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

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Abstract

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 re-stretch maneuver on single muscle fiber segments biopsied from the vastus lateralis of young (~23y), older non-athlete (NA) adults (~80y) and age-matched world class masters athletes (MA; ~80y) to assess the rate of force re-development (k_{tr}) and cross-bridge kinetics. A post-hoc analysis was performed, and only the mechanical properties of 'slow type' fibers based on unloaded shortening velocity measurements are reported. The MA and NA were approximately 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 approximately 43% and 48% weaker, respectively, compared with young ($P<0.05$). Unloaded shortening velocity (V_o) for both MA and NA old groups were 62% and 46% slower, respectively, compared with young. Both MA and NA adults had ~2 times slower values for k_{tr} compared with young. The slower V_o in both old groups relative to young, coupled with a similarly reduced k_{tr} 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.

Key Words: Aging, Muscle, Weakness, Velocity, Sarcopenia

List of Abbreviations

NA – Older non-athlete adults

MA – World-class masters athletes

$\boldsymbol{k_{tr}}$ – Rate of force re-development

$\mathbf{V_o}$ – Unloaded shortening velocity

MHC – Myosin heavy chain isoform

CSA – Cross-sectional area

$\mathbf{Ca^{2+}}$ – Calcium

OCT - Optical coherence tomography

PBS – Phosphate-buffered saline

FFT – Fast Fourier transform

CCD – charge-coupled device

$\mathbf{L_o}$ – Optimal length of force production

SL – Sarcomere length

$\mathbf{P_o}$ – Specific isometric tension

K – Stiffness

EC-coupling – Excitation contraction coupling

SD – Standard deviation

SE – Standard error of the mean

Introduction

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), a decreased neural activation (1) and impaired excitation-contraction (EC) coupling (13, 46). Single muscle fiber contractile function is suggested to be dependent upon the content and expression of myosin heavy chain (MHC) isoforms (2). In older adults (~73y) 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 months), 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 cross-bridge kinetics and function are impaired in older adults between 65 and 75 years of age (10, 21, 29, 32, 33). For example, Ochala et al. (32) investigated single muscle fiber properties of the vastus lateralis of ~66 y 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 which 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 65-75 y 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 y, they do not reveal to what extent cross-bridge kinetics is impaired, nor how these changes contribute to contractile dysfunction in aging muscle. Similarly, they do not address ages when muscle weakness becomes most clinically relevant (>75 y old).

Permeabilized muscle fiber segments activated in high [Ca^{2+}] activating solution offers 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 re-stretch maneuver can be used to assess cross-bridge functioning in a fully activated system independently of Ca^{2+} regulation (3). The slacken re-stretch maneuver is designed to detach nearly all cross-bridges. During the shortening phase (*slacken*), force momentarily reaches zero; next the initial fiber length is re-established via a quick stretch (*re-stretch*), 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 non-force-bearing to force-bearing states, contribute to force re-development. Furthermore, the rate constant of force development (k_{tr}) during the transition from non-force-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

161 contributed to their successful aging and athletic prowess relative to their age-matched
162 counterparts.

163 The purpose of this study was to test the hypotheses that single permeabilized muscle
164 fiber segments from older non-athlete adults show an age-related reduction in specific force,
165 shortening velocity, and have a lower k_{tr} than young, while older masters' athletes would not
166 differ from young and thus intrinsic cross-bridge function would be maintained similar to young.
167

168 **Materials and Methods**

169 ***Participants*:** All young (n = 6, 23.4 ± 1.0 y, 178.3 ± 7.9 cm, 79.7 ± 9.6 kg), older non-
170 athlete adults (NA; n = 5, 78.2 ± 9.4 y, 167.8 ± 2.7 cm, 69.7 ± 3.8 kg), and elite masters track
171 and field athletes (MA; n = 6, 78.8 ± 3.6 y, 176.3 ± 5.7 cm, 73.7 ± 12.7 kg) were asked to
172 refrain from unaccustomed and strenuous exercise prior to the muscle biopsy procedure. All
173 participants were male had no known neurological or musculoskeletal conditions. The young
174 adults were recruited from the university population and the NA adults were living
175 independently and recruited from the local community. The MA consisted of male track and
176 field athletes ranked in the top 3 of their respective events at the world masters championships.
177 The MA combined held world records for the: Marathon (80-84 y), 100 m (75-79 y), 100 m
178 hurdles (75-79 y) and were ranked second and third for the 1500 m (75-79 y) and pentathlon
179 (85-89 y), respectively. This study was approved by the McGill Faculty of Medicine
180 Institutional Review Board (IRB) for research involving human subjects (A08-M66–12B) and
181 conformed to the Declaration of Helsinki. Informed written consent was obtained from all
182 participants prior to the study.

***Biopsy procedure and preparation of muscle fibers*:** The suction-modified Bergström muscle biopsy technique (18) was used to obtain a 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 N_2-cooled isopentane for histology. For the portion used in single fiber segment preparation, muscle bundles were tied to wood sticks, and chemically permeabilized by first incubating in rigor solution (pH = 7.0) for ~4 h and subsequently transferring 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 fridge for 1 h 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 chains (MHCs) I, IIA, and IIX using previously described methods (18) (for additional information on antibody specificity please see reference 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: a 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 a rabbit IgG polyclonal anti-laminin (Sigma). Muscle cross sections were then washed 3 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, USA), 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 3 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, USA). Slides were imaged with a Zeiss Axio Imager M2 fluorescence microscope (Zeiss, Oberkochen, Germany). A systematic sampling approach was taken to analyze 500 fibres per subject across each muscle section.

