6-2020

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American Journal of Physiology: Cell Physiology, Vol. 318, No. 6 (June 2020): C1238-C1251. DOI.

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Abstract

Age-induced declines in skeletal muscle contractile function have been attributed to multiple cellular factors, including lower peak force (P_o), decreased Ca^{2+} sensitivity, and reduced shortening velocity (V_o). However, changes in these cellular properties with aging remain unresolved, especially in older women, and the effect of submaximal Ca^{2+} on contractile function is unknown. Thus, we compared contractile properties of muscle fibers from 19 young (24 ± 3 yr; 8 women) and 21 older adults (77 ± 7 yr; 7 women) under maximal and submaximal Ca^{2+} and assessed the abundance of three proteins thought to influence Ca^{2+} sensitivity. Fast fiber cross-sectional area was ~44% larger in young (6,479 ± 2,487 μm^2) compared with older adults (4,503 ± 2,071 μm^2, $P < 0.001$), which corresponded with a greater absolute P_o (young = 1.12 ± 0.43 mN; old = 0.79 ± 0.33 mN, $P < 0.001$). There were no differences in fast fiber size-specific P_o, indicating the age-related decline in force was explained by differences in fiber size. Except for fast fiber size and absolute P_o, no age or sex differences were observed in Ca^{2+} sensitivity, rate of force development (k_{tr}), or V_o in either slow or fast fibers. Submaximal Ca^{2+} depressed k_{tr} and V_o, but the effects were not altered by age in either sex. Contrary to rodent studies, regulatory light chain (RLC) and myosin binding protein-C abundance and RLC phosphorylation were unaltered by age or sex. These data suggest the age-associated reductions in contractile function are primarily due to the atrophy of fast fibers and that caution is warranted when extending results from rodent studies to humans.

INTRODUCTION

Aging is accompanied by decreases in muscle mass and the ability to generate power (9, 50, 70), which can impair mobility and quality of life for older adults (16, 45, 62). Despite these well-recognized reductions of the whole muscle, the data regarding the effect of aging on single muscle fiber contractile properties in saturating Ca^{2+} (pCa 4.5) are conflicting. Some studies have reported age-related decreases in force, velocity, and power of slow myosin heavy chain (MHC) I and fast MHC II fibers (8, 13, 21, 38, 58), while others have observed no change (20, 56, 69, 74) or even enhanced contractile performance with aging (27, 63). We recently observed an age-induced decline in fiber size, absolute peak force (P_o), and power of fast but not slow fibers in men, whereas all other contractile properties were preserved (69). Importantly, the selective loss of fast MHC II muscle was closely associated with the age-related decline in whole-muscle force and power, suggesting that the atrophy of fast fibers is a primary determinant of age-related decrements in contractile function (69). However, it is not known whether single fiber contractile function is impaired in submaximal Ca^{2+} and if Ca^{2+} sensitivity is compromised in older men and women.

Findings on aging and Ca^{2+} sensitivity of human skeletal muscle fibers are equivocal, with some studies observing decreased Ca^{2+} sensitivity in older compared with young adults (37, 68) and others observing no difference (32, 33). Furthermore, only a single study has tested if there are sex differences in Ca^{2+} sensitivity with aging (68), and no studies have tested the effect of submaximal Ca^{2+} on contractile function of human skeletal muscle fibers from young or older adults. Studying contractile function under submaximal Ca^{2+} is important, because there is evidence of less stored Ca^{2+} in the sarcoplasmic reticulum (37) and a lower amplitude of the intracellular Ca^{2+} transient with age (15). The reduced intracellular Ca^{2+} may result in decreased fiber force, rates of tension development (k_{tr}), and unloaded shortening velocity (V_o) (54, 64), impairing contractile function in fibers from older compared with young adults.

Under submaximal Ca^{2+} conditions, k_{tr} is depressed and V_o is biphasic, showing a slower shortening velocity at lengths >10% of fiber length (43, 52, 54, 71). The slower velocity at longer shortening lengths has been attributed to an increased internal drag from slower cross-bridge turnover due to binding of myosin binding protein-C (MyBP-C) to actin, and/or the cooperative inactivation of thin filaments decreasing the number of strongly bound cross-bridges (31, 34, 44, 52, 54, 71). Importantly, the contribution of myosin strong binding to activation of the thin filaments is greater in submaximal Ca^{2+} (25). Thus, if aging decreases myofibrillar

Ca^{2+} sensitivity, the rate and number of myosin strong binding would decrease and, under submaximal Ca^{2+} conditions, further depress the slow velocity phase of shortening and k_{tr}.

One potential mechanism for age-related differences in Ca^{2+} sensitivity and contractile function in submaximal Ca^{2+} could be alterations in the content and/or phosphorylation of key regulatory proteins, such as MyBP-C, troponin I (TnI), and myosin regulatory light chain (RLC) (31, 46, 48). In rodent models, aging has been associated with decreased MyBP-C content (1, 17, 60) and phosphorylation (1), but no age-differences were observed in content and phosphorylation of TnI (76). However, whether the differences, or lack thereof, observed in the content and phosphorylation of these proteins in rodents are reflective of what happens in aging human skeletal muscle is unknown. Moreover, findings on the content and phosphorylation of RLC from aging humans and rodents are equivocal (8, 23, 24, 49, 76). For example, slow RLC (RLCs) content and phosphorylation have been shown to increase in aged rats (23), while fast RLC (RLCf) content and phosphorylation have been shown to decrease with aging in humans (24). In contrast, Brocca et al. (8) found no changes in RLCf content or phosphorylation in older men but found an increase in RLCs phosphorylation, whereas Miller et al. (49) found a decreased RLCf phosphorylation in older women but not men. The explanation for the discrepancies between studies is unclear but may involve the large heterogeneity in fiber type distribution between individuals, as some studies report concurrent changes in both MHC and RLC content with aging (23, 24). Understanding whether aging decreases RLC content and/or phosphorylation is important, because phosphorylation of RLC has been shown to increase *1*) Ca^{2+} sensitivity and k_{tr} (46, 59, 72) and *2*) force in submaximal Ca^{2+} by increasing the disordered array of myosin and thereby the probability of myosin binding to actin (12, 39, 42).

