Age- and Gender-Related Differences in Maximum Shortening Velocity of Skeletal Muscle Fibers

ABSTRACT


Objective: To determine age- and gender-related differences in maximum unloaded shortening velocity (Vo) of Type I and IIA single muscle fibers. Muscle fibers must have a broad range of contractile velocities to generate the full range of power required for varied activities.

Design: Percutaneous needle biopsies of the vastus lateralis were obtained from 31 healthy subjects (n = 7 young men [YM], n = 7 young women [YW], n = 12 older men [OM], n = 12 older women [OW]). The slack test was used to determine Vo of individual fibers; 916 muscle fibers were chemically skinned. Fiber type was determined by myosin heavy chain isoform identification.

Results: Among men, Vo (fiber lengths/sec) was reduced with age in Type IIA fibers (OM vs. YM: 1.78 vs. 2.14; P < 0.05) but unchanged in Type I fibers. Among women, Vo was reduced with age in Type I fibers (OW vs. YW: 0.70 vs. 0.75; P < 0.05) but not IIA. OW had a lower Vo than did OM in both fiber types (Type I: OW = 0.70, OM = 0.77; Type IIA: OW = 1.51, OM = 1.78; P < 0.05). YW did not differ from YM.

Conclusions: Both age and gender affect Vo. Age- and gender-related differences in Vo may partially explain the impairments in muscle function that occur with aging and the greater impairment in muscle function observed in OW compared with that observed in OM.

Key Words: Single Muscle Fiber, Shortening Velocity, Aging, Muscle Power
The velocity of muscle contraction and the ability of skeletal muscle to generate power are important to perform many daily activities, ranging from instrumental tasks such as rising from a chair or crossing a street to the more recreational activities like playing basketball. In vivo measurements of muscle contraction velocity assess the mean velocity of a heterogeneous tissue, thus ignoring the diversity in velocity attributable to multiple muscle fiber types. The skinned single muscle fiber preparation allows investigation of the force-generating capacity and contractile velocity of individual muscle cells in a fiber type specific manner. The effect of gender and age on the force-generating capacity (specific force) of single muscle fibers has been previously reported by our group, but the effect of gender and age on maximal unloaded shortening velocity (Vo) has not been systematically studied.

Vo has been described as a design parameter of skeletal muscle. Muscle fibers must have a broad range of contractile velocities to generate the full range of power required for varied activities. Vo varies within and between fiber types and is determined, to a large extent, by the myosin heavy chain (MHC) isoforms present in each individual fiber. In whole muscle, Vo decreases with aging. Thus, we expect that Vo will also decline at the single fiber level.

In humans, only one study has been published previously using the skinned single fiber preparation to explore the effect of aging on Vo of the vastus lateralis muscle. This study had eight subjects (n = 4 young; n = 4 old) and found a reduction in Vo for both Type I and Type IIA fibers in older men when compared with the fibers in younger men. Other single fiber studies in humans have explored the effects of endurance training and bedrest on Vo in a variety of muscles. In general, these studies have reported an increase in Vo of Type I fibers in response to both endurance training and bedrest. Because women were not included in these studies, there is no literature regarding gender differences in Vo. A limitation of all of the above-mentioned studies is the small number of subjects (n = 3–11).

Rat skeletal muscle has also been used to study the effect of aging on Vo. Several studies have demonstrated a decrease in Vo of Type I fibers from the soleus muscle of older rats. The two studies presenting data from Type II rat fibers showed no change in Vo of Type IIX or Type IIB fibers; Type IIA fibers were not reported. Age-related changes in Vo were similar in male and female rats.

The objective of this study is to compare Vo of Type I and IIA single muscle fibers from the vastus lateralis muscle of healthy young adults to fibers from older men and women. This is the first study of human single muscle fiber contractile properties to include women as subjects. We have elected to study the vastus lateralis muscle because of its functional importance in gait and the activities of daily living. We hypothesize the following: (1) older adults have lower Vo than younger adults for both Type I and IIA fibers, and (2) there will be no gender-related differences in Vo of Types I and IIA single muscle fibers. However, if Vo declines with aging, or if gender differences are identified, Vo may be an appropriate target for modification via an exercise or other rehabilitative intervention. Theoretically, an intervention that increases Vo at the cellular level should improve the ability of whole muscle to generate power which can enhance functional performance. Leg muscle power has been shown to be a strong predictor of functional status in elderly women.

**METHODS**

**Subjects.** A total of 38 healthy subjects volunteered for the study. The volunteers received a complete explanation of the purposes and procedures for the study and they gave their written consent. A comprehensive medical evaluation including a medical history, physical examination, routine blood and urine tests, and a resting electrocardiogram was performed before their participation in the study. Subjects with conditions that could interfere with neuromuscular function were excluded. The study was approved by the Human Investigation Review Committees of Spaulding Rehabilitation Hospital and the Tufts USDA Human Nutrition Research Center.

