ABSTRACT: The distribution of myosin heavy chain (MHC) isoforms, fiber type composition, and fiber size of the vastus lateralis muscle were analyzed by sodium dodecylsulfate polymerase gel electrophoresis (SDS-PAGE), ATPase histochemistry, and immunocytochemistry in a group of adult sedentary men before and after 3 months of heavy-load resistance training and, subsequently, after 3 months of detraining. Following the period of resistance training, MHC IIX content decreased from 9.3 ± 2.1% to 2.0 ± 0.8% ($P < 0.01$), with a corresponding increase in MHC IIA (42.4 ± 3.9% vs. 49.6 ± 4.0% [$P < 0.05$]). Following detraining the amount of MHC IIX reached values that were higher than before and after resistance training (17.2 ± 3.2% [$P < 0.01$]). Changes in fiber type composition resembled the changes observed in MHC isoform content. Significant hypertrophy was observed for the type II fibers after resistance training. Maximal isometric quadriceps strength increased after resistance training, but returned to pretraining levels after detraining. The present results suggest that heavy-load resistance training decreases the amount of MHC IIX while reciprocally increasing MHC IIA content. Furthermore, detraining following heavy-load resistance training seems to evoke an overshoot in the amount of MHC IIX to values markedly higher than those observed prior to resistance training.
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MYOSIN HEAVY CHAIN IIX OVERSHOOT IN HUMAN SKELETAL MUSCLE

JESPER L. ANDERSEN, PhD,[1] PER AAGAARD, PhD[2]

[1] Copenhagen Muscle Research Centre, Department of Molecular Muscle Biology, Rigshospitalet Section 9312, Juliane Mariesvej 20, First Floor, DK-2100 Copenhagen, Denmark
[2] Department of Neurophysiology, Institute of Medical Physiology, Panum Institute, Copenhagen, Denmark

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Human skeletal muscle contains three major fiber types based on their expression of one of the three myosin heavy chain isoforms (MHC I, IIA, and IIX).[35] In addition, hybrid fibers can be found, co-expressing MHC I/IIA and MHC IIA/IIX, the latter being the most common.[8] A close coupling exists between MHC isoforms and velocity of shortening, with the pure type IIX fibers being about five- to tenfold faster than the pure type I fibers.[11,20,22,28] Also, for human skeletal muscle in vivo, a relationship exists between the proportion of MHC isoforms and contractile power generated at high contraction velocity.[1] Muscle usage does not seem to affect the relationship between the MHC isoforms and velocity of shortening,[21] whereas usage may change the MHC isoform expressed by the individual muscle fiber.[5,20] Thus, by increasing muscle activity, either through resistance or endurance training, the MHC IIX gene is turned off in most IIX fibers, resulting in an elevated proportion of IIA fibers at the expense of IIX or IIA/IIX fibers.[5,20,27] In contrast, inactivity seems to reverse this process, making the occurrence of MHC IIX fibers more common.[6,20,38,40] The extent to which the latter occurs in normal human skeletal muscle has not been studied adequately.

In a recent study, analysis of MHC isoform transcript and protein expression in individual fibers showed indications of an increased MHC IIX expression when disuse/detraining was followed by a period of intense training.[7] Thus, the aim of the present study was to test the hypothesis that markedly reduced muscle activation, if preceded by a period of intense usage, leads to an overshoot in the amount of MHC IIX. This question is of general biological interest, because it bears on the issue of identifying

Abbreviations: MHC, myosin heavy chain; RM, repetition maximum; ROM, range of motion; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis
Key words: detraining; myosin heavy chain (MHC) IIX; MHC isoforms; resistance exercise; sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE)
Correspondence to: J. L. Andersen; e-mail: jla@rh.dk

the stimulus for turning on and off the various genes for MHC isoforms. In addition, such an elevation in MHC IIX may play a significant role in the contractile characteristics of the muscle in vivo,[1] and has clinical implications because MHC IIX fibers usually have a low capacity for lipid oxidation and a low insulin sensitivity.[31,44]

METHODS

Subjects. Nine men volunteered to participate in the study (age: 27 ± 3 years [mean ± SE]; height: 184 ± 2 cm; body weight pretraining: 72.6 ± 1.6 kg; body weight post-resistance training: 75.7 ± kg [$P < 0.05$, compared to posttraining], body weight post-detraining: 74.7 ± 2.6 kg [$P < 0.05$, compared to posttraining]). Prior to the study all subjects had a normal sedentary lifestyle including occasional participation in recreational sports activity. None of the subjects had performed resistance training previously, or engaged in any type of regular or organized sports activity for at least 1 year prior to the study. All participants gave their written consent to participate in the study, which was approved by the local ethics committee.

