Single Skeletal Muscle Fiber Elastic and Contractile Characteristics in Young and Older Men

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The current investigation was designed to: (a) assess the impact of aging on elastic characteristics of single skeletal muscle fibers from young ($N = 6$) and older men ($N = 6$); and (b) correlate the potential changes, with the fiber contractile properties. Chemically skinned single muscle fibers ($n = 235$) from vastus lateralis muscle were maximally activated. Maximal force and cross-sectional area were measured, and specific force calculated. The slack test was used to measure maximal unloaded shortening velocity. A quick release of 0.15% of fiber length was applied to determine instantaneous stiffness. The myosin heavy chain isoform composition of each single fiber was determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Aging induces changes in both fiber elasticity (i.e., increased instantaneous stiffness) and contractility (i.e., reduced specific force and unloaded shortening velocity) in type I and IIa fibers. However, the changes in fiber stiffness may not directly influence contractile characteristics alterations.

SARCOPENIA is a term referring to a loss of skeletal muscle mass and strength in older humans [for review, see (1)]. Physiological studies of single muscle fibers have shown impairments in the contractile properties that may explain, in part, why older individuals have muscle weakness. More specifically, an aging-related decline in the ability of single muscle fibers to generate force independent of changes in fiber size has been reported (2,3). Moreover, single muscle fiber maximum unloaded shortening velocity (V_0) has also been reported to decrease with aging in human skeletal muscle (3–5). Taken together, these findings suggest that the intrinsic contractile characteristics related to cross-bridge mechanics of single fibers are altered with aging.

Although the intrinsic contractile properties have been shown to decline, there have been no reports characterizing single muscle fiber elastic properties in humans with aging. The force responses following stepwise length changes have been used to provide information on isolated muscle fiber elasticity in frogs (6–8), rats and rabbits (9–11), and young humans (12). Applying a length step release to a fiber contracting isometrically causes an immediate, synchronous drop in force (phase 1) followed by a kinetically controlled return to the initial isometric force (Figure 1). Most of the return to isometric force occurs rapidly during phase 2. Elastic properties are commonly quantified in terms of instantaneous stiffness (i.e., ratio between the force change during phase 1 and the corresponding length change) or compliance (i.e., ratio between the length change and the corresponding force change during phase 1). Therefore, the first aim of the present study was to evaluate the impact of aging on elastic properties by quantifying the instantaneous

stiffness of single muscle fibers from young and older humans. Knowing fiber elastic behavior with aging may lead to a better understanding of the mechanical correlates of sarcopenia. Based on studies of whole muscle (13–16), it can be hypothesized that an aging-related increase in single muscle fiber instantaneous stiffness occurs.

The second aim of the present study was to correlate the aging-related potential changes in fiber elastic properties with fiber contractility as measured using the slack-test procedure, that is, V_0 (17) and during the quick release method, that is, half-time ($t_{1/2}$) of phase 2 force recovery (18). Taking into account that fiber instantaneous stiffness may increase with aging, it can be hypothesized that it influences contractility during the force-generating process following length steps by diminishing V_0 and increasing $t_{1/2}$ of phase 2 force recovery.

