Changes in myosin heavy chain composition with heavy resistance training in 60- to 75-year-old men and women

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Abstract  The purpose of this investigation was to assess the myosin heavy chain (MHC) expression in the vastus lateralis muscle from elderly men and women, and to determine whether heavy resistance training influences its expression. Twenty healthy, mildly physically active subjects gave their informed consent to participate in the study. The experimental group consisted of seven men and seven women [mean (SD) age 65.5 (4.1) years] and the control group consisted of three men and three women [mean (SD) age 62.3 (3.6) years]. The 6-month resistance training program was divided into two phases with weeks 1–12 consisting of high-intensity resistance training, and weeks 13–24 involving power training. Muscle biopsy samples were taken from the vastus lateralis muscle at week 0 and week 24 using the needle biopsy technique. The male and female experimental groups both exhibited a significant decrease ($P \leq 0.05$) in the percentage of MHC IIb, while the experimental female group also demonstrated a significant increase ($P \leq 0.05$) in the expression of MHC Ila, after 24 weeks of heavy resistance training. There was no change in MHC expression within the control group. The male [130.4 (25.3) kg vs 171.1 (30.5) kg] and female [58.2 (8.3) kg vs 77.9 (11.1) kg] experimental groups exhibited a significant increase ($P \leq 0.05$) in the maximal strength values for the 1 repetition maximum (1RM) squat exercise. The control group showed no change in strength for the 1RM squat exercise for either the male [115.8 (35.10 kg vs 123.8 (47.2) kg] or female [57.5 (99.0) kg vs 58.3 (2.9) kg] groups. The results clearly show that elderly subjects undergoing heavy resistance training have the ability to produce a similar shift in the expression of MHC isoforms from MHC IIb to MHC IIa, as has been shown to occur in younger subjects. This highlights the plasticity of human skeletal muscle in response to heavy resistance training, even at older ages.

Key words  Aging · Myosin · SDS-PAGE · Strength

Introduction

While examining the plasticity of skeletal muscle to chronic changes in the amount of neuromuscular activity, adaptations have been seen at the level of the myosin heavy chains (MHCs; Talmadge and Roy 1993). Changes in the expression of these MHC isoforms have also been observed with aging (Klitgaard et al. 1990b; Larsson et al. 1993). Analysis of myosin expression in skeletal muscle has attracted a great deal of attention because changes in the phenotypic expression appear to be related to muscle function and adaptations to a variety of physiological stimuli (Periasamy et al. 1989; Pette and Staron 1990).

There have been no long-term studies that have examined the use of very heavy resistance training protocols in inducing changes in the expression of MHC isoforms in aged skeletal muscle. A recent study by Williamson et al. (2000) using a lower-intensity resistance training program found that there was a reduction in the coexpression of hybrid MHCs (i.e., MHC I/IIa,
I/IIa/IIx, IIA/IIX), while favoring a significant increase in MHC I (10.4%) and a non-significant increase in MHC IIa (8.7%) in single fibers from older men (mean age 74 years) following 12 weeks of training. In another study, Willoughby and Pelsue (1998) looked at the MHC mRNA expression after moderate- and high-intensity resistance training in a group of elderly subjects (mean age 69 years). However, they did not report the specific expression of the MHC isoforms. In a cross-sectional study by Klitgaard et al. (1990a), the MHC composition was compared across five groups; three of trained elderly men (around 69 years of age), and a young and aged-matched control group. The trained elderly subjects were swimmers, runners or were strength-trained. It was found that the elderly control subjects had a 27% higher content of MHC I and corresponding lower content of MHC IIA and MHC IIB. The swimmers and runners had nearly identical values to those in the age-matched control group, while the strength-trained subjects had a MHC composition similar to those observed in the young control group.

Previous studies involving both humans and rodents have shown that heavy resistance training induces adaptations in MHC isoforms. These shifts predominantly involve a rearrangement in the pattern of expression involving the fast MHC isoforms, from MHC IIB to MHC IIA in humans and MHC IIB to MHC IIX in rodents (Adams et al. 1993; Bamman et al. 1998; Staron et al. 1991, 1994). It was first proposed by Goldspink et al. (1991) that the gene responsible for encoding for MHC IIB may be a default gene that provides a readily available pool of fibers that transform into IIA fibers with increases in activity, regardless of the type. We hypothesized that a transformation from MHC IIB toward MHC IIA would also occur in the elderly, although this relationship has not yet been verified with long-term heavy resistance training. Therefore, the purpose of this investigation was to determine the changes in MHC expression in the vastus lateralis muscle of elderly men and women in response to heavy resistance training.

**Methods**

**Subjects**

Twenty healthy, mildly physically active subjects gave their informed consent to participate in the study. The subject characteristics are given in Table 1. Approval for this study was granted by the Southern Cross University Ethics Committee on Human Experimentation.