Solutions: The rigor solution (pH 7.0) was composed of (in mM) 50 Tris, 100 NaCl, 2 KCl, 2 $MgCl_2$, and 10 EGTA. The relaxing solution used for muscle storage and dissection (pH 7.0) was composed of (in mM) 100 KCl, 2 EGTA, 20 imidazole, 4 ATP, and 7 $MgCl_2$. The experimental activating solution with pCa^{2+} of 4.5 (pH 7.0) contained (in mM) 20 imidazole, 14.5 creatine phosphate, 7 EGTA, 4 MgATP, 1 free Mg^{2+}, free Ca^{2+} 32 μM (pCa^{2+} 4.5), and KCl to adjust the ionic strength to 180 mM. A pre-activating solution (in mM: 68 KCl, 0.5 EGTA, 20 imidazole, 14.5 creatine phosphate, 4.83 ATP, 0.00137 $CaCl_2$, 5.41 $MgCl_2$ and 6.5 HDTA; pH 7.0, pCa^{2+} 9.0) with a reduced Ca^{2+} buffering capacity was used immediately before activation to minimize delays in diffusion.

***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·sec^{-1} 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 μm × 3.2 μm).

Two experiments were performed during the single fiber study: first, a slack test to determine peak tension and unloaded shortening velocity (V_o), and secondly a slacken re-stretch test to measure the rate of isometric force re-development (k_{tr}) 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; L_o) which then shortened to 2.7-2.6 μm upon activation. All experiments were performed at 10°C. Control contractions at a pCa^{2+} 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 SL, the experiment was terminated.

Muscle contraction was induced by first transferring the single muscle fiber preparation from a relaxing to a pre-activating bath for ~10 s, 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 (P_o) 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 cross-sectional area of the muscle fiber, determined via circularity assumed [$(\pi \cdot (r^2)]$] or angularity data from histology. To account for increased fiber angularity in older adults (47) angularity was derived from the histological preparations – determined as [$(4 \cdot \pi \cdot \text{area})/(\text{perimeter}^2)$] 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 (V_o) 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% L_o. The fiber was allowed to reach P_o, 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 SL was adjusted to ~2.8 μm, prior to the subsequent contraction. The resulting data were plotted as the time required to re-develop 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 (V_o; in $L_o \cdot s^{-1}$).

The kinetics of force re-development was assessed using a slack re-stretch method (3). After full force development and P_o was reached the fiber was shortened by 15% with a 10 $L_o \cdot s^{-1}$ ramp, this reduced the force momentarily to zero, and 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 $L_o \cdot s^{-1}$) re-stretch to the initial L_o allowing for the dissociation of any remaining attached cross bridges. The force re-development following the

slack re-stretch test is related directly to the re-attachment of myosin to actin and a redistribution of cross-bridges from pre-power stroke into force-generating states (3). To determine the proportion of attached cross-bridges throughout the slacken re-stretch maneuver, fiber instantaneous stiffness was measured before, during, and following force re-development. This stiffness (K) was assessed by applying a fast (500 $L_o \cdot s^{-1}$) length step (Δlength = 0.3% L_o) 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 re-development following the slacken re-stretch protocol. For each contraction, k_{tr} was analyzed using the following bi-exponential equation: $F = (a*(1-exp(-k_{tr}*t)-exp(-l*t)) + b)$; where *'F'* is force, *'a'* is the amplitude of the exponential (s), *'t'* is time, k_{tr} 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 V_o values obtained in each of the three groups (n fibers for: Young; 8, NA; 12, 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 $V_o < 0.5L_o/s$. 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

297 data were further analyzed with respect to the above mentioned contractile properties to assess
298 potential age and activity dependent differences of 'slow' type muscle fiber properties.

299 ***Statistical analysis:*** Unless otherwise mentioned in figure legends, comparisons
300 between subjects were performed using unpaired bilateral Student's *t* tests. The level of
301 significance was set at $P \leq 0.05$. Data in the text is presented as mean ± SD and in the figures as
302 mean ± SE.

303

304 **Results**

305 ***Muscle fiber type proportion:*** Muscle fiber type composition (immunohistochemical
306 analysis as seen in Figure 1B) indicated NA adults had a 25% lower type I fiber composition
307 compared with MA and young ($P<0.05$), while MA and young did not differ ($P>0.05$; Figure
308 1A.). The relative distributions of type IIA did not differ across the three groups ($P>0.05$), while
309 type IIX were higher in the young compared with both older groups ($P<0.05$). Additionally, NA
310 adults exhibited significantly more co-expressing type IIA/IIX fibers compared with both MA
311 and young ($P<0.05$; Figure 1A.). There were virtually no pure type IIX fibers in the muscle
312 sections of the two older age groups. Instead, type IIX was almost exclusively expressed in
313 conjunction with type IIA MHC, as depicted in figure 1 (type IIA/IIX).

314 ***Cross sectional area:*** With the assumption of circularity, and angularity derived values,
315 cross sectional area of the permeabilized single muscle fiber segments were similar across
316 groups ($P>0.05$; Table 1).

