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ORIGINAL ARTICLE

Passive force and viscoelastic properties of single fibers in human aging muscles

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Abstract

Purpose Changes in stiffness or extensibility of the muscle or muscle–tendon unit with aging could lead to impaired function and an increased vulnerability to injury. We aimed to investigate the passive force and viscoelastic properties of single muscle fibers in older adults.

Methods Seven older adults (mean age 79.0 ± 3.8 years) and 10 young control (mean age 25.6 ± 4.5 years) were recruited. Biopsy specimens were obtained percutaneously from m. vastus lateralis and skinned single fibers were used for the experiments. Slack tests were performed to determine maximal force and maximal unloaded shortening velocity. Passive force was measured in pCa 9.0 solution using a stepwise stretch technique with increment of sarcomere length from 2.4 to 4.2 µm. Myosin heavy chain (MHC) isoform was determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Specific force was calculated as maximal force divided by cross-sectional area. Passive force, peak passive force, time to half stress relaxation ($T_{1/2}$) and force decay index (a force time integral under a stress relaxation curve) were measured.

Results No difference between the groups were found in specific force and shortening velocity. Passive force and peak passive force were greater in both MHC I and IIa fibers of older adults ($p < 0.001$, $p = 0.012$, respectively, at 4.2 mm SL). Force decay index was higher in older adults. ($p = 0.001$ at 4.2 µm SL). There were no significant differences in passive force and viscoelastic properties between fiber types.

Conclusion We demonstrated greater passive force and viscoelastic properties at the level of single fibers in older adults.

Keywords Passive force · Mechanical properties · Aging muscle fiber · Sarcopenia

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Introduction

Sarcopenia is characterized by the loss of muscle mass, strength, and function with advanced adult age. For example, elderly have muscle mass that is 70–80% that of younger people (Frontera et al. 2001; Krivickas et al. 2001). Older men and women show reductions in strength, movement velocity, and power (Candow and Chilibeck 2005; Frontera et al. 2008; Krivickas et al. 2001,2006). Further, older muscles are vulnerable to injury and show impaired regenerative capacity (Dow et al. 2005; Payne and Delbono 2004; Siu et al. 2005,2006). All of the above changes result in the deterioration of physical performance, decline in function, and loss of independence (Walston and Fried 1999). However, the cellular mechanisms underlying these changes are complex and not well understood.

It has been reported that the intrinsic mechanical characteristics of human single myofibers are altered with aging (Ochala et al. 2007). Both muscle fiber size (a proxy for the

number of cross-bridges) and the force-generating capacity per cross-bridge appear to be reduced (Frontera et al. 2001; Krivickas et al. 2001,2006). However, single muscle fiber contractile function was preserved or even improved with advancing age in other studies (Frontera et al. 2008; Grosicki et al. 2016). Moreover, instantaneous stiffness in both type I and IIa fibers is altered in elderly (Ochala et al. 2007). However, this parameter reflects the elasticity of activated muscles, but it does not evaluate the passive components of stiffness and/or elasticity.

Passive mechanical properties are thought to relate to the properties of the sarcolemma, connective tissue, and titin filaments present in sarcomeres. Elasticity is an important property of muscle tissue that allows movement and contributes to tissue integrity. Increased stiffness or decreased elasticity of muscle can lead to impaired muscle function and an increased susceptibility to injury (Gajdosik et al. 1999). Sarcomere proteins contribute to elasticity (Horowits 1992) and it has been suggested that titin is the most important determinant of the intracellular component of passive elasticity (Granzier and Labeit 2007). Some conflictive reports have been published regarding the age-related changes in stiffness. For example, one report showed no change in whole muscle stiffness but in that study stiffness in older muscle accounted for a greater proportion of total passive force because of a significant decline in muscle mass and active force associated with age (Brown et al. 1999). One animal experiment using mice suggested that the intrinsic stiffness of aging muscle fibers was not different from that of young muscle fibers (Wood et al. 2014). Others have postulated that impaired elastic properties of animal muscles may lead to increased stiffness because of the limited extensibility (decreased sarcomere elongation) and increased relaxation time (loss of spring function) (Toursel et al. 2002). To our knowledge, these experiments have not been conducted in human single muscle fibers.

The purpose of the study is to investigate active and passive mechanical properties (force production, stiffness, and viscoelasticity) in single muscle fiber of older humans. Two sets of experiments were conducted to compare these properties in fibers expressing different myosin heavy chain isoforms. We hypothesize that MHC I and IIa older human single muscle fibers have an increased stiffness and viscoelasticity resulting in altered force production at different sarcomere lengths.

Methods

Subjects

A total of seven community-dwelling healthy elderly (age range 73–87) men ($n=5$) and women ($n=2$) and ten healthy young (age range 21–40) control men ($n=6$) and women ($n=4$) were recruited for the study. Participants were not participating in any formal exercise training program. Subjects with unstable chronic disease, neurologic problems, severe arthritis, bleeding conditions, and those using medications that could impair muscle performance were excluded from the study. Functional performance level was assessed using the short physical performance battery (SPPB) for older adults (Guralnik et al. 2000). We used the short form of the International Physical Activity Questionnaire (IPAQ) to assess physical activity levels in the healthy young participants because the SPPB is an inappropriate tool in this population (Craig et al. 2003). Older participants showed SPPB scores between 10 and 12 which indicated they were physically healthy and independent. All young adults participated in moderate-to-vigorous physical activity. All participants gave informed consent and the study was approved by the Institutional Human Investigation Review Board.

