Effects of isometric training on skeletal myosin heavy chain expression

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Haddad, Fadia, Anqi X. Qin, Ming Zeng, Sam A. McCue, and Kenneth M. Baldwin. Effects of isometric training on skeletal myosin heavy chain expression. J. Appl. Physiol. 84(6): 2036–2041, 1998.—This study tested the hypothesis that an isometric resistance-training program induces upregulation of slow myosin heavy chain (MHC) expression in a fast-twitch skeletal muscle. Thus we studied the effects of two resistance-training programs on rodent medial gastrocnemius (MG) muscle that were designed to elicit repetitive isometric contractions (10–12 per set; 4 sets per session) of different duration (8 vs. 5 s) and activation frequency (100 vs. 60 Hz) per contraction during each training session (total of 6 and 12 sessions). Results showed that both training paradigms produced significant increases in muscle weight (~11–13%) after completion of training (P < 0.05). Significant transformations in MHC expression occurred and involved specifically a decrease in the relative expression of the fast type IIb MHC and concomitant increased expression of the fast type Ix MHC. These adaptations were observed in both the “white” and “red” regions of the MG, and they occurred at both the mRNA and protein levels. These adaptations were detected after only six training sessions. Neither of the training programs produced any change in the relative expression of either the slow type I MHC or the moderately fast type IIa MHC, which can be upregulated in the red MG by chronic functional overload. These findings show that the isometric protocols used in this investigation were not sufficient to induce the hypothesized changes in the myosin heavy chain isoform expression in rodent skeletal muscle.

myosin heavy chain isoforms; messenger ribonucleic acid; resistance training; medial gastrocnemius muscle; Sprague-Dawley rats

PREVIOUS STUDIES involving both humans and rodents clearly show that high resistance-training paradigms of either the concentric or eccentric type induce adaptations in skeletal myosin heavy chain (MHC) isoform expression. These shifts predominantly involve rearrangement in the pattern of expression involving the fast MHC isoforms, i.e., type IIx to IIa in humans and IIb to Ix in rodents (1, 4–6, 10, 12, 13). These findings also suggest that such adaptations can occur after only a few training sessions when evaluated at the mRNA level of analysis (5). Furthermore, in these particular rodent studies, the analyses were performed on muscles normally expressing a high predominance of fast type II fibers (4–6). In contrast, Diffee et al. (10) reported that a resistance-training paradigm of the isometric type appeared to be more effective than one consisting of high-force (90% maximum) isovelocity-concentric contractions in terms of maintaining expression of the type I or slow MHC in a predominantly slow (soleus) muscle undergoing a chronic state of unloading even though the two programs produced similar effects on augmenting muscle mass. In the context of these isomyosin adaptations in response to resistance training, other studies have shown that the model of functional overload, which involves the chronic overloading of a targeted fast muscle by the removal of its synergists, induces the upregulation of both the slow I and fast type IIa MHCs while concomitantly downregulating the two fastest MHCs, i.e., type IIb and Ix (3, 17, 19). Furthermore, the functional-overload model essentially defines the upper limits of the adaptive potential of a fast muscle to transform its structural/functional properties in response to mechanical stimuli. In this model, the mechanical stimulus is chronically imposed on the muscle, and thus it undergoes significantly greater increments in its mass compared with muscles undergoing repetitive resistance-training paradigms (4–6, 10).

The mechanism(s) for such adaptations as muscle enlargement and the upregulation of slower MHC expression remains unknown for all models of mechanical loading. However, the fact that slow isoforms can be upregulated in response to mechanical stress suggests that either a chronic mechanically loaded contractile activity paradigm or one possibly biased to isometric activity may be an important prerequisite stimulus for inducing upregulation of the slower MHC genes. Therefore, the present study was undertaken to test the hypothesis that repetitive isometric contractions, of varying duration and consisting of relatively high frequencies of direct muscle activation, play a central role in inducing transformations in MHC plasticity whereby the slower MHC isoforms become upregulated at the expense of the faster isoforms. Surprisingly, the findings reported herein suggest that, although isometric contractions of high activation frequency induce significant transformations in type IIb to Ix MHC expression and a relatively high degree of muscle enlargement, they are ineffective, based on the experimental paradigms utilized herein, in inducing the fast-to-slow transformations typically seen in models of chronic functional overload.

