Modulation of myosin isoform expression by mechanical loading: role of stimulation frequency

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Caiozzo, Vincent J., Michael J. Baker, and Kenneth M. Baldwin. Modulation of myosin isoform expression by mechanical loading: role of stimulation frequency. J. Appl. Physiol. 82(1): 211–218; 1997.—This study tested the hypothesis that mechanical loading, not stimulation frequency per se, plays a key role in determining the plasticity of myosin heavy chain (MHC) protein isoform expression in muscle undergoing resistance training. Female Sprague-Dawley rats were randomly assigned to resistance-training programs that employed active (n = 7) or 2) lengthening contractions (n = 8). The medial gastrocnemius (MG) muscles in each group trained under loading conditions that approximated 90–95% of maximum isometric tetanic tension but were stimulated at frequencies of 100 and ~25 Hz, respectively. Lengthening and shortening contractions were produced by using a Cambridge ergometer system. The MG muscles trained every other day, performing a total of 16 training sessions. Both training programs produced significant (P < 0.01) and similar reductions in the fast type IIB MHC protein isoform in the white MG muscle, reducing its relative content to ~50% of the total MHC protein isoform pool. These changes were accompanied by increases in the relative content of the fast type IIX MHC protein isoform that were of similar magnitude for both groups. The results of this study clearly demonstrate that stimulation frequency does not play a key role in modulating MHC isoform alterations that result from high-resistance training.

The findings of this study demonstrate that training paradigms involving high-loading conditions (~90–95% P0) can produce alterations in MHC protein isoform composition that are independent of stimulation frequency (at least above ~25 Hz).

METHODS

Animal care and experimental groups. Female Sprague-Dawley rats (~250–300 g) were randomly assigned to the various experimental groups shown in Table 1. The experiments reported in this study were conducted in two phases. Phase 1 examined the MHC isoform alterations produced by Actshort and Actlength training programs that employed similar loading conditions but markedly different stimulation frequencies. Experiments conducted in phase 2 were designed to determine whether the responses observed in phase 1 might be attributable to muscle injury occurring at early stages of the training program. All animals were housed individually and given access to food and water ad libitum. All procedures involving animal welfare were approved by our institutional review board before these experiments were conducted.

Surgical implantation of stimulation electrodes and training programs. Each animal was anesthetized (acepromazine = 4.5 mg/kg; ketamine = 75 mg/kg), and stimulating electrodes were surgically implanted in the left hindlimb of the animal so that the activation pattern of the MG muscle could be controlled. A sham operation was performed on the right leg, which served as the control.

After 1 wk of recovery, the two groups of animals in phase 1 began their respective training programs (see Table 1). Approximately 20 min before each training session, the animals were lightly anesthetized with acepromazine (5 mg/kg) and ketamine hydrochloride (20 mg/kg). Shortening or lengthening contractions were produced by using a computer-controlled Cambridge ergometer system (6, 7). The major components of this system include 1) a Cambridge ergometer (model 310, Cambridge Instruments, Watertown, MA) that was used to control the mechanical loading conditions imposed on the target muscle during training; 2) a computer that activates the stimulator and controls the parameters of the ergometer; and 3) a training platform that translates the moment at the ankle into a linear force. In the present study, the ergometer was used to impose a slow constant angular velocity of 12°/s that caused the MG muscles to train under loading conditions that briefly approximated 90–95% P0. Each Actshort contrac-

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tion began with the ankle in a neutral position (i.e., 90° relative to the tibia). In contrast, each Act\textsubscript{length} contraction began with the ankle at 48° of plantar flexion relative to the neutral position (see Fig. 2). The combination of these conditions (i.e., angular velocity and range of motion) resulted in negative/positive strain rates that approximated 0.04 muscle length ($L_0$)/s. It should be noted that a positive strain rate of 0.04 $L_0$/s is very similar to that used to produce the Act\textsubscript{length} data shown in Fig. 1. Muscles in the Act\textsubscript{short} and Act\textsubscript{length} groups were stimulated at frequencies of 100 and 25 Hz, respectively. Each contraction was 4 s in duration and was followed by a 6-s rest interval. Each group of animals performed 4 sets of 10 contractions during a training session and trained every other day for 4 wk (see Table 1). Twelve hours after the last training session, the left and right MG muscles were removed from each animal, weighed, and separated into regions designated as red and white (7). Once the muscles had been removed, the animal was killed by using a lethal injection of pentobarbital sodium. The types of analyses that were performed on each muscle group are reported in Table 1. The portion of each region that was used for myofibrillar and MHC protein isoform analyses was

