Influence of mechanical loading on myosin heavy-chain protein and mRNA isoform expression

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CAIOZZO, VINCENT J., FADIA HADDAD, MICHAEL J. BAKER, and KENNETH M. BALDWIN. Influence of mechanical loading on myosin heavy-chain (MHC) protein and mRNA isoform expression. J. Appl. Physiol. 80(5): 1503–1512, 1996.—The overall objective of the studies reported herein was to examine the effects of high-resistance training on myosin heavy-chain (MHC) protein and mRNA isoform expression. The findings from these studies can be summarized as follows: 1) there was a substantial increase in the fast type IIX MHC protein isoform content of the trained red and white medial gastrocnemius muscles, but this did not occur until after the eighth training session (i.e., 16 days); 2) single-fiber analyses demonstrated that many so-called fast type IIB fibers contained small amounts of the fast type IIX MHC protein isoform and that the high-resistance training program altered the bias of fast type IIB-type IIX MHC protein isoform distribution in these fibers but did not increase the number of fibers that could be categorized as exclusively fast type IIX fibers; 3) the high-resistance training program produced a rapid (i.e., after two training sessions) elevation in the fast type IIX MHC mRNA isoform and a corresponding repression of the fast type IIB MHC mRNA isoform; and 4) the dose-response study revealed that as few as 10 contractions (40 s) per training session were capable of elevating the expression of the fast type IIB MHC protein isoform and concomitantly upregulated the expression of the fast type IIA MHC protein isoform in the white (WMG) and red (RMG) regions of the medial gastrocnemius (MG) and tibialis anterior (TA) muscles. These findings are consistent with the hypothesis that mechanical loading upregulates the expression of slower MHC protein isoforms.

The objective of this study was to extend these earlier findings in several important ways. First, in our earlier study (7), the fast type IIA and type IIX MHC protein isoforms comigrated, and it was impossible to differentiate which of these two isoforms was truly influenced by the high-resistance training program. Hence, in this study, we have used a new gel system that clearly separates all four MHC protein isoforms (21). Second, a time-course study was conducted to examine how rapidly a high-resistance training program altered the whole muscle MHC protein isoform distribution. Third, in the present study, we used an electrophoretic technique to examine the single-fiber distribution of MHC protein isoforms after high-resistance training. Fourth, to explore the possible role of pretranslational events, the time course of alterations in whole muscle MHC mRNA isoforms was examined. Finally, a dose-response study was performed to identify the optimal number of contractions necessary to induce alterations in the whole muscle MHC mRNA isoform distribution.

METHODS

Animal Care and Experimental Groups

Female Sprague-Dawley rats (250–300 g) were used in each of the studies. Table 1 describes key aspects of the four major studies reported herein. All animals were housed individually and given food and water ad libitum. The experiments described in this study were approved by our Institutional Review Board.

Immediately before surgery, the animals were anesthetized by using a mixture of acepromazine (4.5 mg/kg) and ketamine (75 mg/kg). Each animal underwent surgery and had stimulating electrodes implanted on both sides of the left MG muscle. These wires extended from a connector that was attached to the skull by screws and bone cement. A sham operation was performed on the right leg. The wires that were implanted adjacent to the right MG muscle were not, however, connected to the head plug. The animals were allowed 1 wk to recover from surgery before the training program began.

Approximately 20 min before each training session, the animals were anesthetized with acepromazine (3 mg/kg) and ketamine (50 mg/kg). Animals were subsequently trained by using a computer-controlled Cambridge ergometer training apparatus (6, 10). The key components of this system included 1) a Cambridge ergometer (model 310, Cambridge
Adenosinetriphosphatase (ATPase) Activity 1 mg/ml and at -20°C in a solution containing 50% glycerol by using the biuret technique. Myofibrils were then stored at a pellet was resuspended in a solution (solution B; pH 7.0) described above. The resulting pellet was again suspended in a solution determined by either slot blot or Northern blot analyses. Myofibrillar Purification and Myofibrillar ATPase activity was determined by using an Mg<sup>2+</sup>-activated Ca<sup>2+</sup>-regulated system described previously (6).