317 ***Isometric force:*** Absolute force was 53% and 57% lower in both MA and NA adults,
318 respectively, compared with young ($P<0.05$; Table 1) while MA and NA adults did not differ
319 ($P>0.05$). When force was normalized to circularity assumed cross sectional area, specific force
320 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 cross sectional area determined via the angularity data, specific force was 43% and 48% lower for MA and NA, respectively, as 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 (Figure 2) while the older groups did not differ (P>0.05).

Rate of force redevelopment kinetics (k_{tr}): Rate of force redevelopment was ~2 times lower in both MA and NA adults (P<0.05) compared with young (Figure 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 (Figure 4) and was lower in all groups during force re-development compared with pre- and post- measurements, indicating fewer attached cross-bridges during the slacken re-stretch procedure. When stiffness was compared across age groups during force re-development and following force re-development, NA adults appear to have 22-29% lower stiffness values than Young (P<0.05), (indicating less attached cross-bridges during force re-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, older non-athlete (NA) adults and age-matched world class masters athletes (MA) to elucidate to what extent aging and world class athletic performance affects

cross-bridge functioning. The slacken re-stretch maneuver was used to assess cross-bridge kinetics (rate of attaching)(3), allowing us to measure the rate of force re-development in a fully activated system independent of Ca^{2+} -troponin-tropomyosin regulation of actin-myosin interactions and other confounding contraction coupling factors which 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 re-established is the transition from non-force 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 re-development (k_{tr}) (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_{tr} 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 immunolabelled; Figure 1.) indicated NA adults had a ~25% lower type I fiber composition compared with MA and young. The NA adults exhibited significantly more co-expressing type IIA/IIX fibers compared with both MA and young, a finding which 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 (%) (Figure 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 co-expressing fibers compared with myofibular ATPase staining (44). Thus, there does not appear to be a tight coupling between MHC isoform expression and contractile properties that is observed in young

(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) which 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 fibres (thus, a greater maintenance of muscle fibre 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 in aged rat muscle previously (7), contractile properties of aged muscle is 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 (65-81y) (10, 11, 17, 23, 24, 33, 53) and others reporting no change in force or shortening velocity (15, 45, 49) between the ages of 60-80 y. These disparate findings could be related to varying ages tested (i.e., age range tested spans 2 decades; ~60-80y) and the participants' habitual levels of physical activity, both factors which 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. Further, 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 masters athletes 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 normalised by fiber CSA, the athletes' specific force was similar to age-matched controls (50). Additionally, sprint trained masters athletes typically have larger single muscle fiber diameters compared to 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 (Figure 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 were 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 versus 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 versus 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

413 aged samples is likely a common occurrence in single fiber segment studies since a maintained
414 fiber size with aging is usually seen in studies using this approach (16, 48, 49).

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

428 ***Molecular mechanisms of age-related muscle weakness:*** The decrease in isometric
429 specific tension in both older groups could be due to an impaired intrinsic ability of the muscle to
430 produce force either through a lower number of available cross-bridges, and reduced force
431 generated per bridge or a combination of both. Changes to the myosin molecule through
432 oxidation or glycation inhibits ATPase activity, and this in turn, affects the transition from a
433 strongly bound to detached cross-bridge state, resulting in a greater binding affinity of actin for
434 myosin in the weakly bound state ultimately slowing the whole actomyosin ATPase cycle (42).
435 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 to 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, 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 (~65-75y), 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, 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^{2+} confounding factors, and given the isometric tensions were similar or elevated in the old compared to 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 y old).

Cross-bridge attachment distribution as assessed via instantaneous stiffness measures: In the present study, as cross-bridge kinetics (k_{tr}) and V_o were reduced in both older groups compared with young, the age-related impairments could be owing to: (i) less attached cross-bridges and (ii) 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 (Figure 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 non-force producing cross-bridges. As indicated by similar stiffness values during force re-development between MA and Young groups (Figure 4) and a 2 times slower k_{tr} 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 MA group. However, for the NA group, both stiffness and k_{tr} were reduced, suggesting an overall loss of cross-bridge interactions and not just an increase in the weakly bound state as 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 young. However, relative to their age-matched counterparts, this "cross-bridge" resilience could also have contributed to MA's athletic performance.

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 upon the content and expression of myosin heavy chain (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 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 re-development in healthy older participants and elite world champion master athletes. We found for slow type fibers, cross-bridge kinetics was similarly impaired in both groups of older adults as compared with young. Based on the measure of instantaneous stiffness during force re-development we suggest that there were a similar number of attached cross-bridges for both MA and young, 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

505 interactions and not just an increase in the weakly bound state compared young. This finding
506 would also suggest the NA group had fewer available cross-bridges for force production, perhaps
507 owing to reduced myosin content or an inhibition of cross-bridge attachments. These results
508 challenge the widely accepted resilience of slow type fibers to cellular aging. With respect to the
509 high athletic performance of the masters athletes, success in high-performance sport in old age,
510 at least for this study, do not appear to be due to maintained kinetics of cross-bridge state
511 transitions of slow type fibers.
512

Disclosures

No Conflict of interest, financial or otherwise, is declared by the authors.

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References

1. **Aagaard P, Suetta C, Caserotti P, Magnusson SP, and Kjaer M.** Role of the nervous system in sarcopenia and muscle atrophy with aging: strength training as a countermeasure. *Scand J Med Sci Sports* 20: 49-64, 2010.