The purpose of the present study was to *1*) compare the contractile mechanics of muscle fibers from young and older men and women under both maximal and submaximal Ca^{2+} conditions and *2*) assess the abundance and phosphorylation states of three regulatory proteins thought to influence Ca^{2+} sensitivity. Because the loss of mobility and risks of debilitating falls are more dependent on age-related changes of large lower limb muscles, we chose to study single fibers isolated from the vastus lateralis muscle. On the basis of our previous findings from men (69), we expected to observe marked atrophy of fast muscle fibers but an overall preservation of contractile function in saturating Ca^{2+} conditions with age. In contrast, we hypothesized that aging would decrease Ca^{2+} sensitivity and inhibit k_{tr} and V_o in submaximal Ca^{2+}, with the effect on velocity only observed at shortening lengths >10% of fiber length.

MATERIALS AND METHODS

Subjects.

Nineteen young adults (11 men and 8 women; ages 20–33 yr) and 21 older adults (14 men and 7 women; ages 68–90 yr) volunteered to participate in this study. Participants were given a general health screening that included an assessment of body composition and thigh lean mass with dual X-ray absorptiometry (Lunar iDXA; GE, Madison, WI) (69). Participants were healthy, community-dwelling adults free of any known neurological, musculoskeletal, and cardiovascular diseases and were excluded from participation if they had any major health concerns. All subjects provided written informed consent, and procedures were approved by the Marquette University Institutional Review Board and conformed to the principles in the *Declaration of Helsinki*.

Physical activity assessment.

Physical activity was quantified for each participant with a triaxial accelerometer (GT3X; ActiGraph, Pensacola, FL) worn around the waist for at least 4 days (2 weekdays and 2 weekend days) as reported previously (30, 69). The data were recorded for each participant if the accelerometer was worn for a minimum of 8 h on at least 3 days (29). Physical activity and anthropometric measurements for the participants are reported in Table 1.

Table 1. Anthropometries of the young and older adults

	Men		Women		PValue		
	Young (11*)	Old (14)	Young (8)	Old (7)	Age	Sex	AgexSex
Age, yr	25 ± 4	76 ± 7	23 ± 2	77 ± 7	**0.000**	0.757	0.383
Height, cm	178.4 ± 9.8	171.9 ± 7.5	168.9 ± 7.2	162.8 ± 4.1	**0.018**	**0.001**	0.944
Weight, kg	78.7 ± 16.3	77.9 ± 8.6	62.6 ± 9.2	69.4 ± 9.1	0.435	**0.002**	0.317
Body mass index, kg/m^2	24.5 ± 2.8	26.4 ± 3.2	21.8 ± 1.6	26.5 ± 4.3	**0.002**	0.199	0.176
Whole-body fat, %	18.7 ± 5.3	29.4 ± 7.4	25.6 ± 3.3	38.3 ± 7.4	**0.000**	**0.000**	0.619
Thigh lean mass, kg	15.0 ± 3.5	11.7 ± 1.4	10.7 ± 1.8	9.2 ± 0.9	**0.002**	**0.000**	0.230
Physical activity, steps/day	8,760 ± 4,597	9,275 ± 5,178	10,151 ± 3,949	5,925 ± 2,569	0.215	0.509	0.116

Data are presented as means ± SD. Body fat percentage and the combined thigh lean mass of both legs were measured via dual X-ray absorptiometry (Lunar iDXA, GE Healthcare). The sample size (*N*) for each cohort is reported in parentheses.

*One young male did not have physical activity measured (*N* = 10).

Boldfaced *P* values highlight statistical significance at $P < 0.05$.

Muscle biopsy.

Muscle biopsies of the vastus lateralis were performed on all participants using the modified Bergström technique as previously described (69). A portion of the biopsy used for single fiber contractile experiments was placed in ice-cold glycerol skinning solution (see below) and stored at −20°C for up to 4 wks. The remaining portion used for molecular studies was flash-frozen and stored in liquid nitrogen.

Solutions.

Solutions for single fiber contractile experiments were derived from an iterative program using the stability constants adjusted for temperature, pH, and ionic strength (18, 19) and contained (in mM): 20 imidazole, 7 EGTA, 4 free MgATP, 1 free Mg^{2+}, 14.5 creatine phosphate. ATP was added as Na_2ATP, Mg as $MgCl_2$, creatine phosphate as Na_2 phosphocreatine, and Ca^{2+} as $CaCl_2$. Sufficient KCl was added to relaxing (79.2 mM) and activating (64 mM) solutions to adjust the ionic strength to 180 mM. All solutions were adjusted to pH 7.0 with KOH. The relaxing solution contained negligible amounts of Ca^{2+} (pCa 9.0, where $pCa = -\log_{10}[Ca^{2+}]$), while the activating solution contained saturating levels of Ca^{2+} (pCa 4.5). A range of activating solutions from pCa 6.5 to 5.4 were made by mixing appropriate volumes of pCa 9.0 and 4.5 solutions (77). Glycerol skinning solution was composed of 50% relaxing and 50% glycerol (vol/vol).

Single fiber preparation.

Fibers were prepared as described previously (69). Briefly, single fibers ~2 mm in length were isolated from a biopsy and tied to a force transducer (400A; Aurora Scientific) and a high-speed servomotor (controller 312B; Aurora Scientific). Fibers were kept at 15°C with a temperature-controlled Peltier unit in 120 µL relaxing solution for the duration of the experiment, except when transferred briefly to a second Peltier unit containing 120 µL of activating solution set at 15°C (57). Sarcomere length was adjusted to 2.5 µM, and the fiber length determined by measuring the distance between the points of attachment to the force transducer and motor. Fiber diameter was determined by taking a digital image while the fiber was briefly suspended in air and then measuring the diameter at three points along the length of the fiber. Cross-sectional area (CSA) was calculated from the mean fiber diameter, assuming the fiber forms a cylinder when suspended in air. Fibers were used for multiple experiments if the force remained at >90% of the initial force measured in the first experiment. After completing

experiments, we determined the MHC composition of each fiber by SDS-PAGE and silver staining as previously described (69).

Force-pCa relationship.

The force-pCa relationship was determined as described previously (14, 77) on a subset of individuals (10 young men, 6 young women, 9 older men, and 7 older women). Single fibers were activated a total of 10–13 times in a series of solutions with Ca^{2+} concentrations between pCa 6.5 and 4.5. The forces at each [Ca^{2+}] were fit with Hill plots to determine the half-maximal activation (pCa_{50}), the lowest concentration of Ca^{2+} that elicits force (activation threshold), and the slope of the pCa-force relationship above (n_1) and below (n_2) pCa_{50}. The first, seventh, and last contractions were performed at pCa 4.5 to ensure fiber force remained >90% of the initial force.

k_{tr} and V_o.

