Effect of endurance exercise on myosin heavy chain gene regulation in human skeletal muscle

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O’Neill, D. Sean, Donghai Zheng, Wade K. Anderson, G. Lynis Dohm, and Joseph A. Houmard. Effect of endurance exercise on myosin heavy chain (MHC) isoform regulation in human skeletal muscle. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R414–R419, 1999.—The purpose of this study was to determine the effect of endurance-oriented exercise on myosin heavy chain (MHC) isoform regulation in human skeletal muscle. Exercise consisted of 1 h of cycle ergometer work per day at 75% maximal oxygen consumption for seven consecutive days. Muscle was obtained before the first bout of exercise, 3 h after the first bout of exercise, and before and 3 h after the final bout on day 7 (n = 9 subjects). No changes in MHC mRNA (I, IIa, IIx) were evident after the first exercise period. There was, however, a significant (P < 0.05) decline (−30%) in MHC IIx mRNA 3 h after the final training bout. An interesting finding was that a higher preraining level of MHC IIx mRNA was associated with a greater decline in the transcript before (r = 0.68, P < 0.05) and 3 h after (r = 0.82, P < 0.05) the final exercise bout. These findings suggest that MHC IIx mRNA is downregulated during the early phase of endurance-oriented exercise training in human skeletal muscle but only after repeated contractile activity. Pretraining MHC IIx mRNA content may influence the magnitude of this response.

contractile activity; muscle fiber type

MYOSIN IS A myofibrillar protein that influences the rate of tension and fatigue development during muscle contraction (12, 16, 18, 19). Mature skeletal muscle is characterized by electrophoretically distinct myosin heavy chain (MHC) proteins, the predominant isoforms being the MHC-β/slow or type I, MHC IIa, MHC IIx, and MHC IIb (4, 18, 19). Contractile activity is a powerful and rapid regulator of the expression of these isoforms. In rodents, as little as 2 days of chronic low-frequency motor nerve stimulation (CMNS) induces a repression in MHC IIb mRNA followed by a slower increase in MHC IIa mRNA (12, 16, 18, 19). The MHC proteins are thought to be primarily transcriptionally controlled (12, 16); the time course and threshold stimulus needed to trigger changes at the mRNA level are thus important aspects of gene regulation.

The CMNS model in rodents is commonly used for defining the cellular events that occur with repeated bouts of contractile activity (i.e., endurance-oriented exercise training; see Refs. 12, 16–19). However, in contrast to the consistent stimulus with CMNS, contractile activity with exercise training is typically separated by 12–24 h. In addition, exercise training involves load bearing and specific patterns of motor unit recruitment, both of which may influence MHC gene regulation (6, 12, 14, 17, 18). It is also of interest to study responses in human skeletal muscle, as a species difference in the expression of the IIx and IIb MHC isoforms may exist between human and rodent tissue (4, 18, 19, 21). To our knowledge, no studies have directly determined the acute responses of the MHC genes in human skeletal muscle to a single or repeated bouts of endurance-oriented (i.e., ≥1 h) exercise training. The influence of exercise on MHC mRNA (I, IIa, and IIx) was therefore examined in human skeletal muscle in an attempt to discern the responses of these genes.

METHODS

Experimental design. All subjects provided written informed consent before inclusion in the study. Subjects were screened with a health questionnaire and were tested for cardiovascular fitness (maximal oxygen consumption, VO2max) and anthropometric characteristics to ensure that only sedentary, nonobese, yet healthy individuals were studied. Muscle samples were obtained from the vastus lateralis with the percutaneous needle biopsy technique. The day 1 biopsy was performed with the subject in the fasted state on the morning of the first day of training. Another sample was obtained 3 h after the first training bout (day 1 + 3 h). Subjects then exercised for a total of seven consecutive days for 1 h/day at 75% VO2max on a cycle ergometer. On the morning of day 7 another muscle sample was obtained (day 7); the biopsy was performed 15–17 h after the preceding exercise bout. The last biopsy was obtained 3 h after the final exercise bout on day 7 (day 7 + 3 h). Muscle was obtained under local anesthesia as described previously (8) from the contralateral leg of the preceding biopsy. Care was taken to achieve a consistent biopsy depth due to differences in fiber type between the superficial and deep vastus. The homogeneity of samples within a subject was demonstrated by high correlations of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA between the pretraining and subsequent biopsies (r values of 0.95–0.99, P < 0.001). The timing of the postexercise biopsies (3 h after exercise) was based on the observation that transcription for the GLUT-4 and hexokinase II genes in skeletal muscle were maximal 3 h after exercise (14, 15). Posttraining body composition and VO2max were determined 1–2 days after the final exercise bout.