Training. The training protocol consisted of 3 months of heavy-load resistance training followed by 3 months of detraining.

Resistance training protocol. Resistance training was conducted three times per week, ensuring that all subjects concluded 38 training sessions within a 90-day period. Five different resistance-training exercises for the legs were performed: hack squat; incline leg press; knee extension; hamstring curl; and calf raise. The initial resistance used for each exercise was based on the subject's one-repetition maximum (1 RM) strength, which was determined prior to training. All training was progressive; that is, loading levels were monitored continuously and adjusted throughout the entire period of training to maintain muscle loading at the values intended. The various exercises were essentially conducted in 4–5 sets of 6–15 repetitions (corresponding to a 6–15 RM loading). In general, in the first weeks (training sessions 0–5) exercises involved 10–15 RM loads, followed by 10 RM loads in early weeks (training sessions 6–15), heavier loads of 6–10 RM in the later weeks (training sessions 16–30), and very heavy loads of 6–8 RM in the final weeks (training sessions 31–38) (see Table 1 for details). All training sessions were surveyed and supervised.

Detraining. After the resistance-training period the subjects entered a period of detraining. Detraining lasted 90 days during which the subjects were instructed to return to their normal lifestyle, resuming the activity level that they had before entering the project. To ensure that the activity level of the subjects during the detraining period was similar to that prior to the resistance training period, the subjects were obligated to report their activity level 1 month prior to commencing the resistance training and throughout the detraining period.

Maximal isometric quadriceps muscle strength. Maximal isometric knee extensor moment (MVC) was measured using a commercial dynamometer (Kinetic Communicator, Chattecx Corp., Chattanooga, TN). Subjects were seated at 10° reclined and firmly strapped at the hip and thigh. The rotational axis of the dynamometer lever arm was visually aligned to the lateral femoral condyle and the lower leg was attached to the distal end of the dynamometer lever arm 2 cm above the medial malleolus. Measurements were preceded by 5-min warm-up and 10-min preconditioning to the testing device. Three

Table 1. Resistance training protocol.

	Training sessions							
Exercise	1–5	6–10	11–15	16–20	21–25	26–30	31–34	35–38
Hack squat								
Sets	3	4	4	4	4	4	4	4
Reps.	10, 10, 10	10, 10, 10, 10	10, 10, 10, 10	10, 10, 10, 10	10, 10, 10, 10	10, 10, 8, 8	8, 8, 6, 6	8, 8, 6, 6
Incline leg press								
Sets	3	4	4	4	5	5	5	4
Reps.	10, 10, 10	10, 10, 10, 10	10, 10, 10, 10	10, 10, 8, 8	10, 8, 8, 6, 6	8, 8, 8, 6, 6	8, 8, 8, 6, 6	8, 8, 6, 6
Knee extension								
Sets	3	4	4	5	5	5	5	4
Reps.	15, 15, 15	10, 10, 10, 10	10, 10, 10, 10	10, 10, 8, 8, 8	10, 10, 8, 8, 8	10, 8, 8, 6, 6	8, 8, 8, 6, 6	8, 8, 6, 6
Hamstring curl								
Sets	3	4	4	4	5	5	5	4
Reps.	15, 15, 15	10, 10, 10, 10	10, 10, 10, 10	10, 10, 10, 10	10, 10, 10, 10, 10	10, 10, 8, 8, 8	10, 8, 8, 8, 6	10, 8, 8, 6
Calf raise								
Sets	—	4	4	5	5	5	5	4
Reps.	—	15, 15, 15, 15	15, 15, 15, 15	12, 12, 12, 12, 12	12, 12, 12, 10, 10	12, 12, 10, 10, 10	12, 10, 10, 10, 8	12, 10, 8, 8

Resistance training was conducted three times per week over a period of 3 months for a total of 38 training sessions for each. The table shows the number of sets and repetitions (reps.) conducted in each of the training sessions.

trials of maximal effort were performed by each subject and the MVC value represented the greatest moment value observed. Each isometric contraction lasted 2–3 s and contractions were separated by a pause of 45 s. Knee-joint angle was 65° (0° = knee fully extended), corresponding to the optimum angle for maximal knee extensor moment.[2,3] All recorded moment signals were corrected for the effect of gravity on the lower leg.[2]

