Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise

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Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise. Am J Physiol Endocrinol Metab 280: E203–E208, 2001.—Experimental evidence indicates that a lower synthesis rate of muscle contractile protein myosin heavy chain (MHC) occurs in age-related muscle wasting and weakness. To determine the molecular mechanism of this lower synthesis of MHC, we measured transcript levels of isoforms of MHC (MHC-I, MHC-IIa, and MHC-IIx) in muscle biopsy samples of 7 young (20–27 yr), 12 middle-aged (47–60 yr), and 14 older (>65 yr) people. We further determined the effect of 3 mo of resistance exercise training (exercise) vs. nonintervention (control) on transcript levels of MHC isoforms on these subjects and the fractional synthesis rate (FSR) of MHC in 39 people aged 46–79 yr. MHC-I mRNA levels did not significantly change with age, but MHC-IIa decreased 38% (P < 0.05) from young to middle age and further decreased 50% (P < 0.05) from middle to old age. MHC-IIx decreased 84% (P < 0.05) from young to middle age and 48% from middle to old age (P < 0.05). Exercise increased FSR of MHC by 47% (P < 0.01) and mixed muscle protein by 56% (P < 0.05). Exercise training results in an increase (85%) in transcript levels of MHCIIa and a decrease in the transcript levels of MHCIIa and MHCIIx. In conclusion, an age-related lowering of the transcript levels of MHCIIa and MHCIIx is not reversed by exercise, whereas exercise results in a higher synthesis rate of MHC in association with an increase in MHC isoform transcript levels.

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LIFE EXPECTANCY has dramatically changed during this century, resulting in an increase of the elderly population in our society (38). Muscle wasting and associated impairment of muscle functions and consequent disabilities are rapidly becoming a major public health problem (9, 10). Because muscle is a major metabolic organ, especially in disposing of orally administered carbohydrates, decreased muscle mass can contribute to the reduced glucose disposal that is observed in elderly people (12). Decreased muscle mass and muscle function can also cause a decrease in energy expenditure, which in turn leads to obesity and insulin resistance (19, 21). The mechanism of this age-related muscle change remains to be clearly defined. Several studies have reported that synthetic rates of mixed muscle proteins and myosin heavy chain (MHC), the main protein in muscle contractile apparatus, and of mitochondrial protein decrease with age (5, 30, 36, 39). The synthesis rate of MHC was correlated to muscle strength, suggesting that muscle strength is related to the ability to synthesize MHC (39). The current study sought to determine whether the decrease in MHC synthesis occurred because of changes at the transcriptional or posttranscriptional level. To accomplish this, we measured the steady-state transcript levels of MHC isoforms (MHC-I, MHC-IIa, and MHC-IIx) in skeletal muscle biopsy samples obtained from young, middle-aged, and older people. In addition, we determined the effect of 3 mo of resistance exercise on MHC synthesis rate and the transcript levels of MHC isoforms.

METHODS

Subjects

We studied 39 subjects, 19 of middle age (46–60 yr, 52 ± 0.8 yr) and 20 older (65–79 yr, 71 ± 1 yr). Because of limited sample availability, 26 of these subjects (12 of middle age and 14 older) were used for the measurement of MHC isoform transcript levels. Seven young subjects (20–27 yr, 24 ± 1 yr) were also added to determine the effect of age on MHC transcript levels. Some aspects of the study, including age-related changes in mitochondrial protein (30) and MHC synthesis (5), were previously reported. The body mass index (kg/m²) values of the young (23 ± 1), middle-aged (25.4 ± 0.8), and older subjects (25.8 ± 0.8) were similar. The muscle mass, based on urinary creatinine output, was lower in the middle-aged (26.9 ± 1.7 kg; P < 0.05) and older (23 ± 1.4 kg; P < 0.01) than in the young subjects (30.6 ± 1.8 kg). All subjects were determined to be healthy on the basis of physical examination and blood tests. Subjects who exercised regularly for ≥2 days/wk and women taking hormone replacement were excluded from the study.