MATERIALS AND METHODS

Participants

A total of 12 healthy men were enrolled in the study (young men [YM]: $N = 6$, age range: 25–36 years, mean age: 31.6 years; older men [OM]: $N = 6$, age range: 60–74 years, mean age: 66.1 years), following a physical examination and an interview to document life history of physical activity. Participants who had a history of any acute or chronic illness (neuromuscular disorder, heart disease, hypertension, hyperlipidemia, arthritis, and/or diabetes) or any medication affecting muscle anatomy and function were excluded. The six young men who were enrolled were not involved in any formal training or competitive sports
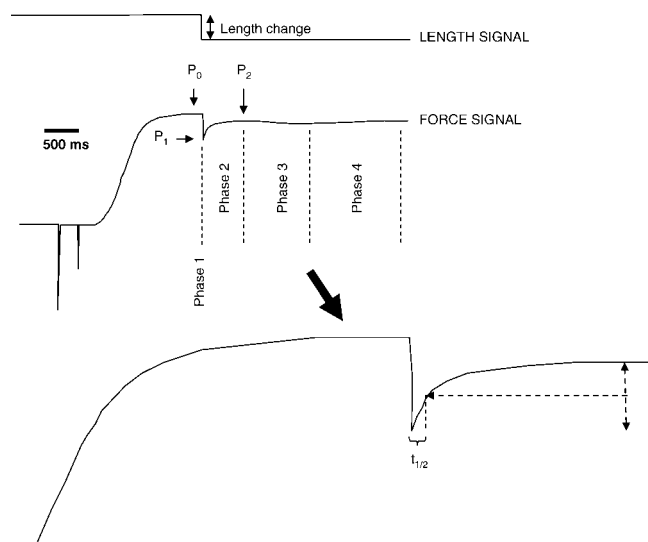
Figure 1. Original recording from a quick-release experiment. When a quick release in length is applied to isometrically activated fiber, force responses can usually be divided into different phases. During the initial quick shortening step (phase 1), the force decreases from the isometric plateau value (P_0) to a minimum value termed P_1. Successively, the force partially recovers (phase 2). Phase 2 is followed by an interruption of the force recovery (phase 3). Finally, a recovery of force (phase 4) takes place, which is considerably slower than that of phase 2. However, in the present study, phases 3 and 4 were difficult to distinguish. During phase 1, the instantaneous stiffness (K) was calculated as the ratio between the force change ($P_0 - P_1$) and the corresponding sarcomere length change (0.15% of fiber length corresponding to an average change of 2 nm per half-sarcomere). During phase 2, the half-time ($t_{1/2}$) of force increase was recorded.

activities; the six older participants had never completed any regular training or exercise activity outside of their activities of daily living (current average walking activity outside home < 1 h/d). The participants received a complete explanation of the purposes and procedures and gave their written consent. A local ethics committee approved the protocol, and the experiments were carried out according to the guidelines of the Declaration of Helsinki.

Muscle Biopsies and Permeabilization of Fibers

Biopsy specimens were obtained percutaneously from muscle vastus lateralis under local anesthesia using Bergstrom needles (19) and suction (20). The specimens were placed in relaxing solution at 4°C. Bundles of ~30-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 hours 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.

Experimental Procedure

On the day of an experiment, fiber segments were placed for 30 minutes in relaxing solution containing 0.5% Brij-58 (polyoxyethylene 20 cetyl ether; Sigma Chemicals, St. Louis, MO) prior to mounting in an experimental apparatus, similar to the one described previously by Moss (21). A fiber segment length of 1.3 ± 0.17 mm was left exposed to the

solution between connectors leading to a force transducer (model 400A; Aurora Scientific, Aurora, Ontario, Canada) and a lever arm system (model 308B; Aurora Scientific). The total compliance of the attachment system was carefully controlled and remained similar for all the single muscle fibers tested (5% ± 0.5). The apparatus was mounted on the stage of an inverted microscope (model IX70; Olympus, Tokyo, Japan). While the fiber segments were in relaxing solution, sarcomere length was set to 2.75–2.85 μm by adjusting the overall segment length. The segments were observed through the microscope at a magnification of ×320.

The sarcomere length, the segment diameter, and the length of fiber segment between the connectors were measured with an image analysis system before the mechanical experiments (Image-Pro Plus; Media Cybernetics, Silver Spring, MD). The sarcomere length was also determined during and after the experiments. If the sarcomere length of a single muscle fiber changed during or after the experiments (> 1% of sarcomere length), the data of this single fiber were not analyzed. Fiber depth was measured by recording the vertical displacement of the microscope nosepiece while focusing on the top and bottom surfaces of the fiber. The focusing control of the microscope was used as a micrometer. Fiber cross-sectional area (CSA) was calculated from the diameter and depth, assuming an elliptical circumference, and was corrected for the 20% swelling that is known to occur during skinning (21).