2. **Bottinelli R, Schiaffino S, and Reggiani C.** Force-velocity relations and myosin heavy chain isoform compositions of skinned fibres from rat skeletal muscle. *J Physiol* 437: 655-672, 1991.

3. **Brenner B and Eisenberg E.** Rate of force generation in muscle: correlation with actomyosin ATPase activity in solution. *Proc Natl Acad Sci U S A* 83: 3542-3546, 1986.

4. **Campbell K.** Rate constant of muscle force redevelopment reflects cooperative activation as well as cross-bridge kinetics. *Biophys J* 72: 254-262, 1997.

5. **Canepari M, Pellegrino MA, D'Antona G, and Bottinelli R.** Single muscle fiber properties in aging and disuse. *Scand J Med Sci Sports* 20: 10-19, 2010.

6. **Canepari M, Rossi R, Pellegrino MA, Orrell RW, Cobbold M, Harridge S, and Bottinelli R.** Effects of resistance training on myosin function studied by the in vitro motility assay in young and older men. *J Appl Physiol* 98: 2390-2395, 2005.

7. **Carter EE, Thomas MM, Murynka T, Rowan SL, Wright KJ, Huba E, and Hepple RT.** Slow twitch soleus muscle is not protected from sarcopenia in senescent rats. *Exp Gerontol* 45: 662-670, 2010.

8. **Claflin DR, Larkin LM, Cederna PS, Horowitz JF, Alexander NB, Cole NM, Galecki AT, Chen S, Nyquist LV, Carlson BM, Faulkner JA, and Ashton-Miller JA.** Effects of high- and low-velocity resistance training on the contractile properties of skeletal muscle fibers from young and older humans. *J Appl Physiol (1985)* 111: 1021-1030, 2011.

9. **Colombini B, Bagni MA, Berlinguer Palmini R, and Cecchi G.** Crossbridge formation detected by stiffness measurements in single muscle fibres. *Adv Exp Med Biol* 565: 127-140; discussion 140, 371-127, 2005.

10. **D'Antona G, Pellegrino MA, Adami R, Rossi R, Carlizzi CN, Canepari M, Saltin B, and Bottinelli R.** The effect of ageing and immobilization on structure and function of human skeletal muscle fibres. *J Physiol* 552: 499-511, 2003.

11. **D'Antona G, Pellegrino MA, Carlizzi CN, and Bottinelli R.** Deterioration of contractile properties of muscle fibres in elderly subjects is modulated by the level of physical activity. *Eur J Appl Physiol* 100: 603-611, 2007.

12. **Dalton BH, Power GA, Vandervoort AA, and Rice CL.** The age-related slowing of voluntary shortening velocity exacerbates power loss during repeated fast knee extensions. *Exp Gerontol* 47: 85-92, 2012.

13. **Delbono O, Renganathan M, and Messi ML.** Excitation-Ca2+ release-contraction coupling in single aged human skeletal muscle fiber. *Muscle Nerve Suppl* 5: S88-92, 1997.

14. **Edman KA.** The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres. *J Physiol* 291: 143-159, 1979.

15. **Frontera WR, Hughes VA, Krivickas LS, Kim SK, Foldvari M, and Roubenoff R.** Strength training in older women: early and late changes in whole muscle and single cells. *Muscle Nerve* 28: 601-608, 2003.

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

17. **Frontera WR, Suh D, Krivickas LS, Hughes VA, Goldstein R, and Roubenoff R.** Skeletal muscle fiber quality in older men and women. *Am J Physiol Cell Physiol* 279: C611-618, 2000.

18. **Gouspillou G, Sgarioto N, Kapchinsky S, Purves-Smith F, Norris B, Pion CH, Barbat-Artigas S, Lemieux F, Taivassalo T, Morais JA, Aubertin-Leheudre M, and Hepple RT.** Increased sensitivity to mitochondrial permeability transition and myonuclear translocation of endonuclease G in atrophied muscle of physically active older humans. *FASEB J* 28: 1621-1633, 2014.

19. **Hancock WO, Martyn DA, Huntsman LL, and Gordon AM.** Influence of Ca2+ on force redevelopment kinetics in skinned rat myocardium. *Biophys J* 70: 2819-2829, 1996.

20. **Hook P, Li X, Sleep J, Hughes S, and Larsson L.** The effect of age on in vitro motility speed of slow myosin extracted from single rat soleus fibres. *Acta Physiol Scand* 167: 325-326, 1999.

21. **Hook P, Sriramoju V, and Larsson L.** Effects of aging on actin sliding speed on myosin from single skeletal muscle cells of mice, rats, and humans. *Am J Physiol Cell Physiol* 280: C782-788, 2001.

22. **Korhonen MT, Cristea A, Alen M, Hakkinen K, Sipila S, Mero A, Viitasalo JT, Larsson L, and Suominen H.** Aging, muscle fiber type, and contractile function in sprint-trained athletes. *J Appl Physiol* 101: 906-917, 2006.

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

24. **Larsson L, Li X, and Frontera WR.** Effects of aging on shortening velocity and myosin isoform composition in single human skeletal muscle cells. *Am J Physiol* 272: C638-649, 1997.

25. **Larsson L, Li X, Yu F, and Degens H.** Age-related changes in contractile properties and expression of myosin isoforms in single skeletal muscle cells. *Muscle Nerve Suppl* 5: S74-78, 1997.

