Skeletal muscle myosin heavy chain composition and resistance training

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ADAMS, GREGORY R., BRUCE M. HATHER, KENNETH M. BALDWIN, AND GARY A. DUDLEY. *Skeletal muscle myosin heavy chain composition and resistance training.* J. Appl. Physiol. 74(2): 911–915, 1993.—We recently reported that 19 wk of heavy resistance training caused a decrease in the percentage of type IIb and an increase in the percentage of type IIa fibers as determined by qualitative histochemical analyses of myofibrillar adenosinetriphosphatase activity of biopsies of musculus vastus lateralis (Hather et al. *Acta Physiol. Scand.* 143: 177–185, 1991). These data were interpretated to suggest that resistance training had caused transformation among the fast-twitch fiber subtypes. To more clearly establish the influence of resistance training on muscle fiber composition, biopsies from the original study were analyzed biochemically for myosin heavy chain (MHC) composition by use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis and histochemically for fiber types by use of myofibrillar adenosinetriphosphatase activity. The results show that after training ($n = 13$), IIb MHC composition decreased ($P < 0.05$) from 19 ± 4 to 7 ± 1%. IIa MHC, in contrast, increased ($P < 0.05$) from 48 ± 3 to 60 ± 2%. These responses were essentially mirrored by alterations in fiber type distribution. The percentage of type IIb fibers decreased ($P < 0.05$) from 18 ± 3 to 1 ± 1%, whereas the percentage of type IIa fibers increased from 46 ± 4 to 60 ± 3% ($P < 0.05$). Neither I MHC composition nor type I fiber percentage changed with training. The control group ($n = 4$) showed no changes in MHC composition or fiber type distribution. These results suggest that heavy resistance training alters MHC composition in human skeletal muscle, presumably reflecting a change in genetic expression.

human skeletal muscle; exercise; muscle fiber type

CLASSIC RESPONSES to endurance training include increases in aerobic power and endurance while alterations in muscle mass and strength are minimal (19). It is also generally accepted that endurance training alters muscle fiber type distribution, especially among the fast-twitch subtypes (12, 17). Resistance training, in contrast, evokes quite different responses in that marked increases in muscle mass and strength are primary objectives of such exercise (15, 26). Furthermore, it has recently been concluded that resistance training does not alter muscle fiber type distribution (12).

We recently completed a study in which males performed 19 wk of heavy resistance training (10, 11). The leg press and knee extension exercises were used to develop the thighs as subjects trained 2 days/wk using concentric only or concentric and eccentric muscle actions. Three to five sets of each exercise (6–12 repetitions per set) were performed each day of training with a load that induced failure within each set. Irrespective of the nature of training, we found a marked decrease in the percentage of type IIb fibers with a concomitant increase in type IIa fibers as determined by standard histochemical methods. Similar results have recently been reported in females performing heavy resistance training (23, 24).

Recent development of sophisticated biochemical techniques now allows separation of skeletal muscle contractile protein isoforms (3, 5, 20, 21, 25). To further understand adaptive responses to exercise, we used one such technique in this study, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), to determine if heavy resistance training alters skeletal muscle myosin heavy chain (MHC) composition. We hypothesized that changes in MHC after resistance training would support previous observations of training-induced alterations among the fast-twitch fiber subtypes.

MATERIALS AND METHODS

Subjects. The subjects in this study were 17 healthy males whose age, height, and weight averaged 36 ± 2 (SE) yr, 178 ± 1 cm, and 89 ± 3 kg, respectively. They were part of a larger group that participated in a 19-wk heavy resistance training program. They were selected based on availability of skeletal muscle biopsy tissue for both histochemical and biochemical analyses (see below). Thirteen subjects were trainees: eight used only concentric actions in training and five used both concentric and eccentric actions. Four subjects served as controls. Like the larger group of subjects, these trainees showed marked changes in fast-twitch fiber type distribution (Table 1; Ref. 11). Likewise, lower limb strength increased from 10 to 30% and average fiber cross-sectional area from 7 to 20%, depending on the nature of training. The procedures, purpose, and risks associated with the study were explained, and written consent was provided. The study was approved by the Human Research Review Board at the Kennedy Space Center, FL.

Muscle biopsies. Two muscle samples were obtained both before and after training from the right musculus vastus lateralis of each subject with the needle biopsy method of Bergström (2). Each biopsy was assessed for MHC composition and muscle fiber type distribution.