Study Protocol

The protocol was approved by the Institutional Review Boards of the University of Vermont and Mayo Clinic and...
Foundation. Informed consent was obtained from the volunteers before the study. The first part of the study was to determine the effect of age on transcript levels of MHC isoforms. The second part of the study was to determine whether 3 mo of resistance exercise stimulates MHC synthesis rate and transcript levels of MHC isoforms in the middle-aged and older people (>46 years), who are reported to have decreased synthesis rate of MHC (5). In the first part of the study, we compared the results of MHC isoform transcripts obtained in the 7 young subjects with those obtained in the 12 middle-aged and 14 older subjects. In the second part of the study, we randomly assigned the 39 middle-aged and older people (46–79 yr) to either a resistance exercise program or a nonintervention (control) program for 3 mo. Muscle strength, fractional synthesis rate (FSR) of MHC, and mRNA levels of isoforms of MHC were measured at baseline and at the end of 3 mo.

The baseline studies and the study at the end of 3 mo were performed in an identical manner. All subjects were given a weight-maintaining diet (protein-fat-carbohydrate = 15:30:55) for 5 days before the study at the General Clinical Research Center. Subjects followed the diet for 2 days as outpatients and were admitted as inpatients on the evening of day 2. Twenty-four-hour urine samples were collected on days 4, 5, and 6 to determine the average 24-h urinary creatinine excretion (18). Muscle strength tests were performed on either day 1 or day 2. The muscle protein synthesis tests and biopsies for mRNA measurements were performed on day 6.

Exercise Training Program

Subjects exercised three times weekly and completed three sets of eight repetitions of the following exercises with Universal Gym apparatus: squat, bench press, lateral pull-down, leg extension, leg curl, arm extension, and arm curl. The subjects were instructed to raise the weight for over 2 s and to resist the lowering of the weight over 4 s. Initially, the resistances were set at a level that represented 50% of the subject’s one repetition maximum strength (1RM). The weight lifted was increased on a progressive basis until by the start of the 9th wk the subjects were exercising at ~80% of their current 1RM. 1RM strengths were reassessed during the 4th, 7th, and 10th wk of the training session. All exercises were completed under the supervision of a qualified exercise physiologist.

Measurement of Muscle Strength

1RM represents the maximum amount of weight that a subject can lift once (13). After a warm-up and instruction regarding proper techniques for completing the movement, subjects were asked to lift progressively heavier weights until they reached a weight they could not lift. The selection of weights and the rate of increase were chosen to ensure that the maximum capacity of the subject was reached in four to six lifts. 1RM tests were performed in this fashion for bench press and leg extension.

Muscle Mass from Urinary Creatinine Output

During the last 3 days (days 4, 5, and 6) of a meat-free diet, muscle mass was computed on the basis of a ratio of 20 kg muscle mass ⋅ g urinary creatinine ⋅ day −1 (18). The approach is based on the fact that creatinine is derived almost exclusively from creatinine phosphate in muscle.

Isotope Infusions and Muscle Biopsy Studies

The study was performed after an overnight fast on the 6th day. The last meals of the day were given at 10 PM on all 5 days to acustom subjects to the meal regimen and to minimize the overnight fast on the study day. An intravenous line for infusion was inserted before 10 PM on day 5, and the line was kept patent by saline (0.9 N) infusion. At 1:30 AM, the infusion was changed to isotope administration, which consisted of a primed (7.5 μmol·kg −1 ·h −1) continuous infusion of t-[1-13C]leucine (7.5 μmol·kg −1 ·h −1), as previously described (27), for 10 h. Muscle biopsies were performed from the vastus lateralis under local anesthesias with a percutaneous needle, as previously described (27), at 5 h (6 AM) and 10 h (11 AM) (27). Muscle samples (150 mg for MHC, 50 mg each for mRNA and mixed muscle protein (MMP)) were immediately frozen in liquid nitrogen and kept at −80°C until analysis.

Measurement of Muscle Protein Synthesis

MMP in the biopsy sample (25–50 mg) was separated and hydrolyzed as described previously (5, 7, 28). MHC purification was performed using 100- to 150-mg muscle samples as previously described (2–4). Both MHC and MMP were hydrolyzed, and amino acids were purified as previously described (2, 28). Isotopic enrichment in leucine was measured as previously described (2). Muscle tissue fluid leucine enrichment was also measured as previously described (25).