**Muscle Biopsies and Permeabilization of Fibers.** Using biopsy needles and suction, we obtained specimens
from the vastus lateralis muscle of subjects who received local anesthesia.\textsuperscript{14} The specimens were placed in relaxing solution at 4°C. Bundles of \(-30\) fibers were dissected free from the samples and then tied with surgical silk to glass capillary tubes at slightly stretched lengths. The fibers were chemically skinned for \(24\) hr in relaxing solution containing \(50\%)\ (v/v) glycerol at 4°C and were subsequently stored at \(-20°C\) before use.\textsuperscript{15}

**Experimental Procedure.** On the day of an experiment, we placed the fibers for \(30\) min in relaxing solution containing \(0.5\%)\ Brij-58 (polyoxethylene 20 cetyl ether; Sigma Chemical Company, St. Louis, MO) before mounting them in an experimental apparatus, similar to the one described previously.\textsuperscript{16} A 1.5-mm fiber segment length was left exposed to the solution between connectors leading to a force transducer (400A, Cambridge Technology, Cambridge, MA) and a DC torque motor (308B, Cambridge Technology). The apparatus was mounted on the stage of an inverted microscope (Olympus IX70, Tokyo, Japan). Figure 1 depicts a segment of a single muscle fiber mounted on the experimental apparatus and demonstrates preservation of the sarcomere patterns. The fibers were in relaxing solution; sarcomere length was set to \(2.75\) to \(2.85\) \(\mu\)m by adjusting the overall segment length. The segments were observed through the microscope at a magnification of \(320\times\).

Relaxing and activating solutions (in nM) contained the following: \(4\) MgATP, \(1\) free Mg\(^{2+}\), \(20\) imidazole, \(7\) EGTA, \(14.5\) creatine phosphate, and sufficient KCl to adjust the ionic strength to \(180\) nM. The pH was adjusted to \(7.0\). The concentration of free Ca\(^{2+}\) was \(10^{-9}\) M (relaxing solution) and \(10^{-4.5}\) M (maximum activating solution) and was expressed as pCa (\(-\log[Ca^{2+}]\)). Immediately preceding each activation, the fiber was immersed for \(10\) to \(20\) sec in a solution with a reduced Ca\(^{2+}\)-EGTA-buffering capacity.\textsuperscript{17} This solution was identical to the relaxing solution except that EGTA was reduced to \(0.5\) mM, which resulted in a more rapid steady tension during subsequent activation.

Maximal Vo was measured by the slack test procedure.\textsuperscript{18, 19} Fibers were activated at pCa \(4.5\); once steady tension was reached, various amplitudes of slack (\(\Delta L\)), ranging from \(7\%)\ to \(13\%)\ of the fiber segment length, were rapidly introduced (within \(1–2\) msec) at one end of the fiber (Fig. 2A). The time (\(\Delta t\)) required to take up the imposed slack was measured from the onset of the length step to the beginning of the tension redevelopment (Fig. 2B). For each amplitude of \(\Delta L\), the fiber was reextended while relaxed to minimize nonuniformity of sarcomere length. A straight line was fitted to a plot of \(\Delta L\) vs. \(\Delta t\) using least-squares regression, and the slope of the line divided by the segment length was recorded as Vo for that fiber (Fig. 2C). All contractile measurements were carried out at \(15°C\).

**Myosin Heavy Chain Composition.** After mechanical measurements, each fiber was placed in sodium dodecyl sulfate (SDS) sample buffer in a plastic microfuge tube and stored at \(-20°C\) for up to \(1\) wk or at \(-80°C\) if the gels were to be run later. The MHC composition of single fibers was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).\textsuperscript{20} The acrylamide concentration was \(4\%)\ (v/v) in the stacking gel and \(6\%)\ in the running gel, and the gel matrix includes \(30\%)\ glycerol. Sample loads were kept small (\(-0.05\) mm of fiber segment) to improve the resolution of the MHC bands (Types I, IIA, and IIB). Gels were silver-stained to determine the MHC isoforms present (Fig. 3). Proteins were identified using a combination of purified human myosins from the vastus lateralis muscle.\textsuperscript{19}

**Statistical Analysis.** Analysis of variance was used to compare Vo in Type I and IIA fibers between the four subject groups. Because the data were not normally distributed, the Kruskal-Wallis one-way analysis of variance on ranks was used. Post hoc comparisons between groups were performed using Dunn’s method. The Mann-Whitney rank-sum test was used to compare Type I and IIA fibers within each subject group. The statistical analysis was performed using Sigma Stat software (SPSS, Chicago, IL).