### Electrophoretic Separation of MHC Isoforms

MHC protein isoforms in whole muscles and single fibers were separated by using techniques described by Talmadge and Roy (21). The separating gel solution contained 8% acrylamide, 0.16% bis-acrylamide, 30% glycerol, 0.4% sodium dodecyl sulfate (SDS), 0.2 M Tris (pH 8.8), and 0.1 M glycine. This solution was degassed for ~15 min. Polymerization was then initiated by the addition of N,N,N',N'-tetramethylethylenediamine (0.05% final concentration) and ammonium persulfate (0.1% final concentration) to the separating gel solution. After the separating gel had been poured, it was layered with ethyl alcohol and allowed ~30 min to polymerize. Once the separating gel was polymerized, the stacking gel was poured. The composition of the stacking gel was 4% acrylamide, 0.08% bis-acrylamide, 30% glycerol, 70 mM Tris (pH 6.7), 1 mM EDTA, and 0.4% SDS. This solution was also degassed for 15 min before the addition of N,N,N',N'-tetramethylethylenediamine (0.05% final concentration) and ammonium persulfate (0.1% final concentration). The composition of the running buffer was 0.1 M Tris, 0.15 M glycine, and 0.1% SDS. Myofibrillar samples were denatured by using a sample buffer solution containing 5% β-mercaptoethanol, 100 mM Tris-base, 5% glycerol, 4% SDS, and bromophenol blue. Approximately 1 μg of protein was loaded into each well. Electrophoresis was performed by using a 5G-200 vertical slab gel system (CBS Scientific, Del Mar, CA). Gels were run by using a constant voltage of 275 V for ~24 h. This method separated the fast type IIA, fast type IIX, and slow type I MHC isoforms (order of migration). MHC protein isoform bands were stained by using Coomassie blue G-250.

### Purified myofibril preparations

Purified myofibril preparations were extracted by using techniques described previously (6). In brief, this included homogenization of the muscle in a solution (solution A; pH 6.8) containing (in mM) 250 sucrose, 100 KCl, 20 tris(hydroxy-methyl)aminomethane (Tris), and 5 EDTA. The homogenate was centrifuged at 1,000 g for 10 min at 4°C. The resulting pellet was resuspended in a solution (solution B; pH 7.0) containing 175 mM KCl and 20 mM Tris and centrifuged as described above. The resulting pellet was again suspended in solution B and adjusted to a protein concentration of 6 mg/ml by using the biuret technique. Myofibrils were then stored at 1 mg/ml and at -20°C in a solution containing 50% glycerol and (in mM) 50 Na<sub>2</sub>P<sub>2</sub>O<sub>7</sub>, 2.5 ethylene glycol-bis(β-amino-ethyl ether)-N,N,N',N'-tetraacetic acid, and 1 β-mercaptoethanol (pH 8.8).

### Myofibrillar ATPase Activity

Myofibrillar ATPase activity was determined by using an Mg<sup>2+</sup>-activated Ca<sup>2+</sup>-regulated system described previously (6).
Isolation of Single Fibre

Before the muscle was removed from the animal, the muscle was immersed in glycerol-relaxing solution [50% glycerol and (in mM) 2 ethylene glycol-bis(β-aminoethyl ether)-N,N′,N′,N′-tetraacetic acid, 1 MgCl₂, 4 ATP, 10 imidazole, and 100 KCl, pH 7.0]. The muscle was quickly cut into small strips and stored in glycerol-relaxing solution overnight at 0°C. Segments of single fibers were isolated by placing the muscle strips in a small dissection chamber. Dissection was performed by using a microscope (Technival 2, ausJena, Germany) with back lighting and microsurgical forceps (Super Finc Dumont tweezers, Biomedical Research Instruments, Rockville, MD). Single fibers were transferred to 500-µl polypolyethylene microcentrifuge tubes that contained 30 µl of a solution containing 62.5 mM Tris (pH 6.8), 1.0% (wt/vol) SDS, 0.01% (wt/vol) bromophenol blue, 15.0% (vol/vol) glycerol, and 5.0% (vol/vol) β-mercaptoethanol. These samples were then heated at 70°C for 2 min and placed into a sonicator for 60 min. This method is a modification of that initially described by Giulian et al. (11). The distribution of MHC protein isoforms in single fibers was determined by using the electrophoretic techniques described above, except that MHC protein isoform bands were identified by using a silver-stain kit (Bio-Rad, Richmond, CA).

