Reduction in single muscle fiber rate of force development with aging is not attenuated in world class older masters athletes

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Submitted 1 October 2015; accepted in final form 20 November 2015

Power GA, Minozzo FC, Spendiff S, Filion ME, Konokhova Y, Purves-Smith FM, Pion C, Aubertin-Leheudre M, Morais JA, Herzog W, Hepple RT, Taivassalo T, Rassier DE. Reduction in single muscle fiber rate of force development with aging is not attenuated in world class older masters athletes. Am J Physiol Cell Physiol 310: C318–C327, 2016. First published December 2, 2015; doi:10.1152/ajpcell.00289.2015.—Normal adult aging is associated with impaired muscle contractile function; however, to what extent cross-bridge kinetics are altered in aging muscle is not clear. We used a slacken restretch maneuver on single muscle fiber segments biopsied from the vastus lateralis of young adults (~23 yr), older nonathlete (NA) adults (~80 yr), and age-matched world class masters athletes (MA; ~80 yr) to assess the rate of force redevelopment (ktr) and cross-bridge kinetics. A post hoc analysis was performed, and only the mechanical properties of “slow type” fibers based on unloaded shortening velocity (V0) measurements are reported. The MA and NA were ~54 and 43% weaker, respectively, for specific force compared with young. Similarly, when force was normalized to cross-sectional area determined via the fiber shape angularity data, both old groups did not differ, and the MA and NA were ~43 and 48% weaker, respectively, compared with young (P < 0.05). V0 for both MA and NA old groups was 62 and 46% slower, respectively, compared with young. Both MA and NA adults had approximately two times slower values for ktr, compared with young. The slower V0 in both old groups relative to young, coupled with a similarly reduced ktr, suggests impaired cross-bridge kinetics are responsible for impaired single fiber contractile properties with aging. These results challenge the widely accepted resilience of slow type fibers to cellular aging.

Ageing; muscle; weakness; velocity; sarcopenia

NORMAL ADULT AGING IS ASSOCIATED with impairments of the neuromuscular system (39). Such impairments include a loss of isometric force production, shortening velocity, and power at the whole muscle (12, 40) and single fiber level (5, 25, 43). These impairments in muscle function can be attributed to many factors including reduced contractile mass, alterations in muscle architecture (31, 41), decreased neural activation (1), and impaired excitation-contraction (EC) coupling (13, 46). Single muscle fiber contractile function is suggested to be dependent on the content and expression of myosin heavy chain (MHC) isoforms (2). In older adults (~73 yr), however, even when matched for MHC isoform expression (10) and normalized to fiber cross-sectional area (CSA) to account for the reduction in myofibrillar content (i.e., specific force), there are still age-associated impairments in muscle contractile function. In other words, the shifts of fiber type proportion as well as the decrease in fiber CSA are partially dissociated from the contractile property changes commonly observed in aging muscle. Ultimately, these impairments manifest as reduced force values per unit of muscle (i.e., muscle quality) (36) and indicate possible impairments in cross-bridge kinetics of aging muscle contributing to impaired function.

At the filamentous level, an age-associated loss of myosin content relative to CSA results in a decreased number of actomyosin interactions (10). As shown in old rats (32–36 mo), the loss of myosin content, coupled with a lower percentage of strongly bound cross bridges during contraction (28), contributes to reduced force production in aging muscle. Additionally, aging muscle shows a decreased actin sliding speed independent of MHC isoform expression (20, 21, 27), as assessed with in vitro motility assays (10), and is associated with reductions in maximal unloaded shortening velocity. These findings point to either a slowing of the kinetic steps within the cross-bridge cycle (20) or a decrease in the step size per myosin step cycle, contributing to a slowed maximal shortening velocity. Taken together, these investigations suggest that cross-bridge kinetics and function are impaired in older adults between 65 and 75 yr of age (10, 21, 29, 32, 33). For example, Ochala et al. (32) investigated single muscle fiber properties of the vastus lateralis of ~66-yr-old men and suggested that actin-myosin cross-bridge kinetics were slowed, but the authors did not identify the specific steps of the cross-bridge cycle that were affected. Recently, Miller et al. (29), using a similar preparation, applied sinusoidal length perturbations and modeled the cross-bridge cycle in an attempt to identify specifically which steps of the cross-bridge cycle are affected in 65- to 75-yr-old males and females. It was suggested that older adults had an increased proportion of strongly bound cross bridges and a longer myosin attachment rate (slowed detachment) compared with young. This combination of factors would result in slowed cross-
bridge kinetics and potentially explain the decreased maximal shortening velocity in older adults. However, single fiber maximal unloaded shortening velocity was not reported, bringing into question whether cross-bridge kinetics were indeed “functionally” slower in the muscles of older adults (29). Specifically, a faster myosin attachment rate could have offset the slower detachment rate to maintain the same duty ratio [time attached/(time attached + time detached)] of attaching cross bridges. Whereas the aforementioned studies all corroborate that cross-bridge kinetics are affected with aging up to 75 yr, they do not reveal to what extent cross-bridge kinetics is impaired or how these changes contribute to contractile dysfunction in aging muscle. Similarly, they do not address ages when muscle weakness becomes most clinically relevant (>75 yr old).