Subjects. Subjects were nine young (mean ± SE, age, 22.3 ± 0.5 yr) men (height, 178.6 ± 2.2 cm; weight, 77.0 ± 2.5 kg). Inclusion criteria were no medications, which could affect metabolism, abstinence from a regular exercise program for 1–2 days before and after the study period.
at least 1 yr before initiating the study, and nonobesity. Body fat percentage was determined from seven skinfolds (9).

Testing and training procedures. VO\textsubscript{2max} was determined on an electrically braked cycle ergometer (Lode, Groningen, The Netherlands) with a protocol in which workload was incrementally increased every 3 min until voluntary exhaustion was achieved (8). Results from the VO\textsubscript{2max} test were used to verify that the subjects were within normative ranges for young, sedentary individuals (<55 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}) and 2) to provide oxygen consumption and heart rate data for the exercise training prescription. The VO\textsubscript{2max} test was performed 10–14 days before the first training bout to minimize any residual effects of prior exercise. Oxygen consumption was determined from expired gases using a metabolic cart (Sensor Medics, Anaheim, CA), and heart rate was determined with a 12-lead electrocardiogram. Subjects trained daily (1 h/day) for 7 consecutive days on a cycle ergometer (Monark) at a workload eliciting 75% VO\textsubscript{2max}. Oxygen consumption and heart rate (Polar XL) were monitored every 15 min to verify training intensity.

Muscle analysis. After the biopsy, any connective and adipose tissue was moved, and the muscle was quick-frozen and stored in liquid nitrogen. Total RNA was isolated using TRIzol reagent (GIBCO-BRL, Gaithersburg, MD). Briefly, the tissue was homogenized in 1 ml of TRIzol. Chloroform (200 µl) was added, and the sample was vortexed vigorously for 15 s and incubated at room temperature for 5 min. Samples were microfuged for 15 min (12,000 g) at 4°C, and 400 µl of the top aqueous layer was transferred to a fresh microfuge tube. RNA pellets were washed with 1 ml of 70% ethanol. After a brief air dry, pellets were resuspended in 50 µl of diethyl pyrocarbonate-treated water.

Muscle was analyzed for MHC mRNA (I, IIa, IIx) using the RNase protection assay. All samples for a subject were analyzed at the same time in the same batch and on adjacent lanes. The cDNA probes for the DNA complementary to MHC IIa and IIx were obtained from Leslie Leinwand (University of Colorado, Boulder, CO; see Ref. 21). The cDNA for MHC I was obtained from Kirti Bhatt (University of Rochester, Rochester, NY; see Ref. 23). Plasmids containing MHC IIa and IIx were linearized with Xba I and Xho I, respectively. Plasmids containing MHC I were linearized with Xho I. Labeled antisense RNA probes for MHC I, IIa, and IIx and GAPDH were synthesized using \textsuperscript{32}P-UTP and an RNA polymerase T3/T7 MAXscript in vitro transcription kit (Ambion, Austin, TX). The RNase protection assay was done with a commercially available kit (RPA II; Ambion). The protected RNA samples were electrophoretically separated on a 6% polyacrylamide gel containing 7 M urea, visualized by autoradiography, and quantitated using phosphorimager analysis (Molecular Dynamics, Sunnyvale, CA). A representative gel for MHC IIx mRNA is presented in Fig. 1. MHC mRNA was normalized to GAPDH mRNA; there was no change in GAPDH mRNA (see RESULTS) in agreement with other exercise studies (11). Assuming uniform loading, data were also normalized by assigning the day 1, non-GAPDH corrected mRNA reading within a subject a value of 1.0, and relative changes were calculated. Values are expressed as dimensionless ratios, and interpretation was identical using either normalization technique.

Statistics. Variables were compared with one-way, repeated-measures analysis of variance at the P < 0.05 level. Post hoc comparisons were performed using a Fisher’s protected least significant difference test. Single-order Pearson-product correlations (P < 0.05) were used to examine relationships between given variables.