Dynamic power-output test. In an isokinetic dynamometer (see earlier), a 45-s test of maximal effort continuous knee extension–flexion cycles (range of motion [ROM] 80° to 20°, angular velocity 180°/s) was employed to measure the maximal contractile power-output of the knee extensors. We examined whether peak power or the decline in instantaneous power throughout the 45-s period was altered after the training period. The test was identical to previously reported isokinetic tests of the dynamic continuous power-output of the knee extensors.[33,43] Subjects were seated according to the previously described procedures for MVC. Two tests were performed by each subject, separated by 1 h of rest that included 5 min of ergometer cycling at low intensity. The joint extension power within each cycle was found by integrating the knee extension moment with respect to knee-joint angle to yield the work produced, which subsequently was divided by the specific time interval of the extension movement. All recorded moment signals were corrected for the effect of gravity throughout the entire range of motion.[2] In addition, the motor-driven movement of the dynamometer lever arm, with phases of controlled acceleration and deceleration, prevented impact artifacts.[16] Moments (M) attributed to acceleration or deceleration of the lower leg were calculated ($M = I \cdot \alpha$) and added/subtracted to the moment recorded.[2,33] As described in detail elsewhere,[2] angular acceleration (α) of the lower limb was obtained by double differentiation of the knee joint angle as recorded by a flexible goniometer (Type G180, Penny and Giles, Christchurch, Dorset, UK). The moment of inertia (I) of the lower limbs was calculated by $I = m \cdot \rho^2$, where m (mass of the shank) was estimated as 0.061 · body mass,[47] and the radius of gyration ρ was calculated from the length of the lower limb segment multiplied by 0.735.[47] A movement range of 80° to 20° knee angle was chosen so that all subjects were able to perform the intended movement frequency without any difficulty.

Signal treatment. During isometric MVC, the force (strain gauge) signal of the dynamometer as well as the lever arm position were synchronously recorded using an external analog-to-digital sampling board (Model dt2801, Datatranslation, Marlboro, MA) at a 1000-Hz sampling rate. The recorded force signal was smoothed using a digital lowpass filter with a 20-Hz cutoff frequency (fourth order, zero-lag Butterworth filter). For the dynamic power test, all signals were synchronously recorded at a 100-Hz sampling rate. The knee goniometer signal was smoothed using a digital lowpass filter with a 3-Hz cutoff. Knee-joint angular velocity and acceleration were calculated by finite-element differentiation.

Muscle biopsies. Needle biopsies were obtained from the middle section of the vastus lateralis muscle.[10] The muscle samples were immediately mounted with Tissue-Tek and frozen in isopentane cooled with liquid nitrogen, and stored at −80°C until further investigation. Biopsies were obtained before and after the period of resistance training and subsequently after the detraining period. In recognition of possible variation in MHC isoform distribution along the length and depth of the vastus lateralis muscle and the limitations of estimating MHC distribution from single biopsies,[14,30] utmost caution was taken in obtaining pretraining, posttraining, and post-detraining biopsies in the same area and depth of the muscle. Posttraining and post-detraining biopsies were obtained at −0.75 cm either distally or proximally (randomized) of the pretraining biopsy scar.

ATPase histochemistry. Serial sections (10 μm) of the muscle biopsy samples were cut in a cryostat (−20°C), and routine ATPase histochemistry analysis was performed after preincubation at pH of 4.37, 4.60, and 10.30.[12] Immunocytochemistry was performed with the following antibodies: BA-F8, reactive with MHC I; and SC-71, reactive with MHC IIA *and* MHC IIX.[37] Five different fiber types were defined (types I, I/IIa, IIa, IIab, and IIb) according to Staron et al.,[40,41] with the modification that fibers termed by Staron et al.[40,41] as Ic and IIac (IIc) were pooled into one group designated I/IIa. Furthermore, to be classified as a I/IIa fiber, the fiber had to fit the ATPase histochemical reaction pattern of a IIc fiber,[40,41] and be reactive with both BA-F8 as well as SC-71. Cross-sections from pre- and posttraining, and post-detraining biopsies from the same subject were placed on the same slide and processed simultaneously for ATPase histochemistry. Fibers determined to be type II fibers, but showing an intermediate staining with pH 4.6 preincubation, were categorized as type IIab fibers.[40,41] These type IIab fibers covered a wide range from fibers with only a light staining (i.e., fibers with predominately MHC IIA content) to fibers with a darker staining (i.e., fibers with predominantly MHC IIX content). Typi-

cally, in the pH 4.6 stainings, the majority of the post-resistance training IIab fibers stain lightly, whereas the majority of the pre-resistance training and post-detraining type IIab fibers stain more darkly. The reason for this is that most posttraining IIab fibers contain a relatively minor amount of MHC IIX compared to the amount of MHC IIA, whereas the majority of the pre-resistance training and post-detraining type IIab fibers contain more equal amounts of MHC IIX and MHC IIA.[40] An average of 319 ± 18 fibers were examined in each of the biopsies. The number of minor fiber types (I/IIa, IIab, and IIb) was so small in some individuals, especially in the post-resistance training biopsies, that a reliable statistical comparison of changes in fiber size of these minor fiber types was impossible. Therefore, calculations of fiber type size were performed only for the two major fiber types (I and II). Furthermore, only truly horizontally cut fibers were used in the determination of fiber size. Thus, a restricted number of fibers, including at least 50 type I and 50 type II fibers, was used for this analysis. On average, 185 ± 31 fibers were used for determining fiber size in each biopsy.