Rate of force development following a slack re-extension (k_{tr}) and unloaded shortening velocity (V_o) were measured as described previously (69) on fibers at both maximal (pCa 4.5) and submaximal (pCa_{50}) Ca^{2+}. For k_{tr}, slack duration before re-extension was set at 20 ms. For V_o, fibers were activated six to eight times for both pCa 4.5 and pCa_{50} and slacked to varying distances (100–400 µm), and time of force redevelopment was determined. Slack distances never exceeded >20% of fiber length. V_o was calculated using all slack distances, as well as from slack distances below and above 10% of shortening length (e.g., slack distances of 100–200 µm and 250–400 µm for a 2 mm fiber). Each V_o measurement was calculated as the slope of the least squares regression line between the slack distance and the time required to begin the redevelopment of force. The solution necessary to elicit half-maximal force was determined empirically for each fiber but generally fell between pCa 5.7 and 6.0.

Protein content.

Western blotting was performed on a subset of subjects to examine the content of three proteins that influence Ca^{2+} sensitivity: RLC, TnI, and MyBP-C. Fast and slow isoforms were compared in the vastus lateralis from the dominant leg of young men (n = 5), young women (n = 6), old men (n = 6), and old women (n = 6). Flash-frozen tissue was weighed (10–30 mg) and homogenized on ice in RIPA buffer (1:30 wt/vol, Thermo Scientific) with a protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific). Protein concentration was determined using the Bradford assay (7), and samples were diluted to 0.5 µg/µL in SDS sample buffer (10% SDS, 23 mM EDTA, 50% glycerol, 0.4% bromophenol blue, and 5.1% β-mercaptoethanol, pH 6.8) and boiled for 3 min.

After verifying that 4 µg of protein was in the linear range for all antibodies used, we separated proteins on precast polyacrylamide gels (Bio-Rad) with a Mini-PROTEAN Electrophoresis Cell (Bio-Rad). Precast 4–15% polyacrylamide gels (Mini-PROTEAN TGX, Bio-Rad) were run for 1 h at 150 V in Tris-glycine running buffer and then transferred at 100 V for 50 min to 0.2 µM nitrocellulose membranes with a Mini Trans-Blot cell (Bio-Rad) containing Tris-glycine buffer plus 20% methanol. Total protein transferred to the membrane was determined following the procedure for Sypro Ruby Protein Blot Stain (Thermo Fisher Scientific). After being stained, membranes were blocked for 1 h in 5% nonfat dry milk in TBS-0.1% Tween, pH 7.6 (TBST), and rinsed three times for 5 min each in TBST, before being probed with antibodies for fast isoforms: TnIf (Abcam Cat. no. ab184554, 1:2,500), RLCf (Abcam Cat. no. ab135404, 1:2,000), and MyBP-Cf (Thermo Fisher Scientific Cat. no. PA5-20917, RRID: AB 11154456, 1:2,000), or slow isoforms: TnIs (Novus Biologicals Cat. no. NBP1-56641, RRID: AB 11035917, 1:4,000), RLCs (Abcam Cat. no. ab92721, RRID: AB 10563535, 1:5,000), and MyBP-Cs (Novus Biologicals Cat. no. NBP2-41157, 1:10,000). All antibodies were added to 5% nonfat dry milk in TBST, and membranes were incubated overnight at 4°C. Following another set of rinses with TBST as described above, membranes were probed with a horseradish peroxidase-conjugated secondary antibody (Thermo Fisher Scientific Cat. no. G-21234, RRID: AB 2536530, 1:5,000) for 1 h at room temperature in 5% nonfat dry milk in TBST. Membranes were

given a final set of rinses before protein signals were detected on autoradiography film (Hyblot CL, Fisher Scientific) by chemiluminescence.

ImageJ (National Institutes of Health) was used for densiometric analysis of the films. To compare data between gels, signals were normalized to total protein and then expressed relative to the pooled homogenate control quantified with Image Studio Lite (Li-Cor) based on calculations in Taylor and Posch (73). Total protein was used as a suitable alternative to housekeeping proteins, such as GAPDH, which may be too abundant for accurate normalization or altered with aging (3, 41, 75). Each sample was run a minimum of two times, and the signal averaged for each participant to compare between cohorts. To minimize the influence of variability between gels, we loaded as many individuals from each cohort on one gel as possible (e.g., 2 young men, 3 old men, 3 young women, and 3 old women), and any participant with considerable differences between gels was rerun. Additionally, the same pooled control homogenate consisting of samples from all four cohorts was loaded at least twice on every gel.

To examine whether variations in the regulatory protein content were due to differences in fiber type distribution, we quantified each participant's MHC composition by running their homogenate in quadruplicate (50, 60, 80, and 100 ng) on 5% SDS-PAGE gels and silver stained as previously described (69). The dilution series used was based on the stain's linear range (51). Densitometry analysis was performed in ImageJ (National Institutes of Health) to determine the percentage of MHC slow (I) and fast (IIa and IIa/IIx) in each lane [e.g., $\%MHC\ I = Signal_{MHCI}/(Signal_{MHCI} + Signal_{MHCIIa\ \&\ IIa/IIx})$], and the four replicates averaged per individual.

To evaluate whether age alters the content of the slow and/or fast isoforms of RLC, MyBP-C, or TnI, the Western blot data were analyzed in two ways. First, we compared the protein content between cohorts without accounting for the potential influence of differences in fiber type distribution. As a secondary assessment and to ensure the results were not influenced by variability from the Western blot procedure, we also compared the protein content of RLC from the SYPRO Ruby-stained gels, where the slow and fast isoforms are readily distinguishable based on molecular weights. The results did not differ between the two analyses, and thus, only the Western blot data are presented. And second, to account for the potential influence of differences in fiber type distribution, we expressed each participant's slow and fast isoforms of the three regulatory proteins relative to the corresponding slow and fast MHC isoform percentages. The results did not differ between these two methods; thus, Western blot data are shown expressed relative to MHC content.

Protein phosphorylation.

Pro-Q Diamond staining was performed to test for differences in the phosphorylation status of RLC, TnI, and MyBP-C between young and old men and women. After determining the load was in the linear range for both stains, we prepared five samples from each cohort as above, loaded at 6 μg on 4–15% gradient precast polyacrylamide gels, and run in Tris-glycine buffer using a Mini-PROTEAN Electrophoresis Cell (Bio-Rad) for 1 h at 150 V. Gels were then stained with Pro-Q Diamond phosphoprotein gel dye (Invitrogen) based on the manufacturer's instructions and imaged using the UV GelDoc-It2 Imager. The PeppermintStick phosphoprotein molecular weight standard (Invitrogen) was used to verify appropriate exposure time. Following imaging, gels were stained overnight with the SYPRO Ruby protein gel stain (Invitrogen), destained according to the manufacturer's instructions, and imaged for total protein.