FSR of Muscle Protein

FSR of MHC and MMP was calculated using the following equation (14, 27)

\[
FSR = \frac{E_t - E_i}{E_p} \times \frac{t}{2} \times 100
\]

where \(E_t\) and \(E_i\) represent the enrichments as atom percent excess of 13C derived from decarboxylation of leucine from MHC and MMP at the final and initial muscle biopsies. \(E_p\), the precursor pool (plasma α-ketosacaproic acid) enrichment, and \(t\) represents the time interval (5 h) between initial and final biopsies in hours.

RNA Isolation and cDNA Synthesis

Total RNA was extracted from skeletal muscle tissue (~10 mg) by the guanidium method (TrizReagent, Molecular Research Center, Cincinnati, OH). One microgram of total RNA was treated with DNase (Life Technologies, Gaithersburg, MD) and then reverse transcribed using the TaqMan Reverse Transcription Reagents (PE Biosystems, Foster City, CA) according to the manufacturer’s instruction.

Real-Time PCR

Primers and probes were selected using the Primer Express software (PE Biosystems) and screened for mispriming of other MHC isoforms by use of the Oligo Primer Analysis Software v.5.0 (National Biosciences, Plymouth, MN). The internal probe was labeled at the 5′ end with a reporter dye FAM (6′-carboxyfluorescein) and at the 3′ end with a quencher dye TAMRA (6′-carboxytetramethyl-rhodamine) and was phosphate-blocked at the 3′ end to prevent extension. The 28S probe was constructed identically, with the exception that the 5′ end of the probe was labeled with a fluorescent dye (PE Biosystems). The reporter and quencher dyes are in close proximity on the probe, resulting in suppression of reporter fluorescence by Forster energy transfer (24). The probe hybridizes to a specific sequence within the
PCD product. The 5′-to-3′ exonuclease activity of the Taq DNA polymerase allows for separation of the reporter from the close proximity of the quencher dye, resulting in fluorescence of the reporter dye (17). The resulting fluorescence was measured at each cycle of amplification on the ABI Sequence Detection System (PE Biosystems). The primers were not labeled.

The following primer and probe sequences were used. MHCI (GeneBank Accession no. M21665) forward primer: AAGGTCAAGGCTCACAAGC; reverse primer: CGGAACCTTGGACAGTTTGGG; probe: TGGCTTGCTCCTCCGGCTCCT. MHCIIa (GeneBank Accession no. AF111784) forward primer: CAATTCTAGCTAAATTCCGGAAG; reverse primer: TCACCTATGACTTTTGTTGGAACCT; probe: TTCACCCGAGTTTGTTGACCTGGA. MHCIIx (GeneBank Accession no. AF111785) forward primer: GAGGAGCAATTCACACGTCA; reverse primer: TGACTGGACTGCAATG; probe: CAAATTCGAGGATCCAGCAGGA. 28S (GeneBank Accession no. AF111784) forward primer: GGAGGAACAATCCAACGTCAA; reverse primer: CCTTACGGTACTTGTTGAC; probe: TTCACCCGAGTTTGTTGACCTGGA. MHCIIx (GeneBank Accession no. M11167) forward primer: TGGGAATGCAGCGCTAATTCCGGAGGATCCAGCACGA. 28S (GeneBank Accession no. AF111785) forward primer: GGAGGAACAATCCAACGTCAA; reverse primer: CGGAACTTGTACGCTAAATTCCGGCAAGC; reverse primer: CCATTCTAGCTAAATTCCGGAAG; reverse primer: CCTTACGGTACTTGTTGACCTGGA. MHCIIa (GeneBank Accession no. AF111784) forward primer: TGGGAATGCAGCGCTAATTCCGGAGGATCCAGCACGA. 28S (GeneBank Accession no. AF111785) forward primer: GGAGGAACAATCCAACGTCAA; reverse primer: CGGAACTTGTACGCTAAATTCCGGCAAGC; reverse primer: CCTTACGGTACTTGTTGACCTGGA. MHCIIx (GeneBank Accession no. M11167) forward primer: TGGGAATGCAGCGCTAATTCCGGAGGATCCAGCACGA. 28S (GeneBank Accession no. AF111785) forward primer: GGAGGAACAATCCAACGTCAA; reverse primer: CGGAACTTGTACGCTAAATTCCGGCAAGC; reverse primer: CCTTACGGTACTTGTTGACCTGGA.