Analysis of serial cryosections. The serial sections were visualized and analyzed using an Olympus BX40 microscope (Olympus Optical Co., Tokyo, Japan), a Sanyo Hi-resolution Color CCD camera (Sanyo Electronic Co., Osaka, Japan), and an eight-bit Matrox Meteor Framegrabber (Matrox Electronic Systems, Quebec, Canada), combined with image-analysis software (Tema, Scanbeam, Hadsund, Denmark). Using ATPase 4.6 staining, a fiber mask was drawn along the cell borders of the desired number of fibers. Images of the remaining ATPase stainings, and the immunocytochemical stainings, were then fitted into the fiber mask. A number was assigned by the computer to each specific fiber and the fibers were then displayed on the screen in multiple images. The individual fibers were therefore easily identified and assigned to a specific group of fiber type. Descriptive statistical analysis by the computer allowed determination of the relative proportion of the various fiber types, fiber type areas, and fiber type sizes.

Homogenate electrophoresis. MHC analysis was performed on the muscle biopsies using sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). From each biopsy 10–20 serial cross-sections (20 μm) were cut and placed in 100–200 μL of lysing buffer and heated for 3 min at 90°C.[18] Between 5 and 20 μL of the myosin-containing samples were loaded on a SDS-PAGE gel containing 6% polyacrylamide and 30% glycerol. Gels were run at 70 V for 42 h at 4°C. Subsequently, the gels were Coomassie stained and MHC isoform content was determined with a densitometric system (Cream 1D, KemEnTec Aps, Copenhagen, Denmark). Using SDS-PAGE, three different MHC bands can be separated in normal adult human skeletal muscle. These bands correspond to the MHC isoforms I, IIA, and IIX.[26] When referring to the MHC isoforms, we have chosen the term "IIX" and not "IIB" for the fastest contracting human MHC isoform, according to the recent findings of consistent homology between this human fast isoform and the rat IIX isoform.[15,37]

Statistics. All values are reported as mean ± standard error (SE). The Friedman test for multiple paired samples was used to evaluate the group of subjects before and after the periods of resistance training and after detraining. The Pearson product-moment relation was used to evaluate the relationship between MHC composition and percentage fiber type area. A confidence level of $P < 0.05$ was chosen to indicate statistical significance.

RESULTS

Maximal Isometric Quadriceps Strength. Maximal isometric strength increased 16.7% after the period of heavy resistance training (294 ± 14 N vs. 342 ± 18 N) ($P < 0.01$). After the detraining period isometric strength returned to pretraining values (298 ± 15 N).

Dynamic Power-Output Test. Maximal contractile muscle power increased following resistance training ($P < 0.05$) (Fig. 1). After detraining, power was reduced ($P < 0.05$), except during the first 15 s, in which there was no difference between posttraining and post-detraining values (Fig. 1). Power declined continuously throughout the work period except during the first 10 s. The relative decline in power (%/s) was not different before and after training or detraining (1.42 ± 0.10%/s, 1.51 ± 0.12%/s, and 1.46 ± 0.22%/s, respectively). However, in absolute terms (watt/s), the decline was greater after resistance training and detraining than before training (pretraining [1.83 ± 0.10 watt/s] vs. posttraining [>2.26 ± 0.23 watt/s] and post-detraining [2.10 ± 0.38], $P < 0.05$). In the first 15 s of contraction, maximal contractile power did not differ between posttraining and post-detraining conditions (Fig. 1).

MHC Content. Marked alterations were demonstrated for the MHC composition after 3 months of resistance training. The proportion of MHC IIA in-