Each sample was run in duplicate on two separate gels, and the same pooled homogenate control was included for comparison across all gels. Densitometry was performed using ImageJ (National Institutes of Health), and phosphorylation level was determined by taking the ratio of each individual's Pro-Q phosphorylated band intensity by their SYPRO Ruby protein abundance band intensity. After validating the phosphorylation level of the pooled homogenate control was similar between gels for all bands measured ($SD < 0.04$), we divided the

phosphorylation level of each individual by the corresponding phosphorylation level of the control: % Control = (Pro-Q_{Ind}/Sypro $Ruby_{Ind}$)/(Pro-$Q_{Control}$/Sypro $Ruby_{Control}$).

Statistical analysis.

Due to the small number of hybrids (MHC I/IIa and IIa/IIx), only pure MHC I and IIa fibers were included in the analysis. Additionally, some fibers were excluded from analysis if they did not meet quality criteria (see above), which resulted in varying fiber and/or subject number between experiments. A nested ANOVA was used for single fiber data to test for differences in age and sex. To test for an effect of Ca^{2+} on contractile mechanics, a repeated-measures nested ANOVA was used. Total protein, phosphorylation levels, and MHC content were compared between cohorts using a two-way ANOVA. When necessary, data were transformed to meet assumptions of normality and homogeneity of variance. Statistical analyses were performed using Minitab, version 18.1 (Minitab Inc., State College, PA). Statistical significance was set at $P < 0.05$. Data are presented as means ± SD in the text and tables. Unless otherwise noted, results comparing the two age groups have men and women combined, sexes have young and old combined, and fiber types have young and old men and women combined.

RESULTS

Cross-sectional area.

There were no differences in cross-sectional area (CSA) for the MHC I fibers between young (6,116 ± 2,049 μm^2) and old (5,870 ± 2,310 μm^2, $P = 0.543$), but men (6,433 ± 2,473 μm^2) had ~21% larger MHC I fibers compared with women (5,329 ± 1,648 μm^2, $P = 0.032$) (Fig. 1). CSA was ~44% larger in MHC IIa fibers from young (6,479 ± 2,487 μm^2) compared with old (4,503 ± 2,071 μm^2, $P < 0.001$) and ~60% larger in men (6,358 ± 2,483 μm^2) compared with women (3,970 ± 1,604 μm^2, $P < 0.001$). Young men (8,599 ± 1,857 μm^2) had a larger CSA than young women (4,909 ± 1,515 μm^2), old men (5,404 ± 2,049 μm^2), and old women (3,174 ± 1,177 μm^2). Old men and young women had similar CSAs of MHC IIa fibers, and both were larger than old women.

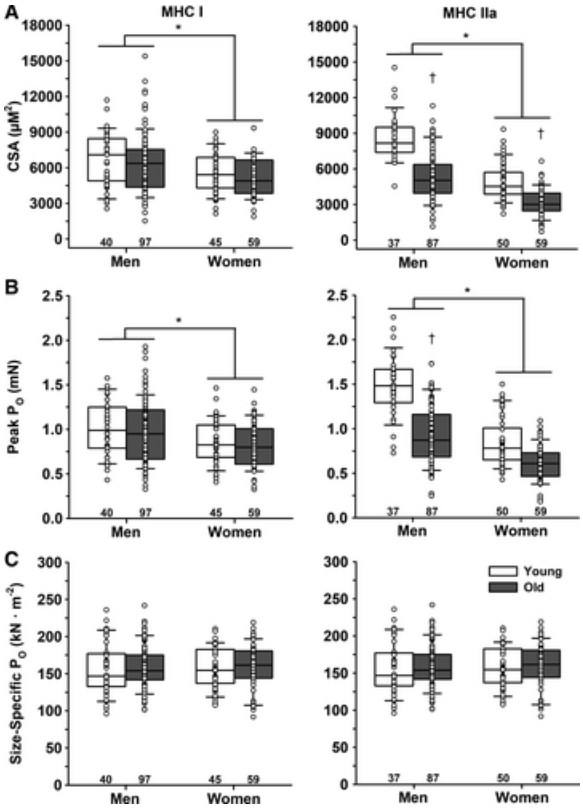

Fig. 1. Cross-sectional area (CSA), peak force (P_o), and size-specific force of single muscle fibers. *A*: CSA. Men had greater CSA in both slow MHC I and fast MHC IIa fibers compared with women. There was no age difference in CSA of MHC I fibers; however, MHC IIa fibers from old men and women were smaller than their younger cohort. *B*: peak force (P_o) in maximal Ca^{2+} (pCa 4.5). Men had greater P_o in both MHC I and IIa fibers compared with women. There was no age difference in P_o for MHC I fibers; however, P_o of MHC IIa fibers was lower in old compared with young, and young men had a greater P_o than all other groups. *C*: size-specific P_o in maximal Ca^{2+} (pCa 4.5). There were no age or sex differences in size-specific P_o for either MHC I or IIa fibers, indicating that the age and sex differences in absolute P_o were explained by fiber size. *Significant effect of sex. †Significant effect of age. Significance level $P < 0.05$. The horizontal line in each box plot indicates the median, while the whiskers represent 1.5 times the upper- and lower-interquartile range. Each dot is an individual fiber, with the number of fibers (*n*) displayed below the box plots. Data were analyzed using a nested ANOVA.

Peak force.

There were no differences in peak force (P_o) for the MHC I fibers between young (0.96 ± 0.28 mN) and old (0.91 ± 0.32 mN, $P = 0.462$), but men (0.98 ± 0.33 mN) had greater P_o compared with women (0.83 ± 0.25 mN, $P = 0.031$) (Fig. 1). P_o was greater in MHC IIa fibers from young (1.12 ± 0.43 mN) compared with old (0.79 ± 0.33 mN, $P < 0.001$) and greater in men (1.09 ± 0.42 mN) compared with women (0.72 ± 0.27 mN, $P < 0.001$). P_o of MHC IIa fibers from young men (1.48 ± 0.34 mN) was higher compared with young women (0.85 ± 0.27 mN), old men (0.92 ± 0.33 mN), and old women (0.60 ± 0.20 mN), but old men, young women, and old women were not different from each other.

Size-specific force.