Permeabilized muscle fiber segments activated in high [Ca^{2+}]-activating solution offer a preparation model free from neural and activation issues, which is useful in investigating cross-bridge function in muscles of older adults. However, any inferences on cross-bridge functioning during muscle force development in the course of activation can be confounded by Ca^{2+} troponin-tropomyosin regulation of actin-myosin interactions (19). To overcome this limitation, a slacken restretch maneuver can be used to assess cross-bridge functioning in a fully activated system independently of Ca^{2+} regulation (3). The slacken restretch maneuver is designed to detach nearly all cross bridges. During the shortening phase (slacken), force momentarily reaches zero; next, the initial fiber length is reestablished via a quick stretch (restretch), not allowing enough time for Ca^{2+} regulation of regulatory (troponin, tropomyosin) proteins to take place. Therefore, in this experiment only the cross-bridge attachments, followed by their transition from nonforce-bearing to force-bearing states, contribute to force redevelopment. Furthermore, the rate constant of force development (k_f) during the transition from nonforce-bearing to force-bearing states reflects the rate of cross-bridge reattachment and the power stroke (4), thus providing a means of assessing the role of cross-bridge kinetics in contractile dysfunction of aging muscle.

Many of the typical age-related impairments to the neuromuscular system are absent in highly trained older masters athletes (37). Additionally, masters athletes provide a model to explore successful aging in a population of older adults free from many confounding factors (i.e., age-related disease, immobility) that may accelerate cellular aging in the “typical” North American older person. Although their physical activity habits are certainly involved in maintaining function with age, genetic influences, independent of physical activity level, are also likely to contribute to successful aging and maintenance of intrinsic muscle contractile function in these exceptional individuals and may thus provide novel insights into mechanisms of successful aging. In this respect, it is unknown whether these exceptional older athletes have maintained cross-bridge kinetics and muscle function similar to younger adults, which may have contributed to their successful aging and athletic prowess relative to their age-matched counterparts.

The purpose of this study was to test the hypotheses that single permeabilized muscle fiber segments from older non-athlete adults show an age-related reduction in specific force and shortening velocity and have a lower k_f than young, while older masters athletes would not differ from young and thus intrinsic cross-bridge function would be maintained similar to young.

MATERIALS AND METHODS

Participants. All young adults (n = 6; 23.4 ± 1.0 yr, 178.3 ± 7.9 cm, and 79.7 ± 9.6 kg), older nonathletic adults (NA; n = 5, 78.2 ± 9.4 yr, 167.8 ± 2.7 cm, and 69.7 ± 3.8 kg), and elite masters track and field athletes (MA; n = 6, 78.8 ± 3.6 yr, 176.3 ± 5.7 cm, and 73.7 ± 12.7 kg) were asked to refrain from unaccustomed and strenuous exercise before the muscle biopsy procedure. All participants were male and had no known neurological or musculoskeletal conditions. The young adults were recruited from the university population, and the NA adults were living independently and recruited from the local community. The MA consisted of male track and field athletes ranked in the top three of their respective events at the world masters championships. The MA combined held world records for the marathon (80–84 yr), 100 m (75–79 yr), and 80 m hurdles (75–79 yr) and were ranked second and third for the 1,500 m (75–79 yr) and pentathlon (85–89 yr), respectively. This study was approved by the McGill Faculty of Medicine Institutional Review Board for research involving human subjects (A08-M66-12B) and conformed to the Declaration of Helsinki. Informed written consent was obtained from all participants before the study.