METHODS

Animal care and experimental groups. Female Sprague-Dawley rats (250–260 g; n = 24) were used in this study. All animals were housed individually and given food and water ad libitum. The experiments described were approved by our institutional animal care committee. Animals were randomly allocated into two major groups that underwent different training programs: isometric resistance-training program I (IT-I; n = 12) and isometric resistance-training program II (IT-II; n = 12). In each program, rats were trained for either 6 sessions (65; n = 6) or 12 sessions (12S; n = 6). This design thus resulted in four groups of six rats per group: IT-I (65), IT-I (125), IT-II (65), and IT-II (125).

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For IT-I (n = 12) electrodes were implanted into the medial gastrocnemius (MG) muscle so that the target muscle could be stimulated (see below) at an activation frequency of 100 Hz. The training paradigm consisted of ten 8-s contractions per set with a rest interval of 12 s between each contraction. The animals performed a total of four sets per session with a rest interval of 10 min between each set. Consequently, this protocol activated the muscle for a total of ~5.3 min during each training session. This protocol was essentially the same as that used by Diffee et al. (10) to induce hypertrophy in the soleus muscle and the maintenance of a slow MHC phenotype. The training regimen involved a sequence of 2 days of training followed by a day of rest, and the tissues were harvested from subgroups (n = 6) ~15 h after either 6S or 12S. These training durations were selected on the basis of previous studies that revealed that MHC-specific transformations could be detected at both the mRNA and protein level of analyses after as little as three to four training sessions (5).

For IT-II, the MG muscles (n = 12) were stimulated with a modified protocol that involved both a lower activation frequency (60 Hz) and a shorter isometric contraction-to-rest interval (5 s on: 5 s off) spanning a period of 2 min for each set (i.e., twelve 5-s contractions per set). Each training session consisted of five sets with a rest interval of 10 min between each set. It should be noted that, despite the shorter contraction-to-rest interval, the total activation time during each training session approximated that of the IT-I program, i.e., ~5 min of muscle activation per session. As in the other program, animals were processed after 6S and 12S. This latter program was selected to 1) lower the activation frequency to a range more commonly observed in slower motor units (2) and 2) reduce the time interval for applying each mechanical stimulus.

Electrode implantation and training apparatus. Each animal was anesthetized (4.5 mg/kg acepromazine and 75 mg/kg ketamine), and stimulating electrodes were surgically implanted into the left MG and connected to a head plug cemented to the skull of the rat so that the activation pattern of the muscle could be controlled. A sham operation was performed on the right leg, which served as a control. The specific details for these procedures have been described previously (5, 6, 10).

After 1 wk of recovery, the animals began their respective training programs as described in Animal care and experimental groups. Approximately 20 min before each training session, the animals were lightly anesthetized with acepromazine (5 mg/kg) and ketamine hydrochloride (20 mg/kg). Isometric contractions were produced by using a computer-controlled Cambridge ergometer system (10). The major components of the system include 1) a Cambridge ergometer (model 310, Cambridge Instruments, Watertown, MA) that was used to control the mechanical loading conditions on the target muscle during training, 2) a computer that activates the stimulus and controls the parameters of the ergometer, and 3) a training platform that translates the moment at the ankle into a linear force (4–6, 10). In these particular experiments the computer was programmed so that no length changes in the ergometer system were elicited during each contraction cycle.

Muscle processing. Approximately 15 h after the last training session, the animals were anesthetized by a lethal injection of pentobarbital sodium (120 mg/kg); the left and right MG muscles were rapidly removed, weighed, and quickly dissected into superficial “white,” mixed, and deep “red” regions (4–6); and the samples were quick frozen on dry ice and stored at −70°C for subsequent biochemical analyses.

The muscle was partitioned into these regions because previous studies have shown that there are potentially differential adaptations in MHC transformation, particularly in the superficial white (consisting predominantly of types IIb and IIx MHC) and the deep red (consisting predominantly types IIx and IIa MHC) regions (5). In view of previous findings on functionally overloaded skeletal muscle (3, 16, 17), we reasoned that fibers in the red region would be more likely to transform MHC in the direction of the slower MHCs (i.e., types IIa and I) compared with the superficial white region.