![Fig. 1. Force-frequency relationships for a medial gastrocnemius muscle (MG) under isometric and active lengthening (Act\textsubscript{length}) conditions. Isometric measurements were made at a length ($L_0$) where muscle produced maximal tetanic tension ($P_O$). Act\textsubscript{length} data were collected by using a positive strain rate of 0.12 $L_0$/s. This strain rate is slightly greater than strain rate that was used for training Act\textsubscript{length} group (0.04 $L_0$/s). Importantly, both strain rates produce similar force-frequency curves. Measurements were centered around $L_0$ and occurred in plateau region of length-tension relationship. Each data point represents peak force measured under a given condition. Note that under Act\textsubscript{length}, a tension equivalent to 100% $P_O$ can be generated by using a stimulation frequency of ~25–30 Hz. This approach was used to alter the relationship between stimulation frequency and mechanical loading.]

![Fig. 2. Forces produced under active shortening (Act\textsubscript{short}; A; solid line; 100 Hz) and Act\textsubscript{length} (B; dotted line; 27 Hz) conditions. C: y-axis, angular position (°) of ankle during each contraction and subsequent relaxation phase, with 0 as ankle in a neutral position (i.e., 90° relative to tibia). Muscles in each group were activated throughout entire Act\textsubscript{short} or Act\textsubscript{length} phase (i.e., 4 s) (see METHODS). Note that muscles were required to generate a contraction every 10 s.

Table 1. Summary of studies conducted, types of analyses, and training programs

<table>
<thead>
<tr>
<th>Phase</th>
<th>Objective Analysis</th>
<th>Type of contraction</th>
<th>Training sessions</th>
<th>Sets/ session</th>
<th>Contractions/ set</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Employ Act\textsubscript{short} and Act\textsubscript{length} contractions to explore importance of mechanical loading on MHC isoform expression</td>
<td>Hematoxylin and eosin Myofibrillar protein concentration MHC isoforms</td>
<td>Act\textsubscript{short}</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Act\textsubscript{length}</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Determine whether muscle injury is produced by Act\textsubscript{short} and/or Act\textsubscript{length} contractions</td>
<td>Native myosin isoforms Hematoxylin and eosin BrdU labeling of activated satellite cells</td>
<td>Act\textsubscript{short}</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Act\textsubscript{length}</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

n = No. of animals; Act\textsubscript{short}, active shortening; Act\textsubscript{length}, active lengthening; MHC, myosin heavy chain; BrdU, 5-bromo-2’-deoxyuridine.
placed into cooled glycerol and stored at −20°C until analy-
yzed. Those sections that were used for histological analyses
were frozen in isopentane cooled by liquid nitrogen and stored
at −70°C until sectioned.

The purpose of the experiments in phase 2 was to deter-
mine whether injury was induced at early time points in the
Act<sub>long</sub> training program. Animals in this phase were trained
under identical conditions, except that they performed either
one or two training sessions (see Table 1). Muscles from these
groups were removed 24 h after the last training session.

Tissues were stored as described above for phase 1.

Myofibrillar protein content. Purified myofibril prepara-
tions were extracted by using techniques described previ-
sely (5, 7). This included homogenization of the muscle in a
solution (solution A; pH 6.8) containing (in mM) 250 sucrose,
100 KCl, 20 tris(hydroxyethyl)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, 50 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, 2.5 mM
ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N''-tetraacetic
cacid, and 1 mM 2-mercaptoethanol (pH 8.8).