Muscle biopsy and preparation of single muscle fibers

Biopsy specimens were obtained percutaneously from muscle vastus lateralis under local anesthesia using Bergstrom needles and suction. The specimens were placed in relaxing solution at 4 °C. Bundles of ~ 50 fiber segments were dissected free from the samples and then tied with surgical silk to glass capillary tubes at slightly stretched lengths. The fiber segments were chemically skinned for 24 h in relaxing solution containing 50% (vol/vol) glycerol at 4 °C and were subsequently stored at − 20 °C for up to 4 weeks before use (Larsson and Salviati 1992). On the day of the experiment, fibers were placed for 30 min in relaxing solution containing 0.5% Brij-58 (polyoxyethylene 20 cetyl ether; Sigma, St. Louis, MO) prior to mounting in the experimental apparatus. A fiber length of 1–2 mm was left exposed to the solution between connectors leading to a force transducer (Model 400A; Aurora Scientific, Aurora, ON, Canada) and a direct current torque motor (Model 300H; Aurora Scientific). While the fibers were in relaxing solution, sarcomere length (SL) was set to 2.75–2.85 µm. The sarcomere length, the diameter, and the length of the fiber between the connectors were measured (ImagePro Plus; Media Cybernetics, Silver Springs, MD). Fiber depth was measured by recording the displacement of the microscope nosepiece while focusing on the top and bottom surfaces of the fiber. Fiber cross-sectional area (CSA) was calculated from the diameter and depth, assuming an elliptical circumference. When calculating specific force (force/CSA), CSA was corrected for the 20% swelling that occurs during skinning (Moss 1979). Relaxing and activating solutions contained 4 mM Mg–adenosine triphosphate (Mg-ATP), 1 mM free Mg^{2+}, 20 mM imidazole, 7 mM ethylene glycolbis-(aminoethylether)-*N*,*N*′ tetraacetate

(EGTA), 14.5 mM creatine phosphate, and KCl to adjust the ionic strength to 180 mM. The pH was adjusted to 7.0. The concentrations of free Ca^{2+} were 10^{-9} M (relaxing) and $10^{-4.5}$ M (activating) expressed as pCa ($-\log [Ca^{2+}]$). Apparent stability constants for Ca^{2+}-EGTA were corrected for temperature and ionic strength.

Mechanical experiments

Active isometric contractile measurements

A total of 275 muscle fibers were used for this set of experiments. Maximum unloaded shortening velocity (Vo) was measured using the slack test procedure. Fibers were activated at pCa 4.5, and once steady tension was reached, various amplitudes of slack, ranging from 7 to 13% of the fiber length, were rapidly introduced (within 1–2 ms) at one end of the fiber. The time required to take up the imposed slack was measured from the onset of the length step to the beginning of the tension redevelopment. For each amplitude of slack, the fiber was re-extended while relaxed to minimize non-uniformity of sarcomere length. A straight line was fitted to a plot of slack length vs. time, using least-squares regression, and the slope of the line divided by the segment length was recorded as Vo for that fiber. Maximal force (Po) was calculated as the difference between the total tension in activating solution (pCa 4.5) and the resting tension measured in the same segment while in the relaxing solution. All contractile measurements were carried out at 15 °C.

Passive force and viscoelasticity measurements

A total of 251 muscle fibers were employed for this set of experiments. Different muscle fibers were used for passive measurements from those used for active contractile measurements. For each stretch experiment, initial sarcomere length was 2.4 µm and estimated 0.2-µm SL increments were applied until the SL reached an estimated 4.2 µm

