Changes in myosin heavy chain mRNA and protein expression in human skeletal muscle with age and endurance exercise training

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Submitted 2 February 2005; accepted in final form 25 February 2005

Short, Kevin R., Janet L. Vittone, Maureen L. Bigelow, David N. Proctor, Jill M. Coenen-Schimke, Paul Rys, and K. Sreekumaran Nair. Changes in myosin heavy chain mRNA and protein expression in human skeletal muscle with age and endurance exercise training. J Appl Physiol 99: 95–102, 2005. First published March 3, 2005; doi:10.1152/japplphysiol.00129.2005.—Aging is associated with reduced muscle strength and atrophy of type II muscle fibers. Muscle fiber type and contractile function are primarily determined by myosin heavy chain (MHC) isoforms. There are few data available on the effects of aging on MHC isoform expression in humans. In the present study, we tested the hypothesis that MHC isoform protein composition and mRNA abundance would favor a fast-to-slow isoform shift with aging and in response to endurance exercise training. Muscle biopsies were obtained from previously sedentary, healthy men and women, aged 21–87 yr before (n = 77) and after (n = 65) 16 wk of bicycle training (up to 45 min at 80% peak heart rate, 3–4 days/wk). At baseline, MHC I mRNA was unchanged with age, whereas IIa and IIx declined by 14 and 10% per decade, respectively (P < 0.001). MHC IIa and IIx protein declined by 3 and 1% per decade with a reciprocal increase in MHC I (P < 0.05). After training, MHC I and IIa mRNA increased by 61 and 99%, respectively, and IIx decreased by 50% (all P < 0.001). The increase in MHC I mRNA was positively associated with age, whereas the changes in MHC IIa and IIx mRNA were similar across age. MHC I protein increased by 6% and was positively related to age, whereas IIx decreased by 5% and was inversely related to age. These results suggest that the altered expression of MHC isoforms with aging is transcriptionally regulated. In response to endurance exercise, regulation of MHC isoform transcripts remains robust in older muscle, but this did not result in corresponding changes in MHC protein expression.

muscle contractile proteins; gene expression; isokinetic strength; muscle size

AGING IS ASSOCIATED WITH REDUCED size and strength of human skeletal muscles (13, 19, 23). With aging, there are also changes in the size, number, and contractile phenotype of individual muscle fibers that may impact muscle function. Classic studies by Larsson et al. (18) and Lexell (22) showed that the decline in size of the vastus lateralis with normal aging could be attributed to fewer total muscle fibers and preferential atrophy of fibers classified as type II (fast twitch) by histochemical staining of myosin ATPase. Those authors also reported that the percentage of each of the three major fiber types in adult muscle, type I (slow twitch), IIa, and IIb, was largely unchanged with age. Subsequent investigations by other groups also showed that the percentage of each fiber type did not appear to change significantly with age in human leg muscles, but that the size of type II fibers was selectively reduced in the vastus lateralis and gastrocnemius of older people (6, 11, 15, 27).

The use of myosin ATPase staining to determine muscle fiber type is based on the relative expression of isoforms of myosin heavy chain (MHC), the major contractile protein in skeletal muscle. Contractile phenotype in adult muscle fibers is primarily determined by the relative expression of the MHC isoforms I, IIA, and IIX (5, 9), which correspond to the histochemically determined fiber types I, IIA, and IIB in humans (29). Han et al. (9) demonstrated that ATP consumption rate and tension cost of single human muscle fibers vary with the expression of MHC isoforms. Whereas most muscle fibers appear to express a single MHC isoform, isoform coexpression has been demonstrated in up to ~30% of fibers and may vary with age and exercise training (1, 16, 38). Thus measurement of fractional MHC isoform composition of muscle provides an important index of the regulation of contractile phenotype.