Sample preparation. A portion of each biopsy deemed appropriate for transverse sectioning was oriented, af-

TABLE 1. *MHC composition and fiber type percentage in biopsies of musculus vastus lateralis before and after 19 wk of heavy resistance training*

	Before		After	
	% MHC	% Fiber	% MHC	% Fiber
Trainees (n = 13)				
IIb*	19±4	18±3	7±1	1±1†
IIa*	47±3	46±4	60±2	60±3
I‡	34±3	36±4	33±3	39±3
Controls (n = 4)				
IIb	22±3	17±1	23±4	21±4
IIa	42±2	39±6	46±4	41±3
I‡	36±4	44±6	31±5	38±5

Values are means ± SE; n, no. of subjects. MHC, myosin heavy chain. * Significant group-by-time interaction with a decrease (IIb) or an increase (IIa) in the trainees after training. † Significant method-by-time interaction with the lesser training response found in MHC. ‡ Significant method effect with lesser values in MHC.

fixed to wooden splints with a mixture of O.C.T. compound (Tissue-Tek, Miles, Elkhart, IN) and tragacanth gum, frozen in 2-methylbutane precooled with liquid nitrogen (−160°C), and stored at −70°C until processed for histochemical analyses. The remainder of the biopsy was frozen in liquid nitrogen, stored at −70°C, and subsequently assessed for MHC composition.

Myofibril extraction. Frozen muscle samples (90 ± 4 mg) were homogenized in ~20 vol of a solution containing (in mM) 250 sucrose, 100 KCl, and 5 EDTA. The homogenate was washed successively in sucrose solution, 0.5% Triton X, and 150 mM KCl. The final myofibril pellet was resuspended in 1 ml of 150 mM KCl. The protein concentration (9.8 ± 0.4 mg/ml, n = 34) of this solution was determined with the biuret method (9). An aliquot of myofibril suspension was added to a solution containing 50% vol/vol glycerol, 100 mM $Na_4P_2O_7$, and 5 mM EDTA at a concentration of 1 mg/ml and stored at −20°C.

Myosin analysis. Myofibril fractions were analyzed to determine MHC composition. Five-microliter samples of stored myofibril solution were added to 35 μl of denaturing buffer [62.5 mM tris(hydroxymethyl)aminomethane, 20% glycerol, 1% α-mercaptoethanol, 2.3% SDS, 0.05% bromophenol blue] and heated at 100°C for 2 min. After this treatment an 8-μl aliquot was withdrawn for analysis by SDS-PAGE with the use of a modification of the methods of Danieli-Betto et al. (6). Stacking gels were prepared with 40% glycerol and 4% total acrylamide concentration at pH 6.8. Separating gels had 40% glycerol content and 7% total acrylamide at pH 8.8. Gels were run at 120 V until the dye front ran off the bottom of the gel (~7 h). Gels were stained for 1 h with Brilliant Blue G 250 dye (Sigma Chemical, St. Louis, MO) and then destained with 25% methanol and 5% acetic acid. MHC bands were scanned using a Zenith soft laser densitometer (BioMedical Instruments, Fullerton, CA) interfaced with a personal computer. The peaks of interest, as determined from standards, were identified in the digitized densitometric data sets, and the area of each peak was determined by integration. The total integrated area of all peaks was set to 100, and each individual area was expressed as a percentage of total MHC to determine the relative amounts of MHC isozymes. MHC isozymes were identified as I, IIa, or IIb on the basis of migration patterns published by others that were substantiated by immunocytochemical techniques (4, 13, 14).

Histochemistry. Serial sections (12 μm) were cut in a cryostat at −22°C. Adjacent muscle sections were assayed for myofibrillar adenosinetriphosphatase (mATPase) at pH 9.4 after acid (pH 4.3 or 4.6) and alkaline (pH 10.4) preincubation to identify the three major fiber types (5) as described in detail elsewhere (10). All fibers within a given section were used to determine fiber type percentages. Fifty of each type were used for cross-sectional area measurements.

Statistical analyses. All values are reported as means ± SE. Histochemical and MHC data for each fiber type were analyzed using a two-way analyses of variance with repeated measures for subjects to examine the influence of training or the method of analyses. Pearson's product correlations were used to examine MHC composition vs. fiber type distribution. All tests of significance were made at $\alpha = 0.05$.