26. **Lexell J.** Human aging, muscle mass, and fiber type composition. *J Gerontol A Biol Sci Med Sci* 50 Spec No: 11-16, 1995.

27. **Li M, Ogilvie H, Ochala J, Artemenko K, Iwamoto H, Yagi N, Bergquist J, and Larsson L.** Aberrant post-translational modifications compromise human myosin motor function in old age. *Aging Cell*, 2015.

28. **Lowe DA, Surek JT, Thomas DD, and Thompson LV.** Electron paramagnetic resonance reveals age-related myosin structural changes in rat skeletal muscle fibers. *Am J Physiol Cell Physiol* 280: C540-547, 2001.

29. **Miller MS, Bedrin NG, Callahan DM, Previs MJ, Jennings ME, 2nd, Ades PA, Maughan DW, Palmer BM, and Toth MJ.** Age-Related Slowing of Myosin-Actin Cross-Bridge Kinetics Is Sex-Specific and Predicts Decrements in Whole Skeletal Muscle Performance in Humans. *J Appl Physiol*, 2013.

30. **Minozzo FC and Rassier DE.** The effects of Ca2+ and MgADP on force development during and after muscle length changes. *PLoS One* 8: e68866, 2013.

31. **Narici MV, Maffulli N, and Maganaris CN.** Ageing of human muscles and tendons. *Disabil Rehabil* 30: 1548-1554, 2008.

32. **Ochala J, Dorer DJ, Frontera WR, and Krivickas LS.** Single skeletal muscle fiber behavior after a quick stretch in young and older men: a possible explanation of the relative preservation of eccentric force in old age. *Pflugers Arch* 452: 464-470, 2006.

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

34. **Parente V, D'Antona G, Adami R, Miotti D, Capodaglio P, De Vito G, and Bottinelli R.** Long-term resistance training improves force and unloaded shortening velocity of single muscle fibres of elderly women. *Eur J Appl Physiol* 104: 885-893, 2008.

35. **Piazzesi G, Reconditi M, Linari M, Lucii L, Bianco P, Brunello E, Decostre V, Stewart A, Gore DB, Irving TC, Irving M, and Lombardi V.** Skeletal muscle performance determined by modulation of number of myosin motors rather than motor force or stroke size. *Cell* 131: 784-795, 2007.

36. **Power GA, Allen MD, Booth WJ, Thompson RT, Marsh GD, and Rice CL.** The influence on sarcopenia of muscle quality and quantity derived from magnetic resonance imaging and neuromuscular properties. *AGE 36*: 9642, 2014.

37. **Power GA, Dalton BH, Behm DG, Doherty TJ, Vandervoort AA, and Rice CL.** Motor unit survival in lifelong runners is muscle dependent. *Med Sci Sports Exerc* 44: 1235-1242, 2012.

634 38. **Power GA, Dalton BH, Behm DG, Vandervoort AA, Doherty TJ, and Rice CL.**
635 Motor Unit Number Estimates in Masters Runners: Use It or Lose It? *Medicine and Science in*
636 *Sports and Exercise* 42: 1644-1650, 2010.

637 39. **Power GA, Dalton BH, and Rice CL.** Human neuromuscular structure and function in
638 old age: A brief review. *Journal of sport and health science* 2: 215-226, 2013.

639 40. **Power GA, Dalton BH, Rice CL, and Vandervoort AA.** Power loss is greater
640 following lengthening contractions in old versus young women. *Age* 34: 737-750, 2012.

641 41. **Power GA, Makrakos DP, Rice CL, and Vandervoort AA.** Enhanced force production
642 in old age is not a far stretch: an investigation of residual force enhancement and muscle
643 architecture. *Physiol Rep* 1: e00004, 2013.

644 42. **Prochniewicz E, Thomas DD, and Thompson LV.** Age-related decline in actomyosin
645 function. *J Gerontol A Biol Sci Med Sci* 60: 425-431, 2005.

646 43. **Prochniewicz E, Thompson LV, and Thomas DD.** Age-related decline in actomyosin
647 structure and function. *Exp Gerontol* 42: 931-938, 2007.

648 44. **Purves-Smith FM, Sgarioto N, and Hepple RT.** Fiber typing in aging muscle. *Exerc*
649 *Sport Sci Rev* 42: 45-52, 2014.

650 45. **Reid KF, Doros G, Clark DJ, Patten C, Carabello RJ, Cloutier GJ, Phillips EM,**
651 **Krivickas LS, Frontera WR, and Fielding RA.** Muscle power failure in mobility-limited older
652 adults: preserved single fiber function despite lower whole muscle size, quality and rate of
653 neuromuscular activation. *Eur J Appl Physiol* 112: 2289-2301, 2012.

654 46. **Renganathan M, Messi ML, and Delbono O.** Dihydropyridine receptor-ryanodine
655 receptor uncoupling in aged skeletal muscle. *J Membr Biol* 157: 247-253, 1997.

656 47. **Rowan SL, Purves-Smith FM, Solbak NM, and Hepple RT.** Accumulation of severely
657 atrophic myofibers marks the acceleration of sarcopenia in slow and fast twitch muscles. *Exp*
658 *Gerontol* 46: 660-669, 2011.

659 48. **Slivka D, Raue U, Hollon C, Minchev K, and Trappe S.** Single muscle fiber
660 adaptations to resistance training in old (>80 yr) men: evidence for limited skeletal muscle
661 plasticity. *Am J Physiol Regul Integr Comp Physiol* 295: R273-280, 2008.