Size-specific P_o of MHC I fibers did not differ between young (157 ± 31 kN/m^2) and old (159 ± 29 kN/m^2, $P = 0.944$) or men (158 ± 31 kN/m^2) and women (158 ± 28 kN/m^2, $P = 0.668$) (Fig. 1). Similarly, there was no difference in size-specific P_o of MHC IIa fibers between young (175 ± 27 kN/m^2) and old (183 ± 38 kN/m^2, $P =$

0.154) or men (175 ± 34 kN/m^2) and women (186 ± 34 kN/m^2, P = 0.286). Irrespective of age or sex, MHC I fibers had a 12% lower size-specific P_o compared with IIa fibers (158 ± 30 vs. 180 ± 34 kN/m^2, P < 0.001).

pCa_{50}.

There were no differences in pCa_{50} of MHC I fibers between young (5.85 ± 0.12) and old (5.84 ± 0.12, P = 0.601) or men (5.83 ± 0.13) and women (5.87 ± 0.11, P = 0.153) (Fig. 2). Similarly, there were no differences in pCa_{50} of MHC IIa fibers between young (5.96 ± 0.10) and old (5.96 ± 0.09, P = 0.714) or men (5.96 ± 0.12) and women (5.96 ± 0.08, P = 0.834). Irrespective of age or sex, MHC I fibers had a lower pCa_{50} compared with IIa fibers (5.85 ± 0.12 vs. 5.96 ± 0.10, P < 0.001).

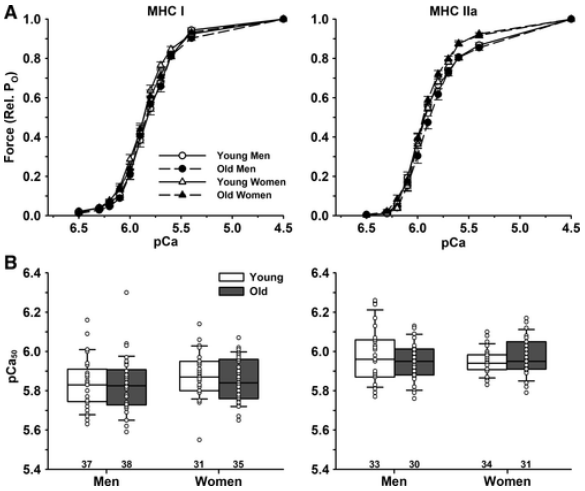

Fig. 2. Mean force-pCa relationship of single muscle fibers. Single muscle fibers were activated in solutions containing free Ca^{2+} concentrations ranging from pCa 6.5 to 4.5. *A*: pCa-force relationship where peak force is expressed as a fraction of maximal Ca^{2+} activated force. *B*: pCa_{50} calculated by fitting the data with Hill plots. There were no age or sex differences for pCa_{50} in slow MHC I or fast MHC IIa fibers. Significance level P < 0.05. The horizontal line in each box plot indicates the median, while the whiskers represent 1.5 times the upper- and lower-interquartile range. Each dot is an individual fiber, with the number of fibers (*n*) displayed below the box plots. Data were analyzed using a nested ANOVA.

Activation threshold.

There were no differences in activation threshold (pCa) for MHC I fibers between young (6.75 ± 0.18) and old (6.73 ± 0.17, P = 0.745), but men (6.69 ± 0.18) had a lower activation threshold compared with women (6.79 ± 0.15, P = 0.011). There were no differences in activation threshold for MHC IIa fibers between young (6.43 ± 0.11) and old (6.43 ± 0.12, P = 0.535) or men (6.44 ± 0.13) and women (6.42 ± 0.09, P = 0.801).

n_1 and n_2.

There were no differences in n_1 for MHC I fibers between young (2.09 ± 0.81) and old (2.12 ± 0.72, P = 0.893) or men (2.11 ± 0.80) and women (2.10 ± 0.74, P = 0.876). There were no differences in n_1 for MHC IIa fibers between young (1.50 ± 0.62) and old (1.59 ± 0.73, P = 0.528), but men had a lower n_1 compared with women (1.27 ± 0.60 vs. 1.81 ± 0.63, P < 0.001). There were no differences in n_2 for MHC I fibers between young (2.66 ± 0.63) and old (2.68 ± 0.67, P = 0.983) or men (2.78 ± 0.73) and women (2.55 ± 0.52, P = 0.060). There were also no differences in n_2 for MHC IIa fibers between young (4.92 ± 1.00) and old (4.99 ± 1.05, P = 0.868) or men (4.72 ± 0.98) and women (5.17 ± 1.01, P = 0.143). Irrespective of age or sex, MHC I fibers had a lower n_2 compared with IIa fibers (2.67 ± 0.65 vs. 4.95 ± 1.02, P < 0.001).

k_{tr}.

There were no differences in k_{tr} at pCa 4.5 or pCa_{50} between young and old or men and women for either fiber type (Table 2, Fig. 3). Submaximal Ca^{2+} decreased k_{tr} for both MHC I and IIa fibers ($P < 0.001$). There were no age differences in the percent decrease in k_{tr} from pCa 4.5 to pCa_{50} for either MHC I or IIa fibers. However, MHC IIa fibers from men had a lower percent decrease compared with women (74.2 vs. 77.1%, $P = 0.033$). Compared with MHC IIa fibers, MHC I fibers had a lower k_{tr} at pCa 4.5 (2.10 ± 0.39/s vs. 6.87 ± 1.50/s, $P < 0.001$) and at pCa_{50} (0.56 ± 0.16/s vs. 1.64 ± 0.49/s, $P < 0.001$). MHC I fibers also had a lower percent decrease in k_{tr} from pCa 4.5 to pCa_{50} compared with IIa fibers (72.5 ± 8.9 vs. 75.6 ± 6.9%, $P = 0.012$).