Immunohistochemistry

WMG muscle samples taken from animals that trained for 8 and 16 days were frozen in isopentane cooled by liquid nitrogen. The muscle samples were serially sectioned in a cryostat (−20°C) at 10-µm thickness and placed on glass slides. The tissue sections were allowed to air dry and then were probed by using a monoclonal antibody (MAb) specific to the fast type IIB MHC protein isoform (BF-F3). Additionally, MAb BF-35 was used to identify muscle fibers that expressed only the type IIX MHC protein isoform. MAbs BF-F3 and BF-35 were kindly supplied by Regeneron Pharmaceuticals (Tarrytown, NY) through an agreement with Dr. S. Schiaffino. The specificity of MAbs BF-F3 and BF-35 has been reported previously by Schiaffino et al. (20). MAbs BF-35 recognizes all the MHC protein isoforms except for the fast type IIX. Hence, a fast type IIX fiber was identified by the absence of staining with the use of this particular MAb. The primary MAbs were exposed to tissue sections for 60 min at 37°C. The slides with tissue sections were then gently washed twice with a Tris-buffered saline solution. Tissue sections were then exposed to a solution containing the appropriate second antibody labeled either with fluorochrome or biotin (60 min; 37°C). After exposure to the second antibody, the slides were again gently washed by using a Tris-buffered saline solution. Tissue sections probed with the biotinylated second antibody were treated further by using the avidin-biotin complex detection method (Vector Laboratories, Burlingame, CA). The staining intensity of a muscle fiber was categorized as light, intermediate, or dark.

The fiber type percentage was determined by counting all of the fibers within a given tissue section. The mean number (n) of fibers counted for each 8-day control (n = 6) and trained (n = 6) WMG muscle were 1,091 ± 148 and 1,547 ± 710 (SD) fibers, respectively. The mean number of fibers counted for each 16-day control (n = 5) and trained (n = 5) WMG muscle were 1,419 ± 287 and 1,345 ± 429 fibers, respectively.

RNA Isolation and Blotting

The RNAzol method (TEL TEST, Friendswood, TX) was used to isolate total cellular RNA from the RMG and WMG of the trained and control MG muscles. This method is based on the procedures described by Chomczynski and Sacchi (8). RNA concentration was determined by measuring the optical density at a wavelength of 260 nm. For the slot blots, -5 µg of total RNA were heat denatured in 50% Formalin and 10× saline-sodium citrate buffer (SSC) and loaded directly onto a nylon membrane (GeneScreen Plus; New England Nuclear, Boston, MA) by using a filtration manifold apparatus (Schleicher and Schuell, Keene, NH). For Northern blots, 5 µg of total RNA were fractionated on 1% agarose denaturing gel (20 mM 3-(N-morpholino)propanesulfonic acid, 5 mM sodium acetate, 0.5 mM EDTA, 6% formaldehyde, pH 7.0) by electrophoresis for 6 h at 30 V. The RNA was transferred to nylon membrane (GeneScreen Plus) by using the capillary method and 10× SSC as a transfer buffer. Both slot blots and Northern blots were ultraviolet fixed to immobilize the RNA onto the membrane, dried at 80°C for 30 min, and then stored dry at 4°C until subsequent hybridization.

Oligonucleotide Probes

Oligonucleotides complementary to the 3′ nontranslated sequences of the four different adult skeletal muscle MHC mRNA isoforms found in rat were purchased from Chemgene (Waltham, MA). The oligonucleotide sequences used to identify the slow type I, fast type IIA, and fast type IIB MHC mRNA isoforms are those reported by Gustafson et al. (12). The fast type IIX MHC mRNA isoform was detected by using a 20-base oligonucleotide sequence complementary to the 3′ nontranslated sequence of a cDNA clone described by Schiaffino (see Refs. 1 and 9 for sequence). The specificity of these probes has been shown previously (5).