662 49. **Trappe S, Gallagher P, Harber M, Carrithers J, Fluckey J, and Trappe T.** Single
663 muscle fibre contractile properties in young and old men and women. *J Physiol* 552: 47-58,
664 2003.

665 50. **Trappe SW, Costill DL, Vukovich MD, Jones J, and Melham T.** Aging among elite
666 distance runners: a 22-yr longitudinal study. *J Appl Physiol* 80: 285-290, 1996.

667 51. **Walker SM and Schrodt GR.** I segment lengths and thin filament periods in skeletal
668 muscle fibers of the Rhesus monkey and the human. *Anat Rec* 178: 63-81, 1974.

669 52. **Williamson DL, Godard MP, Porter DA, Costill DL, and Trappe SW.** Progressive
670 resistance training reduces myosin heavy chain coexpression in single muscle fibers from older
671 men. *J Appl Physiol (1985)* 88: 627-633, 2000.

672 53. **Yu F, Hedstrom M, Cristea A, Dalen N, and Larsson L.** Effects of ageing and gender
673 on contractile properties in human skeletal muscle and single fibres. *Acta Physiol (Oxf)* 190:
674 229-241, 2007.

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684 difference both age groups†
685
686
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694 raw data traces. Mean ± SE, Significantly different than young*
695
696
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700 traces. After full force development the fiber was shortened by 15% with a 10 $L_o \cdot s^{-1}$ ramp, and
701 was then followed by a rapid (500 $L_o \cdot s^{-1}$) re-stretch to the initial L_o. **(C.)** Same as in B, but with
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703 corresponding peak force. Mean ± SE, Significantly different than young*
704
705
706 **Figure 4. (A.)** Stiffness measurements - The instantaneous stiffness (*k*) was assessed by
707 applying a fast length step (Δlength = 0.3% L_o) and is expressed during the initial force
708 development, during force re-development, and following force re-development. Solid bars are
709 the mean data for each group (Young in white; NA-older adults in grey; MA- Master Athletes in
710 black **(B.)** Stiffness measurements during initial force development. **(C.)** Stiffness measurements
711 during force re-development. **(D.)** Stiffness measurements post force re-development. Mean ±
712 SE, Significantly different from young*. Solid bars represent stiffness values following force
713 development, upward hashed lines represents stiffness during force re-development, downward
714 hashed lines represents stiffness following force re-development.
715

716
717 **Table 1.** Fiber segment cross-sectional area (CSA) derived from circularity assumed and fiber
718 angularity, force, and specific force
719
720

	Young	Older Non-Athlete	Masters Athlete
Force (mN)	0.31 ± 0.06	0.13 ± 0.02*	0.15 ± 0.02*
CSA (mm^{-2})			
Circularity Assumed	0.008 ± 0.002	0.006 ± 0.001	0.008 ± 0.001
Fiber Angularity	0.007 ± 0.002	0.005 ± 0.0001	0.005 ± 0.001
Specific Force ($mN \cdot mm^{-2}$)			
Circularity Assumed	41.23 ± 5.83	23.49 ± 5.56*	19.06 ± 2.81*
Fiber Angularity	49.44 ± 7.09	25.73 ± 3.52*	28.39 ± 3.96*