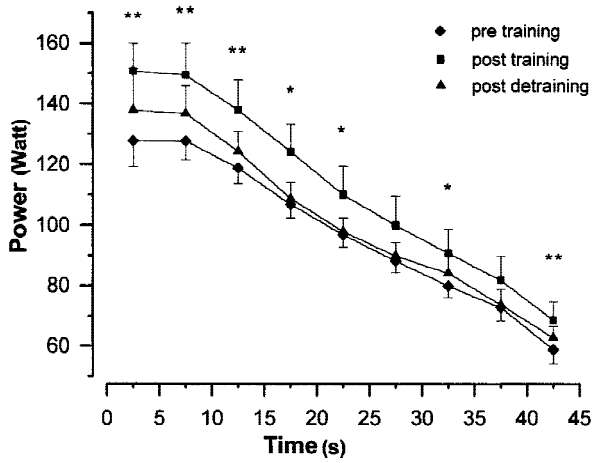

FIGURE 1. Maximal contractile knee extensor power recorded during a 45-s all-out test. Double asterisk (**): posttraining values > pretraining values ($P < 0.01$), but no difference between post-detraining and posttraining values; asterisk (*): posttraining values > pretraining and post-detraining values ($P < 0.05$). The decline in contractile power over the 45-s period was greater after training and after detraining than prior to training (see text). Values are mean ± SE ($N = 9$).

creased from 42.4 ± 3.9% to 49.6 ± 4.0% ($P < 0.05$), whereas the proportion of MHC IIX decreased from 9.3 ± 2.1% to 2.0 ± 0.8% ($P < 0.01$), with no change in MHC I (48.3 ± 4.1% vs. 48.4 ± 3.9%) (Fig. 2). After detraining, the amount of MHC IIX was significantly higher than that observed before and after resistance training: 17.2 ± 3.2% vs. 9.3 ± 2.1% ($P < 0.01$) and 2.0 ± 0.8%, respectively ($P < 0.01$) (Fig. 2). Correspondingly, the proportion of MHC IIA was higher after resistance training (49.6 ± 4.0%) than before

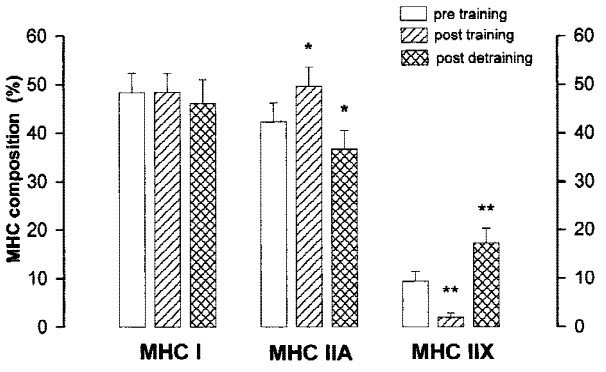

FIGURE 2. The relative distribution of MHC isoforms. Open bars: pretraining; hatched bars: post-resistance training; solid bars: post-detraining. Asterisk (*): significant difference for MHC IIA distribution, post-detraining < pretraining < post-resistance training ($P < 0.05$); double asterisk (**): significant difference ($P < 0.01$) for MHC IIX distribution, post-detraining > pretraining > post-resistance training. Values are mean ± SE ($N = 9$).

training (42.4 ± 3.9%, $P < 0.05$) and after detraining (36.7 ± 3.8%, $P < 0.01$) (Fig. 2). It should be noted that all nine subjects showed a decrease in MHC IIX content after resistance training, and that all nine also showed an increased MHC IIX content after detraining compared to both pre- and post-resistance training (Fig. 3).

Fiber Types. After resistance training, there was a significant decrease in type IIb fibers and a corresponding increase of type IIa fibers, regardless whether fiber type composition was determined as percentage fiber type in area or as percentage fiber type in number (Table 2). After detraining, both the percentage area and number of type IIb fibers increased to values higher than both those observed after resistance training and those observed prior to training, with a corresponding decrease in type IIa percentage (Table 2). Furthermore, the percentage of type I/IIa fibers increased after the detraining period as compared to that seen after resistance training.

Fiber Size. Significant hypertrophy was observed for type II fibers (16%) after resistance training, whereas no significant hypertrophy was observed for type I fibers (Table 3). After the period of detraining, muscle fiber size showed a tendency to decrease toward pretraining values, although this decrease did not reach significant levels. Hence, the post-detraining fiber size was not significantly different from either pretraining or post-resistance training fiber size (Table 3). In further support of a selective type II hypertrophy the average size of the type II fibers was significantly larger than that of the type I fibers after resistance training, whereas no difference in size between the two major fiber type sizes was observed prior to training or after detraining (Table 3).