Biopsy procedure and preparation of muscle fibers. The suction-modified Bergström muscle biopsy technique (18) was used to obtain an average sample of muscle tissue from the belly of the vastus lateralis muscle (mid-thigh). One portion of the biopsy was used for single fiber segment preparation, and a second portion was mounted in cross section in OCT and frozen in liquid N2-cooled isopentane for histology. For the portion used in single fiber segment preparation, muscle bundles were tied with suture and chemically permeabilized by first being incubated in rigor solution (pH 7.0) for ~4 h and subsequently being transferred to a rigor-glycerol (50:50) solution for 15 h. Single fibers were prepared as described previously (30). Briefly, samples were placed in a fresh rigor-glycerol (50:50) solution with the addition of a cocktail of protease inhibitors (Roche Diagnostics) and stored in a freezer (−20°C) for at least 7 days. Before each experiment, a muscle sample was transferred to a fresh rigor solution in a 1°C bath before use. A ~4-mm strip of the sample was cut and single fibers were dissected carefully in relaxing solution (see below). These fibers were gripped at their ends with T-shaped clips made of aluminum foil and were transferred to a temperature-controlled chamber to be attached between a force transducer (resonant frequency: 2 kHz; model 403A; Aurora Scientific, Toronto, ON, Canada) and a length controller (model 312B; Aurora Scientific).

Fiber type, size, and proportion assessments. Muscle cross sections (10-μm thick) were cut transversely at ~23°C from the portion prepared for histology and immunolabeled for myosin heavy chain (MHC) I, IIA, and IIX using previously described methods (18) (for additional information on antibody specificity, please see Ref. 44). Cross sections were first allowed to reach room temperature and washed with phosphate-buffered saline (PBS; pH 7.2). These sections were then blocked using goat serum (10% in PBS) and incubated for 1 h at room temperature with the following primary antibody cocktail: mouse IgG2b monoclonal anti-MHC type I (BA-F8, 1:25), mouse IgG1 monoclonal anti-MHC type IIa (SC-71, 1:200), mouse IgM monoclonal anti-type IIX MHC (6H1, 1:25), and rabbit IgG polyclonal anti-laminin (Sigma). Muscle cross sections were then washed three times in PBS before being incubated for 1 h at room temperature with the following secondary antibody cocktail: Alexa Fluor 350 IgG2b goat anti-mouse (A-21140, 1:500; Invitrogen, Carlsbad, CA), Alexa Fluor 594 IgG1 (y1) goat anti-mouse (A-21125, 1:100; Invitrogen), Alexa Fluor 488 IgM goat anti-mouse (A-21042, 1:500; Invitrogen), and Alexa Fluor 488 IgG goat anti-rabbit (A-11008, 1:500; Invitrogen). Muscle cross sections were then washed three times in PBS, and coverslips were applied to slides using Prolong...
Gold (P36930; Invitrogen) as mounting medium. All primary antibodies targeting MHCs were purchased from the Developmental Studies Hybridoma Bank (DSHB; University of Iowa, Iowa City, IA). Slides were imaged with a Zeiss Axio Imager M2 fluorescence microscope (Zeiss, Oberkochen, Germany). A systematic sampling approach was taken to analyze 500 fibers per subject across each muscle section.

**Solutions.** The rigor solution (pH 7.0) was composed of the following (in mM): 50 Tris, 100 NaCl, 2 KCl, 2 MgCl₂, and 10 EGTA. The relaxing solution used for muscle storage and dissection (pH 7.0) was composed of the following (in mM): 100 KCl, 2 EGTA, 20 imidazole, 4 ATP, and 7 MgCl₂. The experimental activating solution with pCa²⁺ of 4.5 (pH 7.0) contained the following (in mM): 20 mM imidazole, 14.5 mM creatine phosphate, 7 mM EGTA, 4 mM MgATP, 1 mM free Mg²⁺, and 32 μM free Ca²⁺ (pCa²⁺ 4.5) and KCl to adjust the ionic strength to 180 mM. A preactivating solution (in mM: 68 KCl, 0.5 EGTA, 20 imidazole, 14.5 creatine phosphate, 4.83 ATP, 0.00137 CaCl₂, 5.41 MgCl₂, and 6.5 HTDA at pH 7.0, pCa²⁺ 9.0) with a reduced Ca²⁺ buffering capacity was used immediately before activation to minimize delays in activation.

**Experimental protocol.** The average sarcomere length was calculated in relaxing solution with a high-speed video system (HVSL, Aurora Scientific 901A), wherein images from a selected region of the fibers were collected at 1000-1500 frames/s and used to calculate sarcomere length by fast Fourier transform (FFT) analysis, based on the striation spacing produced by dark and light bands of the thick and thin filaments, respectively. The fiber diameter and length were measured with a charge-coupled device (CCD) camera (Go-3, QImaging; pixel size: 3.2 × 3.2 μm).