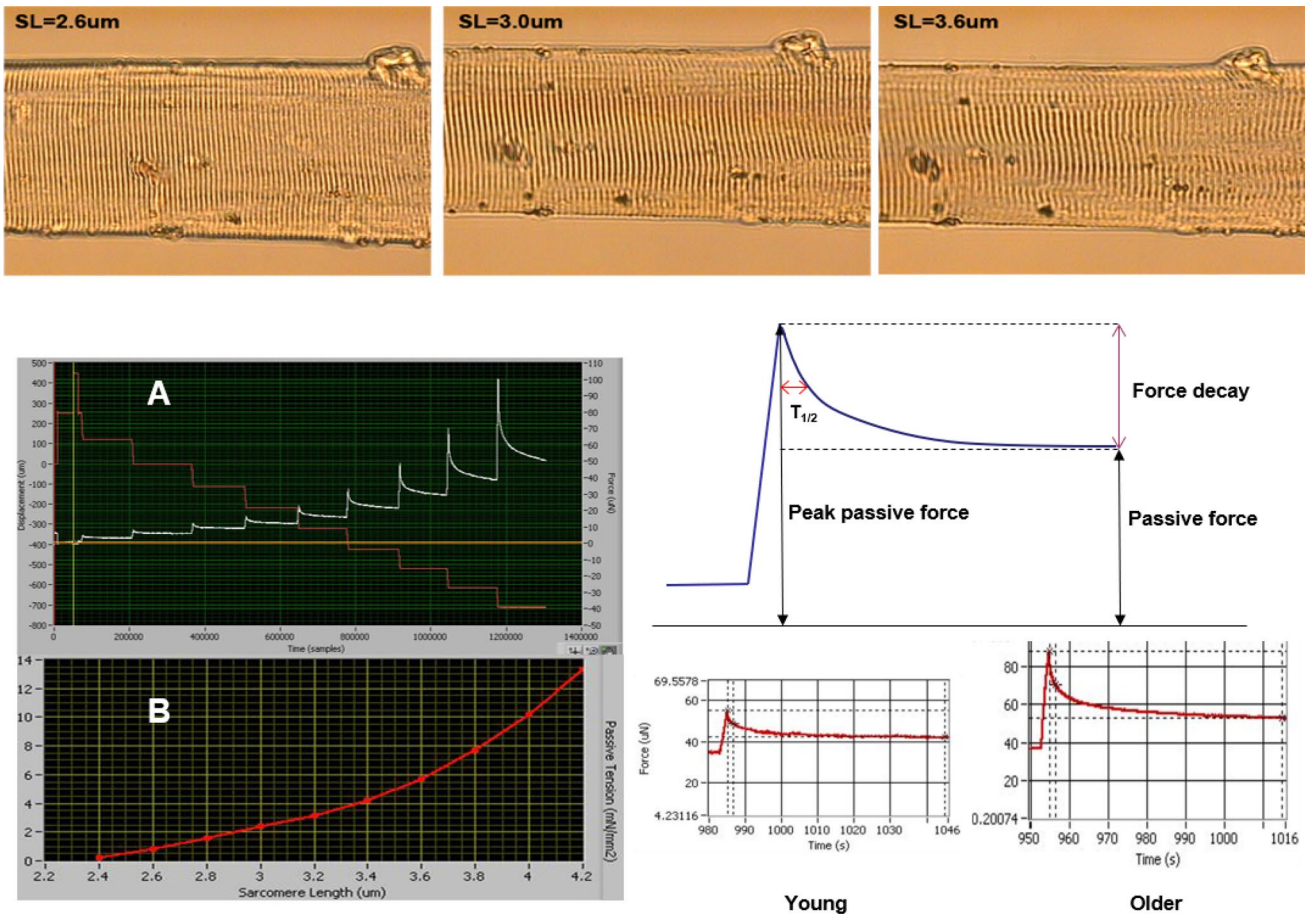

Fig. 1 Lengthening of sarcomeres by stepwise stretching of muscle fiber using the lever arm (upper images). Incremental stretches (lower left A) and corresponding curve of passive force (lower left B). Changes in passive force in one stretch and the representative graphs of a young vs. older single fiber are shown in lower right curve. Peak passive force = maximal passive force after a single stretch; $T_{1/2}$ = time to half stress relaxation, time to reach 50% of the force decay; passive force = steady state force after a single stretch; force decay = difference between maximal force and steady state passive force

(Fig. 1). The fiber was slowly stretched stepwise by constant velocity (0.1 µm/s) for each increment (Trombitas et al. 2003). Each sarcomere length during stepwise stretch was determined by stretching the fibers based on the measurement of SL at the initial fiber length. The fiber length to create the intended SL was calculated by a mathematical equation for rotational motion of the length controller to stretch the fiber (Choi et al. 2011). After each incremental step, stress relaxation (force decay at a constant length) was permitted for 1 min (Prado et al. 2005) before measuring resting (passive) force at the end of stress relaxation. Lever arm position and changes in force were recorded during stress relaxation. Passive force at the end of stretching (after ramp stretching) indicating initial modulus of stress (peak passive force), time to half stress relaxation ($T_{1/2}$), passive force at the end of stress relaxation (after 1 min) indicating steady state modulus of stress, and force decay (the difference between peak passive force and passive force at the end of stress relaxation) were measured. Fiber CSA was measured (see above) after each stretch and force was adjusted for CSA. Time to half stress relaxation ($T_{1/2}$) was defined as the time when the initial modulus (peak passive force) reached the average of the initial modulus and steady state modulus at the end of stress relaxation (passive force), which is one of parameters for viscoelastic properties of the tissues. (Nam et al. 2016) We defined a force decay index as a force time integral under a stress relaxation curve. The force decay index was calculated as the product of force decay (Fd) and time to half stress relaxation ($T_{1/2}$) using the following formula fit for the exponential decay patterns:

$$F = \mathrm{Fd} \cdot 2^{-T/T_{1/2}},$$

$$\int_0^\infty F\mathrm{d}t = \int_0^\infty \mathrm{Fd} \cdot 2^{-T/T_{1/2}} \cdot \mathrm{d}t = \mathrm{Fd} \cdot \frac{T_{1/2}}{\log 2}.$$

This index reflects the exponential decay patterns of time-dependent modulus of viscoelasticity. A higher value of force decay index indicates higher viscosity (Vincent 2012).

Active force measurements with increasing sarcomere length

A total of 91 fibers were examined to measure active force in pCa 4.5 at different sarcomere lengths from 2.4 to 4.2 µm using the same stepwise stretch as used for passive force. The changes in active force were compared to those of passive force with increasing sarcomere length.

Identification of muscle fiber type

At the end of the experiments described above, the fiber was removed from the test apparatus and stored in 30 µl of SDS sample buffer (containing 62.5 mM Tris pH 6.8, 2% SDS, 10% glycerol, 5% betamercaptoethanol, and 0.001% bromophenol blue) at – 20 °C. Myosin heavy chain composition (MHC) of each single fiber segment was determined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE). The polyacrylamide gel system consists of a 6% separating gel and a 4% stacking gel (Larsson and Moss 1993). The gels contained 30% of glycerol to improve separation of MHC isoforms. The gels were run at a voltage 140 for 6 h. A silver staining procedure was used to visualize the protein bands. MHC standards prepared from pooled muscle biopsy samples were run on each gel.

Statistical analyses

Due to the multiple measurements of single muscle fibers taken from each participant, a mixed effects model was used to analyze the hierarchically nested data. It was a generalization of analysis of variance and took into account the intrasubject clustering of the measurements. Briefly, the procedure first included muscle fiber type, age group, and fiber type × age as fixed effects and individual as a random effect. If the interaction term was not significant, it was removed, and only the main effects of fiber types and age were included in the model. Additionally, the different effects of muscle fiber types on dependent variables were separately analyzed in each age group by mixed effects model with Bonferroni post hoc correction. The alpha level was set at $p = 0.05$ and all reported p values are the result of two-sided tests. All statistical analyses were done using the SPSS software program (version 20.0) and values were expressed as means + SE.