Relaxing and activating solutions contained (in mM): 4 Mg-ATP, 1 free Mg^{2+}, 20 imidazole, 7 EGTA, 14.5 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 solution) and $10^{-4.5}$ M (maximum activating solution), and were expressed as pCa (i.e., $-\log [Ca^{2+}]$). Apparent stability constants for Ca^{2+}-EGTA were corrected for temperature (15°C) and ionic strength (180 mM). The computer program of Fabiato (22) was used to calculate the concentrations of each metal, ligand, and metal–ligand complex.

Immediately preceding each activation, the fiber was immersed for 10–20 seconds in a solution with a reduced Ca^{2+}-EGTA buffering capacity. This solution is identical to the relaxing solution except that EGTA is reduced to 0.5 mM, which results in a faster attainment of steady force during subsequent activation. Maximal isometric force was calculated as the difference between the total force in activating solution (pCa 4.5) and the resting force measured in the same segment while in the relaxing solution. Maximal force (P_0) was adjusted for CSA and called specific force (SF). All mechanical measurements were carried out at 15°C.

Mechanical Experiments

For each fiber segment, the quick release experiment (18) and the slack-test procedure (17) were applied. A total of 301 fibers were tested and 235 were further analyzed (66 fibers were rejected because they were damaged or did not meet the inclusion criteria during or after the experiment).

Table 1. Contractile Characteristics of Single Muscle Fibers From Young (YM) and Older Men (OM)

Parameters	Type I					Type IIa				
	YM	OM	b-SD	w-SD	p Value	YM	OM	b-SD	w-SD	p Value
N	62	56				55	62			
CSA (μm^2)	3184.52	3560.974	573.48	1275.91	.380	3655.09	3435.77	485.66	945.24	.525
SF (N · cm^{-2})	165.1	12.38	1.51	4.13	.005	20.88	13.89	1.67	5.12	.001
V_0 (Fl · s^{-1})	0.91	0.70	0.14	0.21	.043	1.66	1.30	0.22	0.29	.027
$t_{1/2}$ (ms)	246.11	234.52	38.70	81.73	.679	87.54	109.68	11.15	31.45	.030

Notes: The values in columns 1 and 2 are weighted means that account for the fact that an unequal number of measurements was made on each participant. All data are expressed as mean.

b-*SD* = standard deviation between individuals; w-*SD* = standard deviation within individuals; *n* = number of single fibers; CSA = cross-sectional area; SF = specific force; V_0 = maximal unloaded shortening velocity; $t_{1/2}$ = half time of phase 2 force recovery following a quick release of approximately 0.15% of fiber length (Fl).

Quick release experiment.—After steady force was reached, a release of approximately 0.15% of fiber length (corresponding to an average change of 2 nm per half-sarcomere) was applied rapidly. As shown in Figure 1, when a quick release in length is applied to an isometrically activated fiber, force responses can usually be divided into different phases (18). During the initial quick shortening step (phase 1), the force decreases from the isometric plateau value (P_0) to a minimum value termed P_1. Successively, the force partially recovers (phase 2) to a value known as P_2. Phase 2 is followed by an interruption of the force recovery (phase 3). Finally, a recovery of force (phase 4) takes place, which is considerably slower than that of phase 2.

The instantaneous stiffness (K) of activated muscle fiber was then calculated as the ratio between the force change ($P_0 - P_1$) and the corresponding sarcomere length change (6) (Figure 1). The unit for instantaneous stiffness was [N · cm^{-2} · (nm per half sarcomere length)$^{-1}$] so [N · cm^{-2} · nm/HSL^{-1}] (10). The $t_{1/2}$ of force increase during the quick recovery of phase 2 after the release was also recorded (18) (Figure 1).