RESULTS

Alterations in fiber type distribution and MHC composition were comparable for the different types of heavy resistance training; thus the results were combined to simplify presentation. MHC composition showed a marked change after 19 wk of training (Fig. 1). The proportion of IIb MHC decreased ($P < 0.05$), whereas that of IIa MHC increased ($P < 0.05$; Fig. 1, Table 1). Likewise, there was a significant decrease in the percentage of type IIb fibers with training, whereas the percentage of type IIa fibers increased (Table 1, Fig. 2). Training did not alter the percentage of type I fibers or the proportion of I MHC (Figs. 1 and 2, Table 1). The decrease in the proportion of IIb MHC with training was less ($P < 0.05$) than the reduction in the percentage of type IIb fibers (Table 1). The controls exhibited no changes in MHC composition or fiber type distribution (Table 1). Overall, the percentage of type I fibers was greater ($P < 0.05$) than the proportion of I MHC (Table 1).

Fiber type percentage and MHC composition were highly correlated both before and after training (Fig. 3). This was true whether the data were pooled or examined by fiber type.

DISCUSSION

Our interest in the present study arose from observations, based on histochemical analyses of mATPase activity of human skeletal muscle biopsy samples, that the percentage of type IIb fibers was markedly reduced after 19 wk of heavy resistance training, whereas that of type IIa fibers changed reciprocally (11). These data were interpreted to reflect a transformation among the fast-twitch fiber subtypes because the percentage of type I fibers did not change with training. The suggestion that resistance training might alter muscle fiber type distribution on the basis of these data could be judged tenuous for several reasons. Differentiation between type IIa and

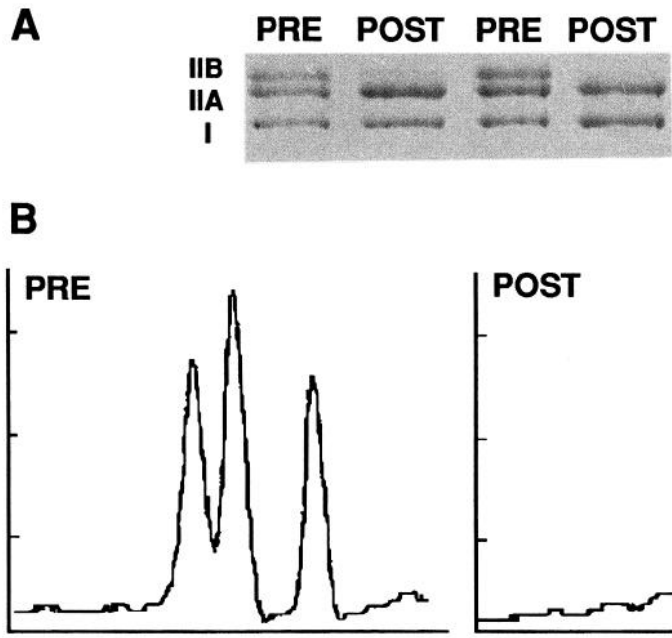

FIG. 1. *A*: representative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel sections showing I, IIa, and IIb myosin heavy chain (MHC) bands from 2 subjects before (Pre) and after (Post) 19 wk of resistance training. *B*: densitometric scans from MHC region of SDS-PAGE gel with pre- and posttraining samples. No IIb MHC was detected posttraining in this sample.

type IIb fibers is done visually by indicating whether a given fiber "stains" intermediate or light when in fact there may be a continuum of staining intensities among the fast-twitch fibers (21). It is generally believed, in addition, that resistance training does not alter fast-twitch fiber subtype populations (12). It may also appear unreasonable that repeated performance of near-maximal voluntary efforts during resistance training would diminish the population of fast-twitch type IIb fibers because it is contended they are mainly used during such activity (1, 17–19, 27, 28).

The results of the present study clearly show an alteration in skeletal muscle MHC after 19 wk of heavy resistance training. The proportion of IIb MHC decreased, IIa MHC increased, and I MHC did not change. The controls, in addition, showed no change in skeletal muscle MHC composition over this time. These are the first data, to our knowledge, to show that heavy resistance training alters MHC composition of human skeletal muscle. It should be appreciated that this type of exercise typically involves the performance of a few hundred intermittent, near-maximal efforts per week (15, 16). The activity pattern of heavy resistance exercise, therefore, is markedly different than that imposed by endurance-type exercise training or chronic electrical stimulation, which have also been shown to alter muscle fiber type distribution (12, 17, 18). Thus, it may be as proposed by Goldspink et al. (8) that IIb MHC is the default gene that provides a readily available pool of fibers that transform into IIa fibers with increases in activity, irrespective of the type.