Relationship between Fiber Types and MHC Content. To perform a correlation analysis between the MHC isoform content of the muscle homogenates and the fiber type area composition evaluated by ATPase histochemistry and immunocytochemistry, the number of fiber types was reduced from five to three. This reduction was based on the fiber type area percentages (Table 3) as previously described.[18] In short, the reduction was based on the following equations: type I = I + ½I/IIa; type IIa = ½I/IIa + IIa + ½IIab; and type IIb = ½IIab + IIb. A strong positive relationship was observed between the fiber type per-

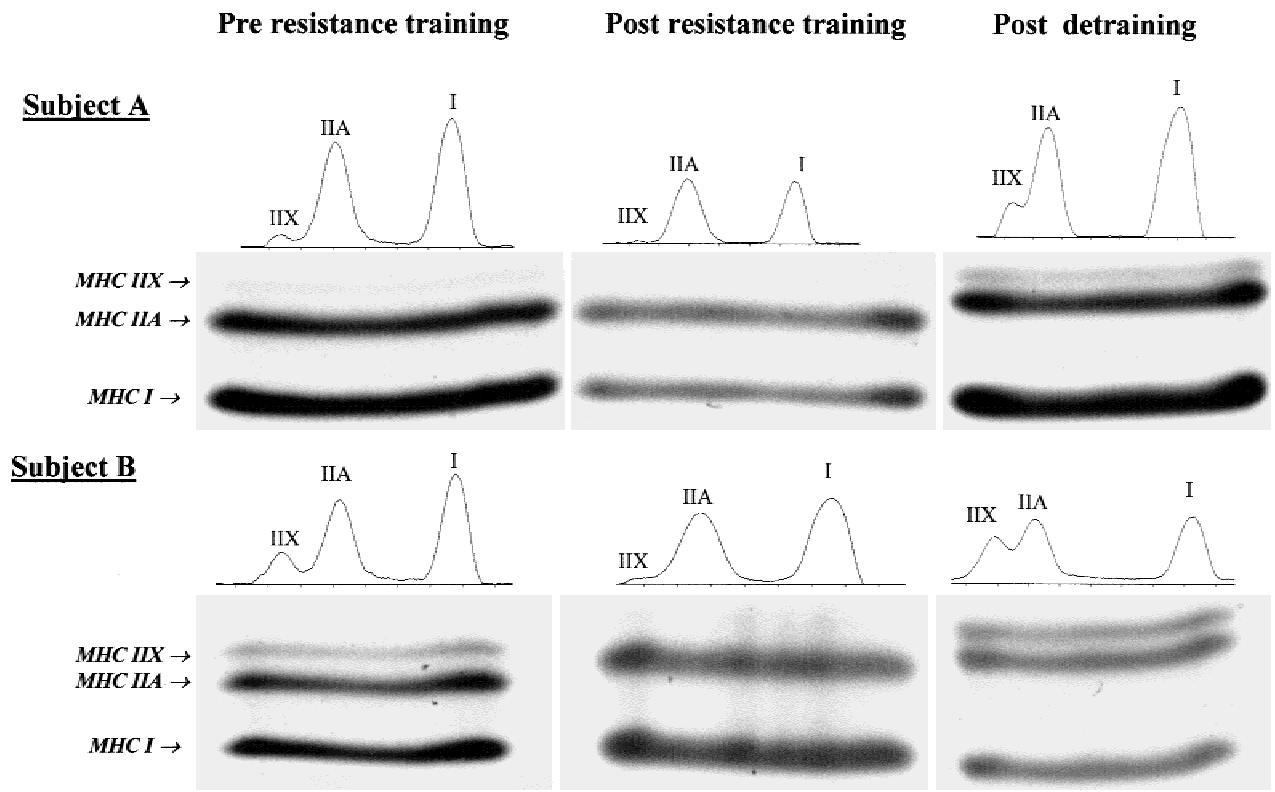

FIGURE 3. SDS-PAGE gel separations showing MHC I, MHC IIA, and MHC IIX bands from two subjects before (Pre resistance training), after 90 days of resistance training (Post resistance training), and after 90 days of detraining (Post detraining). Densitometric scans from the MHC regions of the SDS-PAGE gels are shown above each of the lanes.

centage area and MHC isoform composition for all fiber type/MHC isoform percentages (Table 4).

DISCUSSION

Heavy-loaded muscle contractions have previously been shown to decrease the amount of MHC IIX and, correspondingly, increase the amount of MHC IIA in human skeletal muscle.[4,5,23,40,41] The purpose of the present study was to downregulate the amount of MHC IIX via heavy-load resistance training, followed by an abrupt removal of the high-tension training stimulus to induce a change in MHC IIX content. As expected, the resistance-training regime resulted in a decrease in MHC IIX content, with a corresponding increase in MHC IIA content. More

Table 2. Muscle fiber type composition.