Slack test.—After steady force had been reached, seven slacks of various amplitudes were rapidly introduced at one end of the fiber. Slacks were applied at different amplitudes ranging from 7% to 13% of fiber length. Between slacks the fiber was re-extended while relaxed to minimize changes in sarcomere length (23). During the slack test, the time required to take up the imposed slack was measured from the onset of the length step to the beginning of the force redevelopment. A straight line was fitted to a plot of slack length versus time, using least-squares regression. The slope

of the line divided by the fiber segment length was recorded as V_0 for that fiber segment.

It should be noticed that the results of the slack test procedure and the quick release experiment can be influenced by a variety of experimental parameters which have to be controlled: (a) the compliance of the system; (b) the resting sarcomere length; (c) the composition of the bath solutions if skinned muscle fibers are used (temperature, adenosine triphosphate concentration [ATP], pH). By controlling all these experimental parameters, the results are reproducible (24).

Myosin Heavy Chain Composition

After mechanical measurements, each fiber was placed in sodium dodecyl sulfate sample buffer in a plastic microcentrifuge tube and stored at −20°C for up to 1 week (or at −80°C if the gels were to be run later). The myosin heavy chain (MyHC) composition of single fibers was determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The acrylamide concentration was 4% (wt/vol) in the stacking gel and 7% in the running gel, and the gel matrix included 30% glycerol. Sample loads were kept small (equivalent to ∼0.05 mm of fiber segment) to improve the resolution of the MyHC bands (types I, IIa, and IIx). Proteins were identified using a combination of purified human myosins from vastus lateralis muscles.

Statistical Analysis

Because multiple fibers from each man were studied, mixed linear models were used to analyze the data. In Tables 1 and 2 are the weighted means for young and older men. The *p* value is for the difference between groups and a random intercept for each man. The standard deviations

Table 2. Elastic Characteristics of Single Muscle Fibers From Young (YM) and Older Men (OM)

Parameters	Type I					Type IIa				
	YM	OM	b-SD	w-SD	p Value	YM	OM	b-SD	w-SD	p Value
N	62	56				55	62			
K (N · cm^{-2} · nm/HSL^{-1})	2.58	2.68	0.17	0.77	.564	2.95	2.44	0.39	0.67	.084
K/SF (nm/HSL^{-1})	0.16	0.21	0.01	0.03	.0001	0.15	0.18	0.02	0.03	.013

Notes: The values in columns 1 and 2 are weighted means that account for the fact that an unequal number of measurements was made on each participant. All data are expressed as mean.

b-*SD* = standard deviation between individuals; w-*SD* = standard deviation within individuals; *n* = number of single fibers; K = instantaneous stiffness; K/SF = ratio between instantaneous stiffness and specific force. HSL = half sarcomere length.
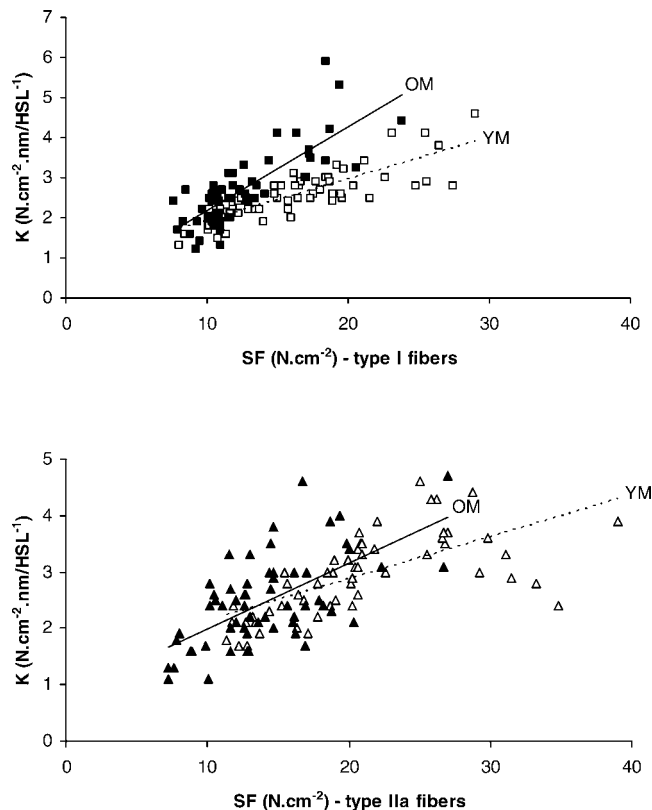
Figure 2. Scatter plots of fiber specific force (SF) and instantaneous stiffness (K) for type I and IIa single muscle fibers from young and older men. On the first plot, type I fibers from young (□) and older individuals (■) are represented, whereas on the second plot, type IIa fibers from young (△) and older individuals (▲) appear. *Solid lines:* best fits for fibers from young men (YM). *Dashed lines:* best fits for fibers from older men (OM).