The 5′ end of the oligonucleotide probes was labeled with [γ-32P]ATP (1–2 × 10⁶ counts min⁻¹ µg⁻¹) by using polynucleotide kinase (17). Labeled probes were separated from unincorporated ATP by gel filtration through a Sephadex G-25 minicolumn (quick spin G-25, Boehringer Mannheim).

Hybridization

Both slot blots and Northern blots were prehybridized (2 h, 5°C less than the melting temperature of the probe) in 10× Denhardt’s solution, 6× sodium chloride-sodium phosphate-EDTA (SSPE), 1% SDS, sonicated salmon sperm DNA (50 µg/ml), and yeast transfer RNA (50 µg/ml). Hybridization was performed overnight at the prehybridization temperature in 6× SSPE and 1% SDS at a probe concentration of 1–2 × 10⁶ counts min⁻¹·µl⁻¹. The blots were then washed in the following 15-min sequences: 1) three times at room temperature; 2) once at the hybridization temperature; and 3) a final wash at room temperature. Each 15-min wash was performed in a solution containing 6× SSPE and 0.1% SDS. After this sequence, the blots were individually wrapped in sealed plastic bags and exposed to an X-OMAT autoradiographic film (Kodak) with intensifying screen (DuPont) at −70°C for 1–3 days depending on the signal intensity. After signal detection by using autoradiography, the probes were washed off the blots by boiling 10–15 min in 1% SDS. For the slot blots, the membranes were rehybridized with an excess of a 32P-labeled oligo(dT) probe (16 mer; Pharmacia LKB, Piscataway, NJ) that hybridizes to poly(A)+ RNA (total mRNA). For the Northern blots, the membranes were rehybridized with an excess of a 32P end-labeled 18S oligoprobe (18) that hybridizes to 18S ribosomal RNA. Band intensities on the autoradiogram were quantitated by using a laser-scanning densitometer (Molecular Dynamics, Sunnyvale, CA), and each specific MHC absorbance was normalized to its corre-
sponding oligo(dT) signal on the slot blots or 18S signal on the Northern blots.

**Statistical Analyses**

All statistical analyses were performed by using a computer program (Systat, Evanston, IL). The time-course data for both the protein and mRNA MHC isoform data were analyzed by using a two-way analysis of variance. The data for each separate MHC isoform were analyzed independently of the other isoforms. If a significant time-group interaction was observed, Dunnett’s test was employed to determine at which time point significant differences between the control and trained groups occurred. With respect to the immunohistochemical data, the percentages of a particular fiber type (e.g., light, intermediate, or dark staining) from the control and trained muscles were compared by using an independent t-test. Statistical comparisons were considered significant when \( P < 0.05 \).

**RESULTS**

**Muscle Weights, Myofibrillar Protein Concentration, and Myofibrillar ATPase Activity**

The changes in muscle mass associated with training are shown in Table 2. Significant differences between the trained and control MG muscles were found after 1 (+8%; \( P < 0.05 \)), 2 (+8%; \( P < 0.01 \)), and 16 training sessions (+11%; \( P < 0.01 \)). With respect to myofibrillar protein concentration, there were no differences between the trained and control MG muscles at any time point. There were no significant changes in the myofibrillar ATPase activities of the WMG or RMG muscles, although a progressive difference between the trained and control WMG muscles was observed (0% after 1 training session; 9% decrease after 16 training sessions). This trend is consistent with the MHC protein isoform changes noted below.

**Time Course of MHC Protein Isoform Alterations in WMG and RMG Muscles**

**WMG muscle.** The time course of altered MHC protein isoform composition is shown in Fig. 1. The control WMG contained very little slow type I (-1%) and fast type IIA (-6%) MHC protein isoforms. In contrast, the fast type IIB MHC protein isoform represented the greatest proportion (~70%) of the MHC pool. The type IIX MHC protein isoform represented ~20% of the MHC isoform pool. Training did not affect the relative content of either the slow type I or fast type IIA MHC protein isoforms. However, training produced a substantial increase (\( P < 0.001 \)) in the relative content of the fast type IIX MHC protein isoform (see Fig. 1C) and a concomitant decrease (\( P < 0.001 \)) in the relative content of the fast type IIB MHC protein isoform (see Fig. 1D). Dunnett’s test demonstrated that a significant increase in the fast type IIX MHC and decrease in fast type IIB MHC protein isoforms did not occur until training session 8 (i.e., day 16).