Table 2. k_{tr} and V_O at maximal and submaximal Ca^{2+} levels

	Young	Old	PValue	Men	Women	PValue
Slow MHC I						
n (N)	83 (15)	155 (19)		135 (20)	103 (14)	
K_{tr} pCa 4.5, /s	2.12 ± 0.37	2.09 ± 0.41	0.564	2.06 ± 0.41	2.15 ± 0.38	0.193
K_{tr} pCa_{50}, /s	0.58 ± 0.17	0.55 ± 0.15	0.793	0.57 ± 0.16	0.56 ± 0.15	0.862
% Change	-72 ± 9	-73 ± 9	0.803	-72 ± 11	-74 ± 6	0.257
n (N)	73 (14)	131 (19)		116 (19)	88 (14)	
V_O pCa 4.5, fl/s	1.14 ± 0.31	1.07 ± 0.29	0.377	1.12 ± 0.35	1.06 ± 0.21	0.368
V_O pCa_{50}, fl/s	0.45 ± 0.18	0.38 ± 0.14	0.138	0.41 ± 0.17	0.41 ± 0.14	0.578
% Change	-61 ± 9	-64 ± 8	0.240	-64 ± 9	-62 ± 8	0.516
Fast MHC IIa						
n (N)	80 (17)	131 (80)		113 (20)	98 (15)	
K_p pCa 4.5, /s	6.59 ± 1.35	7.04 ± 1.57	0.252	6.69 ± 1.54	7.07 ± 1.44	0.250
K_{tr} pCa_{50}, /s	1.60 ± 0.45	1.66 ± 0.51	0.622	1.69 ± 0.55	1.59 ± 0.40	0.199
% Change`	-75 ± 7	-76 ± 7	0.535	-74 ± 8	-77 ± 6	0.033
n (N)	58 (15)	91 (18)		81 (19)	68 (14)	
V_O pCa 4.5, fl/s	3.20 ± 1.07	3.38 ± 1.09	0.563	3.46 ± 1.11	3.13 ± 1.02	0.235
V_O pCa_{50}, fl/s	0.97 ± 0.58	0.97 ± 0.44	0.839	1.03 ± 0.55	0.89 ± 0.41	0.354
% Change	-72 ± 9	-71 ± 9	0.923	-71 ± 10	-72 ± 8	0.736

Data are presented as means ± SD. The number of fibers (*n*) and number of subjects (*N*) are reported for each group and fiber type. Sexes are combined when comparing age groups, and ages are combined when comparing between sexes. k_{tr}, rate of force development; V_O, unloaded shortening velocity. Boldfaced *P* values highlight statistical significance at $P < 0.05$.

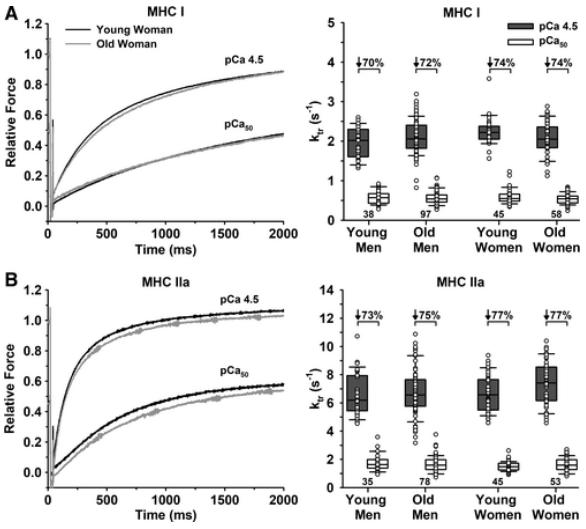

Fig. 3. Rate of force development (k_{tr}) of single fibers measured in maximal (pCa 4.5) and half-maximal (pCa_{50}) Ca^{2+}. *A*: slow MHC I fibers. *Left*, representative force traces of a single fiber from a young and old woman, where k_{tr} is determined from the best fit of an exponential function following a slack and rapid re-extension maneuver. Traces are superimposed to compare between the young and old and between the two Ca^{2+} levels. *Right*, group means for k_{tr}. *B*: fast MHC IIa fibers. *Left*, representative traces of a single fiber from a young and old woman. *Right*, group means for k_{tr}. There were no age or sex differences for k_{tr} in pCa 4.5 or pCa_{50} for either fiber type. Significance level $P < 0.05$. The horizontal line in each box plot indicates the median, while the whiskers represent 1.5 times the upper- and lower-interquartile range. Each dot is an individual fiber, with the number of fibers (*n*) displayed below the box plots. Data were analyzed using a nested ANOVA.

V_o.

There were no differences in V_o at pCa 4.5 or pCa_{50} between young and old or men and women for either fiber type (Table 2, Fig. 4). Submaximal Ca^{2+} decreased V_o for both MHC I and IIa fibers ($P < 0.001$). However, there were no age or sex differences in the percent decrease in V_o from pCa 4.5 to pCa_{50} for either MHC I or IIa fibers. Compared with MHC IIa fibers, MHC I fibers had a lower V_o at pCa 4.5 (1.10 ± 0.30 vs. 3.31 ± 1.08 fl/s, $P < 0.001$) and pCa_{50} (0.41 ± 0.16 vs. 0.97 ± 0.50 fl/s, $P < 0.001$). MHC I fibers also had a lower percent decrease in V_o from pCa 4.5 to pCa_{50} compared with IIa fibers (63.0 ± 8.7 vs. 71.0 ± 9.3%, $P < 0.001$).

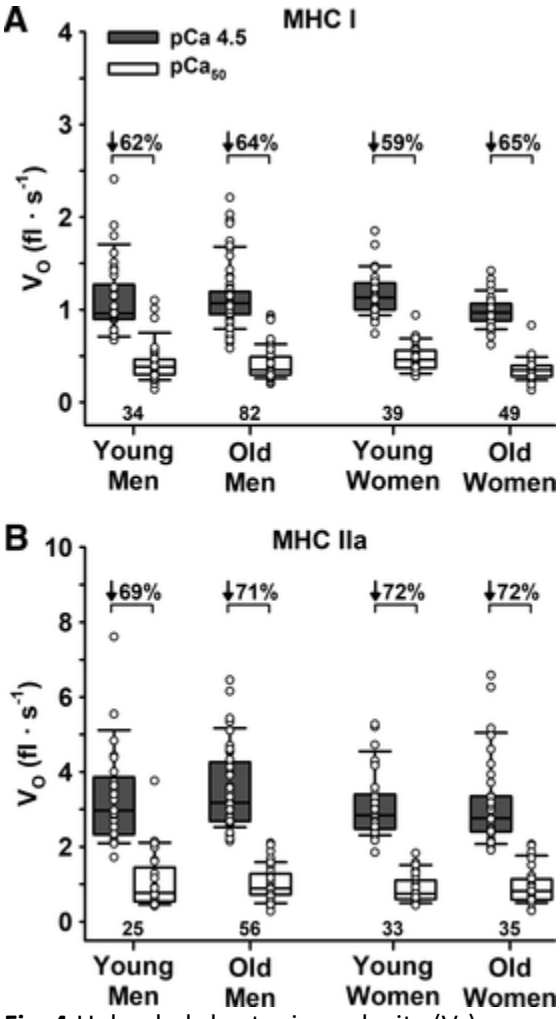

Fig. 4. Unloaded shortening velocity (V_o) measured in maximal (pCa 4.5) and half-maximal (pCa_{50}) Ca^{2+}. *A*: slow MHC I fibers. *B*: fast MHC IIa fibers. There were no age or sex differences for V_o in pCa 4.5 or pCa_{50} for either

fiber type. Significance level $P < 0.05$. The horizontal line in each box plot indicates the median, while the whiskers represent 1.5 times the upper- and lower-interquartile range. Each dot is an individual fiber, with the number of fibers (*n*) displayed below the box plots. Data were analyzed using a nested ANOVA.