Electrophoretic separation of MHC isoforms. MHC protein
isoforms were separated by using techniques described previ-
sely (5, 6, 20). The separating gel solution contained 8%
cracylamide, 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 adding N, N', N''-tetramethylethylenedi-
amine (TEMED; 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 polymer-
ize. Once the separating gel was polymerized, the stacking gel
was poured. The composition of the stacking gel was 4%
cracylamide, 0.08% bis-acrylamide, 30% glycerol, 70 mM Tris (pH 6.7), 4 mM EDTA, and 0.4% SDS. This solution was also
degassed for 30 min before addition of TEMED (0.05% final
concentration) and ammonium persulfate (0.1% final concen-
tration). The composition of the running buffer was 1 M Tris, 0.15 M glycine, and 0.1% SDS. Myofibril 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 SG-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 II<sub>A</sub>, fast type
II<sub>X</sub>, fast type IIB, and slow type I MHC isoforms (order of
migration). MHC protein isoform bands were stained by
using Coomassie blue G-250. The MHC protein isoform bands
were scanned and quantified by using a Molecular Dynamics
Personal Densitometer (Molecular Dynamics, Sunnyvale,
CA).

Electrophoretic separation of native myosin isoforms. Skele-
tal muscle injury can lead to the de novo expression of
neonatal native myosin isoforms (8, 9, 11, 15). Consequently,
muscle samples from the phase 2 experiments were analyzed
for the presence of neonatal myosin isoforms by using nondis-
sociating electrophoretic procedures described previously (7).

Briefly, ~5–10 µg of myofibrillar protein were loaded onto
tube gels (60 × 5 mm). The tube gels were composed of 4.17%
cracylamide, 2.6% bis-acrylamide, 20 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, 10% glyc-

col, and 0.2% TEMED. This solution was adjusted to a pH of
8.8. The running buffer contained 20 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, 10% glycerol, and 0.2 mM cysteine and was adjusted to a pH of 8.8.

Electrophoresis was performed for 22 h by using a constant
voltage of 90 V. After the electrophoresis, the gels were
stained by using a solution containing 0.1% Coomassie blue
R-250. The migration pattern and distance of adult and
neonatal native myosin isoforms were determined by using a
plantaris muscle obtained from a 15-day-old rat.

Histological and immunohistochemical analyses of muscle
injury. Muscle injury can lead to the activation and incorpora-
tion of satellite cells into myofibers (26, 28). These satellite
cells can be identified by using 5-bromo-2'-deoxyuridine

![Image](356x126 to 523x413)

Fig. 3. Electrophoretic gels of control and trained white MG muscles. Lanes 2 and 4 in A and B are taken from control white MG muscles. Trained contralateral muscles are shown in lanes 1 and 3. Note that both Act<sub>long</sub> (A) and Act<sub>long</sub> (B) training downregulated fast type IIB myosin heavy chain (MHC) isoform and a concomitantly upregulated relative amount of fast type II<sub>X</sub> MHC isoform. Act<sub>long</sub>, MG muscles trained at a stimulation frequency of 25 Hz.

<table>
<thead>
<tr>
<th>Group</th>
<th>Muscle Wt, mg</th>
<th>Myofibrillar Protein Concentration, mg/g muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Act&lt;sub&gt;long&lt;/sub&gt;</td>
<td>Con 917 ± 88</td>
<td>105 ± 11</td>
</tr>
<tr>
<td></td>
<td>Trn 1,021 ± 98*</td>
<td>107 ± 13</td>
</tr>
<tr>
<td>Act&lt;sub&gt;long&lt;/sub&gt;</td>
<td>Con 856 ± 56</td>
<td>106 ± 10</td>
</tr>
<tr>
<td></td>
<td>Trn 952 ± 80*</td>
<td>101 ± 11</td>
</tr>
</tbody>
</table>