**RMG muscle.** The time course of altered MHC protein isoform expression for the RMG is also shown in Fig. 1. Note that the relative content of the fast type IIB MHC protein isoform in the control muscles was ~45% of the total MHC protein pool. The fast type IIX isoform also represented a large proportion of the total MHC protein pool (~35%). As with the WMG, training had little affect on the slow type I and fast type IIA MHC protein isoforms. However, changes that mirrored those seen for the WMG were noted for both the fast type IIB (\( P < 0.01 \)) and IIX MHC (\( P < 0.01 \)) protein isoforms.

**Gel electrophoresis.** Figure 2 illustrates the distribution of MHC protein isoforms observed in single fibers taken from a representative control and its contralateral trained WMG muscle. Note that, although some of the fibers taken from the control WMG muscle only expressed the fast type IIB MHC protein isoform, others contained both fast type IIB and fast type IIX MHC protein isoforms. A more quantitative description of the MHC protein isoform distribution of single fibers taken from the control WMG muscles is shown in Fig. 3. This histogram reveals that the majority (i.e., ~62%) of control WMG fibers had a fast type IIB MHC content.

**Table 2. Muscle weights, myofibrillar protein concentration, and myofibrillar ATPase activity**

<table>
<thead>
<tr>
<th></th>
<th>1 Day</th>
<th>2 Day</th>
<th>4 Day</th>
<th>8 Day</th>
<th>16 Day</th>
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<tr>
<td></td>
<td>Trn</td>
<td>Con</td>
<td>Trn</td>
<td>Con</td>
<td>Trn</td>
</tr>
<tr>
<td>Total muscle mass, mg</td>
<td>773 ± 53</td>
<td>715 ± 41*</td>
<td>743 ± 27**</td>
<td>686 ± 27**</td>
<td>741 ± 28</td>
</tr>
<tr>
<td>Myofibrillar protein, mg/g muscle</td>
<td>123 ± 8</td>
<td>115 ± 7</td>
<td>130 ± 10</td>
<td>114 ± 6</td>
<td>126 ± 8</td>
</tr>
<tr>
<td>Myofibrillar ATPase activity, nM Pi·mg⁻¹·min⁻¹</td>
<td>623 ± 24</td>
<td>624 ± 29</td>
<td>567 ± 32</td>
<td>602 ± 16</td>
<td>577 ± 27</td>
</tr>
<tr>
<td>Myofibrillar protein, mg/g muscle</td>
<td>112 ± 4</td>
<td>106 ± 8</td>
<td>116 ± 10</td>
<td>111 ± 6</td>
<td>120 ± 9</td>
</tr>
<tr>
<td>Myofibrillar ATPase activity, nM Pi·mg⁻¹·min⁻¹</td>
<td>459 ± 35</td>
<td>489 ± 36</td>
<td>474 ± 33</td>
<td>476 ± 27</td>
<td>466 ± 44</td>
</tr>
</tbody>
</table>

Values are means ± SE. ATPase, adenosinetriphosphatase; Trn, trained; Con, control. *\( P < 0.05 \); **\( P < 0.01 \).
MECHANICAL LOADING AND MHC PROTEIN AND mRNA ISOFORMS

that was \( \geq 90\% \) of the total MHC pool. Of the 280 control WMG fibers that were analyzed, 39% expressed only the fast type IIB MHC protein isoform. The other 61% of the control WMG fibers expressed varying fast type IIB-to-type IIX ratios that were biased toward the fast type IIB MHC protein isoform. The median fast type IIB MHC protein isoform content was 93% for the entire population of control WMG fibers examined.