Two experiments were performed during the single fiber study: first, a slack test to determine peak tension and unloaded shortening velocity (V₀); and second, a slacken restretch test to measure the rate of isometric force redevelopment (kₒ) with instantaneous stiffness measurements imposed; before, during, and following force redevelopment. Based on thin filament lengths of skinned permeabilized single fiber segments from the human vastus lateralis (51), the optimal length of force production would be ~2.7 μm. Therefore, before activation, the initial sarcomere length was adjusted to ~2.8 μm (optimal length; Lₒ), which then shortened to 2.7-2.6 μm upon activation. All experiments were performed at 10°C. Control contractions at a pCa²⁺ of 4.5 were elicited throughout the experiments and at the end of the experiments to ensure the isometric force never decreased by >10% (actual range: 5.2–8.3%) from the maximal force produced at the beginning of the experiment. As well, if the striation pattern of the muscle fibers became unclear such as to not allow measurements of sarcomere length, the experiment was terminated.

Muscle contraction was induced by first transferring the single muscle fiber preparation from a relaxing to a preactivating bath for ~10 s and then to the activating bath for ~30 s before return to the relaxing bath. Force and length data were sampled at a rate of 10,000 Hz. The specific isometric tension (Pₒ) was calculated as the peak tension amplitude (resting force in relaxing solution subtracted from the force reached while in the activating solution) divided by the CSA of the muscle fiber, determined via circularity assumed [π(π²/4)] or angularity data from histology. To account for increased fiber angularity in older adults (47), angularity was derived from the histological preparations, determined as [4(π²-π)/perimeter³] and multiplied by the circularity assumed. A normal polygonal fiber typically has a shape factor of between 0.7 and 0.8 with angular fibers being less than that. Unloaded shortening velocity (V₀) was determined using the slack test method (14). Three separate slack tests were performed on each fiber, corresponding to length steps of 5, 10, and 15% Lₒ. The fiber was allowed to reach Pₒ and then a predetermined length step was imposed rapidly (2 ms), which allowed the fiber to become slack and tension drop to zero. Force redeveloped over time in proportion to the shortening length change. Following ~30 s in the activating solution, the fiber was returned to the relaxing bath and sarcomere length was adjusted to ~2.8 μm before the subsequent contraction. The resulting data were plotted as the time required to redevelop tension relative to the imposed length step, which was then fitted with a linear least squares error regression line. The slope of this line represents the unloaded shortening velocity (Vₒ in Lₚ/s).

The kinetics of force redevelopment was assessed using a slack restretch method (3). After full force development and Pₒ were reached, the fiber was shortened by 15% with a 10 Lₚ/s ramp, which reduced the force momentarily to zero, and this was then followed by a brief period (25–100 ms) of unloaded shortening; this procedure has been shown to reduce dynamic stiffness and serves as an index of the proportion of attached cross bridges to the thin filament (3). The shortening ramp was followed by a rapid (500 Lₚ/s) restretch to the initial Lₒ allowing for the dissociation of any remaining attached cross bridges. The force redevelopment following the slack restretch test is related directly to the reattachment of myosin to actin and a redistribution of cross bridges from prepower stroke into force-generating states (3). To determine the proportion of attached cross bridges throughout the slacken restretch maneuver, fiber instantaneous stiffness was measured before, during, and following force redevelopment. This stiffness (Kₒ) was assessed by applying a fast (500 Lₚ/s) length step (Δlength = 0.3% Lₒ) to the fibers and dividing the change in force (Δforce) during this step by the length step (Δlength), as described previously (9).

**Data analysis.** The maximal force produced by each fiber was calculated after force development stabilization and after force redevelopment following the slacken restretch protocol. For each contraction, kₒ was analyzed using the following biexponential equation: F = [a × (1-exp(-kₒ × t) − exp(-l × t)] + b, where F is force, a is the amplitude of the exponential (s), t is time, kₒ the first exponential constant, l the second exponential constant, and “b” is the initial force value. In a post hoc analysis, muscle fibers were binned into a slow type group based on Vₒ values obtained in each of the three groups (n fibers for young: 8; NA: 12; and MA: 15). This was performed because there were no apparent “fast fibers” present in the NA group, perhaps owing to selection bias and fragile “fast type” fibers. Therefore, those fibers slower than 40% of the maximum speed of the fastest fiber in each group were considered “slow” and binned into the slow type fiber group; across groups this represented fibers with a Vₒ < 0.5 Lₚ/s. A similar binning procedure based on a percentage of unloaded shortening velocity (8) was used in a previous investigation of single fiber contractile properties in older adults and was validated against MHC isoform analysis. This previous study showed that the error associated with this binning procedure was ~5 and 7% for the fast and slow type fibers, respectively. Therefore, it is possible that some of the fibers in the “slow type” group may express MHC II isoforms. These data were further analyzed with respect to the above mentioned contractile properties to assess potential age- and activity-dependent differences of slow type muscle fiber properties.