We applied this highly sensitive and reproducible real-time PCR method to quantify MHC mRNA. The signal for 28S ribosomal RNA was used to normalize against differences in RNA isolation and RNA degradation and in the efficiencies of the reverse transcription and PCR reactions. All samples were run in triplicate and quantitated by normalizing the MHC signal with the 28S signal. The final quantitation was achieved by a relative standard curve (29).

Statistics

ANOVA was used to detect differences among the three age groups in the mRNA levels of MHC isoforms and muscle mass. When a difference (age effect) was observed, unpaired \( t \)-tests were performed to determine the differences between the specific groups. Multivariate repeated-measures ANOVA with two response variables (exercise and age) was applied for determining training effect. In addition, we determined whether the different pre- and postexercise parameters between middle and older age people are significant by use of ANOVA.

RESULTS

Part I: Age Effect

We measured the transcript levels of MHC isoforms. Figure 1 shows mRNA levels of MHCI, MHCIIa, and MHCIIx in young (20–27 yr), middle-aged (47–54 yr), and older (64–79 yr) people. As noted in Fig. 1, a significant age effect was evident for MHCIIa and MHCIIx. For both MHCIIa and MHCIIx, there was progressive decrease from young to middle and older age groups.

Part II: Exercise Effect

Muscle strength. There was an exercise effect on muscle strength (\( P < 0.001 \)). Whereas in the exercise group leg extension (91 ± 8 lbs to 125 ± 11, \( P < 0.01 \)), leg curl (35 ± 4 lbs to 54 ± 6, \( P < 0.01 \)), and bench press (75 ± 7 lbs to 91 ± 8, \( P < 0.01 \)) showed increased strength, no significant changes in leg extension (68 ± 8 lbs to 70 ± 8), leg curl (24 ± 4 lbs to 25 ± 3), and bench press (60 ± 6 lbs to 63 ± 7) occurred in the control group. There was no interaction between age and exercise effects.

FSR. Figure 2 shows the FSR of MHC and MMP, demonstrating an exercise effect (\( P < 0.01 \)). The control group showed no change in FSR of either MHC or MMP after exercise. In contrast, in the exercise group there was an increase in FSR of MHC (average 47%: \( P < 0.01 \)) and MMP (56%; \( P < 0.05 \)). There was no age effect on any of the responses to exercise.

Transcript levels of MHC isoforms. Figure 3 shows the exercise effect on the mRNA levels of MHC isoforms in middle-aged and older subjects. MHCI mRNA levels significantly increased after 3 mo of exercise. MHCIIa and MHCIIx mRNA levels decreased significantly postexercise. The control group showed no change in transcript levels of MHCI, MHCIIa, and MHCIIx.

DISCUSSION

The current study demonstrated for the first time that, in humans, there is generalized age-related low-
ing of transcript levels of MHC isoforms, with significant changes occurring only for MHCIIa and MHCIIx. A 3-mo resistance exercise program increased muscle strength and stimulated synthesis rate of MHC. The resistance exercise increased mRNA levels of MHCI and further decreased those of MHCIIa and MHCIIx.

**Effect of Age on MHC Isoform Transcript Levels**

The mechanism of senescence remains to be clearly defined. One of the leading hypotheses is that senescence results from a progressive accumulation of damage to DNA and tissues caused by free oxygen radicals (15). In support of this hypothesis, it has been observed that there are age-related deletions and mutations of mitochondrial DNA (26, 32), presumably due to the constant exposure to free oxygen radicals generated in mitochondria during electron chain transport. Studies in rodents demonstrated that a decrease in mitochondrial DNA copy numbers occurs with age (6), a reflection of possible cumulative DNA damage. It is believed that damage to mitochondrial DNA results in a decrease in mitochondrial protein synthesis (30). It is unclear whether similar age-related changes in nuclear DNA also occur. The results from the current study demonstrate that the transcript levels of muscle proteins encoded by the nuclear DNA are also diminished with age. These results are in contrast to the hypothesis that the decrease in synthesis rate of MHC (5) occurs because of posttranscriptional changes (34). The current study demonstrates a substantial reduction in transcript levels of MHCIIa and MHCIIx in the older people compared with the younger people. Even MHCI tends to decrease with age, although this decrease did not reach statistical significance. There is certainly an overall reduction in transcript levels of MHC isoforms. These results suggest that the decreased synthesis rate of MHC in older people (5) occurs because of decreased transcript availability of the adult isoforms of MHC. It is therefore possible that the decreased protein synthesis with age is due either to defects at the transcription level or to DNA damage. However, direct evidence for this will be forthcoming only if synthetic rates of isoforms of MHC are measured and the precise level of defect (DNA or transcription or both) has been demonstrated. Currently, such measurements are not feasible by use of needle biopsy samples.