Results

The results of the fiber type distribution, morphological, and active isometric contractile properties are presented in Table 1. Of the total 275 fibers, 152, 88 and 35 fibers were MHC I, IIa and IIx or hybrid, respectively. Since the number of IIX and hybrid fibers was too small, only MHC I and IIa fibers were included in the comparison between the fiber types. The proportion of MHC I fibers (54%) was higher in both older adults and young controls. No statistically significant differences between groups were seen in CSA, maximal force, specific force, or maximum unloaded shortening

Table 1 Morphological and contractile properties of MHC I and IIa single muscle fibers in older adults and young controls

	Fiber type	Older adults	Young control
Number of fibers (% of total)	I	86 (54.1)	66 (53.6)
	IIa	47 (29.5)	41 (33.3)
CSA (μm^2)	I	5280 ± 2130	4890 ± 1990
	IIa	4620 ± 1540	5120 ± 1790
Maximal force (μN)	I	808 ± 391	652 ± 295
	IIa	601 ± 269	763 ± 361
Specific force (N/cm^2)	I	23.1 ± 11.1	20.5 ± 7.7
	IIa	19.9 ± 9.6	23.0 ± 10
Maximum unloaded shortening velocity (V_0)	I	1.12 ± 0.62	1.01 ± 0.59
	IIa	2.26 ± 1.05*	2.45 ± 1.08*

No significant differences were noted between groups in any of the variables

*$p < 0.05$ significant differences between fiber types in both age groups

velocity. There were no statistical differences between fiber types except for significant difference in maximal shortening velocity ($p < 0.001$, $\beta = 1.36$, SE 0.17, 95% CI 1.00–1.71; $p < 0.001$, $\beta = 1.44$, SE 0.19, 95% CI 1.05–1.84, older and young groups, respectively).

The relationships between sarcomere length and peak passive force and passive force are presented in Fig. 2. A total of 208 fibers (124 MHC I and 84 MHC IIa) were used for this set of experiments. As expected, both force measurements increased with stretch increments. Both MHC I and IIa fibers from elders showed a larger increment in peak passive force and passive force ($p < 0.001$, $p = 0.012$, respectively, at 4.2 mm SL) after stretch when compared to fibers from young controls. There was no significant difference between MHC I and MHC IIa fibers in both age groups.

Figure 3 shows the results for time to half stress relaxation ($T_{1/2}$) and the force decay index at different sarcomere lengths. $T_{1/2}$ decreased with increases in sarcomere length in both groups and fiber types. There were no significant differences of $T_{1/2}$ in both age groups and fiber types except that MHC I fibers showed greater $T_{1/2}$ at 3.4 μm SL ($p = 0.001$). In general, the force decay index was higher in the older group. This difference was statistically significant ($p = 0.02$ at 3.4, $p = 0.000$ 3.8, $p = 0.001$, 4.0 and $p = 0.001$ 4.2 μm SL, respectively) at most, but not all sarcomere lengths.

The relationship between sarcomere length and force during activation (active force) and stretching without activation (passive force) is shown in Fig. 4. Active force continued to decline with increases in sarcomere length in both age groups and fiber types. There were no statistical differences between age groups and fiber types in active force.

Discussion

In this study, we compared the passive mechanical properties of chemically skinned single muscle fibers from healthy young and older humans. The main finding of this study is

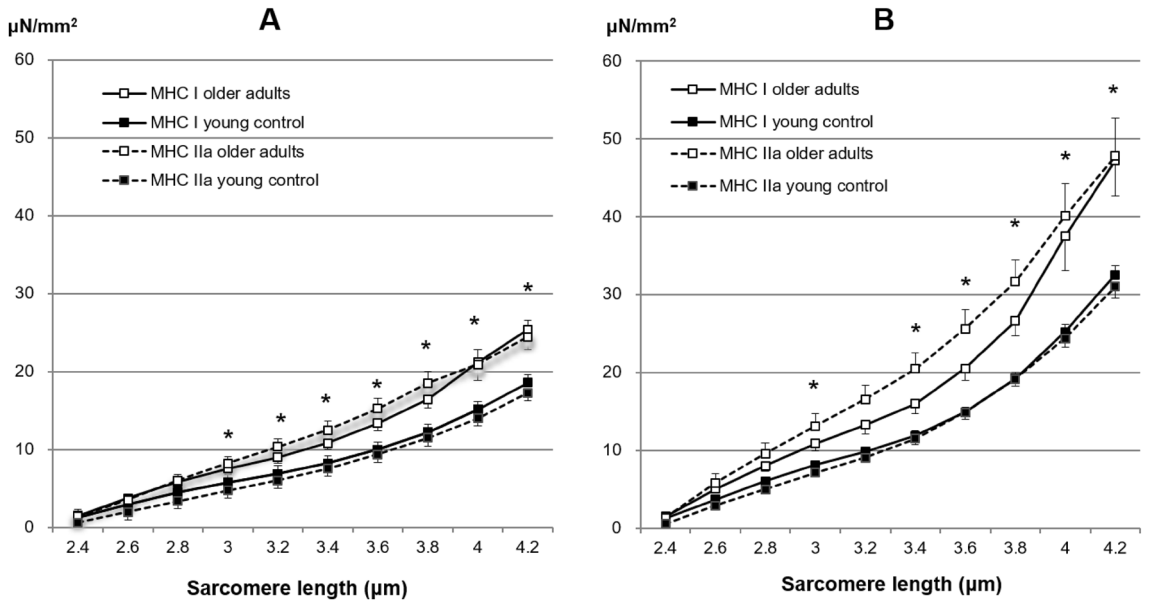

Fig. 2 The length–force curve for passive force (**a**) and peak passive force (**b**) for type I and IIa fibers in older adults and young controls. All data are adjusted for fiber cross-sectional area. *$p < 0.05$, statistical difference between older adults and young controls by mixed effects model with Bonferroni post hoc correction. †$p < 0.05$, statistical difference between MHC I and IIa fiber by mixed effects model with Bonferroni post hoc correction