The mechanisms that regulate the expression of MHC isoforms in aging muscle are not yet clear. It has been reported that the synthesis rate of MHC protein is reduced with age in humans (2, 10), but it is not known whether this is a global change that affects all MHC isoforms or whether there is isoform-specific regulation. Isoform-specific regulation may occur at the pretranslational level. Previous work from our laboratory demonstrated that the mRNA abundance of MHC IIA and IIX was reduced in vastus lateralis biopsies from healthy older vs. younger people, whereas MHC I mRNA content was unchanged (3). This finding is consistent with a report by Klitgaard et al. (15) in which older men were found to have smaller type II muscle fibers than young men and a corresponding decrease in the fractional amount of MHC IIX protein. In contrast, in studies by Welle and colleagues (36, 37) and Marx et al. (24), there was no difference detected between groups of younger and older people (n = 8–16 people per group) in mRNA abundance of MHC isoforms. The study by Marx et al. also reported that the fractional protein content of MHC isoforms was also unchanged with age.

Therefore, it is not yet resolved whether expression of MHC mRNA and protein is altered with age in human skeletal muscle, and to our knowledge only one study has examined the age effect on both MHC mRNA and protein (24). Some of the variation among studies may be due to the use of a small number of subjects, different methods of sample analysis, or...
variable control for physical activity status. Thus the first purpose of the present study was to test the hypothesis that expression of MHC II mRNA and protein is reduced with age in human skeletal muscle. To address this question, muscle biopsies of the vastus lateralis were obtained from 77 healthy, non-exercise-trained men and women between the ages of 21 and 80 yr. We also examined the strength of the association between MHC expression and muscle strength in these people. Larsson et al. (18) reported that the total cross-sectional area of type II fibers was significantly related to muscle strength in younger and older people. Klitgaard et al. (15), however, did not find a significant relationship between muscle strength and fiber area or MHC isoform content in young and older untrained men, but this negative finding may be due to the use of a small number of subjects (7–8 per group).

The second purpose of the study was to determine whether an endurance exercise training program would alter MHC isoform expression and whether the effect would vary with age. We are aware of only one study that prospectively examined the effect of endurance exercise training on MHC mRNA or protein expression in human skeletal muscle. O’Neill and colleagues (25) found that the mRNA abundance for MHC IIX was reduced in vastus lateralis muscle in young men after performing 7 consecutive days of bicycle training. This appears to be in agreement with previous studies that have reported that endurance training results in a decrease in the percentage of fibers classified as type IIB by myosin ATPase histochemistry (7, 12). These changes in fiber type percentcs were observed after 6 wk of bicycle training in young men (12) or after a 9- to 12-mo walking and jogging program in older men and women (7). To our knowledge, however, the effects of endurance exercise training on MHC mRNA or protein expression have not been compared in younger and older people. Therefore, subjects in the present study were tested before and after completion of a 16-wk program of standardized bicycle exercise training to determine whether the hypothesized shift from fast to slow MHC isoform expression would vary with age.

METHODS

Participants. Healthy men and women who exercised <30 min on two or fewer occasions per week during the previous 9 mo were recruited from the local community. Physical activity levels were confirmed by questionnaire (33). Health status was assessed by medical history, physical examination, blood chemistries (complete blood count and comprehensive chemistry panel, including liver enzymes, creatinine, electrolytes, and glucose), urinalysis, and resting electrocardiogram. Exclusion criteria included body mass index >32 kg/m², tobacco use, use of β-blockers, diabetes or other metabolic or endocrine disorders, history of alcohol or substance abuse, and debilitating chronic illness. Thirty-nine women and 38 men between the ages of 21 and 87 yr met these criteria and were enrolled after providing written and oral consent. Consent was obtained before any tests were performed. The Mayo Foundation Institutional Review Board approved the study. Physical characteristics of the men and women in each decade have been published elsewhere (30, 31). A summary version of those data is provided in Table 1, in which the participants were grouped as young (21–37 yr), middle aged (40–56 yr), or older (60–87 yr).

Study protocol. Participants were randomized to either a 16-wk endurance exercise or control program. A similar 5-day protocol was completed at baseline and again within 1 wk of completion of the training or control phases. During each study period, a weight-maintaining diet (55% carbohydrate, 30% fat, and 15% protein) was provided for the first 4 days. On the morning of day 4, subjects were admitted to the General Clinical Research Center (GCRC). The following morning (day 5), muscle biopsy samples from the vastus lateralis (33) were obtained.