It has previously been demonstrated that there is a decrease in the total number of muscle fibers in older people and that the relative decrease of type II fiber size is more pronounced than that of type I fibers in the elderly (23). Lexell (22), on the basis of an extensive review of publications in this area, concluded that the age-related reduction in fiber number of vastus lateralis muscle affects both types of fibers, although type I fibers are relatively higher in number. It is now well known that the fiber classification based on myosin ATPase histochemistry depends on the differences in myosin ATPase activity of various isoforms of MHC (11, 31, 33). Human muscle fibers are composed of MHCI, MHCIIa, and MHCIIb (8) in different stoichiometric ratios. Because the dominant MHC isoforms determine fiber type on the basis of the ATPase staining technique, the fiber classification is not sufficiently sensitive to demonstrate the changes in muscle MHC isoforms in skeletal muscle, especially in elderly people (1). Analysis of vastus lateralis muscle samples from the elderly subjects demonstrated that younger subjects had a higher proportion of fibers containing only MHCI and MHCIIa than the older subjects (20). The current study demonstrated that the underlying molecular mechanism of this was the lower transcript levels of these two isoforms of MHC in older than in younger people. The maximal difference was noted for
MHCIIx transcript levels, which may explain a substantial reduction in fiber type IIb in the older people (23). There are many potential functional changes that could occur with the changes in the composition of MHC isoforms that occur with aging. The lower levels of MHCIIa and MHCIIx that are noted with aging could be the cause of lower fast-twitch muscle fibers in the elderly people.

Effect of Exercise on Age-Induced Changes in Transcript Levels, MHC Isoforms, and MHC Synthesis Rate

The current study demonstrated that resistance exercise stimulated MHC synthesis rate in the middle-aged and older subjects. These results are consistent with the demonstration of increased synthesis rate of MMP reported previously in the elderly people after a 2-wk resistance training program (39). Another study failed to see this stimulatory effect on myofibrillar protein synthesis rate in the elderly people after 3 mo of resistance exercise (37). A recent study by the same group found a stimulation of myofibrillar protein synthesis on 3 days of exercise over a week, but this was not associated with any changes in gene transcript concentrations (35). Recently, another study (16) demonstrated that a 2-wk resistance training program increases MHC synthesis rate. The current study demonstrated that this increased MHC synthesis persists even after 3 mo. In addition, the current study demonstrated that the observed changes are not simply time-related changes, because there were no changes in MHC synthesis rate in the placebo group after 3 mo of nonintervention. As we previously noted (5), muscle strength may be related to the ability to synthesize MHC. The current study demonstrated that this increased MHC synthesis occurs by increasing transcript levels of MHCIIa and MHCIIx that occur with aging cannot be reversed by resistance exercise alone. Two previous reports represent average measures of synthesis rates of several muscle proteins (35, 39). The present study also demonstrated that resistance training-induced increase in MHC synthesis occurs primarily by increasing the synthesis rate of MHC. We originally hypothesized that the age-related changes in muscle protein synthesis might be due to changes in lifestyle (5). A resistance exercise program increased the synthesis rate of MHC in the middle-aged and older people, but this increase only made them equal to the sedentary young subjects (5). The current study also demonstrated that an exercise program does not reverse all the changes occurring at a transcriptional level with aging. It remains to be determined whether resistance training in the older people decreases synthesis rates of the isoforms MHCIIa and MHCIIx.

In summary, the current study demonstrated that the transcript levels of MHCIIa and MHCIIx mRNA become lower progressively with age. These observations may explain the molecular basis of metabolic and functional changes that occur in human muscle with advancing age. Although a 3-mo resistance-training program increased muscle strength and synthesis rate of MHC, it did not reverse the decrease in transcript levels of MHCIIa and MHCIIx that we observed in the middle-aged and older subjects. However, resistance training increased MHCI transcript levels and may explain why older people have relatively higher amounts of type I fibers in their skeletal muscle.

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