**Statistical analysis.** Unless otherwise mentioned in figure legends, comparisons between subjects were performed using unpaired bilateral Student’s t-tests. The level of significance was set at P ≤ 0.05. Data in the text are presented as means ± SD and in the figures as means ± SE.

**RESULTS**

Muscle fiber type proportion. Muscle fiber type composition (immunohistochemical analysis as seen in Fig. 1B) indicated NA adults had a 25% lower type I fiber composition compared with MA and young (P < 0.05), while MA and young did not differ (P > 0.05; Fig. 1A). The relative distributions of type IIa did not differ across the three groups (P > 0.05), while type IIX were higher in the young compared with both older groups (P < 0.05). Additionally, NA adults exhibited significantly more coexpressing type IIA/IIX fibers compared with...
both MA and young (P < 0.05; Fig. 1A). There were virtually no pure type IIX fibers in the muscle sections of the two older age groups. Instead, type IIX was almost exclusively expressed in conjunction with type IIA MHC, as depicted in Fig. 1 (type IIA/IIX).

Cross-sectional area. With the assumption of circularity, and angularity derived values, the CSA of the permeabilized single muscle fiber segments was similar across groups (P > 0.05; Table 1).

Isometric force. Absolute force was 53 and 57% lower in both MA and NA adults, respectively, compared with young (P < 0.05; Table 1) while MA and NA adults did not differ (P > 0.05). When force was normalized to circularity assumed CSA, specific force was 54 and 43% lower for both MA and NA (P < 0.05), respectively, compared with young (Table 1), while both older groups did not differ (P > 0.05). Conversely, when force was normalized to CSA determined via the angularity data, specific force was 43 and 48% lower for MA and NA, respectively, compared with young (P < 0.05; Table 1), while both old groups did not differ significantly (P > 0.05).

Unloaded shortening velocity. Unloaded shortening velocity was 62% and 46% slower in both MA adults and NA (P < 0.05), respectively, compared with young (Fig. 2), while the older groups did not differ (P > 0.05).

Rate of force redevelopment kinetics (ktr). Rate of force redevelopment was approximately two times lower in both MA and NA adults (P < 0.05) compared with young (Fig. 3), while MA and NA adults did not differ (P > 0.05).

Instantaneous stiffness. Stiffness measurements did not differ between groups for initial force development (Fig. 4) and was lower in all groups during force redevelopment compared with pre- and postmeasurements, indicating fewer attached cross bridges during the slacken restretch procedure. When stiffness was compared across age groups during force redevelopment and following force redevelopment, NA adults appear to have 22–29% lower stiffness values than young (P < 0.05; indicating less attached cross bridges during force rede-

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<th>Table 1. Fiber segment cross-sectional area derived from circularity assumed and fiber angularity, force, and specific force</th>
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Values are means ± SE. CSA, cross-sectional area. *Significantly different from young.
Fig. 2. A: mean unloaded shortening velocity data. Solid bars are the mean data for each group (young in white; NA: nonathlete older adults in grey; MA: masters athletes in black). B: unloaded shortening velocity raw data traces of a typical older nonathlete adult. Three separate slack tests were performed on each fiber corresponding to length steps of 5, 10, and 15% \( L_o \). The data were plotted as the time required to redevelop tension relative to the imposed length step, which was then fitted with a 1st-order least squares regression line, the corresponding slope of this line represented unloaded shortening velocity. C: zoomed in view of unloaded shortening velocity raw data traces. Data are means ± SE. *Significantly different than young.

Development). However, there was no difference between young and MA or between NA and MA groups.

DISCUSSION

This study examined the contractile properties of single permeabilized muscle fiber segments from young adults, older NA adults, and age-matched world class MA to elucidate to what extent aging and world class athletic performance affects cross-bridge functioning. The slacken restretch maneuver was used to assess cross-bridge kinetics (rate of attaching) (3), allowing us to measure the rate of force redevelopment in a fully activated system independent of Ca\(^{2+}\)/troponin-tropomyosin regulation of actin-myosin interactions and other confounding contraction coupling factors that are known to be impaired in aging muscle (13, 46). Essentially, this test provides an environment in which no cross bridges are generating force. The rate at which force is reestablished is the transition from nonforce bearing to force bearing cross-bridge states, thus allowing for the assessment of one aspect of cross-bridge kinetics, i.e., the rate of force redevelopment (\( k_{un} \)) (4). To our knowledge, this was the first time such an assessment was performed on muscle from older adults. The main finding was that both older groups had a considerably slower \( k_{un} \) than the young group, indicating that cross-bridge kinetics in both older groups was twice as slow as young for slow type fibers.