RESULTS

Skeletal muscle. There were no statistically significant alterations with exercise training in either MHC I or MHC IIa mRNA concentrations (Fig. 2). The percent changes for MHC I mRNA for day 1 + 3 h, day 7, and day 7 + 3 h compared with day 1 were 104 ± 6.0, 94 ± 6, and 109 ± 12%, respectively. The percent changes for MHC IIa mRNA compared with day 1 were 107 ± 6, 99 ± 12, and 91 ± 10%, respectively.

There was a decrease in MHC IIx mRNA after the final bout of exercise (day 7 + 3 h) compared with the sedentary, preexercise condition and 3 h after the first exercise bout (day 1 + 3 h, Fig. 3). MHC IIx mRNA concentration was depressed by ∼30% compared with before training; percent changes compared with pretraining values (day 1) were 105 ± 9, 99 ± 11, and 70 ± 7 for day 1 + 3 h, day 7, and day 7 + 3 h, respectively. There were no alterations in GAPDH mRNA with training (%change from day 1, 114 ± 15, 115 ± 13, and 94 ± 11 for day 1 + 3 h, day 7, and day 7 + 3 h, respectively).

Fig. 1. Detection and measurement of myosin heavy chain (MHC) IIx mRNA in human muscle. Presented is an RNase protection assay from one subject with \textsuperscript{32}P-labeled riboprobes, yielding a 122-base pair protected fragment for human MHC IIx mRNA and a 316-base pair fragment for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Total RNA (2 µg) from each individual sample was incubated with MHC IIx and GAPDH riboprobes, digested with RNase, resolved on polyacrylamide gels, and identified by phosphorimagery. Lane A: before training (day 1); lane B, 3 h after a single training bout (day 1 + 3 h); lane C, after the 7th training bout (day 7 + 3 h); lane D, after 6 days of training (day 7). Individuals with high initial levels of the transcript, such as the subject presented in this figure, demonstrated a trend for MHC IIx mRNA to decrease at day 7 (lane D) and to demonstrate relatively large decreases at day 7 + 3 h (lane C; see Fig. 4). There was, however, no mean reduction in MHC IIx mRNA at day 7 and a mean reduction of −30% (P < 0.05) 3 h after the final training bout (day 7 + 3 h) in the 9 subjects studied.

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The relative alteration in mRNA for each subject was calculated by subtracting the mRNA value for the respective postexercise time point (day 1, day 7, and day 7 + 3 h) from the pretraining value (day 1). Relationships were evident between the pretraining level of MHC IIx mRNA and relative alterations at 7 days ($r = 0.68$) and 7 days + 3 h ($r = 0.82$) of training (Fig. 4). These correlations suggest that the individuals with a higher pretraining MHC IIx mRNA level displayed a more substantial decrease in MHC IIx mRNA with training.

Exercise variables. There were no alterations in $V\dot{O}_2\text{max}$ or body composition with the 7 days of training, as indicated in Table 1. Heart rate during training was 143.5 ± 3.7 beats/min; oxygen consumption was 2.6 ± 0.11 l/min, which represented 76.2 ± 0.9% of $V\dot{O}_2\text{max}$.

**DISCUSSION**

The intent of this study was to determine the effect of endurance-oriented exercise training on MHC isoform regulation in human skeletal muscle. To our knowledge, there are no data on this topic; MHC gene regulation has been studied in rodents with CMNS and findings generalized to exercise training (12, 16–19). It is important, however, to compare and define the MHC responses to exercise training, as the contractile activity is intermittent, involves specific motor unit recruitment patterns, and is load bearing. We observed that MHC isoform (I, IIa, IIx) mRNA concentrations were not altered significantly 3 h after a single bout of cycle ergometer exercise. There was a significant decrease in MHC IIx mRNA, however, after the final exercise bout of the 7-day training period (Fig. 3). No mean change in MHC IIx mRNA was evident before the exercise bout on...
day 7, despite the previous 6 days of training (Fig. 3). Thus the main finding of this study was that, during the early phase of endurance-oriented exercise training, MHC IIx mRNA content begins to decrease in human skeletal muscle but only after repeated exercise.

In rodents, a similar model was used to discern the adaptive ability of the insulin-responsive glucose transporter protein (GLUT-4) to endurance-oriented contractile activity (14). After 7 days (80 min/day) of treadmill running, GLUT-4 transcription increased by 1.8-fold at 3 h postexercise but returned to control levels 24 h after this final training bout. In animals that performed only a single bout of exercise, GLUT-4 transcription also increased at 3 h after exercise; the elevation, however, was not to the extent (1.4-fold) observed in the trained animals.