721
722 *Significant different from young (Mean ± SE).
723
724

Figure 1

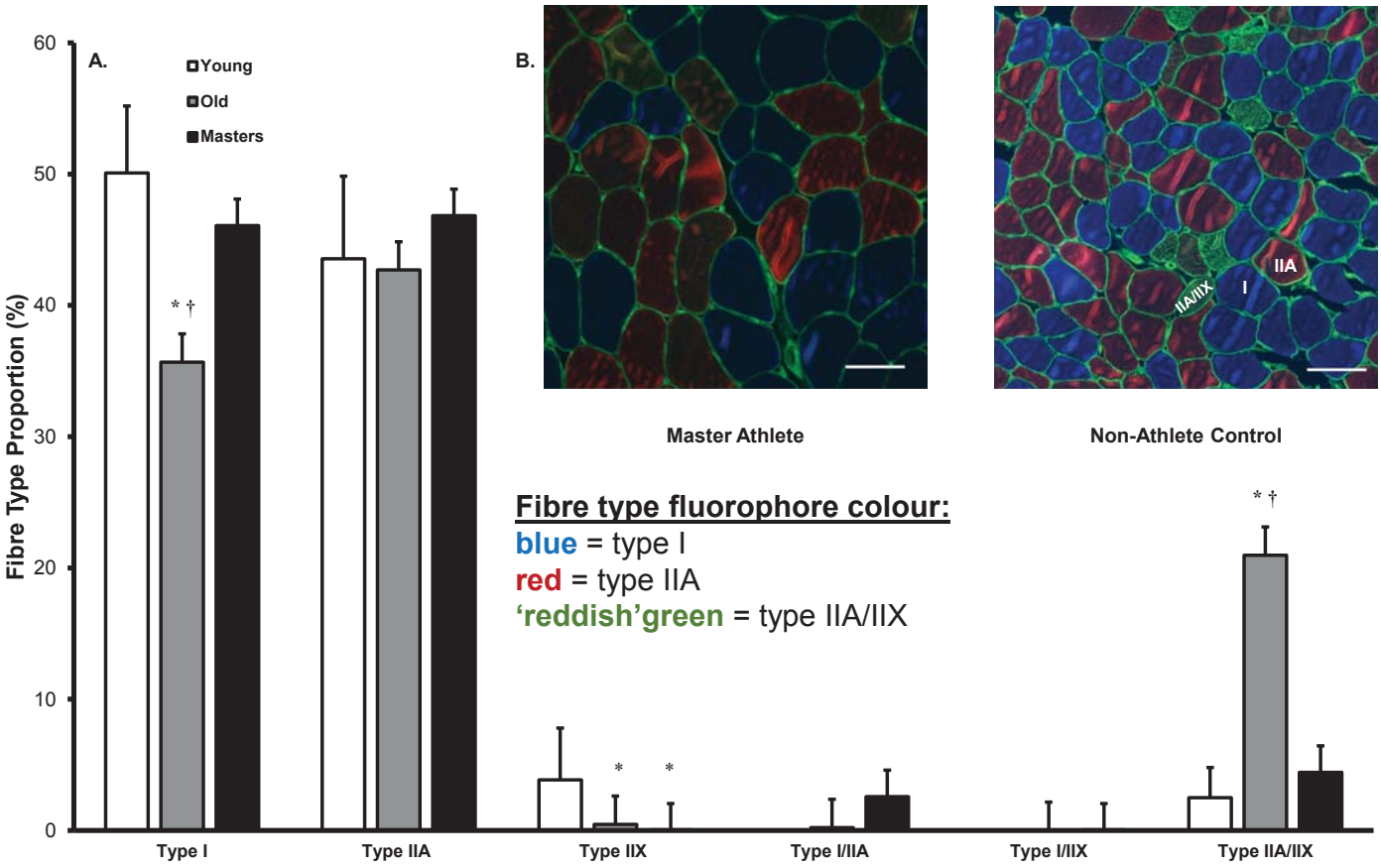

A.
Young
Old
Masters
Fibre Type Proportion (%)
60
50
40
30
20
10
0
* †
*
*
* †
Type I
Type IIA
Type IIX
Type I/IIA
Type I/IIX
Type IIA/IIX
B.
IIA
I
IIA/IIX
Master Athlete
Non-Athlete Control
Fibre type fluorophore colour:
blue = type I
red = type IIA
'reddish'green = type IIA/IIX

Figure 2

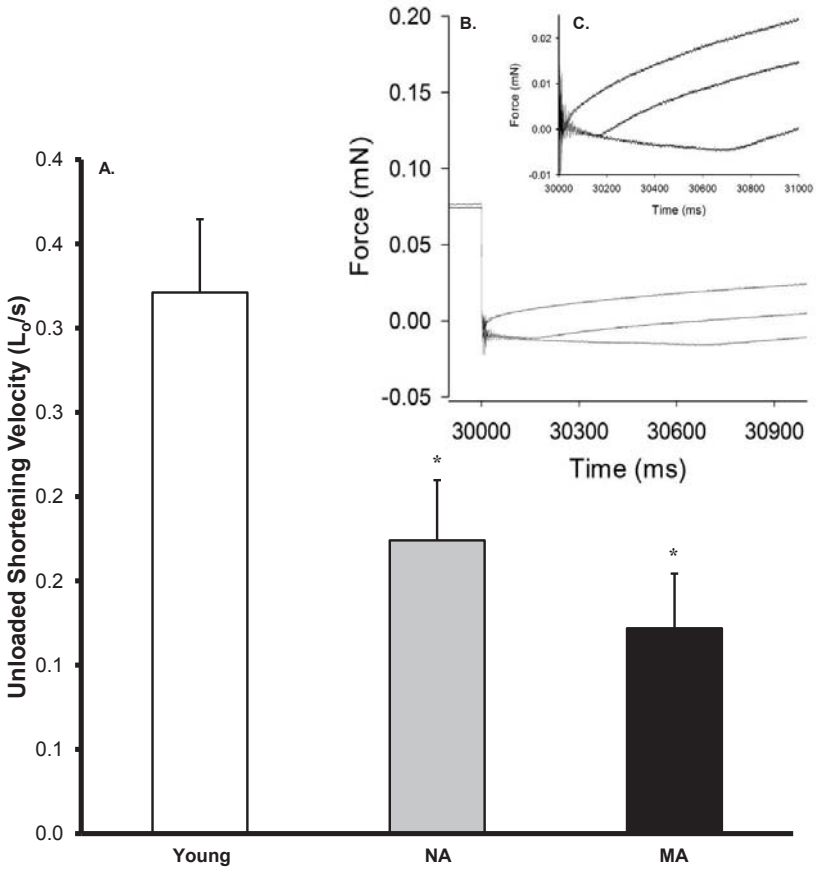

A.
B.
C.
Unloaded Shortening Velocity (L_o/s)
0.4
0.3
0.2
0.1
0.0
Young
NA
MA
*
*
Force (mN)
0.20
0.15
0.10
0.05
0.00
-0.05
30000
30300
30600
30900
Time (ms)
Force (mN)
0.02
0.01
0.00
-0.01
30000
30200
30400
30600
30800
31000
Time (ms)