The exercise program was performed on a stationary bicycle. Training started with three sessions per week, lasting 20 min each, at an intensity eliciting 70% of the peak heart rate. Peak heart rate during cycling was measured during an aerobic capacity test as described below. Intensity, duration, and number of sessions were gradually increased so that the final month of training consisted of four sessions per week at 80% of maximal heart rate for 40 min. Exercise specialists supervised each session and recorded heart rates. Compliance with the target workloads and number of sessions was >90%. The exercise protocol was completed by 41 of 47 participants originally assigned. The control group was taught a series of flexibility exercises and encouraged to perform them at home while maintaining their regular lifestyle. Follow-up tests were available for 37 of the 43 people in the control group. Subsequently, 24 control group members opted to complete the exercise program, yielding a total of 65 people studied before and after exercise training.

Because the goal of the study was to examine effects of the exercise program, participants were instructed to maintain body weight. Weight was recorded weekly, and the GCRC dietary staff provided further guidance if weight changed >2%. Only one person in the exercise group discontinued the study because of excessive weight loss.

Body composition and aerobic capacity. Fat and fat-free mass were determined by dual-energy X-ray absorptiometry (DPX-L, Lunar, Madison, WI). Thigh muscle area was measured by using a single-slice (6-mm thickness) computed tomography scan (model C-150, Imatron, San Francisco, CA) at the midpoint between the knee and hip. Areas of muscle, fat, and bone were estimated by manual planimetry using custom software (21).

A standard treadmill stress test was performed initially to ensure cardiovascular health and was followed on another day with measurement of peak oxygen uptake (VO₂peak) on a bicycle ergometer (26). The volume and composition of expired gases using breath-by-breath analysis, heart rate by 12-lead electrocardiogram, and blood pressure were continuously monitored throughout the tests (26). The posttraining assessment was made within 3 days of the completion of the last training bout.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Gender</th>
<th>Younger (21–37 yr)</th>
<th>Middle-aged (40–58 yr)</th>
<th>Older (60–87 yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participants</td>
<td>W</td>
<td>10</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>M</td>
<td>12</td>
<td>11</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>W</td>
<td>68.7±1.8</td>
<td>69.1±2.2</td>
<td>69.5±2.0</td>
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<tr>
<td>M</td>
<td>85.7±2.7</td>
<td>90.4±2.8</td>
<td>86.0±2.8</td>
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</tr>
<tr>
<td>BMI, kg/m²</td>
<td>W</td>
<td>24.2±0.5</td>
<td>25.1±0.6</td>
<td>26.3±0.6</td>
</tr>
<tr>
<td>M</td>
<td>25.8±0.6</td>
<td>28.1±0.6</td>
<td>27.6±0.7</td>
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</tr>
<tr>
<td>Body fat, %</td>
<td>W</td>
<td>34.1±1.2</td>
<td>37.2±1.1</td>
<td>39.3±1.1</td>
</tr>
<tr>
<td>M</td>
<td>23.9±1.0</td>
<td>25.8±1.7</td>
<td>27.1±1.3</td>
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</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>W</td>
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<td>39.9±0.9</td>
<td>38.7±0.9</td>
</tr>
<tr>
<td>M</td>
<td>60.4±1.7</td>
<td>60.9±2.6</td>
<td>56.6±1.4</td>
<td></td>
</tr>
<tr>
<td>VO₂peak, l/min</td>
<td>W</td>
<td>1.95±0.05</td>
<td>1.66±0.06</td>
<td>1.26±0.05</td>
</tr>
<tr>
<td>M</td>
<td>3.15±0.11</td>
<td>2.73±0.19</td>
<td>1.96±0.12</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. W, women; M, men; BMI, body mass index; VO₂peak, peak oxygen uptake. Statistically significant differences between men and women were present for each variable. There was not a statistically significant change in BMI with age. Body fat increased (r = 0.40), whereas fat-free mass (r = −0.46), and VO₂peak (r = −0.81) decreased with age, P < 0.01.
Muscle strength. Isokinetic knee extensor strength was measured on a Cybex II dynamometer (Ronkonkoma, NY). Subjects were seated with the hip joint angle between 90 and 100° of flexion and had stabilizing straps over the chest, hips, and thigh. The knee joint was aligned with the axis of the lever arm, and the leg was attached to the lever arm just above the ankle. Each subject was given a familiarization and brief warm-up before testing. Testing was performed at a speed of 180°/s (3.14 rad/s). From a starting point of 90° of knee flexion, subjects were instructed to perform a maximal force voluntary contraction. Isokinetic contractions were performed with each leg separately. The highest torque production recorded from the five attempts using the self-reported dominant leg was used for data analysis. Calibration of the machine was performed before testing by hanging standard weights from the lever arm.