V_o below and above 10% shortening.

There were no age or sex differences in V_o below or above 10% shortening for either Ca^{2+} level or fiber type (Table 3, Figs. 5 and 6). For all cohorts and both fiber types, V_o decreased with increased shortening length and decreased [Ca^{2+}], such that the V_o below 10% in pCa 4.5 > V_o above 10% in pCa 4.5 > V_o below 10% in pCa_{50} > V_o above 10% in pCa_{50}. Similarly, the percent decrease in V_o at longer compared with shorter slack distances did not differ with age for either Ca^{2+} level or fiber type. Specifically, the percent decrease in V_o in pCa 4.5 for young and old adults was 21 ± 17 and 20 ± 17% in slow MHC I ($P = 0.672$) and 35 ± 14 and 32 ± 13% in fast MHC IIa fibers ($P = 0.115$), while in pCa_{50} it was 32 ± 17 vs. 38 ± 14% ($P = 0.308$) for MHC I and 42 ± 16 vs. 41 ± 13% ($P = 0.470$) for MHC IIa fibers.

Table 3. V_O below and above 10% shortening at maximal and submaximal Ca^{2+} levels

	Young	Old	PValue	Men	Women	PValue
Slow MHC I						
n (N)	73 (14)	131 (19)		116 (19)	88 (14)	
<10% shortening						
pCa 4.5, fl/s	13.6 ± 0.45	1.30 ± 0.54	0.228	1.40 ± 0.60	1.22 ± 0.33	0.825
pCa_{50}, fl/s	0.60 ± 0.26	0.55 ± 0.26	0.358	0.59 ± 0.30	0.54 ± 0.19	0.834
% Change	-55 ± 12	-56 ± 14	0.922	-56 ± 15	-55 ± 12	0.562
>10% shortening						
pCa 4.5, fl/s	1.04 ± 0.32	0.98 ± 0.26	0.223	1.02 ± 0.34	0.99 ± 0.21	0.473
$pCa_{50,}$ fl/s	0.40 ± 0.16	0.33 ± 0.12	0.076	0.35 ± 0.15	0.35 ± 0.13	0.714
% Change	-61 ± 12	-67 ± 8	0.217	-65 ± 10	-64 ± 10	0.743
Fast MHC IIa						
n (N)	58 (15)	91 (18)		81 (19)	68 (14)	
<10% shortening						
pCa 4.5, fl/s	4.25 ± 1.62	4.37 ± 1.46	0.942	4.62 ± 1.52	3.97 ± 1.46	0.064
$pCa_{50.}$ fl/s	1.51 ± 1.17	1.38 ± 0.56	0.865	1.57 ± 1.02	1.26 ± 0.54	0.176
% Change	-65 ± 17	-68 ± 11	0.719	-66 ± 16	-68 ± 10	0.783
>10% shortening						
pCa 4.5, fl/s	2.69 ± 0.88	2.90 ± 0.91	0.275	2.90 ± 0.94	2.72 ± 0.85	0.635
$PCa_{50,}$ fl/s	0.82 ± 0.47	0.82 ± 0.41	0.901	0.86 ± 0.47	0.77 ± 0.37	0.551
% Change	-71 ± 9	-72 ± 10	0.550	-71 ± 11	-72 ± 8	0.638

Data are presented as means ± SD. The number of fibers (*n*) and number of subjects (*N*) are reported for each group and fiber type. Sexes are combined when comparing age groups, and ages are combined when comparing between sexes. Significance level $P < 0.05$.

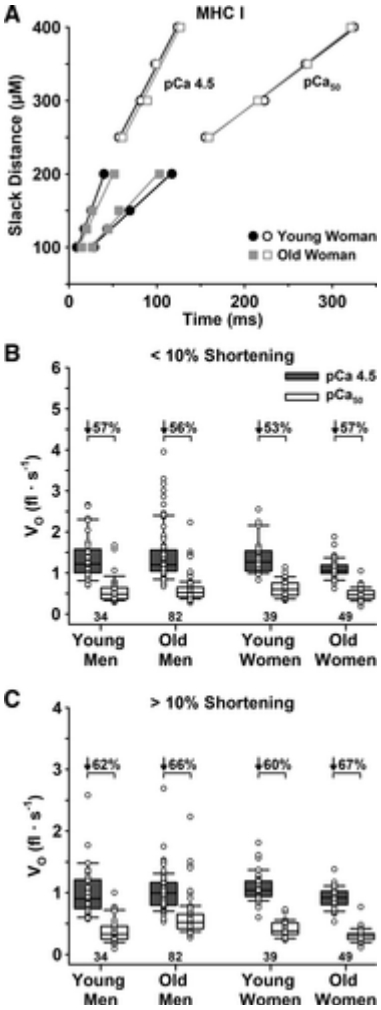

Fig. 5. Unloaded shortening velocity (V_o) of slow MHC I fibers measured below and above 10% shortening in maximal (pCa 4.5) and half-maximal (pCa_{50}) Ca^{2+}. *A*: representative plot of individual fibers from a young and old woman for V_o below (filled symbols) and above (open symbols) 10% shortening in both pCa 4.5 and pCa_{50}. *B*: group means for V_o below 10% shortening in both pCa 4.5 and pCa_{50}. *C*: group means for V_o above 10% shortening in both pCa 4.5 and pCa_{50}. There were no age or sex differences in V_o below or above 10% shortening in either pCa 4.5 or pCa_{50}. Significance level $P < 0.05$. The horizontal line in each box plot indicates the median, while the whiskers represent 1.5 times the upper- and lower-interquartile range. Each dot is an individual fiber, with the number of fibers (*n*) displayed below the box plots. Data were analyzed using a nested ANOVA.