Muscle fiber type composition. Muscle fiber type composition analysis (muscle cross sections immunolabeled; Fig. 1) indicated NA adults had a ~25% lower type I fiber composition compared with MA and young. The NA adults exhibited significantly more coexpressing type IIA/IIX fibers compared with both MA and young, a finding that likely is a function of both their relatively low physical activity status (52) as well as a greater influence of dysfunctional neuromuscular junction remodeling resulting in muscle fiber denervation (47). The finding of a reduced type I fiber proportion (%; Fig. 1) in NA adults relative to young is consistent with a recent review on fiber type changes in aging muscle (44), despite the current dogma of a preferential loss of type II area in old age. The immunohistochemical staining procedure used in the present study has a greater sensitivity to detect coexpressing fibers compared with myofibular ATPase staining (44). Thus, in older adults there does not appear to be a tight coupling between MHC isoform expression and contractile properties that is observed in young adults (2). Thereby, grouping the fibers based on the mechanical parameter \( V_o \) (8) allowed for the investigation of age-related impairments in cross-bridge kinetics on specifically mechanically slow contracting single fiber segments and not on a factor (i.e., MHC) that could be confounded by aging.

Muscle fiber contractile properties. The lack of difference between NA adults and MA in contractile function at the single fiber segment level suggests that a greater preservation of muscle fiber number rather than contractile function may be contributing to the MA exceptional athletic performance. Additionally, fewer denervated muscle fibers (thus a greater maintenance of muscle fiber number) and the ability of the MA to activate their muscle to a greater extent than NA may influence EC coupling and Ca\(^{2+}\) kinetics, which could have
been hidden in the present "fully activated" model. As reported previously in aged rat muscle (7), the contractile properties of aged muscle are partially dissociated from fiber type composition. The literature is divergent regarding age-related changes to isometric single fiber specific force and shortening velocity, with some studies showing specific force and unloaded shortening velocity to be decreased with aging (65–81 yr) (10, 11, 17, 23, 24, 33, 53) and others reporting no change in force or shortening velocity (15, 45, 49) between the ages of 60 and 80 yr. These disparate findings could be related to varying ages tested (i.e., age range tested spans 2 decades; 60–80 yr) and the participants’ habitual levels of physical activity, both factors that are known to influence muscle contractility (5). For example, when older adults were grouped relative to their levels of daily physical activity (11), contractile performance progressively declined from highly active to sedentary. Furthermore, two longitudinal training studies showed an increase in specific force, unloaded shortening velocity (34), and actin sliding velocity (6), highlighting a considerable plasticity for contractile performance to improve in old age. Additionally, the biopsy procedure and fiber dissection may be intrinsically biased towards sampling only from those most robust single fiber segments capable of surviving the procedure (discussed below).

Absolute single muscle fiber force of endurance-trained MA is typically reduced compared with age-matched controls owing to a smaller fiber diameter (50). However, as reported previously, and as shown in the present study, when force is normalized by fiber CSA, the athletes’ specific force was similar to age-matched controls (50). Additionally, sprint-trained MA typically have larger single muscle fiber diameters compared with endurance trained and similar specific force (22). In the present study, upon normalizing force to CSA and binning into slow type fibers, we report here that the slow type fibers from NA were weaker than young and not different from MA adults (Table 1). Similarly, when circularity was not assumed and CSA was calculated based on fiber angularity, upon normalizing force, NA had a lower specific force than young and not different from MA adults (Table 1). Unloaded shortening velocity for both MA and NA adults was slower compared with young while both older groups did not differ (Fig. 3). These findings for unloaded shortening velocity may not only be related to the $k_tr$ values but the myosin step size (discussed below). CSA (circularity assumed) of the permeabilized single muscle fiber segments was similar across group. Interestingly, these results at the single fiber segment level contrast sharply with morphological measurements in the MHC-labeled cross sections in that the latter method revealed marked fiber atrophy of all fiber types in the NA adults vs. young subjects (Table 1). There remained a significantly lower fiber size for type IIA, I/IIA, and IIA/X fibers in muscle cross sections from MA vs. young. This indicates that the fiber segments analyzed in the mechanical contractile function experiments represent the larger fibers, perhaps more capable of "surviving" the dissection and harvesting from the aged subjects. The selective harvest of the larger fibers from aged samples is likely a common occurrence in single fiber segment
studies since a maintained fiber size with aging is usually seen in studies using this approach (16, 48, 49).