The alterations in muscle fiber type distribution found in the present study are comparable to those reported previously for the larger group of trainees (11). The selection of subjects based on availability of sufficient tissue for fiber type distribution and MHC composition analyses, therefore, did not bias the results of this study. Our results are substantiated by the observation that females show a decrease in the percentage of type IIb fibers after heavy resistance training with a concomitant increase in the percent type IIa fibers (22, 23). Likewise, the results of cross-sectional studies have been interpreted to suggest that heavy resistance training alters muscle fiber type distribution (13, 22). Transformation among the fast-twitch subtypes has been put forth as the mechanism responsible for these responses, as the proportion of type I fibers remains unaltered (23, 24). Degeneration of fast-twitch glycolytic fibers and regeneration of fast-twitch oxidative-glycolytic fibers, as has been reported after chronic electrical stimulation, would also alter the fast-twitch fiber subtype distribution (16). Muscle fiber degeneration/regeneration, however, was not evident in toluidine blue O histological preparations of biopsies used in the present study (unpublished observations). Furthermore, the decrease in IIb fibers in the present study did not require performance of eccentric muscle actions, which would be expected to lead to the greatest extent of muscle damage and trauma (11). It is suggested, therefore, that fiber type transformation was responsible for the observed alterations in muscle fiber composition.

The larger decrease in the percentage of type IIb fibers than in the relative proportion of IIb MHC in the present study suggests that the transformation of fiber types was not as complete as judged by histochemical means. This probably occurred because some of the fibers that were histochemically typed as IIa after training were also expressing IIb MHC to some extent (21). The overall greater percentage of type I fibers than proportion of I MHC probably reflects the smaller size of type I than type II fibers in musculus vastus lateralis of males (e.g., see Ref. 11). Fiber type percentage is based on relative number and is not influenced by fiber size, whereas the proportion of a given MHC is relative to the total amount of MHC detected in the gel. It might also be expected that I MHC composition would decrease after training as the ratio of fast-twitch to slow-twitch fiber areas increases with resistance training (11, 26). For the subjects used in this study, however, the increase in the ratio (11%) was offset by an 8% increase in the percentage of

type I fibers such that the relative proportion of I MHC did not change.

It has been shown previously in humans and lower mammals that individual muscle fibers of a given type express mainly the analogous MHC (3, 20, 21, 25). It was suggested, therefore, that muscle fiber type was conferred by MHC composition. If this were true, analyses of mixed muscle samples for fiber type and MHC should be comparable. The results of the present study support this contention. Fiber type distribution and MHC composition of mixed fiber biopsies were correlated both before and after training. These data lend credence to previous studies that have suggested, on the basis of histochemical analyses, that resistance training alters muscle fiber composition (23, 24). They also suggest that histochemical analyses can provide insight into alterations in skeletal muscle MHC composition after a given intervention in addition to the well-accepted morphological measures.

An intriguing aspect of the present and previous studies that have reported a decrease in type IIb muscle fiber percentage after resistance training (23, 24) is that IIb fibers are often held to be critical for optimal performance of such activity. Staron et al. (22) reported some years ago that experienced weight trainees had exceptionally large type IIa fibers and a lower percentage of type IIb fibers than sedentary controls. They concluded that weight lifters must rely to a greater extent on type IIa fibers than generally believed. Resistance training, in fact, appears to decrease IIb MHC expression while at the same time enhancing performance during near-maximal voluntary efforts. Thus, type IIb fibers are probably used to meet the demands of unaccustomed physical activity. If the activity becomes routine, such as during resistance training, a portion of IIb fibers appears to transform into IIa fibers.