These findings (14) and the present data suggest that the induction of some skeletal muscle genes is a function of a cumulative effect from previous contractile activity. Such observations suggest that a training-induced transcription factor is produced but not significantly expressed until repeated bouts of contractile activity are performed. In support, in rodents, GLUT-4 and cytochrome c mRNA increase transiently during the early phase of training (1 wk); when training is extended (2 wk or more), mRNA remains elevated (13, 14). We cannot discern if a similar time course is evident with the MHC isoforms in human skeletal muscle due to the limited time period studied. It is tempting, however, to postulate that the genes of the energy-producing (i.e., GLUT-4 and cytochrome c) and contractile machinery (MHC IIx) are coregulated and influence muscle function in a synchronized manner. The novel aspect of the current study is that this cumulative nature of the training response can be extended to the MHC IIx isoform in human tissue using a physiologically relevant exercise protocol (exercise for 1 h/day over 7 days) and mode (cycling).

Fig. 4. Relationship between pretraining MHC IIx mRNA and individual alterations in MHC IIx mRNA at day 7 (A) and day 7 + 3 h of training (B). Data suggest that a higher pretraining MHC IIx mRNA concentration was associated with a greater decline in the transcript with training.

Table 1. \( \dot{V}O_2 \) max and body composition before and after the 7 days of training

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before Training</th>
<th>After Training</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \dot{V}O_2 ) max, l/min</td>
<td>3.4 ± 0.2</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>( \dot{V}O_2 ) max, ml·kg(^{-1})·min(^{-1})</td>
<td>45.0 ± 2.0</td>
<td>46.1 ± 1.7</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>77.0 ± 2.5</td>
<td>76.7 ± 2.6</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>9.2 ± 1.4</td>
<td>10.6 ± 1.4</td>
</tr>
<tr>
<td>Skinfold sum, mm</td>
<td>70.7 ± 9.3</td>
<td>72.5 ± 10.2</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 9 subjects. \( \dot{V}O_2 \) max, maximum rate of oxygen consumption. There were no changes in any of these parameters with training.
This has led to the hypothesis that the type IIb gene is a “default” gene that provides a pool of fibers available for transformation with increases in contractile activity (6). Our observed reduction in MHC IIx mRNA supports this theory.

In human striated muscle, fiber type and MHC expression can be extremely heterogenous (1, 2, 4, 16, 18, 19). In a sedentary population, relative type IIb fiber distribution in the vastus lateralis can vary from 0 to 44% with a coefficient of variation of ~66% (20). An interesting finding of the present study was that pretraining muscle phenotype may influence the magnitude of the change in MHC mRNA with contractile activity. We observed that a higher pretraining concentration of MHC IIx mRNA was associated with a more pronounced decrease in the transcript with training (Fig. 4). This was particularly evident on day 7 as subjects with high initial levels tended to decrease concentration of the transcript (Figs. 1 and 4), despite no mean change in MHC IIx mRNA (Fig. 3). Pretraining MHC isoform content may thus be a factor to consider when examining responses to contractile activity in human skeletal muscle.

A limitation in studying human muscle is that numerous samples are impractical to obtain. We chose to focus on the acute responses of the MHC genes, since contractile activity is a powerful and rapid modulator of the isoforms (12, 16–19). The 7-day training model was selected as it has been used to discern early responses to exercise training (8, 13, 14). The training regimen selected approximated the intensity (75% Vo2max), duration (1 h), and mode (cycling) of a training prescription used in a clinical or intervention setting to provide relevant and applicable findings.

No changes in the MHC I or IIa mRNA were evident (Fig. 2). It is unlikely that exercise training would induce a type II to I isoform transition in human tissue (12, 16–19). With either endurance- or resistance-oriented training, there is, however, a type IIb to IIa transition (1–3). The current data suggest that the increase in MHC IIa expression is not evident during the early stages of endurance-oriented exercise training in human skeletal muscle. This suggests either 1) a different transcription factor or 2) that a higher threshold stimulus is required compared with the MHC IIx gene.

In summary, no changes in MHC isoform mRNA were evident in human skeletal muscle 3 h after a single bout of endurance-oriented exercise training. In contrast, there was a decline in MHC IIx mRNA after seven consecutive days of training. These findings suggest that MHC IIx mRNA is downregulated during the early phase of endurance-oriented training but only after repeated contractile activity. Pretraining MHC IIx mRNA content may influence the magnitude of this response.

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REFERENCES