Quantification of MHC mRNA. A real-time quantitative polymerase chain reaction (PCR) system (ABI Prism 7700, PE Biosystems, Foster City, CA) was used to measure the abundance of mRNAs in muscle tissue (3). RNA was extracted from 25 mg of skeletal muscle from individual subjects by TR1zol method (Life Technologies, Gaithersburg, MD), treated with DNase (Life Technologies), and then reverse transcribed using the TaqMan Reverse Transcription Reagents (PE Biosystems). The primer and probe sequences used for human MHC I, MHC IIa, MHC IIx, and 28S ribosomal RNA (used for normalization because it does not change with age in our hands) were previously described (3). Samples were run in triplicate with comiplification of the target gene and 28S rRNA within each well and quantified by normalizing the target signal for the 28S rRNA signal. Finally, for each gene, the average transcript level of the young group was divided by the average transcript level of the old group and multiplied by the expression level of the target gene and 28S rRNA within each well and quantified by normalizing the target signal for the 28S rRNA signal. Finally, for each gene, the average transcript level of the young group was transformed relative to this value and expressed in arbitrary units (AU).

Quantification of MHC protein. The relative protein abundance of MHC isoforms was measured using SDS-PAGE. Muscle samples (150 mg) from each participant were homogenized in 250 mM sucrose, 2 mM EDTA, and 10 mM Tris-EDTA, pH 7.4, and then centrifuged at 1,000 g as previously described (28). The resulting pellet, containing the myofibrillar fraction, was rehomogenized in 176 mM KCl, 10 mM Tris-HCl, and 2 mM EDTA, pH 7.2. After determination of the protein concentration (DC Protein Assay, Bio-Rad, Hercules, CA), an aliquot was adjusted to a concentration of 3 mg/ml and then diluted fourfold in sample loading buffer (66 mM Tris, 19 mM EDTA, 1.05% vol/vol SDS, 0.008% vol/vol bromophenol blue, 810 mM 2-mercaptoethanol, and 40% vol/vol glycerol, pH 6.7).

Samples were heated to 95°C for 4 min, cooled to room temperature, centrifuged briefly, and then loaded on 0.75-mm-thick polyacrylamide gels for protein separation. The discontinuous gel recipe of Talmadge and Roy (32) was used, consisting of an 8% separation gel, a 4% stacker gel, and 30% glycerol in the gel matrix. Samples were run in duplicate with pre- and poststudy samples in adjacent lanes. A semipurified MHC preparation from rabbit muscle (Sigma Chemical, St. Louis, MO) was loaded on each gel as a molecular weight standard and for quality control monitoring. Gels were run for 24 h at 275 V at 5°C using a Hoefer SE600 electrophoresis unit (Amersham Biosciences, Piscataway, NJ). The upper running buffer contained 0.08% vol/vol 2-mercaptoethanol as recommended by Blough et al. (4) for improved separation of MHC isoforms.

Gels were fixed overnight in 50% vol/vol methanol and 10% vol/vol acetic acid. A silver staining protocol was used to visualize protein bands (Fig. 1). The following incubation steps were performed after the gels were washed briefly in deionized water: 1) 5 min in 8 mM Na2S2O3 and then wash three times for 30 s in deionized water; 2) 25 min in 12 mM AgNO3 and then wash three times for 1 min in deionized water; 3) 10 min in 280 mM Na2CO3, 0.16 mM Na2S2O3, and 0.2% vol/vol formaldehyde; and 4) 10 min in 37.2 mM Na2-EDTA and then wash in deionized water. Stained gels were dried between cellophane sheets and digitally imaged on a Kodak Image Station 1000 (Kodak Scientific, Rochester, NY). The relative density of each myosin isoform band was measured using Kodak Image Analysis Software 3.5 (Kodak Scientific) and expressed as the fraction of the total MHC density in each gel lane. Therefore, with this procedure, it is possible to determine whether there is a change in the

Fig. 1. Representative electrophoretic gel separation of myosin heavy chain (MHC) isoforms in human skeletal muscle by SDS-PAGE. Results from duplicate analysis from 2 participants (samples 1 and 2) are shown.
proportional content of each of the three MHC isoforms, but it was not possible to determine whether the total MHC concentration varied with age or exercise training.