Values are means ± SD. MG, Medial gastrocnemius; Con, contralat-
eral side that underwent sham electrode placement; Trn, trained.
*Significantly different, P < 0.001.
(BrdU), which is a thymidine analogue. Previous reports (16) have shown that muscle injury can be detected as early as 8–12 h after mechanical overload of skeletal muscle. Additionally, satellite cell activation has been detected 24 h after injury to the rat soleus muscle and peaks 3 days after the onset of injury (18). Animals in phase 2 were injected with BrdU (100 µg/100 g body weight ip) 12 h after each training session. Relative to the time course of satellite cell activation described above for the rat soleus muscle, the labeling scheme used in the present study included early (i.e., 12 h after the onset of training) and peak time points (i.e., 2½ days after the onset of training). At the time of death, a section of intestine and the midportion of the red and white regions of the trained and contralateral control muscles were frozen in isopentane cooled by liquid nitrogen. The tissue was then sectioned (10 µm) in a cryostat. Approximately 50 µl of solution containing a primary monoclonal antibody specific for BrdU (1:12; Becton-Dickinson) was placed on the tissue sections for 1 h at 37°C. The tissue sections were then analyzed by using a charge-coupled device camera (XC-77, Sony) attached to a Nikon fluorescent microscope. Images of the tissue sections were captured and analyzed by using the public-domain National Institutes of Health Image program (written by Wayne Rasband). The number of BrdU-positive nuclei was expressed relative to the number of muscle fibers.

Statistical analyses. All statistical analyses were performed by using a computer program (Systat, Evanston, IL). The myofibril and MHC 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. Statistical comparisons were considered significant when \( P < 0.05 \).

**Fig. 4.** Effects of Actshort and Actlength training programs on MHC isoform composition of white MG. White MG only expressed fast type IIX (A) and IIB MHC (B) isoforms, hence absence of any data related to slow type I and fast type IIA MHC isoforms. Data are means ± SD. Note that both training programs had similar effects on fast type IIX and IIB MHC isoforms while similar loading conditions but very different stimulation frequencies were used. A two-way analysis of variance demonstrated that differences between control (open bars) and trained muscles (filled bars) were significant at \( P < 0.001 \) for both fast type IIX and IIB MHC isoforms. Group (Actshort, Actlength)-training (control, trained) interactions were not significant for fast type IIX and type IIB MHC isoforms, demonstrating that training had a similar effect on both Actshort and Actlength groups.

**Fig. 5.** Effects of Actshort and Actlength training programs on MHC composition of red region of MG muscle. Data are means ± SD. Note that neither training program affected slow type I (A) or fast type IIA (B) MHC isoform content. However, both training programs produced similar changes in fast type IIX (C) and IIB (D) MHC isoform contents. These results are consistent with those observed for white region of MG muscle. Differences between control (open bars) and trained (filled bars) muscles for fast type IIX and type IIB MHC isoforms were significant at \( P < 0.001 \). Group (Actshort, Actlength)-training (control, trained) interactions were not significant for fast type IIX and type IIB MHC isoforms, demonstrating that training had a similar effect on both Actshort and Actlength groups.
RESULTS

Muscle weight and myofibrillar protein. The data for muscle weight and myofibrillar protein concentration are reported in Table 2. Both the Act_short and Act_length training programs produced significant increases in muscle mass that were similar in magnitude (i.e., +11%). Neither of the training programs altered the myofibrillar protein concentration in either the white or red regions of the MG muscle.

MHC isoforms. The MHC protein isoform alterations produced by the different training programs are shown in Figs. 3-5. With respect to the white region of the MG muscle (see Fig. 4), the fast type IIB MHC protein isoform comprised ~75–80% of the total MHC protein isoform pool in each of the control groups. Importantly, both the Act_short and Act_length training programs produced similar reductions in the fast type IIB MHC protein isoform, reducing the relative content in each group to ~50% of the total MHC protein isoform pool. The fast type IIX MHC protein isoform contents of the white MG control groups were ~20–25% of the total MHC protein isoform pool. Both training programs produced an approximately twofold increase in the relative content of the fast type IIX MHC protein isoform.