(*SD*) represent the *SD* for multiple observations on a single individual "within *SD* (w-*SD*)" and the *SD* for measurements of different individuals "between *SD* (b-*SD*)." The models assume that each participant has his own mean measurement (with a normal distribution between participants) and that each measurement within a participant is also normally distributed around this mean. The *p* value tests the hypothesis that there is a difference in these mean measurements between older and young participants. Given the small number of hybrid (I/IIa and IIa/IIx) fibers studied in these experiments, they were not included in the analysis. In addition, no pure IIx fibers were identified among the fibers studied. Thus, comparisons are limited to type I and IIa fibers. Finally, the degree of association between variables was evaluated (K with SF, K and K/SF with V_0, $t_{1/2}$) also using mixed linear models to test whether the relationships had nonzero slopes.

RESULTS

Contractile Properties of Single Fibers

The results for CSA, SF, V_0, and $t_{1/2}$ of phase 2 force recovery after a quick release are presented in Table 1. Both age groups showed similar CSA in type I and IIa fibers. SF was significantly lower in type I (-25%, $p < .01$) and IIa (-33%, $p < .01$) fibers in older men. Older men had significantly lower V_0 in type I (-23%, $p < .05$) and IIa (-22%, $p < .05$) fibers. In type I fibers, $t_{1/2}$ was not related to age whereas in type IIa fibers, $t_{1/2}$ was significantly longer in older men ($+25\%$, $p < .05$).

Elastic Characteristics of Single Fibers

The results for the instantaneous stiffness (K), and K/SF ratio of type I and IIa single muscle fibers are presented in Table 2. K was unrelated to age in type I and IIa fibers but linearly related to SF as seen in Figure 2 ($p < .001$). Therefore, the K/SF ratio was calculated to overcome the influence of SF on K. This ratio corresponded to the instantaneous stiffness per force unit. K/SF was significantly greater in type I ($+31\%$, $p < .001$) and IIa ($+20\%$, $p < .05$) fibers in older men.

Relationship Between Elastic and Contractile Parameters of Single Fibers

No significant correlations were seen between the elastic parameters K and K/SF and the contractile parameters: V_0 and $t_{1/2}$ in type I and IIa fibers.

DISCUSSION

The intent of the present study was to (a) evaluate the influence of aging on elastic characteristics of single muscle fibers and (b) correlate the elastic properties with the fiber contractile parameters. The most important findings of the present investigation were: (a) an increase in the instantaneous stiffness per force unit (K/SF) in type I and IIa fibers with aging; and (b) the absence of a strong relationship between the stiffness (K, K/SF) and contractile characteristics (V_0, $t_{1/2}$) in type I and IIa fibers.

Contractile Properties of Single Fibers

SF was reduced in older men in type I and IIa fibers. This observation is consistent with most previous experiments (2–4) and suggests that the intrinsic ability of muscle fibers to generate force is affected by aging. The decrease in SF can be due to either a lower number of strongly bound actin–myosin cross-bridges during maximal activation and/or a reduced force-generating capacity per cross-bridge. According to D'Antona and colleagues (3), the strong correlation between myosin concentration in human single fibers and SF suggests that a major determinant of the lower SF in older men is a decrease in the contractile material per CSA, that is, a reduced number of cross-bridges. In contrast, maximally activated skinned fibers of older rats have a reduced fraction of myosin heads in the strong-binding structural state (25,26) resulting in a reduced force-generating capacity per cross-bridge. It might well be that both a decrease in myosin concentration and a reduction in the fraction of strongly bound myosin heads affect SF in single skinned muscle fibers.