Total RNA and protein isolation. Total cellular RNA and total muscle proteins were simultaneously extracted from frozen muscle samples by using the TRI reagent (Molecular Research Center, Cincinnati, OH) according to the company’s protocol, which is based on the method described by Chomczynski (7). Total proteins were separated in the organic phase and subsequently precipitated with isopropanol, washed with guanidine hydrochloride and ethanol, and suspended in 1% SDS (7). Protein concentration was determined by the Bio-Rad protein assay kit, by using gamma globulin as standard. Samples were adjusted to protein concentration of 1 mg/ml with 1% SDS and were stored at −20°C until later analysis for MHC distribution pattern by SDS-PAGE (18). Total RNA was precipitated from the aqueous phase with isopropanol, and after being washed with ethanol, it was dried and suspended in a small volume of 0.5% SDS in Tris-EDTA buffer (10 mM Tris, pH 8.0, and 1 mM EDTA). This method of RNA extraction can yield high-quality undegraded RNA, free of protein and DNA, on the basis of a ratio of optical density at a wavelength of 260 nm to that at 280 nm of ~2 and as tested by agarose gel electrophoresis with ethidium bromide staining, showing both 28S and 18S ribosomal RNA with an intensity ratio of ~2:1 (8). The RNA concentration was determined by optical density at a wavelength of 260 nm (by using a unit of optical density at a wavelength of 260 nm equivalent to 40 µg/ml). The RNA samples were stored frozen at −70°C until being subsequently analyzed for MHC mRNA expression by Northern hybridization.

MHC mRNA analysis. MHC mRNA expression was analyzed by using Northern blot hybridization. Because four different probes needed to be tested (types I, IIa, IIx, and IIb), equal amounts of RNA were loaded on four separate gels. Stage 1 samples were run on a 1% agarose gel for many hours, and the gel was dried and stored at 4°C until later analysis. For MHC types IIa and I, the RNA samples were loaded on four separate gels. Stage 2 samples were run on a 1% agarose gel for many hours, and the gel was dried and stored at 4°C until later analysis. For MHC types IIx and IIa, the RNA samples were loaded on four separate gels. Stage 3 samples were run on a 1% agarose gel for many hours, and the gel was dried and stored at 4°C until later analysis. For MHC types IIx and IIa, the RNA samples were loaded on four separate gels. Stage 4 samples were run on a 1% agarose gel for many hours, and the gel was dried and stored at 4°C until later analysis.

For a given muscle sample, the RNA for all experimental groups, i.e., control vs. stimulated, was simultaneously pooled and hybridized with a specific probe. The probes were hybridized with the RNA samples that had been run on the gel. The RNA samples were first denatured and then hybridized overnight, washed, and exposed to autoradiographic film with an intensifying screen as described in detail previously (5, 17).

For a given muscle sample, the RNA for all experimental groups, i.e., control vs. stimulated, was simultaneously processed (prehybridized, hybridized, washed, and exposed to film) under the same conditions for a specific MHC probe. Therefore, within-group comparisons are valid for a given muscle for a specific MHC mRNA. After signal detection by using autoradiography, the probes were washed off the blots and rehybridized with an excess of a 32P-end labeled 18S oligoprobe (14), which hybridizes to 18S ribosomal RNA. Band intensities on the autoradiogram were quantified by 31.
using a laser scanning densitometer (Molecular Dynamics, Sunnyvale, CA), and each specific MHC was normalized to its corresponding 18S signal. In determining each band signal, we used the method of volume integration with local background correction (Image Quant software, Molecular Dynamics). This method is more accurate and quantitative regardless of the shape of the bands. The 18S ribosomal RNA is directly proportional to the amount of total RNA; therefore, correcting to the 18S rRNA can validate the Northern blot by correcting to the amount of RNA loaded or transferred for each sample. The values reported for each MHC mRNA are related to total RNA, and they can be considered valid regardless of possible variation in total mRNA yield due to experimental conditions (i.e., training paradigm, etc.). Although other investigators have used a correction strategy involving other mRNA species such as β-actin and glyceraldehyde-3-phosphate dehydrogenase, these have been shown to change in skeletal muscle in response to altered loading state (19) and thus were inappropriate to use for the present study.