In contrast to the white MG region, the control Act_short and Act_length red MG regions contained all four MHC protein isoforms (see Fig. 5). Neither training program influenced the slow type I and fast type IIA MHC protein isoform contents of the red MG region. However, consistent with the data from the white MG region, both the Act_short and Act_length training programs produced similar increases in the fast type IIX MHC protein isoform content of the red MG regions, increas-
ing the fast type IIX MHC protein isoform content from ~35 to 55% of the total MHC protein isoform pool. Concomitantly, the Act\textsubscript{short} and Act\textsubscript{length} training programs reduced the fast type IIB MHC protein isoform content to a similar degree.

Muscle injury. The potential presence of muscle injury in the Act\textsubscript{length} group at early time points of the training program was evaluated in phase 2 by using three criteria: 1) the presence of neonatal native isoforms; 2) the histological presence of inflammatory cells, degenerating muscle fibers, and internal nuclei; and 3) positive staining of nuclei for BrdU. As shown in Fig. 6, the muscles from the phase 2 Act\textsubscript{short} and Act\textsubscript{length} groups only expressed adult native myosin isoforms. Consistent with these data, necrotic or degenerating fibers were rarely evident in either the phase 2 Act\textsubscript{short} or Act\textsubscript{length} groups (see Fig. 7). Finally, there were no differences between the percentage of BrdU-labeled nuclei in a comparison of the trained phase 2 Act\textsubscript{short} and Act\textsubscript{length} muscles with their respective control samples (see Table 3, Fig. 8). As shown in Table 3, very few BrdU-labeled cells were found in both the trained and control muscles. Typically, ~0.15% of fibers examined contained BrdU-labeled cells. If the BrdU-labeled cells were expressed relative to the total number of nuclei, then the importance of the BrdU-labeled cells would be diminished further, given that there were ~4–5 nuclei/fiber. Collectively, these data provide strong evidence to suggest that the MHC alterations produced by the Act\textsubscript{length} training program in phase 1 were not the result of muscle fiber degeneration/regeneration induced by injury.

**DISCUSSION**

There are two key findings in this study. First, both the Act\textsubscript{short} and Act\textsubscript{length} training programs produced similar alterations in the MHC isoform expression that were characterized by a downregulation of the fast type IIB MHC isoform and a concomitant upregulation of the fast type IIX MHC isoform. This finding suggests that previous results (6, 7) were not due to the high stimulation frequency employed in these earlier studies. Second, the Act\textsubscript{length} training paradigm, as used in the present study, did not produce signs of muscle injury. Hence, the similarity in MHC isoform alterations produced by the Act\textsubscript{length} and Act\textsubscript{short} training programs cannot be ascribed to MHC isoform plasticity linked to muscle fiber degeneration/regeneration.

Mechanical loading of skeletal muscle has been shown to play an important role in the modulation of MHC isoform composition. A large amount of information has

![Fig. 8. Tissue sections stained with monoclonal antibody specific for BrdU. A: intestinal section showing uptake of BrdU by nuclei (arrow). B and C: Act\textsubscript{length} control (B) and trained (C) white MG muscle after 2 training sessions (total of 4 days). Note absence of satellite cells staining positive for BrdU in B and C.](image-url)

<table>
<thead>
<tr>
<th>Group</th>
<th>Muscle</th>
<th>No. of BrdU-Labeled Cells</th>
<th>No. of Muscle Fibers Counted</th>
<th>%Fibers With BrdU-Labeled Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Act\textsubscript{short}</td>
<td>Con</td>
<td>1</td>
<td>10</td>
<td>3,724</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
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<td>3</td>
<td>42</td>
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<td>Trn</td>
<td>1</td>
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<td></td>
<td>3</td>
<td>10</td>
<td>6,864</td>
<td>0.15</td>
</tr>
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</table>

Data for red region and muscles that performed 1 training session are not included because they appeared similar to those for muscles presented herein.
been obtained (17) from three key experimental manipulations: 1) hindlimb suspension; 2) exposure to microgravity; and 3) compensatory overload. Studies employing either hindlimb suspension or microgravity to unload skeletal muscles have reported an increased expression of fast MHC isoforms at both the protein and mRNA levels (5, 10). In contrast, increased mechanical loading, as induced by compensatory overload, upregulates the slower forms of MHC protein and mRNA isoforms (19).