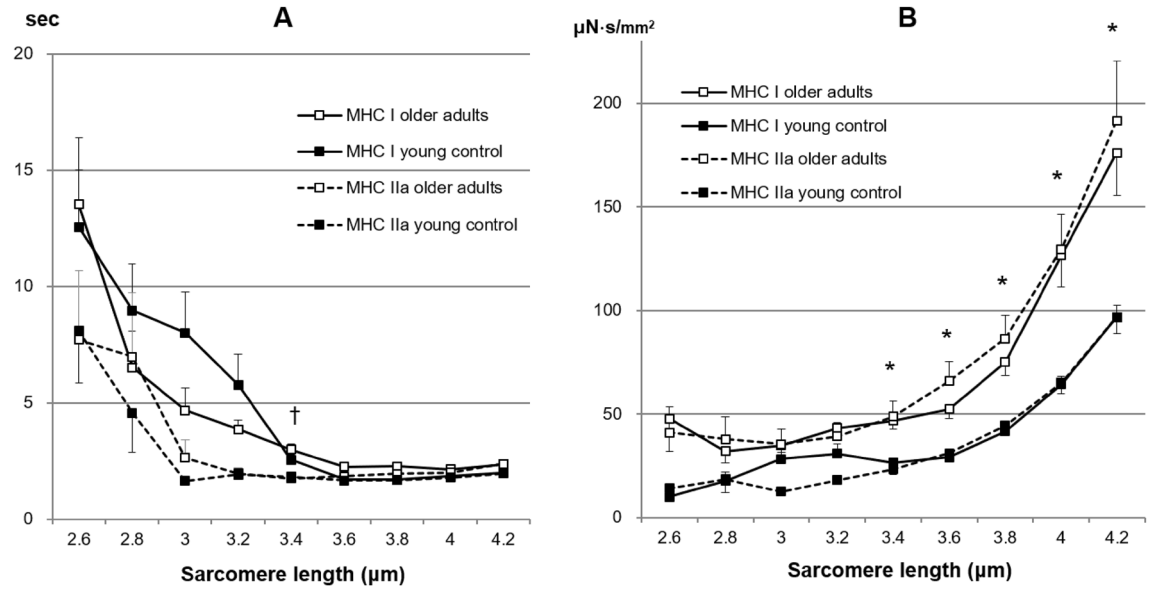

Fig. 3 Half relaxation time (**a**) and force decay index (**b**) as a function of sarcomere length in MHC I and IIa fibers in older adults and young controls. $*p < 0.05$, statistical difference between older adults and young controls by mixed effects model with Bonferroni post hoc correction. $^{\dagger}p < 0.05$, statistical difference between MHC I and IIa fiber by mixed effects model with Bonferroni post hoc correction

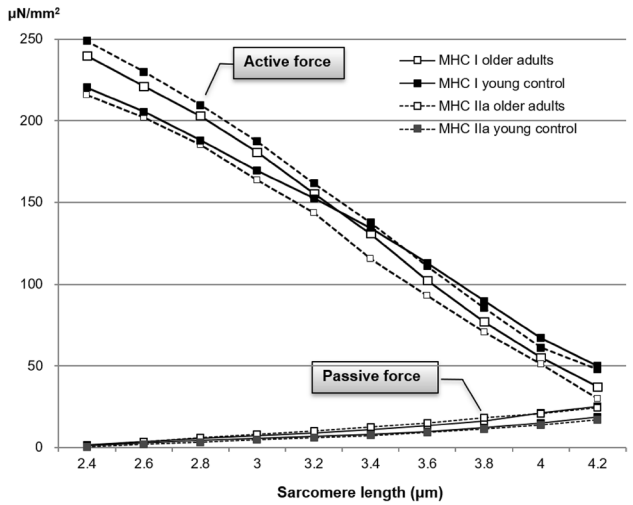

Fig. 4 Relationship between sarcomere length and force during activation (active force) and stretching without activation (passive force at the end of stress relaxation) in MHC I and IIa fibers in older adults and young controls. No statistical difference between aging and fiber types in active force by mixed effects model

the greater passive force and increased viscoelastic function in isolated single muscle fibers in older adults compared to younger participants; these findings were similar in both MHC I and IIa fibers. On the other hand, no differences were seen in passive force and viscoelastic properties between fiber types in both age groups. Active specific force obtained at each sarcomere length declined as the sarcomere length increased. Our results suggest that intracellular changes contribute to increased passive force with viscoelasticity in aging skeletal muscles. These intracellular changes may, in combination with the previously reported changes in the extracellular matrix and connective tissue, may contribute to an age-related increase in muscle stiffness.

Stiffer muscles have been recognized as a typical age-related alteration of the musculoskeletal system (Brown et al. 1999). However, the effect of age on passive mechanical properties is still controversial. For example, some studies have shown that passive stiffness increases with age (Blanpied and Smidt 1993; Gajdosik et al. 2005; Gao et al. 2008; Palmer and Thompson 2017) but other studies have shown no change (Brown et al. 1999; Chesworth and Vandervoort 1989; Winegard et al. 1997). These conflicting results may be due to the fact that various factors determine muscle stiffness and elasticity in elderly including reduced range of motion, reduced muscle mass, shortened muscle length, decreased contractile force, increased extracellular matrix, and poor muscle quality (i.e., intramuscular fat infiltration) (Brown et al. 1999; Canon and Goubel 1995; Gajdosik et al. 1999). One study reported that passive stiffness did not increase with age, but muscle mass decreased; therefore, the remaining muscle tissue was likely to be stiffer per gram of tissue (Brown et al. 1999). In another study, decreased muscle quality, as indicated by increases in ultrasonographic echo intensity was suggested to contribute to the greater passive stiffness observed in older adults (Palmer and Thompson 2017). To investigate the intramuscular change of passive stiffness, in vitro muscle fiber studies are useful because the contribution of extra-muscular factors such as

muscle–tendon unit and connective tissues can be eliminated. In vitro studies using whole muscles or muscle fibers have been reported to evaluate the age-related differences in passive force and elasticity (Brown et al. 1999; Gao et al. 2008; Wood et al. 2014), but to our knowledge, no studies using human single muscle fibers have been reported. The results of our study provide evidence of age-related changes in passive force and viscoelasticity at the muscle cell level that are independent of changes in the extracelluar matrix.