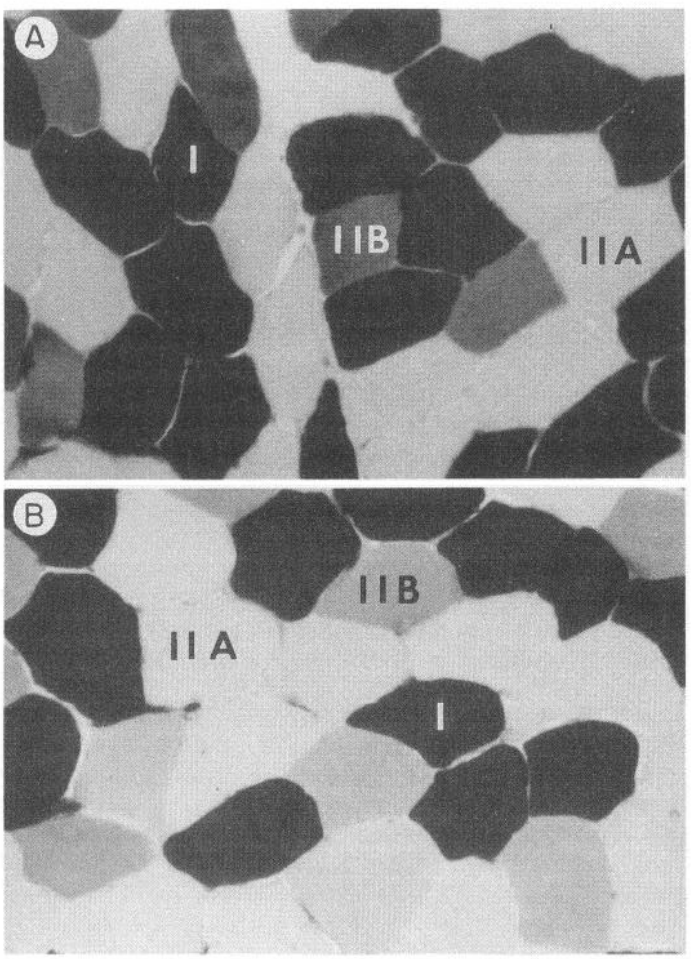

FIG. 2. Representative cross section of biopsy of musculus vastus lateralis histochemically assayed for myofibrillar actomyosin adenosinetriphosphatase activity (preincubation pH 4.6) before (*A*) and after (*B*) 19 wk of resistance training. I, IIa, and IIb, type I, type IIa, and type IIb fibers.

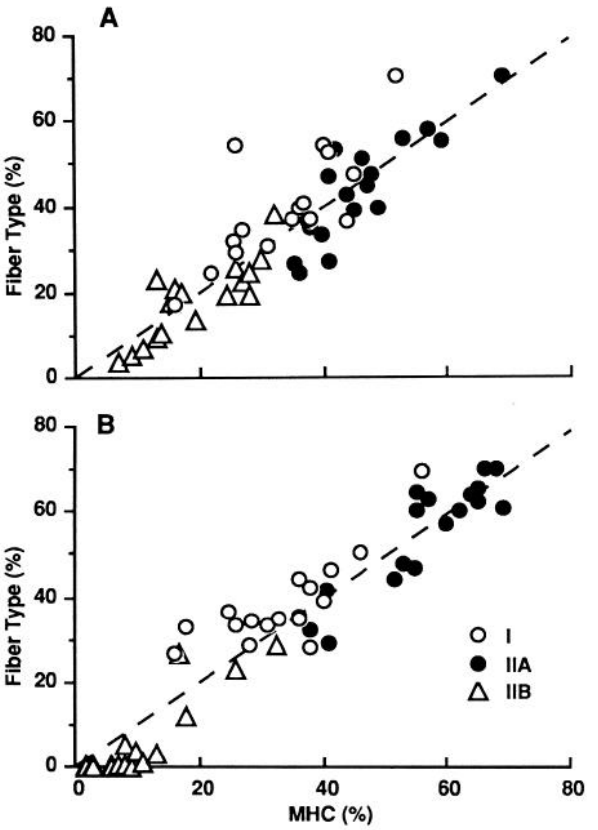

FIG. 3. Percentage of type I, IIa, or IIb fibers of a given biopsy of musculus vastus lateralis plotted against MHC composition (I, IIa, or IIb) of same sample before (*A*) and after (*B*) 19 wk of resistance training. Correlations overall and for I, IIa, and IIb fibers before training were 0.88, 0.75, 0.87, and 0.84, respectively ($P < 0.05$). Corresponding values for after training were 0.96, 0.87, 0.90 and 0.89, respectively ($P < 0.05$). Dashed line is that of identity.

In summary, the results of the present study suggest that heavy resistance training alters the MHC composition of human skeletal muscle as reflected by a reduction in IIb MHC and a concomitant increase in IIa MHC. These changes in MHC composition were reflected by alterations in fiber type distribution as determined by histochemical analyses of mATPase. On the basis of the results of the present and previous studies, it is suggested that increases in physical activity, whether resistance or endurance in nature, can alter expression of fast MHC isoforms.

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