V_0 of type I and IIa fibers is lower in older men. This observation is in agreement with the results of previous studies (3–5). To assess whether changes in V_0 were due to an alteration of the myosin function, previous experiments

used the in vitro motility assay (27–29). Isoform-specific pure myosin molecules were extracted from single fibers, and the sliding of actin on myosin (V_f) was calculated. In humans, a lower V_f in type I myosin (3,28) and a trend toward a lower V_f in type IIa myosin (3) from older men has been observed. Therefore, aging appears to alter the function of a given myosin isoform without changing isoform type (29,30). It has recently been suggested that glycation of myosin might be involved in such phenomenon (31). Therefore, the decrease in V_0 with aging may be due to a slowing of some kinetics steps of the cross-bridge cycling such as the actin-myosin cross-bridge detachment rate (4).

In type I fibers, $t_{1/2}$ was not affected by aging whereas in type IIa fibers, $t_{1/2}$ was significantly longer in older men compared to young men. To the best of our knowledge, no study has examined the effect of aging on this contractile characteristic of single muscle fibers. According to Irving and colleagues (32,33), the quick force recovery after a step release (Figure 1, phase 2) results from the force-generating movements of the myosin heads bound to actin. In fact, during phase 1, the step release induces conformational changes of myosin heads and the detachment of only the weakly attached cross-bridges (34). During phase 2, the myosin heads still attached to actin recover their initial conformation, and new cross-bridges are formed (18). Consequently, the longer $t_{1/2}$ in type IIa fibers in older men confirms that some force-generating kinetics steps of the actin–myosin cross-bridge cycling are modified with aging (3,28).

Taking into consideration V_0 and $t_{1/2}$ data, it appears that in addition to a possible decrease in myosin concentration and a reduction in the fraction of strongly bound myosin heads, the actin–myosin cross-bridge cycling is slowed down and becomes less efficient for both type I and IIa muscle fibers with aging. However, the kinetics steps of the actin–myosin cross-bridge cycling may be affected differently according to fiber type. Type I fibers may be slowed down only at the end of the process, that is, during the detachment phase (decrease in V_0), whereas type IIa fibers can be less efficient during both the force-generating and detachment steps (increase in $t_{1/2}$ and decrease in V_0). In human skeletal muscle, fibers consist of fast-twitch (type II) fibers, and slow-twitch (type I) fibers. Type I fibers are responsible for slow, repetitive movements; type II fibers are responsible for fast movements. Based on these different functions, one can suspect that type II fibers may be more affected with aging because of decreased fast motor activities. This can partly explain why type IIa fibers are more affected that type I fibers during the aging process.

Elastic Properties of Single Fibers

We are not aware of other experiments that have examined the effect of aging on human elastic characteristics of single muscle fibers. In the present study, the K/SF ratio was greater in type I and IIa fibers in older men compared to young men. The observation of a higher stiffness per force unit with aging is novel. Stiffness of activated single muscle fibers depends on the number of attached actin–myosin cross-bridges, on the compliance of the cross-bridges, and on the compliance of the structures in series with the cross-

bridges (the thick filament, proteins along the thin filament, probably also Z-disks) (6,18,34–40). Thus, the increase in stiffness per force unit with aging observed in the present study could be due to several mechanisms. First, an increase in the number of attached actin–myosin cross-bridges required to sustain the same SF, that is, an increased number of low-force state cross-bridges, would increase the stiffness. In fact, during phase 1, a few cross-bridges are detached, that is, only the weakly attached cross-bridges (34). The larger number of low-force state actin–myosin cross-bridges with aging may increase the force change during phase 1, thus the instantaneous stiffness (i.e., ratio between the force change during phase 1 and the corresponding length change). In former studies, electron paramagnetic resonance spectroscopy has been extensively used for quantifying the structural states of actin–myosin cross-bridges (41). In fact, it has been shown that the myosin head has two primary structural states: a strong-binding (to actin) structural state that is rigid and stereospecific and a weak-binding structural state that is dynamically disordered. According to the observations of Lowe and colleagues (25), during a maximal isometric contraction, 32% of myosin heads are in the strong-binding (force-generating) structural state in fibers from young rats, but only 22% of myosin heads are in that state in fibers from older rats. Others have also suggested such result (42,43), but the electron paramagnetic resonance spectroscopy study (25) provided the first, and to date only, direct evidence supporting this hypothesis.