Increased stiffness of skeletal muscle extracelluar matrix could be a major mechanism responsible for stiffer muscles with advanced adult age. Age-associated increases in stiffness of extracelluar matrix were observed during direct in vitro biomechanical testing by uniaxial extension of the epimysium of rat tibialis anterior (Gao et al. 2008). Wood et al. reported that the extracellular matrix of tibialis anterior muscles from old mice had a higher intrinsic stiffness than that of adult muscles, but the intrinsic stiffness of individual muscle fibers from adult and old muscles were not different (Wood et al. 2014). Their results in muscle fibers are in conflict with our findings of greater passive force in aging single muscle fibers. However, direct comparison of the results may not be possible due to different species and muscles and different strain–stress protocols used. On the other hand, observations in human spastic muscle support our findings (Friden and Lieber 2003; Olsson et al. 2006). The global passive muscle stiffening in patients with spasticity is partially caused by elevated passive tension of the skeletal muscles themselves. In one study of muscle biopsies from patients with chronic spasticity, increased passive tension was shown to arise not only from extracellular matrix remodeling, but also from structural and functional adaptations inside the muscle cells (Olsson et al. 2006).

Viscoelastic properties of human skeletal muscle have been investigated through the viscoelastic stress relaxation (force decay at a constant length) during static stretch (Magnusson 1998) and can be expressed as a percentage of peak force [(peak force − steady state force)/peak force)]. Ideal pure elastic function indicates both minimal force decay and quick return to steady state (Weppler and Magnusson 2010). We developed a force decay index (a force time integral under a stress relaxation curve) to reflect the exponential decay patterns of time-dependent modulus of stress relaxation. We acknowledge there are several models that can be used to determine viscoelasticity (Meyer et al. 2011; Minajeva et al. 2001). Stress relaxation fits by Minajeva et al. (2001) is also an appropriate model for viscoelastic properties. However, these models cannot be applied directly to our stress relaxation data due to differences in test parameters used in our model. Force decay index in our study seems to reflect well the exponential decay patterns of time-dependent modulus of stress. This index includes changes of both viscosity and elasticity. A higher force decay index indicates higher viscosity. On the other hand, a lower force decay index and higher steady state force means high elastic modulus, that is, good muscle spring (George et al. 2013; Linke 2000). In the present study, the force decay index of older muscle fibers was significantly greater than in the younger group when the fibers were stretched to 3.4 μm of sarcomere length or longer. These findings indicate an increased viscoelasticity with aging which may be due to an increased proportion of non-elastic and non-contractile elements such as collagen depositions, amorphous materials, or intramyocellular lipid droplets (Olsson et al. 2006). The current skinning procedure used in our study allows the study of the intrinsic ability of the actomyosin cross-bridges in the absence of nervous influence by disrupting all membrane-bound structures such as sarcolemma and sarcoplasmic reticulum without damaging the myofilament structure (Larsson and Salviati 1992). However, it is not very clear how, or to what extent, other cellular elements and the structural (not contractile) proteins are also affected.

Although we did not measure titin concentrations, it is necessary to briefly comment on its potential role in stiffness. Titin has a functional role because sarcomere elongation with less stiffness and enough elasticity may be necessary for proper contraction. Decreased passive elasticity due to titin and increased non-titin-based stiffness may explain age-related changes in passive force and viscoelasticity at the muscle cell level. Increased proportion of non- or less-extensile structures such as thin, thick and intermediate filaments in aging muscles indicates poor quality of elastic function (Prado et al. 2005). A difference in passive stiffness among muscle fiber types has been suggested based on the findings that predominantly slow muscles have long titin and low titin-borne passive stiffness, whereas fast muscles have relatively short titin and high titin-based stiffness (Prado et al. 2005). Therefore, titin-based stiffness, but not extramyofibrillar stiffness, correlates with muscle type. Olsson et al. reported that the elevated passive tension was found only in fast-type fibers of chronic spastic muscles, but there were no differences in passive tension among fiber types in healthy athletes (Olsson et al. 2006). In our study, there were no significant differences in passive force and force decay index between MHC I and MHC IIa in both young and older group. These findings suggest that non-titin-based stiffness, which is independent of fiber type, may significantly affect the stiffness of aging muscle. Taken together, the effect of age-related changes of titin-based and non-titin-based stiffness on intracellular stiffness and elasticity are still unclear. Furthermore, we need to better understand the influence of titin on the contractility of muscle fibers in aging skeletal muscles.

This study has several limitations. First, the protocol to develop passive force used in the present study was modified from the fiber stretch protocol of previous studies

(Olsson et al. 2006; Prado et al. 2005; Toursel et al. 2002; Trombitas et al. 2003). Each sarcomere length during stepwise stretch was determined by stretching the fibers based on the measurement of SL at the initial fiber length. It is not guaranteed that the change of fiber length always matches that of sarcomere length because sarcomere length is not uniform over the entire length of the fibers mounted between the connectors. The fiber could have been stretched more than intended during stretching protocols perhaps because the length controller rotates to alter fiber length, even though a mathematical model was used to minimize the potential errors by the rotational motion during stretching (Choi et al. 2011). Second, in our study, the active specific force declined starting at a SL of 2.4 µm. This finding is in conflict with those of other studies indicating that the force plateau should extend to a SL of ~2.8 µm (Gollapudi and Lin 2009; Maganaris 2001). In the present study, active forces at different sarcomere lengths were obtained in the same fibers with sequential sarcomere lengths from 2.4 to 4.2 µm. It is possible that this could cause fiber damage due to repeated stretches and contractions. This may have resulted in a slightly lower value for active force between 2.6 µm and 2.8. In addition, in our experiments, we did not monitor sarcomere length in real time and the degree of slacking was determined by mathematical equations based on the initial fiber length. Therefore, it is possible that sarcomere length was not exactly 2.4 or 2.6 µm after slacking. Third, we examined 275 fibers and 251 fibers for active and passive force measurement, respectively, which roughly corresponds to 15–16 fibers per participant. The number of fibers, although similar to that of several other published studies, may not be enough to represent the behavior of all fibers in the vastus lateralis muscle. The differences in mechanical properties of single muscle fibers between males and females need to be evaluated. Our sample size was too small to allow us to study sex-related differences. Men showed greater muscle strength, power and stiffness compared to women in in vivo studies, but single muscle fiber quality in women was reported to be equivalent to that of men (Krivickas et al. 2006). Lastly, we excluded individuals with physical impairments using the SPPB in the older group and the IPAQ in the younger group. Our intent was to show that participants in the study were healthy and functional. However, we did not quantify specifically the levels of physical activity in both groups using the same instrument. It needs to be recognized that others have suggested that physical activity can influence muscle fiber mechanical properties (Canepari et al. 2010).