	Type I	Type I/IIa	Type IIa	Type IIab	Type IIb
Pre-resistance training					
% fiber type area	48.0 ± 4.5	2.3 ± 2.0	33.0 ± 3.4	7.1 ± 3.0	9.6 ± 2.5
% fiber types	48.8 ± 4.5	2.4 ± 2.0	30.9 ± 3.0	7.7 ± 2.9	10.2 ± 2.5
Post-resistance training					
% fiber type area	50.4 ± 4.2	1.0 ± 0.7	39.9 ± 3.3*	5.3 ± 1.9	3.4 ± 1.1*
% fiber types	50.8 ± 4.5	1.2 ± 0.8	37.9 ± 3.2†	6.0 ± 2.1	4.1 ± 1.2*
Post-detraining					
% fiber type area	44.8 ± 6.2	4.3 ± 1.4§	27.8 ± 6.0‡	6.1 ± 1.6	17.0 ± 3.4*,§
% fiber types	45.7 ± 5.4	4.3 ± 1.3§	25.1 ± 5.1§	6.1 ± 1.7	18.8 ± 3.5*,§

Values are mean ± SE in % fiber type area and in % fiber types (N = 9).
**Significantly different from pre-resistance training (P < 0.05).*
†Significantly different from pre-resistance training (P < 0.01).
‡Significantly different from post-resistance training (P < 0.05).
§Significantly different from post-resistance training (P < 0.01).

Table 3. Fiber size.

	Type I (μm^2)	Type II (μm^2)
Pre-resistance training	3660 ± 349	4104 ± 369
Post-resistance training	4114 ± 315	4881 ± 244*,†
Post-detraining	3993 ± 403	4512 ± 255

Values are mean ± SE (N = 9).
**Significantly different from pre-resistance training (P < 0.01).*
†Significant difference between type I and type II fiber size (P < 0.01).

unexpectedly, detraining following the period of heavy-load resistance training evoked an overshoot in MHC IIX content.

Physiological Stimuli Triggering Shift in Expression between MHC IIX and MHC IIA. Endurance training seems to suppress the amount of MHC IIX as does resistance training, although the patterns of muscle activity obviously differ.[38] Thus, it is startling that only very few near-maximal muscle contractions performed against heavy external loads three times per week are effective in suppressing the amount of MHC IIX almost completely.[39] This indicates that the total number of contractions (or nerve impulses) is not the sole determining factor when high-tension muscle loading regimes are applied, as is supposedly the case with repetitive low-tension loading patterns (i.e., endurance training).[25] More likely, the maximal tensile load exerted on the muscle is a governing factor as well. It has been suggested that powerful muscle contractions may lead to structural deformation of the sarcolemma and the cytoskeleton, thereby activating presently unidentified stretch-dependent signaling pathways affecting gene regulation of the nucleus.[46] It may be hypothesized that the mechanical tensile load through the aforementioned pathway would switch off or downregulate the IIX gene, while upregulating the MHC IIA gene, in all of the type II fibers. In support of this hypothesis, the subjects in the present study responded unambiguously to resistance exercise and detraining by a consistent decrease and increase in MHC IIX content, respectively.

Table 4. Correlation analysis between fiber type area and MHC isoform composition before training, after resistance training, and after detraining.

	MHC I:type I	MHC IIA:type IIa	MHC IIX:type IIb
Pretraining	0.94	0.86	0.90
Post-resistance training	0.90	0.90	0.73
Post-detraining	0.88	0.89	0.94

All values are given as correlation coefficients (N = 9). All correlation values were significant (P < 0.05).

Time Perspective for Reappearance of MHC IIX. In the present study, MHC IIX was elevated after 90 days of detraining, to reach values exceeding those observed prior to training. In an earlier study, 19 weeks of resistance training decreased the amount of type IIb fibers and, correspondingly, increased the amount of type IIa fibers, as evaluated by ATPase histochemistry.[23] However, after 4 weeks of detraining the IIa/IIb ratio was not reestablished.[23] Likewise, 14 days of detraining following resistance training yielded no increase in type IIb fiber proportions in a group of heavy-resistance–trained power athletes.[24] It is not unlikely that the lack of increase in type IIb fibers observed in the aforementioned studies is the result of detraining periods of insufficient length. In a more prolonged study, a group of sedentary women performed resistance training over a 20-week period, resulting in an almost total disappearance of the type IIb fibers.[40] The training period was then followed by 30 weeks of detraining after which the percentage of type IIb fibers reached 24%, as compared to the 16% observed prior to resistance training, although levels of statistical difference were not reached. This observation may be interpreted to indicate an overshoot in MHC IIX content due to the regime of resistance training followed by detraining. Consequently, MHC IIX content would, in week 30 of detraining, be on the decline toward pretraining values after having peaked earlier in the course of detraining. Thus, given a turnover time of ~3–4 weeks[9,29,42] for the MHC isoform proteins, it is likely that any major change in MHC composition due to physical activity or inactivity will need several weeks or even months to be fully initiated.

