletal myofilament proteins; alternatively, use of the SDS-PAGE silver staining technique provided a more accurate portrayal of the alterations occurring in skeletal muscle of older men.

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