MHC protein analysis. Skeletal MHC isoforms were separated by using a SDS-PAGE technique (18). The gels were run at 275 V for 22 h under refrigeration. After electrophoresis, the gels were stained for 1 h with Brilliant blue G 250 (Sigma Chemical) and destained with 25% methanol and 10% acetic acid. The separated MHC bands were scanned and quantified by using a Molecular Dynamics densitometer. The peaks of interest representing the distinct MHC isoforms were identified in the digitized densitometric data sets. The area of each peak was determined by integration (Image Quant Software, Molecular Dynamics). The MHC identity on the resulting banding pattern was verified on the basis of Western blotting techniques by using a battery of MHC antibodies as obtained from the laboratory of Dr. S. Schiaffino (data not shown).

Statistical analyses. All data are reported as means ± SE. All statistical analyses were performed by using a computer software package (Prism, GraphPad Software). For each training paradigm, each MHC was analyzed separately by using either a one-way analysis of variance test or a t-test for paired samples, with statistical significance set at the P, 0.05 level.

RESULTS

Muscle weight. After 6S, there was no significant change in the trained MG muscle weight undergoing the IT-I program, whereas IT-II caused a 6% increase (P < 0.01) relative to the nontrained muscle (Table 1). 12S of both the IT-I and IT-II training programs increased the MG wet muscle weight by 11 and 13%, respectively, in the stimulated vs. nonstimulated muscles (P < 0.05). These increments in muscle mass are similar in magnitude to previous reports in which both fast and slow rodent skeletal muscles were trained under conditions involving high-force-output paradigms of either the isometric or concentric-isovelocity type (4–6, 10).

Fig. 1. Representative myosin heavy chain (MHC) mRNA signals and MHC proteins as separated by SDS-PAGE in both white medial gastrocnemius (A) and red medial gastrocnemius (B) muscles for isometric resistance-training program I (IT#I; 100 Hz) and isometric resistance-training program II (IT#II; 60 Hz) after 12 sessions of training. Also shown is signal after hybridization with 18S rRNA probe. C, control; T, trained.

MHC expression. In the superficial white region of the MG only the fast types IIb and IIx MHCs were expressed at both the protein and mRNA levels, with the type IIb being the more predominant isoform (i.e., ~75% of the pool), whereas, in the deep red region, all four adult MHCs were abundantly expressed, with the type IIx MHC being the most predominant isoform (e.g., accounting for ~50% of the total MHC pool) (Fig. 1).

Both training programs induced a remarkably similar training response involving both the white and red regions of the MG. After 6S, there was no significant change in the trained MG muscle weight undergoing the IT-I program, whereas IT-II caused a 6% increase (P < 0.01) relative to the nontrained muscle (Table 1).
regions of the MG muscle. This was manifest as chiefly a decrease in the relative expression of the type IIb MHC with a concomitant increase in the type IIx MHC (Figs. 2 and 3). These changes occurred at both the mRNA and protein level of analyses, although the changes in the protein data were more predominant after 12S.

Neither training program had any significant impact on the pattern of expression of either the slow type I or the fast IIa MHC. Consequently, these adaptive changes in MHC plasticity in response to these isometric contraction protocols are consistent with findings obtained in previous training programs that focused on both concentric-isovelocity and eccentric-isovelocity paradigms at either high (concentric) or low (eccentric) activation frequencies (10).

**DISCUSSION**

Previous studies clearly show that increasing the mechanical stress imposed on skeletal muscle induces...
significant enlargement of the fiber population and a transformation in MHC phenotype in a subset of the fiber pool (4–6, 10). In animal models, two primary approaches have been used to elevate the mechanical stress imposed on the muscle. The first involves resistance-training programs that are predicated on classic training paradigms used to train human subjects (1, 12, 13). However, the unique feature of the animal model is that, by using stimulating electrodes, the activation pattern of the muscle can be controlled to both regulate the active state of the muscle and ensure that all the motor units in the muscle participate in the contraction response (4–6, 10). In this way one can examine the adaptive response by focusing on different regions of the muscle with known differences in fiber type (15). In these modes of training rodent muscle, there has been relatively good agreement with that reported in human subjects with regard to the degree of enlargement and the modest fast-to-slow transformation in MHC phenotype that are induced (1, 12, 13).