In future investigations, the correlation of age-related changes in titin and extramyofibrillar contents with viscoelastic and elastic properties of single muscle fibers and the role of titin in age-related changes of skeletal muscles needs to be studied. This knowledge may help us understand the vulnerability of aging muscles to injury, disuse, and chronic disease.

Conclusions

We demonstrated the greater passive force and increased viscoelastic function in isolated single muscle fibers in older individuals in comparison to younger individuals. There were no significant differences in passive force and viscoelastic properties between fiber types. The results of our study provide evidence of age-related changes in passive force and viscoelasticity at the muscle cell level that are independent of changes in the extracelluar matrix.

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Author contributions FWR and LJY designed and coordinated the study. LJY and PEM collected the clinical samples and clinical data. LJY and CSJ performed the laboratory work and analyzed the data. WJJ and FWR were involved in the planning and supervised the work. All the authors discussed the results and contributed to the manuscript.

Compliance with ethical standards

Conflict of interest The author(s) declare that they have no competing interests.

References

Blanpied P, Smidt GL (1993) The difference in stiffness of the active plantarflexors between young and elderly human females. J Gerontol 48(2):M58–M63

Brown M, Fisher JS, Salsich G (1999) Stiffness and muscle function with age and reduced muscle use. J Orthop Res 17(3):409–414. https://doi.org/10.1002/jor.1100170317

Candow DG, Chilibeck PD (2005) Differences in size, strength, and power of upper and lower body muscle groups in young and older men. J Gerontol A Biol Sci Med Sci 60(2):148–156

Canepari M, Pellegrino MA, D'Antona G, Bottinelli R (2010) Single muscle fiber properties in aging and disuse. Scand J Med Sci Sports 20(1):10–19. https://doi.org/10.1111/j.1600-0838.2009.00965.x

Canon F, Goubel F (1995) Changes in stiffness induced by hindlimb suspension in rat soleus muscle. Pflugers Arch 429(3):332–337

Chesworth BM, Vandervoort AA (1989) Age and passive ankle stiffness in healthy women. Phys Ther 69(3):217–224

Choi SJ, Kim S, Lim JY (2011) Potential errors in fiber length measurements resulting from lever arm rotation during mechanical testing of muscle cells. J Biomech 44(9):1797–1800. https://doi.org/10.1016/j.jbiomech.2011.03.027

Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, Oja P (2003) International physical activity

questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 35(8):1381–1395. https://doi.org/10.1249/01.MSS.0000078924.61453.FB

Dow DE, Dennis RG, Faulkner JA (2005) Electrical stimulation attenuates denervation and age-related atrophy in extensor digitorum longus muscles of old rats. J Gerontol A Biol Sci Med Sci 60(4):416–424

Friden J, Lieber RL (2003) Spastic muscle cells are shorter and stiffer than normal cells. Muscle Nerve 27(2):157–164. https://doi.org/10.1002/mus.10247

Frontera WR, Hughes VA, Krivickas LS, Roubenoff R (2001) Contractile properties of aging skeletal muscle. Int J Sport Nutr Exerc Metab 11(Suppl):S16–20

Frontera WR, Reid KF, Phillips EM, Krivickas LS, Hughes VA, Roubenoff R, Fielding RA (2008) Muscle fiber size and function in elderly humans: a longitudinal study. J Appl Physiol 105(2):637–642. https://doi.org/10.1152/japplphysiol.90332.2008

Gajdosik RL, Vander Linden DW, Williams AK (1999) Influence of age on length and passive elastic stiffness characteristics of the calf muscle-tendon unit of women. Phys Ther 79(9):827–838

Gajdosik RL, Vander Linden DW, McNair PJ, Riggin TJ, Albertson JS, Mattick DJ, Wegley JC (2005) Viscoelastic properties of short calf muscle-tendon units of older women: effects of slow and fast passive dorsiflexion stretches in vivo. Eur J Appl Physiol 95(2–3):131–139. https://doi.org/10.1007/s00421-005-1394-4

Gao Y, Kostrominova TY, Faulkner JA, Wineman AS (2008) Age-related changes in the mechanical properties of the epimysium in skeletal muscles of rats. J Biomech 41(2):465–469. https://doi.org/10.1016/j.jbiomech.2007.09.021

George NT, Irving TC, Williams CD, Daniel TL (2013) The cross-bridge spring: can cool muscles store elastic energy? Science 340(6137):1217–1220. https://doi.org/10.1126/science.1229573

Gollapudi SK, Lin DC (2009) Experimental determination of sarcomere force-length relationship in type-I human skeletal muscle fibers. J Biomech 42(13):2011–2016. https://doi.org/10.1016/j.jbiomech.2009.06.013

Granzier H, Labeit S (2007) Structure-function relations of the giant elastic protein titin in striated and smooth muscle cells. Muscle Nerve 36(6):740–755. https://doi.org/10.1002/mus.20886

Grosicki GJ, Standley RA, Murach KA, Raue U, Minchev K, Coen PM, Health ABCS (2016) Improved single muscle fiber quality in the oldest-old. J Appl Physiol (1985) 121(4):878–884. https://doi.org/10.1152/japplphysiol.00479.2016

Guralnik JM, Ferrucci L, Pieper CF, Leveille SG, Markides KS, Ostir GV, Wallace RB (2000) Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery. J Gerontol A Biol Sci Med Sci 55(4):M221–231