**Statistical analysis.** Summary data are reported as means ± SE. Differences between men and women and between pre- and posttesting within groups were analyzed by using unpaired and paired t-tests, respectively. Pearson correlation coefficients were used to measure association among selected variables. Multiple regression analysis was used to determine the contributions of leg muscle size and MHC protein content to variance in knee extensor torque. Covariate adjustment of knee extensor torque for muscle cross-sectional area was performed (34) so that the residual effect of age could be graphically represented. A P value < 0.05 was considered statistically significant for all tests.

**RESULTS**

Subject characteristics at baseline are shown in Table 1. The data are summarized into three age groups to simplify the presentation, but analysis of age effects was performed by linear regression. As expected, there were significant differences between men and women for most variables. With the exception of body weight and body mass index, there were significant age-related changes in body composition, reflecting increasing adiposity and decreasing fat-free mass. The peak aerobic capacity (VO₂ peak) declined with age by 8% per decade in both men and women.

There were no statistically significant changes in the physiological characteristics of the control group between measurements at baseline and 16 wk later. In exercisers, there were small but statistically significant (P < 0.001) reductions in body weight (0.6 ± 0.2 kg) and body mass index (0.2 ± 0.1 kg/m²) but no change in dual-energy X-ray absorptiometry-determined body fat or fat-free mass. VO₂ peak during cycling improved by 9.5% on average (P < 0.001). The magnitude of posttraining changes in body mass, body mass index, and maximal oxygen uptake in the exercise participants was not statistically different between men and women and did not vary with age of the participant. These physiological characteristic data have been presented previously (30, 31).

Peak isokinetic knee extensor torque was on average 48% higher in men than women (P < 0.001) and declined with age at a rate of 13% per decade in men and 8% per decade in women (Fig. 2A). Cross-sectional area of the midthigh muscle was on average 32% higher in men than women (P < 0.001) and declined 5% per decade in men and 4% per decade in women (Fig. 2B). Peak torque was strongly associated with thigh muscle area in men and women combined (r = 0.88, P < 0.001). After covariate adjustment of peak torque for differences in muscle area, differences in knee extensor torque between men and women were eliminated, but there was still a significant decline in peak torque with age of 5% per decade (Fig. 2C). In the control group, there was no change from baseline to follow-up in muscle size or strength (data not shown). The exercise group had an average 6% increase in peak isokinetic knee extensor torque (75.7 ± 4.8 N·m pretraining, 78.5 ± 5.2 N·m posttraining; P < 0.01) after training, but the change was similar in young (7%), middle-aged, (4%), and older (6%) groups. Leg muscle area did not change significantly in response to training in the exercise group (overall average, 129 ± 5 cm² pretraining, 133 ± 4 cm² posttraining; P = 0.17).

Values for muscle MHC isoform mRNA and protein content at baseline are shown in Fig. 3. At the mRNA level, MHC I abundance did not vary with age, whereas both MHC IIA and IIX transcripts declined by 14 and 10% per decade, respectively. At the protein level, the relative content of MHC I increased with age by 3.5% per decade, whereas there was a decline of 3% per decade for MHC IIA and 1% per decade for MHC IIX. There were no differences between men and women in average MHC mRNA abundance or fractional protein content nor any significant differences in the changes in MHC expression with age.

The association between the fractional MHC protein content and peak knee extensor torque is shown in Fig. 4. Peak torque was negatively related to the fraction of MHC I protein and positively related to the fraction of MHC IIA protein. After covariate adjustment of peak torque values for differences in thigh muscle cross-sectional area, however, the fraction of MHC protein isoforms was no longer significantly correlated with peak torque (data not shown). Multivariate regression confirmed that MHC protein did not make a significant contribution to the explanation of the variance in knee extensor torque once thigh muscle area was entered into the model.