As shown in Fig. 2, each of the single fibers taken from a representative trained WMG muscle had an increased fast type IIX MHC protein isoform distribution. Concomitant with this alteration, the distribution of the fast type IIB MHC protein isoform in the trained fibers was reduced, as exemplified by the data shown in Fig. 3. This is illustrated by the fact that the median fast type IIB MHC protein isoform content was reduced from 93 (i.e., control) to 72% (i.e., trained). Of the 289 trained fibers that were analyzed, only 6% of the fibers contained just the fast type IIB protein isoform. The single-fiber analyses demonstrated that the high-resistance training program employed in this study provided a sufficient stimulus to bias the polymorphic MHC protein isoform expression in the direction of the fast type IIX MHC protein isoform, but, importantly, it did not appear to induce a complete transformation of MHC composition.

**Immunohistochemistry.** To complement the electrophoretic analyses of the single fibers, immunohistochemical analyses were also performed by using a MAb specific for the fast type IIB MHC protein isoform (BF-F3) and another MAb (BF-35) that recognized all the adult MHC protein isoforms except for the fast type IIX MHC protein isoform (see Fig. 4). These data demonstrate that eight training sessions did not pro-
produce a significant decrease in the percentage of fibers that had a high content of fast type IIB MHC protein isoform expression (i.e., %dark fibers). With respect to the muscles that performed 16 training sessions, there was a small but statistically significant \( P < 0.05 \) decrease from 79 (control) to 68\% (trained) of fibers that stained dark. Coincident with the decrease in dark-staining fibers, there was a significant \( P < 0.05 \) increase in intermediate-staining fibers in the trained muscles. The 16 training sessions did not alter the percentage of fibers that were categorized as light staining. The fast type IIX data obtained by using MAb BF-35 basically mirrored those observed by using BF-F3.

**Time Course of MHC mRNA Isoform Alterations in WMG and RMG**

**RNA concentration.** The mean values for RNA concentration are reported in Table 2. There were no differences between RNA concentrations of the trained and control WMG muscles after 1, 2, 4, or 8 training sessions. However, after 16 training sessions (32 days total), the RNA concentrations of the trained WMG muscles were significantly greater than those of the control WMG muscles \( P < 0.01 \).

There were no significant differences between the trained and control RMG muscles at any of the time points.

**WMG muscle.** The time course data for the control and trained WMG muscles are shown in Figs. 5–7. As shown in Fig. 7, the expression of fast type IIB MHC mRNA isoform signal in the control muscles was similar across all time points. Training produced a substantial downregulation in the level of the fast type IIB mRNA isoform.
MECHANICAL LOADING AND MHC PROTEIN AND mRNA ISOFORMS

Fig. 5. Slot blots of fast type IIX MHC mRNA content of trained (TRN) and control (CON) WMG muscles. Blots at each time point are from 3 representative animals (i.e., WMG1, WMG2, WMG3). In each case, TRN left WMG muscle is shown with its contralateral CON WMG muscle. Note that after 4 training sessions (8 days total) there was a large increase in fast type IIX MHC mRNA isoform in TRN WMG.

Dose Response of Fast Type IIX MHC mRNA Isoform

WMG muscle. As shown in Fig. 7, two training sessions were sufficient to elevate the fast type IIX MHC mRNA signal by ~120%. The dose response of the fast type IIX MHC mRNA concentration was examined by having different groups of muscles perform 10, 20, 40, or 80 contractions per training session (i.e., 2 training sessions total). As shown in Fig. 8, 10 contractions per training session were sufficient to significantly (+225%; P < 0.05) elevate the fast type IIX MHC mRNA signal in the trained WMG muscles. As shown in Fig. 8, the response of the trained WMG muscles was maximal (+350%) at 20 and 40 contractions per training session. Although 80 contractions per training session also significantly elevated the fast type IIX MHC mRNA content of the trained WMG muscles, the response was not nearly as great as that seen at the other doses of training. This is in contrast to the RMG (see RMG muscle) and MMG muscles (not reported), in which 80 contractions produced a response similar to that produced by 20 and 40 contractions per training session.