Horowits R (1992) Passive force generation and titin isoforms in mammalian skeletal muscle. Biophys J 61(2):392–398. https://doi.org/10.1016/S0006-3495(92)81845-3

Krivickas LS, Suh D, Wilkins J, Hughes VA, Roubenoff R, Frontera WR (2001) Age- and gender-related differences in maximum shortening velocity of skeletal muscle fibers. Am J Phys Med Rehabil 80(6):447–455 **(quiz 456–447)**

Krivickas LS, Fielding RA, Murray A, Callahan D, Johansson A, Dorer DJ, Frontera WR (2006) Sex differences in single muscle fiber power in older adults. Med Sci Sports Exerc 38(1):57–63

Larsson L, Moss RL (1993) Maximum velocity of shortening in relation to myosin isoform composition in single fibres from human skeletal muscles. J Physiol 472:595–614

Larsson L, Salviati G (1992) A technique for studies of the contractile apparatus in single human muscle fibre segments obtained by percutaneous biopsy. Acta Physiol Scand 146(4):485–495. https://doi.org/10.1111/j.1748-1716.1992.tb09450.x

Linke WA (2000) Stretching molecular springs: elasticity of titin filaments in vertebrate striated muscle. Histol Histopathol 15(3):799–811. https://doi.org/10.14670/HH-15.799

Maganaris CN (2001) Force-length characteristics of in vivo human skeletal muscle. Acta Physiol Scand 172(4):279–285. https://doi.org/10.1046/j.1365-201x.2001.00799.x

Magnusson SP (1998) Passive properties of human skeletal muscle during stretch maneuvers. A review. Scand J Med Sci Sports 8(2):65–77

Meyer GA, McCulloch AD, Lieber RL (2011) A nonlinear model of passive muscle viscosity. J Biomech Eng 133(9):091007. https://doi.org/10.1115/1.4004993

Minajeva A, Kulke M, Fernandez JM, Linke WA (2001) Unfolding of titin domains explains the viscoelastic behavior of skeletal myofibrils. Biophys J 80(3):1442–1451. https://doi.org/10.1016/S0006-3495(01)76116-4

Moss RL (1979) Sarcomere length-tension relations of frog skinned muscle fibres during calcium activation at short lengths. J Physiol 292:177–192

Nam S, Hu KH, Butte MJ, Chaudhuri O (2016) Strain-enhanced stress relaxation impacts nonlinear elasticity in collagen gels. Proc Natl Acad Sci USA 113(20):5492–5497. https://doi.org/10.1073/pnas.1523906113

Ochala J, Frontera WR, Dorer DJ, Van Hoecke J, Krivickas LS (2007) Single skeletal muscle fiber elastic and contractile characteristics in young and older men. J Gerontol A Biol Sci Med Sci 62(4):375–381

Olsson MC, Kruger M, Meyer LH, Ahnlund L, Gransberg L, Linke WA, Larsson L (2006) Fibre type-specific increase in passive muscle tension in spinal cord-injured subjects with spasticity. J Physiol 577(Pt 1):339–352. https://doi.org/10.1113/jphysiol.2006.116749

Palmer TB, Thompson BJ (2017) Influence of age on passive stiffness and size, quality, and strength characteristics. Muscle Nerve 55(3):305–315. https://doi.org/10.1002/mus.25231

Payne AM, Delbono O (2004) Neurogenesis of excitation-contraction uncoupling in aging skeletal muscle. Exerc Sport Sci Rev 32(1):36–40

Prado LG, Makarenko I, Andresen C, Kruger M, Opitz CA, Linke WA (2005) Isoform diversity of giant proteins in relation to passive and active contractile properties of rabbit skeletal muscles. J Gen Physiol 126(5):461–480. https://doi.org/10.1085/jgp.200509364

Siu PM, Pistilli EE, Butler DC, Alway SE (2005) Aging influences cellular and molecular responses of apoptosis to skeletal muscle unloading. Am J Physiol Cell Physiol 288(2):C338–349. https://doi.org/10.1152/ajpcell.00239.2004

Siu PM, Pistilli EE, Murlasits Z, Alway SE (2006) Hindlimb unloading increases muscle content of cytosolic but not nuclear Id2 and p53 proteins in young adult and aged rats. J Appl Physiol 100(3):907–916. https://doi.org/10.1152/japplphysiol.01012.2005

Toursel T, Stevens L, Granzier H, Mounier Y (2002) Passive tension of rat skeletal soleus muscle fibers: effects of unloading conditions. J Appl Physiol 92(4):1465–1472. https://doi.org/10.1152/japplphysiol.00621.2001

Trombitas K, Wu Y, McNabb M, Greaser M, Kellermayer MS, Labeit S, Granzier H (2003) Molecular basis of passive stress relaxation in human soleus fibers: assessment of the role of immunoglobulin-like domain unfolding. Biophys J 85(5):3142–3153. https://doi.org/10.1016/S0006-3495(03)74732-8

Vincent J (2012) Structural biomaterials, 3rd edn. Princeton University Press, Princeton

Walston J, Fried LP (1999) Frailty and the older man. Med Clin N Am 83(5):1173–1194

Weppler CH, Magnusson SP (2010) Increasing muscle extensibility: a matter of increasing length or modifying sensation? Phys Ther 90(3):438–449. https://doi.org/10.2522/ptj.20090012

Winegard KJ, Hicks AL, Vandervoort AA (1997) An evaluation of the length-tension relationship in elderly human plantarflexor muscles. J Gerontol A Biol Sci Med Sci 52(6):B337–343

Wood LK, Kayupov E, Gumucio JP, Mendias CL, Claflin DR, Brooks SV (2014) Intrinsic stiffness of extracellular matrix increases with age in skeletal muscles of mice. J Appl Physiol (1985) 117(4):363–369. https://doi.org/10.1152/japplphysiol.00256.2014