**Experimental strength training**

The 6-month resistance training program was divided into two phases and was designed to promote muscle hypertrophy, strength and power. Weeks 1–12 consisted of high-intensity resistance training, and weeks 13–24 involved power training. The power training aspect of the program for weeks 13–24 was included to promote the activation of high-threshold motor units. Training consisted of two sessions per week of three sets of Smith-machine squat, leg press, leg extension, leg curl and deadlift, as well as a number of upper body exercises, thus making it a total-body training program. Only six exercises were performed per workout. The load intensity for the high-intensity resistance training (weeks 1–12) began at 10–12 repetitions maximum (RM) for the first 4 weeks, then 6–8RM for the next 4 weeks, and 3–5RM for the last 4 weeks. The power-training phase (weeks 13–24) involved 3–6 repetitions at 30–50% 1RM only for the Smith-machine squat, leg press and leg curl exercises. The remainder of the exercises were performed non-expectively and involved a repeat of the periodization cycle for weeks 1–12. To maintain strength levels during weeks 13–24, every sixth workout performed was heavy (4–6RM). A warm-up of 3–5 min on an exercise bike was performed prior to each workout. Warm-up sets of increasing percentages were also used for the Smith-machine squat, leg press, leg extension, leg curl, deadlift and upper body exercises.

**Measurement of IRM**

The measurement of maximal strength values was performed with the aid of the Smith-machine during the squat exercise to a knee angle of 110°. In the testing of the maximal load, separate IRM contractions were performed, allowing 1.5 min for recovery between the trials. After each repetition the load was increased until the subject was unable to squat to the required knee angle. The last acceptable squat with the highest possible load was determined as the IRM (Häkkinen et al. 2000).

**Muscle biopsy sampling**

Two muscle biopsy samples (week 0 and week 24) were obtained using the percutaneous needle biopsy technique of Bergström (1962), as modified by Evans et al. (1982). Tissue samples were taken from a site one-third of the length from the proximal lateral edge of the patella to the anterior superior iliac spine of the vastus lateralis muscle. Approximately 60–75 mg of skeletal muscle was removed and frozen in isopentane that had been precooled in liquid nitrogen, and stored at −80 °C for later analysis. The analyses performed during this investigation were part of a larger study, and the histochemical fiber data will be reported in detail elsewhere. However, it should be noted that both type I and II fibers exhibited a significant increase in size for the experimental group, but no changes were observed for the control group.

**MHC analysis**

Muscle samples were sectioned using a cryostat at −20 °C to a thickness of 40 μm. Three to five of these sections were placed into 0.5–1 ml of a lysing buffer containing 36.25% glycerol, 6.25% 2-mercaptoethanol and 2% sodium dodecyl sulfate (SDS) in Tris-HCl buffer (pH 6.8). Small amounts of the extracts (8 μl) were loaded on 6% SDS-polyacrylamide gels with 4% stacking gels and run using a Mini-Protean II electrophoresis system (Bio-Rad, Regents Park, NSW, Australia). The running conditions consisted of 70 V (constant voltage) for 30 min followed by 150 V (constant voltage) for 6 h using a Bio-Rad PowerPac (model 300) power supply. Gels were stained using Bio-Safe Coomassie Blue (Bio Rad). This procedure was based on the SDS-polyacrylamide gel electrophoresis protocol described by Talmadge and Roy (1993) and Humphries et al. (1997).

The gels were scanned electronically and the identification of MHC protein bands was conducted using Scion Image software beta version 3b. The bands were identified as MHC I, MHC IIA and MHC IIB isoforms from a myosin molecular weight standard (Kaleidoscope Prestained Standards, Bio-Rad) and on the basis of previously determined migration patterns, using densitometric and image processing techniques (Klitgaard et al. 1990b; Talmadge and Roy 1993). The integrated peak areas corresponding to each band were summed and are expressed relative to the total area. Results
Table 1  Mean (SD) age, height, body mass, and 1 repetition maximum (1RM) of experimental and control subjects prior to (week 0) and after 24 weeks (week 24) of heavy resistance training (experimental group) or no training (control group)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Experimental group</th>
<th>Control group</th>
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<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 24</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.6 (3.6)</td>
<td>62.0 (3.5)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.4 (6.3)</td>
<td>162.7 (5.9)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>72.3 (9.9)</td>
<td>71.1 (9.8)</td>
</tr>
<tr>
<td>1RM squat (kg)</td>
<td>58.2 (8.3)</td>
<td>77.9 (11.1)*</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>66.4 (4.8)</td>
<td>62.7 (4.6)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172.5 (6.6)</td>
<td>172.4 (6.1)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>83.2 (13.2)</td>
<td>82.3 (12.4)</td>
</tr>
<tr>
<td>1RM squat (kg)</td>
<td>130.4 (25.3)</td>
<td>171.1 (30.5)*</td>
</tr>
</tbody>
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*Significantly different from week 0: \( P \leq 0.05 \)

are expressed as percentages of total MHC in each sample. This imaging procedure was adapted from that described by Humphries et al. (1997). Examples of electrophoretic separation of MHC isoforms and of individual plot profiles are shown in Figs. 1 and 2, respectively.