MHC Isoform Responsiveness to Changes in Muscle Activity. Human skeletal muscle responds to increased or decreased contractile activity by altering the relative distribution of the various MHC isoforms present in the muscle. Recent data suggest that some activity-induced MHC isoform shifts are more easily evoked than others.[17] The content of MHC IIX is highly sensitive to contractile activity per se, regardless of whether this involves repetitive endurance-like contraction patterns or few contractions at heavy external loading.[4,5,38] The present data emphasize that, under normal physiological conditions, an increase in contractile activity induces reductions in MHC IIX in parallel with an upregulation of MHC IIA. In the case of reduced contractile activity, this pattern is reversed. Whether endurance-type train-

ing within a realistic physiological range induces a MHC II → I transformation in human skeletal muscle is still a matter of controversy.[17,20] No longitudinal human training study has yet provided consistent evidence of a shift from MHC IIA → MHC I, although some studies indicate the possibility of such a shift.[13,20,32,36] Thus, the transformation between MHC IIA and MHC IIX isoforms appears far more easily evoked than the transformation between MHC isoforms IIA and I.[17] This notion is emphasized by the present data because no significant changes in MHC I content were observed throughout the periods of resistance training and detraining. Interestingly, the number of type I/IIa fibers increased after detraining, compared to that observed after resistance training. Similar observations have been reported for endurance-trained rats, in which no significant change in MHC I content could be observed using SDS-PAGE, but a thorough histochemical investigation revealed that the number of fiber containing both MHC I and MHC IIA increased.[17] Moreover, increased amounts of mRNA/protein mismatch for the MHC isoforms I and IIA following detraining were observed in our subjects.[7] Taken together, these data may indicate that resistance training does not cause a major switch between MHC I and MHC II content, even though a minor proportion of the fibers turn toward containing both MHC I and MHC IIA. Nevertheless, if the observation of an increased number of I/IIa fibers should bear any overall significance, this would still remain quite low compared to the marked shifts observed in MHC IIA and MHC IIX content.[7,17]

Defaulted MHC IIX Gene. It has been suggested that the MHC IIX gene constitutes a defaulted gene setting, upon which any increase in contractile activity will cause the MHC IIX isoform to decrease in parallel with an increased expression of MHC IIA.[4–6,19] If this hypothesis holds true, the return to sedentary activity levels following a period of heavy resistance training should reestablish the amount of MHC IIX. From the present observations, however, an overshoot in the amount of MHC IIX to values exceeding pretraining levels seems possible in response to intensive resistance training followed by detraining. This overshoot or "boosting" phenomenon probably arises from the abrupt withdrawal of stimulus from the muscle, but the specific mechanisms responsible for triggering this overshoot phenomenon remain unknown. Most likely, MHC IIX content will gradually return to pre-resistance training levels, so that the time after cessation of resistance training at which detraining biopsies are obtained influences the results obtained and their interpretation. For muscle biopsies obtained shortly (2–4 weeks) after the cessation of resistance training, a significant increase in MHC IIX content should not be readily manifested,[23,24] although minor changes between fiber subtypes may possibly occur.[39] However, if biopsies are obtained some weeks later, the MHC IIX content is likely to have returned to pre-resistance training levels and, some additional weeks later, it may reach even higher levels (present study); subsequently, MHC IIX content would gradually return to pretraining values, according to our interpretation of the study by Staron et al.[39]

Changes in Contractile Capacity. The changes in contractile strength (isometric MVC) resembled those observed for muscle fiber size: maximal contraction strength increased after resistance training, while returning to pretraining levels after the period of detraining. In addition, a general increase in maximal contractile power was observed after resistance training. After detraining, however, maximal power exerted within the first 15 s of contraction was not significantly different from posttraining values. The lack of decrease in contractile power after detraining probably occurred because of the enhanced amounts of MHC IIX, which increased by about 100% relative to pretraining levels. In support of this, single muscle fibers dominated by the MHC IIX isoform may have severalfold greater peak power output compared to fibers containing solely MHC I.[22,45]

Clinical Significance. Besides the obvious advantage for athletes competing in sprintlike events involving very high muscle-shortening velocities and explosive contraction forces, the clinical interpretations of the MHC IIX overshooting phenomenon is not straightforward. An increased relative amount of MHC IIX in a muscle would at least theoretically lead to a reduced and impaired energy economy.[35] On the other hand, an increased amount of MHC IIX would, in light of the higher contractile rate-of-force development of muscle fibers containing the fastest MHC isoforms,[20,34] be highly beneficial, not only in sports athletes, but also for the weakened elderly muscle.

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