A second approach to loading the muscle and one that is more or less unique to animal models involves the surgical removal of those muscles that act synergistically to the target muscle so that the latter becomes chronically overloaded whenever the animal is in a weight-bearing mode (3, 16, 17). Fast muscles overloaded in this manner typically hypertrophy by 70–100% and undergo significant transformations in MHC phenotype that involve significant downregulation of type IIb MHC and upregulation of the slow type I and fast type IIa MHCs (17). Interestingly, this upregulation of the type I and type IIa MHCs is largely confined to the inner red core of the muscle (16), which typically contains a predominance of fibers that express the type IIa and IIx isoforms (e.g., Fig. 1).

The rationale of the experiments performed in the present study is based on a previous study by Diffee et al. (10) showing that high-force-output contractions performed in the isometric mode are effective in blunting the decrease in type I MHC expression in the unloaded slow-twitch skeletal muscle (10). Our findings in rodents suggest that although the isometric training paradigms used herein can induce the same types of adaptation in MHC phenotype seen with other resistance paradigms involving either a concentric or eccentric contraction mode (10), they are ineffective in providing a stimulus to increase either pretranslational or translational events affecting expression of both the type IIa and type I MHC genes.

Several factors may account for the ineffectiveness of the training programs used herein for inducing increased expression of the slower MHC phenotypes. First, an insufficient number of contractions could have been performed during each training session to elevate those factors involved in increasing pretranslational/translational events for increasing slower myosin isoform gene expression. This could be an important possibility because the functional-overload model typically places a chronic stress on the target muscle that is sufficient to induce all fibers to essentially double their size. Consequently, a greater number of isometric contractions may have to be elicited each day to more fully transform a fast muscle to express a slower MHC phenotype.

Second, the training program, although of sufficient duration to alter regulation of the type IIb and IIx MHCs, may require a longer duration (i.e., many weeks) to accumulate sufficient levels of factors involved in the regulation of these slower MHC genes. In this context, it is curious that chronic electrical stimulation, a model used to globally induce a fast-to-slow muscle phenotype, requires a longer time course of stimulation to induce changes in the sarcomeric genes than it does for transforming changes in either the mitochondrial or sarcoplasmic reticular systems (2). Thus the two resistance programs used herein may have to be extended for many weeks to achieve a further expression of a slower contractile phenotype compared with that induced in the present study.

Third, the activation frequencies (60 and 100 Hz) used herein to stimulate the muscle may have been too high to achieve a more complete fast-to-slow MHC phenotype. Previous studies have provided evidence to suggest that continuous low-frequency-stimulation protocols (~10 Hz) of fast muscles were effective in causing fast-to-slow MHC transitions (2, 15), whereas stimulation of slow muscles at high frequencies, i.e., 100 Hz, caused an opposite type of transformation (2, 15). In the context of these observations, we have recently determined (retrospectively) that both fast- and slow-twitch skeletal muscles, when stimulated isometrically for durations in excess of 4 s per contraction and at frequencies >50 Hz, undergo a progressive fatigue response during the course of a training session (unpublished observations). This observation raises the possibility that repetitive high-frequency contractions of sufficient duration performed in an isometric mode may actually result in an insufficient amount of force being imposed on those moderately slow motor units, i.e., units expressing IIa and IIx MHC and predominating in the red MG, to enable them to achieve a “slower” phenotype.

In conclusion, the findings reported herein suggest that isometric contraction paradigms, when used in the context of a typical resistance-training mode and normally involving a high frequency of stimulation to fully activate the muscle, are not any more effective than conventional high-frequency concentric-eccentric resistance paradigms in terms of inducing upregulation of expression of the two slower isomyosins expressed in adult skeletal muscle. Despite the fact that our results show that these two training paradigms did not induce an increase in type I MHC expression, important information was obtained that is relevant to designing future experiments to address issues concerning the regulation of MHC plasticity. More research is needed to define the type of mechanical stimuli, its frequency of activation, and the amount of contractile output that must be accumulated to induce the type of MHC transformation seen in models of chronic functional overload.
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