Statistical analysis

Means and standard deviations were calculated for all variables using conventional methods. The General Linear Model for analysis of variance (ANOVA) with repeated measures was used to compare within (pre-, post-training) and between groups (experimental, control) and gender (male, female) subject effects. As there were significant main effects of trial as well as an interaction of trial x group, probability-adjusted paired t-tests and one-way ANOVAs were used for follow up analyses. A criterion alpha level of \( P \leq 0.05 \) was used for all statistical comparisons.

The principle criterion for differentiating the experimental and control groups in this study was the change in MHC IIa and MHC IIb isoforms following the training period. Further aims were to determine whether any change with training was in fact significant without making a Type II error, and whether the percentage change was different between the groups. Based on previous research using younger resistance-trained subjects (Adams et al. 1993) and cross-sectional differences between older sedentary and strength trained subjects (Klitgaard et al. 1990a) it was expected that the effect size (ES) of this training program in MHC IIa and MHC IIb isoforms in these previously untrained subjects would be quite large. Therefore an ES of 1.4 was assumed. In this experiment the researchers wanted to be 80% (power = 0.8, \( \beta = 1-0.8 = 0.2 \) confident that such a difference in the MHC IIa and MHC IIb isoforms pre- to post-training intervention would be detected (i.e., the null hypothesis would be rejected). Therefore, with an ES of 1.4

Fig. 1  Analysis of myosin heavy chain (MHC) expression from the vastus lateralis muscle of aged control and experimental subjects. MHC isoforms were separated using 6% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Three MHC bands were separated (MHC IIb, MHC IIa and MHC I). Lane 1: experimental subject at week 0; Lane 2: experimental subject at week 24, no MHC IIb was detected for this subject; Lane 3: control subject at week 0; Lane 4: control subject at week 24

Fig. 2 A Plot profile for an experimental subject prior to the commencement of the heavy resistance training (Pre, week 0). B Plot profile for an experimental subject at the completion of training (Post, week 24). The three peaks correspond to the three MHC isoforms found in human skeletal muscle and are labeled accordingly at an alpha level of 0.05 (two-tailed test) the sample size required was found to be six subjects in the experimental and control groups, resulting in a power of 0.80 (Cohen 1988).
Results

The male and female experimental groups exhibited a significant decrease ($P \leq 0.05$) in the percentage of MHC IIb, while the experimental female group also demonstrated a significant increase ($P \leq 0.05$) in the expression of MHC IIa after the 24 weeks of heavy resistance training (Figs. 3–5). An example of week 0 and week 24 plot profiles for a representative experimental subject appears in Fig 2, and clearly demonstrates the decrease in MHC IIb with training. In 9 out of the 14 experimental subjects there was no MHC IIb detectable in the week 24 biopsy sample (Fig. 1). There was no change in the MHC expression within the control group for the male or female subjects.

Fig. 3 MHC I expression (mean ± SD) at week 0 (white bars) and week 24 (black bars) for male and female subjects in the experimental and control groups

*Significantly different from Week 0: $p<0.05$

Fig. 4 MHC IIa expression (mean ± SD) at week 0 (white bars) and week 24 (black bars) for male and female subjects in the experimental and control groups

*Significantly different from Week 0: $p<0.05$

Fig. 5 MHC IIb expression (mean ± SD) at week 0 (white bars) and week 24 (black bars) for male and female subjects in the experimental and control groups

*Significantly different from Week 0: $p<0.05$
The male experimental group exhibited a significant increase ($P \leq 0.05$) in the 1RM squat exercise (Table 1) at week 24 [130.4 (25.3) kg vs 171.1 3 (0.5) kg]. The female experimental group also showed a significant increase in the 1RM squat [58.2 (8.3) kg vs 77.9 (11.1) kg]. The control group showed no change in strength for the 1RM squat exercise for either the male [115.8 (35.1) kg vs 123.8 (47.2) kg] or female [57.5 (9.0) kg vs 58.3 (2.9) kg] groups.