Fig. 3. Age effect on expression of MHC mRNA and protein isoforms. The relative abundance of mRNA transcripts for each isoform (left) are shown in arbitrary units (AU) after normalization for 28S rRNA. Protein expression of each isoform (right) is shown as the fraction of each isoform relative to the total MHC in each gel lane. There were no statistical differences in expression between women and men, so a single regression line is shown in each panel except for MHC I mRNA, where there was not a statistically significant association with age.
There were no statistically significant changes in either MHC mRNA or protein expression in the control group. In the exercise group, there were changes in both MHC mRNA and protein (Fig. 5). On average, mRNA abundance was increased by 63% for MHC I (1.07 ± 0.09 AU pretraining, 1.74 ± 0.15 AU posttraining), increased 99% for MHC IIa (0.72 ± 0.08 AU pretraining, 1.43 ± 0.14 AU posttraining), and decreased 50% for MHC IIx (0.78 ± 0.09 AU pretraining, 0.39 ± 0.05 AU posttraining) (all \( P < 0.001 \)). The change in MHC I mRNA abundance showed a modest positive correlation with age (Fig. 5), but changes in MHC IIa and IIx mRNA did not vary with age. Changes in MHC protein content were smaller than the mRNA responses. On average, the fractional content of MHC I protein increased by 6% (0.37 ± 0.02 pretraining, 0.43 ± 0.02 postraining; \( P = 0.004 \)), whereas MHC IIx protein decreased by 5% (0.26 ± 0.01 pretraining, 0.21 ± 0.02 postraining; \( P = 0.001 \)), and MHC IIa protein was not significantly changed (0.37 ± 0.02 pretraining, 0.36 ± 0.02 postraining; \( P = 0.39 \)). The change in MHC I protein was inversely correlated with age, whereas the change in MHC IIx protein was positively correlated with age, as shown in Fig. 5. There were no differences between men and women in the exercise effects on MHC mRNA and protein. Therefore, exercise-induced changes in MHC mRNA and protein were pooled by age group and are shown in Fig. 6. All of the changes in mRNA were statistically significant within the young, middle-aged, and older groups, but statistically significant changes in protein were only present in the young group (increase in MHC I, decrease in MHC IIx) and the middle-aged group (decrease in MHC IIx).

**DISCUSSION**

The major findings from the present study were the age- and exercise-related changes in expression of MHC isoform mRNA and protein in human skeletal muscle. In a relatively large group of healthy men and women, we found that both MHC mRNA and protein expression of MHC II isoforms declined with age, in support of our hypothesis. In response to a 4-mo program of moderate-intensity endurance exercise training, MHC I and IIa mRNA increased and IIx decreased, shifting transcript expression from fast to slow isoforms in all age groups. However, a training effect on MHC isoform protein expression in this same direction was detected in younger people, but there was no change in the composition of MHC isoform protein in older people. This suggests that some adaptations to exercise remain robust in older muscles, i.e.,...
gene expression at the mRNA level, whereas other adaptations may be either prevented or delayed, i.e., alteration in the expression of specific proteins.

Changes in MHC expression with age. The present data demonstrate that with age there is a decrease in the fractional content of MHC IIa and IIX protein in human leg muscle and a corresponding increase in the proportion of MHC I. This finding is consistent with previous reports that the cross-sectional area occupied by histochemically typed fast-twitch fibers is reduced from older people (6, 11, 15, 18, 22, 27). Those studies also suggested that the decline in fast-twitch fiber area is primarily due to a selective reduction in the size of type II fibers because most studies report that the percentage of each fiber type appears not to change significantly with age (6, 11, 15, 18, 22, 27). We are aware of only two other reports in which the effect of age on MHC protein expression in human muscle has been presented, but those results of those studies contradicted one another.

In agreement with our results, Klitgaard et al. (15) found that the fraction of type II MHC was reduced and MHC I was increased in vastus lateralis biopsies obtained from 8 older (68 yr old) compared with 7 young (28 yr old) untrained men. A similar, but nonsignificant, trend was observed in the biceps brachii of these same men. In contrast, Marx et al. (24) studied a larger number (n = 16 per group) of younger (22 yr old) and older (74 yr old) untrained men, but they did not detect any differences in MHC protein isoforms. Other studies have examined the MHC isoform expression within single muscle fibers and found an increase with age in the number of fibers that coexpress multiple isoforms (1, 16, 20). Such information is useful to understand how MHC expression is controlled within single fibers, but the technical demands of such work have meant that most of these studies have analyzed muscle samples from a small number of people. A strength of the present investigation was the inclusion of a large number of subjects (n = 77) across most of the adult life span, thus demonstrating that the effect of age on MHC expression is a continuous process after linear growth potential is achieved.