RMG muscle. The dose-response data for the RMG is shown in Fig. 8. As with the white region, as few as 10 contractions were sufficient (P < 0.05) to elevate the fast type IIX MHC mRNA content. The alterations in fast type IIX MHC mRNA isoform expression produced by 20 (P < 0.01), 40 (P < 0.01), and 80 (P < 0.01) contractions were similar to that produced by 10 contractions per training session.

DISCUSSION

Three key factors suggest that it was mechanical loading and not stimulation frequency that produced the MHC transitions reported in this study. First, whereas it has been suggested previously that high stimulation frequencies produce faster, not slower, muscle fibers (16), a recent study (7) found that the relative content of a slower MHC protein isoform was upregulated when a high stimulation frequency was used in conjunction with a high loading condition. Second, by using an approach by which muscles contracted either concentrically or eccentrically under identical loads but at different stimulation frequencies (i.e., eccentric = 25 Hz; concentric = 100 Hz), the same degree of fast type IIB to IIX MHC protein isoform transitions occurred (unpublished observations). Finally, Baldwin et al. (2) have shown that passive mechanical loading upregulates slower myosin isoforms in electrically silent muscles. Collectively, these findings are consistent with the position that mechanical loading played the key role in dictating the MHC protein and mRNA alterations reported herein.

Fig. 6. Slot blots of fast type IIB MHC mRNA content of TRN and CON WMG muscle. Blots at each time point are from 3 representative animals (i.e., WMG1, WMG2, WMG3). Note that after 1 day of training both TRN and CON WMG have similar fast type IIB MHC mRNA contents. After 4 days of training, however, there is a consistent decrease in fast type IIB MHC mRNA content of TRN WMG.
MECHANICAL LOADING AND MHC PROTEIN AND mRNA ISOFORMS

To What Extent Does High-Resistance Training Affect MHC Protein Isoform Content of Single Fibers in MG Muscle?

The alterations in the whole muscle MHC protein isoform distribution described above can be potentially explained by several different schemes of muscle plasticity at the single-fiber level: 1) complete conversion of some fast type IIB to fast type IIX fibers; 2) the partial conversion of fast type IIB to fast type IIX fibers, yielding fibers that have an increased polymorphism; or 3) a combination of these two different schemes of muscle plasticity. As shown in Figs. 2–4, the high-resistance training program employed in the present study did not produce an increase in the percentage of fast type IIX fibers. Rather, the training program altered the fast type IIB-to-type IIX MHC protein isoform bias such that there was an increase in the relative amount of the fast type IIX MHC protein isoform within a given fiber.

The absence of a complete conversion of fast type IIB to fast type IIX MHC protein isoform content raises the issue of whether the fibers in the MG muscle have the adaptive capacity to make complete transitions in MHC protein isoform expression. It may be possible that a longer training program (e.g., 8 wk) would have produced a complete conversion of MHC protein isoform expression in some fibers. In this regard, however, it should be noted that in a previous study (7) we found that the alterations in MHC protein isoform expression after 8 wk of training were not much greater than those produced by a 4-wk training program. Hence, it is doubtful that a longer training program would have increased the number of fibers that only expressed the fast type IIX MHC protein isoform. Rather, it is more likely that a longer training program would have only modulated the pattern of change observed in the present study. It is possible, however, that a greater number of contractions per training session might have...
How Much High-Resistance Activity Is Required to Alter Fast Type IIX MHC mRNA Isoform Expression in MG Muscle?

As reported previously (6), as few as 40 contractions every other day were sufficient to produce substantial changes in the fast type IIB MHC protein isoform expression. The question remains, however, as to the amount of mechanical activity that is required to alter MHC mRNA isoform expression. The dose-response data shown in Fig. 8 demonstrate that as few as 10 contractions per training session are sufficient to produce significant alterations in the expression of the fast type IIX MHC mRNA isoform. This was true for both the WMG and RMG muscles. The dose-response data also demonstrate that, in the WMG muscles, 20–40 contractions per training session appear to produce an optimal increase in the fast type IIX MHC mRNA isoform expression. Beyond this number of contractions, there appeared to be a decrease in the response. It is not clear whether this represents an injury response or just an artifact. Interestingly, in the RMG muscles, the response was similar regardless of the number of contractions used per training session. Additionally, data obtained from the mixed region of the MG (not shown) indicated that the response obtained with 80 contractions was similar to that observed at 20 and 40 contractions. The dose-response data from the WMG and RMG indicate that even <10 contractions every other day might be sufficient to alter the fast type IIX MHC mRNA isoform expression in the MG.