**Discussion**

The purpose of this study was to evaluate the changes in the expression of MHC isoforms as a result of heavy resistance training in elderly subjects. To our knowledge this is the first study to examine these changes utilizing a very heavy resistance training program. The results show clearly that elderly subjects undergoing heavy resistance training have the ability to produce the same shifts in the expression of MHC isoforms from MHC IIb to MHC IIa as has been shown to occur in younger subjects (Adams et al. 1993; Fry et al. 1994; Jurimae et al. 1996). The expression of the MHC isoforms did not change for the control group, but the heavy resistance training protocol caused a significant decrease for the male [12.2 (7.4)% vs 3.9 (5.3)%] and female [16.7 (11.5)% vs 7.0 (12.2)%] experimental groups in the percentage of MHC IIb from week 0 to week 24. This decrease in the MHC IIb expression is consistent with previous findings with younger subjects (Adams et al. 1993; Jurimae et al. 1996; Staron et al. 1994). This adds to the growing evidence that the transformations of the MHC IIb isoform toward MHC IIa may be due to the readiness and ability of MHC IIb to offer a pool of fibers that transform toward more metabolically oxidative fibers as a consequence of activation via heavy resistance training. The changes occurring as a result of the heavy resistance training demonstrate the preservation of plasticity in aged muscle as a response to altered functional demands.

The shift from MHC IIb to IIa as a result of training in younger subjects has been demonstrated extensively (Adams et al. 1993; Carroll et al. 1998; Harridge et al. 1998; Jurimae et al. 1996). Studies in which the MHC expression of young trained subjects has been examined are all consistent, reporting small percentages of the MHC IIb isoform (Andersen et al. 1999). However, in contrast to this, studies examining MHC expression with aging do not show large increases in the MHC IIb isoform. Recent research shows that the myogenic regulatory gene MyoD, may play a crucial role in the activation of MHC IIb expression in response to altered physiological demand in the soleus muscle of rats (Wheeler et al. 1999). The apparent significance of the role of these myogenic regulatory genes such as MyoD, myogenin, MRF4 and myf-5, and their control over the transcription of MHC mRNAs is still unclear and requires further investigation.

In the study reported here, a significant increase in MHC IIa was only found for the female experimental group. This may be due to the finding that the occurrence of hybrid MHC isoforms in single muscle fibers is significantly increased in elderly subjects. Andersen et al. (1999) found that the number of single fibers that coexpress MHC I and MHC IIa were significantly larger in elderly subjects (around 28.5%). While in younger adults, the proportion of fibers coexpressing MHC I and MHC IIa rarely exceeds 10% of the fibers in the vastus lateralis muscle (Andersen et al. 1999). Another reason for no increase being found in the MHC IIa isoform in the male experimental group may be due to such factors as the intra-subject variability, the difference in the hypertrophy of type II fibers, and the availability of type II motor units. The increase in MHC IIa expression shows an adaptation toward more efficient metabolic properties within the fiber after training the higher-threshold motor units using heavy and more powerful training protocols.

A study by Williamson et al. (2000) showed a significant increase in the expression of MHC I as a result of 12 weeks of progressive resistance training involving low-volume, low-intensity training using only three sets of leg extensions. The first two sets used 10 repetitions and the last set was to volitional exhaustion at 80% of the 1RM. Their finding of an increase in MHC I indicates that the lower intensity of the training program may not be capable of recruiting higher-threshold motor units. This would explain the observed increase in MHC I expression. The increase in MHC I was found to be non-significant in the present study, indicating that training protocols may differentially interact with the MHC transformations that occur. This is most likely due to a lower amount of hypertrophy in type I fibers, intra-subject variability and/or preferential recruitment of type I fibers. Further studies are required to identify the shifts in the coexpression of MHC fiber types that occur as a result of adaptation to a very heavy resistance training program.

In a recent study it was found that high-intensity resistance training was effective in preventing sarcopenia after periods of bed rest unloading in younger subjects (Bamman et al. 1998). This highlights the significance of resistance training for the older members of our community to prevent the muscle atrophy that results with decreased usage during aging. Carroll et al. (1998) suggested that the transformation toward MHC IIa represents a positive strength adaptation. This adaptation toward the MHC IIa isoform may not necessarily be a strength adaptation, but more an adaptation toward a more effective recruitment and utilization of the muscle following the high-threshold motor unit training that occurs with specific heavy-resistance exercise protocols. The heavy resistance training program was successful in increasing the dynamic strength (1RM squat) observed within both the male and female experimental groups.

Much more research will obviously be needed to find the physiological keys that regulate the expression of the contractile proteins with advancing age. The dynamic nature of skeletal muscle allows it to vary the expression
of myosin in response to changes in functional usage. Exercise and detraining, or inactivity will cause specific sequential changes in myosin expression within the fast fibers, and possibly under long-term conditions in the slow fibers. By gaining a better understanding of the morphological changes that impart skeletal muscle its plasticity, we might be better able to counteract the muscle atrophy and frailty that results in old age.

References