The present study provided some indication that age-related changes in muscle fiber type are controlled at the pretranscriptional level. We found that the abundance of mRNA transcripts for MHC IIa and IIX declined with age, whereas MHC I mRNA content was unchanged. This selective alteration in transcript availability for protein synthesis may be one means through which altered protein expression pattern is regulated. These alterations in MHC isoform mRNAs represent either an altered rate of gene transcription or altered stability of the transcripts. The global fractional synthetic rate of MHC protein (a measure of translational rate) has been shown to decline with age in human muscle (2, 10). Further work is needed to determine whether translational regulation of specific MHC isoform transcripts is affected by aging because such mechanisms could also contribute to protein expression pattern. The changes in MHC mRNA we observed are in agreement with a previous report from our laboratory in which muscle biopsies of the vastus lateralis from young (−25 yr old, n = 7), middle-aged (~50 yr old, n = 12), and older (~71 yr old, n = 14) men and women were analyzed by using the same real-time PCR method used in the present study (3). In contrast, in studies by Marx et al. (24) and Welle et al. (36, 37), there were no detectable differences in abundance of mRNA of MHC I, IIa, or IIX in comparisons between younger and older people. The reason for the discrepant findings among these studies is not apparent because it appears that healthy, non-exercise-trained subjects were examined in all of these studies and that biopsy samples were obtained from the vastus lateralis muscle after 2–3 days of diet and physical activity control.

Changes in MHC expression in response to endurance exercise. In response to the endurance exercise program, there was a general shift from fast to slow MHC isoform expression. The increase in MHC I mRNA was modestly higher in older people compared with younger people, whereas the increase in MHC IIa mRNA and decrease in MHC IIX mRNA were similar in young, middle-aged, and older people. The intriguing finding was that changes in MHC protein were age dependent, with a significant increase in MHC I and decrease in MHC IIX in younger people, whereas there were no significant differences in the older group, and intermediate changes occurred in middle-aged people. There are two plausible explanations for these results. First, older muscles at baseline already had a smaller fraction of MHC IIX protein compared with young, so there is a smaller margin for further suppression in response to training. Thus a greater stimulus, in the form of a more vigorous or longer training program, may be required to cause a detectable shift in the MHC isoform protein composition in older people. Second, older muscles may be slower to respond to the exercise stimulus at the level of protein expression. The synthesis rate of MHC protein declines with age in untrained people (2, 10), so it may take longer to upregulate translational machinery and produce a measurable change in concentration of specific proteins in older muscles. It was previously shown that synthesis rate of total MHC protein is increased in response to a
12-wk resistance training program in older people (2), but MHC isoform distribution was not measured, so it is unclear whether there is selective translational regulation of specific isoforms. Our laboratory has reported elsewhere that the subjects in the present study responded to the endurance training with increased mRNA abundance for genes encoding mitochondrial proteins, nuclear transcription factors, and glucose transporter 4, increased activity of mitochondrial enzymes, and increased synthesis rate of mixed (total) muscle proteins, all of which were changed similarly in younger and older people (30, 31). A notable exception was that insulin sensitivity was increased in young but not older people (31), so, despite evidence that many of the adaptive processes remain intact in older people, there are some variables that may take a relatively longer period to respond to the exercise program. Further studies on the time course of adaptations are needed to address this issue.