Alternatively, a decrease in the compliance of the actin–myosin cross-bridges and/or a decrease in the compliance of the myofilaments (10,39) could also increase the instantaneous stiffness per force unit. To our knowledge, no one has raised the question of investigating the aging-related effect on the compliance of the cross-bridges and the structures in series with the cross-bridges, that is, myosin filament backbone and/or actin filament, and probably also titin filaments and nebulin proteins along the thin filament. Changes in such compliance may not be excluded. Further investigations would be necessary to localize the mechanisms responsible for the aging-related differences in muscle fiber elasticity. Nevertheless, the observation of higher fiber stiffness per force unit with aging explains why, in studies of whole muscle (13–16), the muscle–tendon unit was found stiffer in older than in young men.

Relationship Between Elastic and Contractile Characteristics of Single Fibers

Surprisingly, no significant correlations were detected between the elastic parameters K and K/SF and the contractile characteristics V_0 and $t_{1/2}$. Therefore, the observation of a greater instantaneous stiffness per force unit with aging does not influence the less efficient contraction, the slowing of the actin–myosin cross-bridge cycling. As reported by Ochala and colleagues (44), V_0 and $t_{1/2}$ are determined by the mean elongation and/or compression of the single muscle fiber during maximal activation rather than instantaneous stiffness. This mean elongation and/or compression depends on the compliance of the actin–myosin cross-bridges and the myofilaments (6,10). The longer the

elongation and/or compression is, the faster the cross-bridge cycling is, that is, decreasing $t_{1/2}$, increasing V_0 (44). One can speculate that in addition to an alteration of stiffness characteristics, a decrease in the elongation and/or compression may occur with aging. Such decrease in the mean elongation and/or compression of the fiber during activation would disrupt the force-generating phase following steps in length (increasing $t_{1/2}$, decreasing V_0). To confirm this hypothesis, further experiments using x-ray diffraction are needed.

The greater instantaneous stiffness per force unit with aging does not induce less efficient muscle contraction but on the contrary may have some noticeable beneficial impact on the cost of the contraction in terms of energy-saving mechanisms. During human daily motor activities such as terrestrial locomotion the stretch-shortening cycle is present. Active muscle fibers from vastus lateralis muscle are lengthened and rapidly shortened. During the lengthening phase, active muscle fibers store mechanical energy that is released during the shortening phase. The greater fiber stiffness with aging improves the subsequent release of energy during the shortening phase (45). It minimizes the metabolic energy (ATP) cost, which is necessary to induce muscle shortening, that is, cross-bridge formation. This energy-saving mechanism may represent a compensatory mechanism to counterbalance the less efficient muscle contraction with aging.

Summary

Aging induces changes in fiber elasticity in both type I and IIa fibers. A greater instantaneous stiffness per force unit was observed in fibers from older compared to young men. This modification at the cell level explains why the muscle–tendon unit was found stiffer in older compared with young men on studies of whole muscle. One can speculate that the increased stiffness per force unit in type I and IIa fibers is due in part to an increase in the number and proportion of low-force state actin–myosin cross-bridges. However, changes in the compliance of the cross-bridges and structures in series with the cross-bridges can not be excluded. This alteration of stiffness properties with aging may not directly influence contractile characteristics. In fact, K and K/SF were not significantly related to the fiber contractile properties (V_0, $t_{1/2}$).

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