The downregulation of the fast type IIB MHC isoform and the concomitant upregulation of the fast type IIX MHC isoform observed in the present study are consistent with the hypothesis implicating mechanical loading as a key factor modulating MHC gene plasticity. The most likely explanation of our training data is that the alterations in MHC isoform composition induced by the two different training programs were invoked by similar mechanical factors (e.g., stress) acting on similar cellular mechanisms that act to reciprocally regulate fast type IIB and IIX MHC isoform genes. Consistent with the suggestion that our training programs affected MHC protein isoform expression by altering MHC gene expression, we have observed previously (6) that the Act\textsubscript{short} training program used in this study produces substantial alterations in both fast type IIB and IIX MHC mRNA isoform content.

From a historical perspective, the cross-innervation study by Buller et al. (4) has provided a strong argument that some aspect of neural input plays an important role in determining skeletal muscle phenotype. Buller et al. (4) proposed a number of hypotheses (e.g., frequency hypothesis, aggregate hypothesis) to explain the results of their cross-innervation experiments. The “frequency hypothesis” stipulates that muscles stimulated at low frequencies develop phenotypic properties similar to those of slow skeletal muscle, whereas those muscles stimulated at high frequencies exhibit phenotypic properties similar to those of fast skeletal muscle. Although Buller et al. (4) specifically rejected this hypothesis, Lomo et al. (14) reported that denervated soleus muscles stimulated at 100 Hz developed fast contractile properties, whereas those that were stimulated at 10 Hz developed slow contractile properties. More recently, it has been reported that high stimulation frequencies caused the soleus (2, 12) and the extensor digitorum longus (2) muscles to upregulate their fast MHC isoform content. An important distinction needs to be made regarding the approach used in these earlier studies (2, 12, 14) and that used in the present study. These previous studies (2, 12, 14) employed a chronic type of electrical stimulation, causing the target muscle to contract thousands of times per day. In the present study, the muscles produced 40 contractions every other day. Hence, although stimulation frequency might be an important modulator of muscle phenotype when the stimulation pattern is applied in a chronic fashion, under the conditions employed in the present study, stimulation frequency does not appear to play a key role in modulating MHC isoform expression.

Previous studies have shown that, under specific circumstances, active lengthening can produce skeletal muscle injury (13, 21) and that this injury initiates developmental programs of myosin isoform expression (8, 9, 11, 15). Although it is doubtful that such a program could explain the alterations produced by the Act\textsubscript{length} training program used in the present study and the similarity of these changes to those produced by the Act\textsubscript{short} training program, both groups of trained muscles were examined for the presence of muscle injury. On the basis of the collective criteria used in the present study, the muscles that trained under Act\textsubscript{length} conditions did not exhibit any signs of muscle injury. Hence, the alterations produced by the Act\textsubscript{length} training program are not obfuscated by issues related to degeneration/regeneration.

From an applied perspective, it has been shown that human subjects performing high-resistance training programs downregulate the expression of the fast type IIB/IIX MHC isoform while reciprocally upregulating the slower fast type IIA MHC isoform (1). The similarity between this response and that seen in the present study suggests that the rodent high-resistance training model employed in the present study represents a powerful tool for exploring important issues related to humans performing resistance training. In this respect, the cellular/molecular training response of skeletal muscle to Act\textsubscript{length} contractions has rarely been studied. In the present study, we observed that when mechanical loading was held constant, both the Act\textsubscript{short} and Act\textsubscript{length} training programs produced identical responses with respect to muscle mass and MHC isoform expression. Hence, when mechanical loading conditions are similar, it does not appear as though one type of training is more effective than the other in modulating MHC isoform composition.

In summary, the findings of this study clearly indicate that stimulation frequency is not responsible for the MHC isoform alterations observed by us previously, and they refute the concept of a frequency hypothesis as it applies to training conditions used in this study. Although these results are consistent with the mechanical loading hypothesis, further experimentation will be required to better delineate the putative role of mechanical factors in modulating MHC isoform expression and the cellular/molecular mechanisms they invoke.

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