Age-related changes in muscle contractile function are often confounded by health conditions brought about via a sedentary life style which can accelerate the aging phenotype. To account for these factors, MA can be used as a model of natural healthy biological aging (37, 38). In addition to the available literature on age-related changes in force and velocity, little is known on the rate of force generation and whether cross-bridge kinetics are altered during force redevelopment ($k_{tr}$). The proxy of cross-bridge kinetics we used in the present study, $k_{tr}$, indicated that both NA adults and MA were slower than young for the slow type fibers, strengthening the hypothesis that age-related impairments in contractile properties are indeed driven by impaired kinetics affecting the slow type fibers, at least for those fibers binned as slow. A factor that may contribute to the exceptional athletic performance of the MA, but was not mechanically tested in the current study, is the potential that those older individuals maintaining a greater relative percentage of fast type (noncoexpressive) motor units (Fig. 1) may have maintained whole body function in old age.

Molecular mechanisms of age-related muscle weakness. The decrease in isometric specific tension in both older groups could be due to an impaired intrinsic ability of the muscle to produce force through either a lower number of available cross bridges or reduced force generated per bridge or a combination of both. Changes to the myosin molecule through oxidation or glycation inhibits ATPase activity, and this, in turn, affects the transition from a strongly bound to detached cross-bridge state, resulting in a greater binding affinity of actin for myosin in the weakly bound state ultimately slowing the whole actomyosin ATPase cycle (42). Reduced force per cross bridge in aging muscle is consistent with electron paramagnetic resonance spectroscopy imaging to determine the fraction of myosin heads in a strongly bound state (i.e., 32% in young compared with 22% in old) during contraction (28). Additionally, unloaded shortening velocity can decline if the system spends too much time in the strong-binding states such that the detachment rate decreases or if the unitary displacement myosin step is reduced (35). Ochala et al. (33) investigated single fiber properties of the vastus lateralis of older men and suggested that myosin-actin cross-bridge kinetics slowed in old age but ultimately could not identify the specific steps of the cross-bridge cycle impaired. Recently, Miller et al. (29) using a similar muscle preparation, applied sinusoidal length perturbations and modeled cross-bridge function to identify specifically which steps of the cross-bridge cycle are impaired with age. They found a reduced myosin transition rate between the weakly and strongly bound states and an increased average myosin attachment time. This suggested old had an increased proportion of strongly bound cross bridges, which allowed for maintained (or greater) isometric tension in old age (~65–75 yr) but ultimately was assumed to slow cross-bridge kinetics and decrease shortening velocity. Additionally, Miller et al. (29) reported reduced cross-bridge kinetics for type IIA fibers in older men with no change in type I. We report the opposite result for the slow type fibers in the present study, whereby the slow type fibers showed a lower $k_{tr}$ compared with the young.

Fig. 4. A: stiffness measurements. The instantaneous stiffness ($k$) was assessed by applying a fast length step ($\Delta$length = 0.3% $L_o$) and is expressed during the initial force development, during force redevelopment, and following force redevelopment. Solid bars are the mean data for each group (young in white; NA: nonathlete older adults in grey; MA: masters athletes in black). B: stiffness measurements during initial force development. C: stiffness measurements during force redevelopment. D: stiffness measurements post force redevelopment. Data are means ± SE. *Significantly different from young. Solid bars represent stiffness values prior to the slacken-restretch test, upward hashed lines represents stiffness during force redevelopment, and downward hashed lines represents stiffness following force redevelopment.
alterations to cross-bridge kinetics in older adults

suggesting this aspect of cross-bridge kinetics to be impaired for the slow type fibers with aging. Miller et al. (29) did not have a measure of single fiber contractile velocity, or rate of force development independent of regulatory Ca\textsuperscript{2+} confounding factors, and given the isometric tensions were similar or elevated in the old compared with the young this brings into questions whether cross-bridge kinetics were indeed functionally slower in the muscles of older adults or whether the subjects were "old enough" to address ages when muscle weakness becomes most clinically relevant (>75 yr old).