**RESULTS**

The general characteristics of the subjects are presented in Table 1.

**Contractile Properties of Single Muscle Fibers.** The Vo of Type I and IIA single muscle fibers from the four groups is displayed in Table 2. A total of \(669\) Type I and \(247\) Type IIA fibers were studied. Figures 4 and 5 display the distribution of Vo of each gender and age group for Type I and IIA fibers, respectively.

**Age Comparisons.** Post hoc analysis revealed no significant difference between Vo of Type I fibers in older and younger men. However, Figure 1 demonstrates that the distribution of Vo values was broader in older subjects (i.e., more very slow and more very fast Type I fibers). Type IIA fibers had significantly slower Vo in older men (\(P < 0.05\)), and the distribution of Vo values was narrower in older
Type IIA fibers were faster than Type I fibers for both older and younger men \((P < 0.001)\).

The results of age comparison in women were the opposite of those seen in men. Older women had slower Vo than did younger women in Type I fibers \((P < 0.05\) in post hoc analysis), but not Type IIA fibers. The spread of Vo values was similar in the two groups. Type IIA fibers were faster than Type I fibers for both

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**Figure 2:** A, activation of a single muscle fiber with calcium followed by introduction of a slack in fiber length, force redevelopment, and transfer to relaxing solution; B, measurement of time to take up the imposed slack \((\text{Dur, msec})\), lever arm displacement \((\text{Dis, \mu m})\), and maximal force \((\text{MF, mN})\) for an individual slack; C, plot of duration vs. displacement used to determine shortening velocity \((\text{Vo})\). Vo is the slope of the regression line divided by fiber segment length.

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older and younger women ($P < 0.001$).

Gender Comparisons. Post hoc analysis revealed that both Type I and Type IIA fibers had slower Vo in older women than in older men ($P < 0.05$). No gender-related differences were present in the younger subjects.

### DISCUSSION

The impairment in muscle function that occurs with aging is important to physiatrists treating geriatric patients because it produces both primary and secondary disability. Secondary disability occurs when muscle weakness predisposes patients to develop associated disorders, such as osteoporosis or fractures caused by falls, which impair function and independence. The effect of aging on individual skeletal muscle design parameters, such as Vo, must be identified if we are to develop an understanding of the mechanisms underlying the impairment of muscle function. Older women have a higher incidence of functional impairment than do older men, and gender differences may influence the mechanisms underlying skeletal muscle dysfunction. Thus, it is important to characterize gender- and age-related differences in skeletal muscle design parameters if we wish to optimize preventive and rehabilitative strategies.

The main finding of this study is that Vo decreases with age in Type I muscle fibers in women and Type IIA fibers in men. In addition, we found that older women have slower Vo than do older men in both fiber types;

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>General characteristics of subjects</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>YM (n = 7)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>36.5 ± 3.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179.4 ± 9.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.9 ± 11.4</td>
</tr>
</tbody>
</table>

YM, young men; OM, older men; YW, young women; OW, older women. Mean ± SD values.

<table>
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<tr>
<th>TABLE 2</th>
<th>Maximal unloaded shortening velocity (Vo) of single muscle fibers from vastus lateralis muscle</th>
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<tbody>
<tr>
<td>Type I</td>
<td>YM</td>
</tr>
<tr>
<td>n</td>
<td>91</td>
</tr>
<tr>
<td>Vo</td>
<td>0.77 ± 0.22 (0.43 – 1.49)</td>
</tr>
<tr>
<td>Type IIA</td>
<td>n</td>
</tr>
<tr>
<td>Vo</td>
<td>2.14 ± 0.81 (0.83 – 4.30)</td>
</tr>
</tbody>
</table>

YM, young men; OM, older men; YW, young women; OW, older women; n, number of single muscle fibers studied; Vo, maximal unloaded shortening velocity in fiber lengths/sec expressed as mean ± SD (range). P values were determined by Kruskal-Wallis one-way analysis of variance on ranks.

aSignificantly different from both younger women and older men in post hoc analysis.
bSignificantly different from younger men in post hoc analysis.
cSignificantly different from older men in post hoc analysis.
this gender difference is not present in younger individuals.