To our knowledge, this is the first study to directly compare the effects of endurance exercise training on MHC expression in younger and older people. In fact, there is limited information available on the effects of exercise training on MHC isoform expression in human muscle, especially at the mRNA level. In a cross-sectional comparison between sedentary and running-trained older men (n = 8 and 5 per group, respectively), there were no differences in fractional MHC isoform protein composition, although the runners did have a higher percentage of type I fibers by histochemical analysis (15). Another study showed that, in response to a short-term (1 h per day for 7 consecutive days) bicycle training program, MHC IIx mRNA abundance decreased in the muscles of young men, but there were no changes in either MHC I or IIa mRNA (25). Considering that we also observed a decline in MHC IIx mRNA in addition to increases in MHC I and IIa mRNA after 16 wk of training, it is possible that the time course of transcriptional regulation varies among individual MHC isoforms. In agreement with our results, other studies that used the histochemical fiber-typing approach found a decrease in the percentage of fibers expressing type II myosin ATPase and a corresponding increase in type I fibers in a comparison of sedentary vs. chronically endurance-trained men (27), after a 6-wk bicycle training program in young men (12), and after a 9- to 12-mo walking and jogging program in older men and women (7). The finding by Coggan et al. (7) that fiber type was changed after a longer training program than used in the present study further supports the possibility that some adaptations to exercise may be slower in older people.

**Relation of MHC expression and muscle strength.** Thigh muscle cross-sectional area and peak isokinetic knee extensor torque declined with age in both men and women, in agreement with previous observations (13–15, 19, 23). Muscle size is clearly a major determinant of muscle strength, but the decline in knee extensor torque with age persisted after covariate adjustment for thigh muscle cross-sectional area, an index of specific force. This indication that muscle quality declines with age is consistent with previous reports in which knee extensor torque was normalized for thigh muscle size in younger and older people (14, 15, 23). We assessed whether the age-related shift in MHC expression contributed to the decline in muscle quality because MHC isoforms are the major determinant of contractile speed, force, and energy cost as reported in single muscle fibers (5, 9). In multiple regression analysis, Larsson and Karlsson (19) found that the percent and the proportional cross-sectional area occupied by type II fibers were significant determinants of muscle strength and endurance. In the present study, although the fractional content of MHC I and IIA proteins was modestly correlated with knee extensor peak torque, this relationship was eliminated after adjusting peak torque for differences in muscle size. Klitgaard and colleagues (15) also did not find a significant correlation between MHC isoforms and specific force in young and old men, although MHC protein isoform composition was related to muscle speed of movement. Similarly, Jubrias et al. (14) did not detect an association between MHC protein isoforms and specific force, but they examined a smaller age range, from 65 to 80 yr. Thus, the decline in muscle-specific force with age appears to be related to changes in properties other than MHC expression. Contractile studies of single muscle fibers from older people have demonstrated reduced speed and force characteristics compared with young controls that persist after controlling for the smaller size of older fibers (8, 17, 20), although a more recent study did not reach this same conclusion (35).

In conclusion, the present study demonstrates that, in unhealthy untrained men and women, there is a shift with age in the fraction of MHC protein from fast (decrease in MHC I and IIx) to slow (increase in MHC I) isoforms. The mRNA abundance of MHC isoforms shifts with age in the same direction, suggesting a role for transcriptional regulation of fiber type. Although there is an association between fractional MHC protein expression and isokinetic knee extensor strength, MHC protein isoforms do not explain the decline in specific force with age. We also found that, in response to a standardized 4-mo program of moderate-intensity endurance exercise, MHC isoform mRNA profile shifted in favor of fast to slow expression in younger and older people but that this change was evident at the protein-expression level in younger but not older people. These findings suggest that older muscles may require a longer period in translating adaptive responses at the gene-transcript level into changes in the expression of MHC protein isoforms. This may represent reduced translational rate (fractional synthesis rate) in response to stimuli such as endurance exercise.

**ACKNOWLEDGMENTS**

The authors are grateful for the valuable technical support provided by Becca Kurup, Jane Kahl, and Dawn Morse. We also thank the GCRC nurses, dieticians, and support staff, and the staff of the Healthy Living Center for help with completion of the studies and training sessions, as well as the volunteers who completed the protocol.

**GRANTS**

This study was funded by National Institutes of Health Grants MO1-RR-00585 (for the Mayo Clinic General Clinical Research Center) and ROI-AG-09531 (to K. S. Nair). Additional support was provided by the Mayo Foundation and the Dole–Murdoch Professorship (to K. S. Nair) and by National Research Service Award T32-DK-07352 and the Mayo-Thompson Fellowship (to K. R. Short).

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