Cross-bridge attachment distribution as assessed via instantaneous stiffness measures. In the present study, as cross-bridge kinetics (k\textsubscript{a} and V\textsubscript{o}) were reduced in both older groups compared with young, the age-related impairments could be owing to 1) less attached cross bridges, and 2) a shift of the cross-bridge population toward the weakly bound state. The latter appears to be the case for the older group as indicated by a similar stiffness in the older groups and lower force (Fig. 4). Moreover, the age-associated impairments in contractile properties of the slow fibers from both older groups may be due to a longer time attached than unattached and an increase in the number of weakly bound nonforce producing cross bridges. As indicated by similar stiffness values during force redevelopment between MA and young groups (Fig. 4) and a two times slower k\textsubscript{a} in the MA, we can assume that there was a similar number of attached cross bridges for these two groups but suggest the transition between weakly to strongly bound cross bridges was compromised in the MA group. However, for the NA group, both stiffness and k\textsubscript{a} were reduced, suggesting an overall loss of cross-bridge interactions and not just an increase in the weakly bound state compared with young. These findings would suggest the NA group had fewer available cross bridges for force production. For the MA group, exercise training may have preserved the number of attaching cross bridges, whereas the transition rate between weakly to strongly bound states was likely reduced or slowed relative to the young group. However, relative to their age-matched counterparts, this "cross-bridge" resilience could also have contributed to the athletic performance of the MA.

Limitations. In our sample, virtually no fast type fibers survived the isolation procedure for the NA group. Therefore, in the present study, single muscle fibers were binned into a slow type group based on 40% of the maximum unloaded shortening velocity of the fastest fiber in each group. In healthy young adult muscle, it is well accepted that single muscle fiber contractile properties are highly dependent on the content and expression of MHC isoforms (2). With adult aging, however, it has been reported historically that there is a reduction in the relative area of type II to type I muscle fibers directly resulting in a relative loss of MHC IIA and IIX isoforms expression, ultimately contributing to weaker, slower, and less powerful contracting muscles (26), although recently decoupling of fiber type and contractile performance in older muscle brings into question the divergence of fiber type and contractile function in older adults (7). The authors acknowledge here that without biochemical MHC isoform analysis of the mechanically tested single muscle fiber segments, it is impossible to know with certainty whether the fibers binned into the slow group expressed the representative MHC I and other protein isoforms for slow type muscle fibers. Given the fiber type grouping procedure used by Claflin et al. (8) (discussed in MATERIALS AND METHODS), we may only have <10% error in grouping the fibers based on unloaded shortening velocity. Considering the emerging evidence (7) that fiber type and contractile properties are rather divergent in aging muscle, we believe stratifying the muscle fibers based on contractile velocity was appropriate for our particular analysis of cross-bridge kinetics.

Conclusion. In the present study, we provide for the first time a detailed account of the rate of force redevelopment in healthy older participants and elite world champion MA. We found that for slow type fibers cross-bridge kinetics were similarly impaired in both groups of older adults compared with young. Based on the measure of instantaneous stiffness during force redevelopment, we suggest that there were a similar number of attached cross bridges for both MA and young groups; however, the transition from weakly to strongly bound cross bridges was driving the impairment in the MA group. On the other hand, for the NA group, both cross-bridge kinetics and stiffness were reduced, suggesting an overall loss of cross-bridge interactions and not just an increase in the weakly bound state compared young. This finding would also suggest the NA group had fewer available cross-bridges for force production, perhaps owing to reduced myosin content or an inhibition of cross-bridge attachments. These results challenge the widely accepted resilience of slow type fibers to cellular aging. With respect to the high athletic performance of the MA, success in high-performance sport in old age, at least for this study, does not appear to be due to maintained kinetics of cross-bridge state transitions of slow type fibers.

ACKNOWLEDGMENTS
The helpful advice of Dr. Scott Trappe in using a binning approach based on unloaded shortening velocity to permit comparisons between groups of fibers with similar contractile characteristics is gratefully acknowledged.

GRANTS
Funding was provided by an operating grant from the Canadian Institutes of Health Research (MOP 84408 to T. Taivassalo), infrastructure support from the Canadian Foundation for Innovation (T. Taivassalo, R. T. Hepple, and D. Rassier), and support from the Natural Science and Engineering Research Council (NSERC) of Canada (to D. Rassier). R. T. Hepple was supported by a Chercheur Boursier Senior award from the Fond de Recherches du Quebec-Sante. G. A. Power was supported by a Banting postdoctoral fellowship (Canadian Institutes for Health Research; CIHR) and Alberta Innovates Health Solutions (AIHS).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES
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