Figure 3

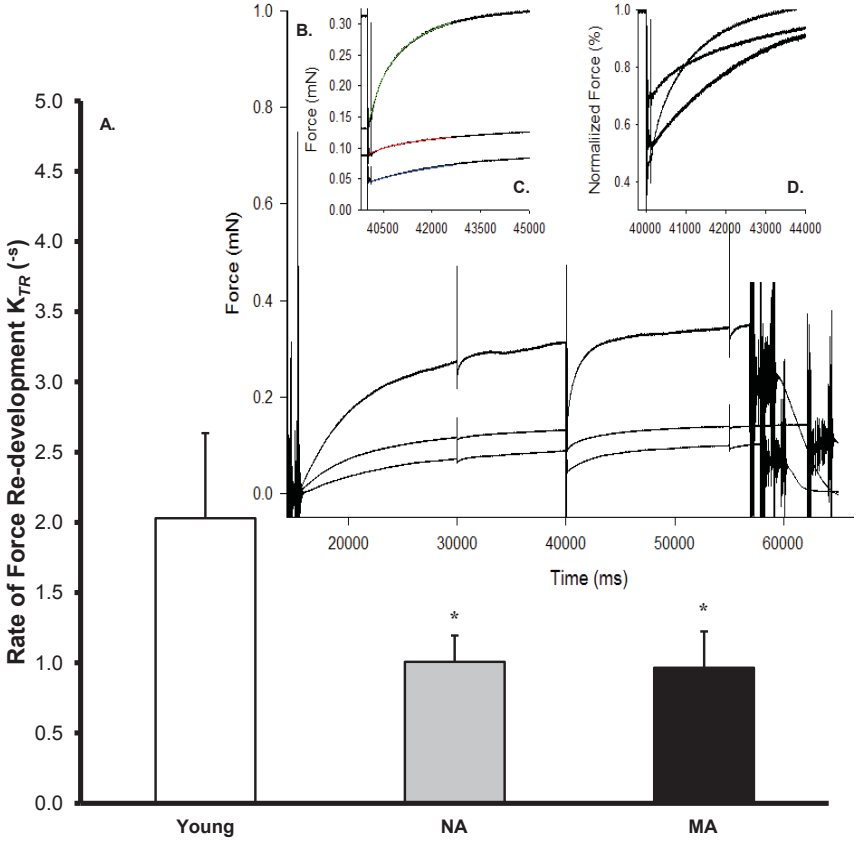
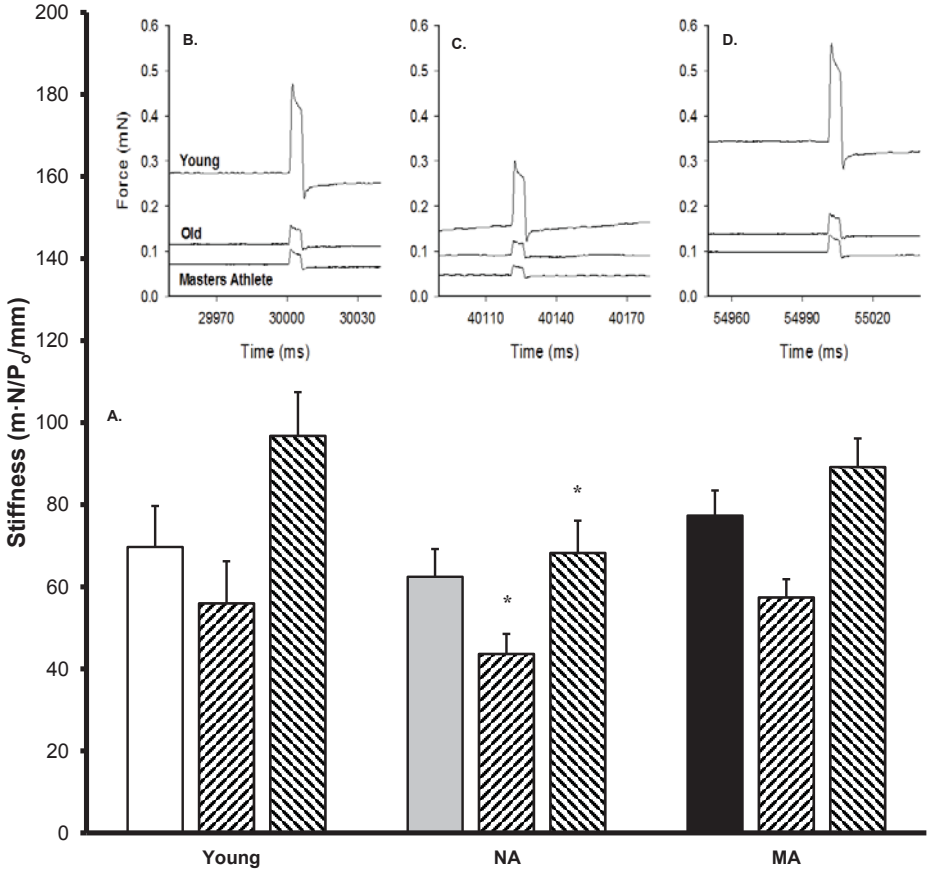

A.
B.
C.
D.
Rate of Force Re-development K_{TR} ($^{-s}$)
Force (mN)
Time (ms)
Normalized Force (%)
Young
NA
MA
*
*

Figure 4


A.
B.
C.
D.
Stiffness (m·N/P$_o$/mm)
Force (mN)
Young
Old
Masters Athlete
Time (ms)
Time (ms)
Time (ms)
Young
NA
MA
*
*