What Is the Physiological Significance of Fast Type IIB-to-Fast Type IIX Transitions Produced by High-Resistance Training?

Although we observed a sizeable decrease in the relative content of the fast type IIB MHC protein isoform, only small decreases (<10%; P < 0.09) in the myofibrillar ATPase activities were observed in the WMG and RMG. This finding is consistent with the single-fiber contractile studies of Bottinelli et al. (3), who reported that the maximal unloaded shortening velocity of fast type IIX fibers was ~20% less than that of fast type IIB fibers. Given the population of IIB/IIX hybrid fibers that were produced in the present study and the relationship between maximal shortening velocity and myofibrillar ATPase activity, it seems reasonable to expect that the high-resistance training program employed in the present study would only produce a small decrease in ATPase activity. The question arises then as to the physiological significance of the types of MHC alterations produced in this study. Why would skeletal muscle undergo a substantial remodeling process when there are no obvious benefits? One possible explanation might be that these types of MHC alterations produce motor units that are less fatigable while maintaining the capacity to produce mechanical work and power. This speculation is based on the findings of Bottinelli et al. (3) described above and of Larsson et al. (15), who reported that fast type IIX

What Is the Time Course of Alterations in MHC Protein and mRNA Isoform Expression Induced by a High-Resistance Training Program?

As stated above, the high-resistance training program employed in the present study was effective in altering the MHC protein isoform expression in the WMG and RMG. However, significant alterations in the MHC protein isoform expression were not observed until eight training sessions (i.e., 16 days). Hence, the alterations in MHC protein isoform expression occurred gradually over this period of time. Analyses of these muscle samples also included examination of alterations in MHC mRNA isoform expression in an attempt to provide some mechanistic analyses of the events occurring at the protein level. The mRNA data illustrated in Figs. 5–7 demonstrate that the high-resistance training program employed in the present study rapidly altered the MHC mRNA isoform expression.

With respect to the rapidity of alterations in the MHC mRNA isoform signal, Fig. 7 demonstrates that only 320 s of training over 4 days (i.e., 2 training sessions) were required to significantly elevate the signal of the fast type IIX MHC mRNA isoform and concomitantly repress the signal of the fast type IIB MHC mRNA isoform. Given that detectable changes in the MHC protein isoforms occurred sometime after eight training sessions (i.e., 16 days total), our data indicate that there is a substantial lag between the modulation of mRNA expression and net accumulation of MHC protein isoform.

To our knowledge, this study is the first to examine the influence of high resistance training on the expression of MHC mRNA isoforms. Importantly, it should be noted that other investigators have also observed rapid changes in MHC mRNA isoform expression after the onset/cessation of chronic electrical stimulation (4, 14). These studies, however, employed a model of chronic electrical stimulation that caused the muscle to contract thousands of times per day. Collectively, the results from the chronic electrical stimulation studies (4, 14) and the results of the present study suggest that the expressions of certain MHC mRNA isoforms are very sensitive to altered mechanical loading and can be rapidly altered.
motor units are less fatigable than fast type IIB motor units. Future studies are required to resolve this issue.

Summary

The findings of this study demonstrate that the fast type IIX and IIB MHC mRNA isoforms are very sensitive to the mechanical loading conditions imposed on the MG muscle. The altered MHC mRNA isoform signals observed in the present study could be due to alterations in transcriptional activity and/or changes in MHC mRNA stability. Future studies employing nuclear run-on assays will be required to better delineate the role of transcription. Finally, the response observed at the single-fiber level demonstrates that high-resistance training causes the single fibers of the MG muscle to alter the MHC protein isoform distribution is a key issue that remains to be determined.

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