Table 3 summarizes the data reported in the literature on Vo in healthy individuals. Only Larsson et al.4, 8 and Harridge et al.21 reported data from the vastus lateralis muscle. However, Harridge et al.21 have shown that single fibers expressing the same MHC isoform, but from different muscles, do not differ in Vo. Larsson et al.4, 8 reported much slower Vo than we reported for Type I fibers. However, in young men, the relationship between the velocity of the two fiber types is similar; in Larsson’s subjects, Type IIA fibers had a Vo 3.3 to 4.1 times faster than Type I fibers, and in our young men, Type IIA fibers were 2.8 times faster. In Larsson’s older men,4 Type IIA fibers were 5 times faster than Type I fibers; in our older men, Type IIA fibers were only 2.3 times faster. The absolute differences in Vo may be related to the differences in experimental techniques. Studies by Larsson et al. were performed at a temperature of 12°C, whereas our experiments were performed at 15°C. Vo is markedly temperature dependent, decreasing by one-half to two-thirds with a temperature change of 10°C.22, 23 However, a relative difference in Vo of the two fiber types should not be produced by differences in temperature. Our older men had a smaller difference in velocity between the two fiber types than did the subjects in the study by Larsson et al. In addition, Larsson’s older men showed a reduction in Vo for both fiber types, whereas we

![Histogram showing the distribution of shortening velocity (Vo) (fiber length/sec) for Type I single muscle fibers from the vastus lateralis muscle in younger men, older men, younger women, and older women.](image-url)
found a reduction of Vo in Type IIA fibers only in our men. However, Larsson et al. only studied four older men (two sedentary and two athletic), so some of these differences may be caused by the small sample size.

Our values for Vo (Table 2) are closer to those reported by Fitts et al.5 and Widrick et al.6, 7 for the deltoid, gastrocnemius, and soleus muscles in young and middle-aged men. These investigators reported Vo values from 0.43 to 1.03 for Type I fibers and 1.90 to 4.85 for Type IIA fibers.

There are three possible explanations for the decline in Vo that occurs with aging. One possibility is that the MHC protein is dysfunctional. MHC protein synthesis decreases with aging,24 and if synthesis lags behind catabolism, it is possible that dysfunctional proteins are not replaced rapidly enough. Alternatively, MHC protein may be replaced by a newly synthesized but dysfunctional protein.

A second explanation for the change in Vo that occurs with aging is that a new, unidentified MHC isoform, which comigrates with known MHC isoforms on SDS-PAGE, is expressed in older individuals altering Vo. Two different MHC I protein isoforms have been identified,25 but multiple MHC IIA isoforms remain to be identified.

A third explanation proposed for the change in Vo that occurs with aging is alteration in myofilament spacing.10 In subjects who have had prolonged bedrest, Vo increases in Type I fibers; this increase correlates well with an increase in space between the thin filaments of muscle fibers.26 Studies using dextran to alter myofilament spacing have shown that Vo of both Type I and IIA fibers decreases with filament lattice com-

**Figure 5**: Histogram showing the distribution of shortening velocity (Vo) (fiber lengths/sec) for Type IIA single muscle fibers from the vastus lateralis in younger men, older men, younger women, and older women.
expression. Theoretically, the decreased spacing of the myofilaments increases the internal drag occurring during cross-bridge cycling, reducing Vo. The above findings suggest the hypothesis that myofilament spacing may decrease with aging, increasing the internal drag during cross-bridge cycling and slowing Vo.

Our finding that Vo declines with aging suggests that the diminished ability of older muscle to produce power is caused by a combination of decreased force production and decreased contraction speed. Thus, interventions to improve muscle function in the elderly should target both of these skeletal muscle design parameters. Two studies have addressed the effect of endurance training on single muscle fiber contractile properties in younger individuals and have found that Vo in Type I fibers increases and Vo in Type IIA fibers decreases. If this trend holds true for older individuals, it does not seem that endurance training can correct the age-related decline in Vo of Type IIA fibers. The effect of resistance or power training on single muscle fiber contractile properties has not been studied and should be investigated as a possible means of reversing the decline in Vo which occurs with aging.

The lower Vo of muscle fibers in older women is an unexpected finding that helps to explain the greater frailty and impairment of muscle function seen in older women compared with older men. The fact that gender differences are present only in old age suggests that they may be related to postmenopausal hormone changes in women. It has been suggested that estrogen may affect \( \text{Ca}^{2+} \) kinetics or myosin ATPase activity. One area for further research is the effect of hormone replacement therapy on single muscle fiber contractile properties and on the gender-related differences in Vo observed in postmenopausal women.

**CONCLUSIONS**

The maximal unloaded shortening velocity of single muscle fibers is influenced by age and gender. In men, aging seems to result in a slowing of Vo in Type IIA muscle fibers without a change in Vo of Type I muscle fibers. In women, Vo decreases in Type I rather than Type IIA fibers. When comparing genders in older adults, women have slower Vo than do men in both Type I and IIA muscle fibers; these differences are not present in younger adults. Age- and gender-related differences in Vo may partially explain the muscle function impairments that occur with aging and the greater impairment in muscle function seen in older women compared with older men. Reversing changes in Vo that occur with aging may be a reasonable target for interventions designed to decrease muscle impairment that occurs with aging.

**ACKNOWLEDGMENTS**

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