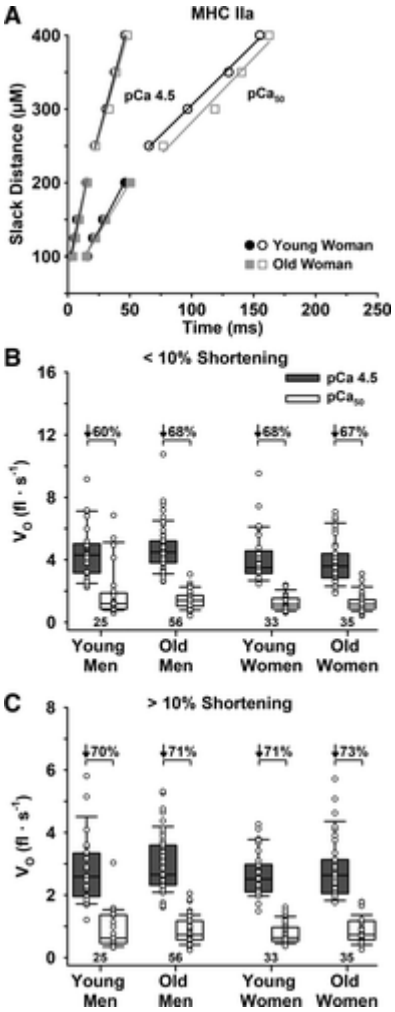

Fig. 6. Unloaded shortening velocity (V_o) of fast MHC IIa fibers measured below and above 10% shortening in maximal (pCa 4.5) and half-maximal Ca^{2+} (pCa_{50}). *A*: representative plot of individual fibers from a young and old woman for V_o below (filled symbols) and above (open symbols) 10% shortening in both pCa 4.5 and pCa_{50}. *B*: group means for V_o below 10% shortening in both pCa 4.5 and pCa_{50}. *C*: group means for V_o above 10% shortening in both pCa 4.5 and pCa_{50}. There were no age or sex differences in V_o below or above 10% shortening in either pCa 4.5 or pCa_{50}. The horizontal line in each box plot indicates the median, while the whiskers represent 1.5 times the upper- and lower-interquartile range. Each dot is an individual fiber, with the number of fibers (*n*) displayed below the box plots. Data were analyzed using a nested ANOVA.

The percent decrease in V_o from below to above 10% shortening did not differ between men and women in MHC I fibers at pCa 4.5 (23 ± 18 vs. 17 ± 15%, $P = 0.068$) and pCa_{50} (37 ± 16 vs. 33 ± 15%, $P = 0.244$), or in MHC IIa fibers at pCa_{50} (43 ± 16 vs. 39 ± 12%, $P = 0.068$). However, the percent decrease in V_o for MHC IIa fibers at pCa 4.5 was greater in men compared with women (36 ± 13 vs. 29 ± 14%, $P = 0.013$).

Irrespective of age or sex, there were several differences in V_o between fiber types and Ca^{2+} levels. At both Ca^{2+} levels, MHC I fibers had a lower V_o below ($P < 0.001$) and above 10% shortening ($P < 0.001$) compared with MHC IIa fibers. The percent decrease in V_o from below to above 10% shortening was greater at pCa_{50} compared with pCa 4.5 for both MHC I (36 ± 16 vs. 20 ± 17%, $P < 0.001$) and IIa fibers (41 ± 15 vs. 33 ± 14%, $P < 0.001$), with a lower percent decrease in MHC I compared with IIa fibers at both Ca^{2+} levels ($P < 0.05$). The percent decrease in V_o from pCa 4.5 to pCa_{50} was lower below compared with above 10% shortening in both MHC I ($P < 0.001$) and

IIa fibers (P = 0.001) (Table 3, Figs. 5 and 6) but was lower in MHC I compared with IIa fibers at both shortening lengths (P < 0.001).

Protein content and phosphorylation.

MHC composition was similar between young and old adults (57 ± 15 and 53 ± 13% MHC I in young and old, respectively; P = 0.568) and between men and women (54 ± 16 and 56 ± 12% MHC I in men and women, respectively; P = 0.769). Aging did not alter protein isoform content of RLC, TnI, or MyBP-C (Fig. 7). The only difference in protein content between sexes was in TnI, where men had less of the slow isoform than women (P = 0.005). Using the ProQ Diamond stain, we were unable to detect phosphorylation of TnI or MyBP-C in any of the samples studied and found no age or sex differences in RLC phosphorylation (Fig. 8). For all measurements and in all cohorts, there was large variability between individuals.

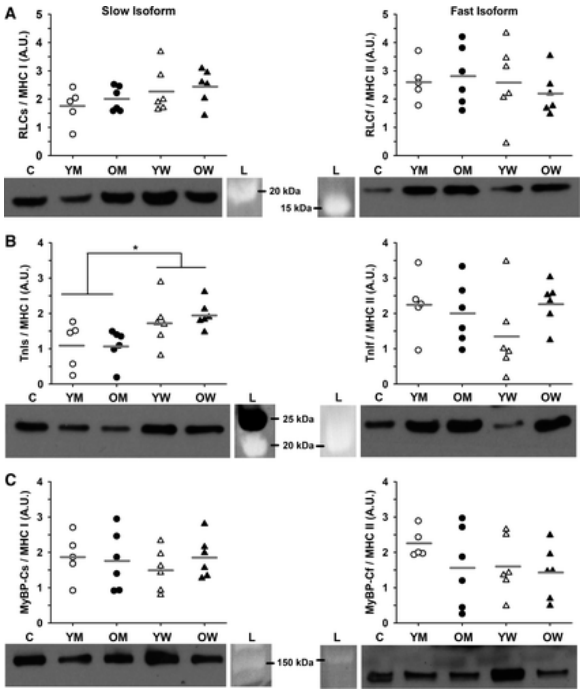

Fig. 7. Relative protein content. *A*: myosin regulatory light chain (RLC). *B*: troponin I (TnI). *C*: myosin binding protein C (MyBP-C). Proteins were identified with antibodies specific to either the slow or fast isoform of each protein. Protein content was normalized to total protein, expressed relative to the pooled homogenate control and then expressed relative to MHC content. Representative Western blots are shown for each protein along with the molecular weight marker from the corresponding Sypro Ruby-stained membrane. There were no age or sex differences except for TnI slow, where men had less content compared with women (P = 0.005). *Significant effect of sex. Significance level P < 0.05. Individual subjects are shown as symbols with group means indicated by gray horizontal lines. N = 6 subjects for each cohort except young men, where N = 5. C, control; L, ladder; OM, old men; OW, old women; YM, young men; YW, young women. Data were analyzed with a two-way ANOVA.

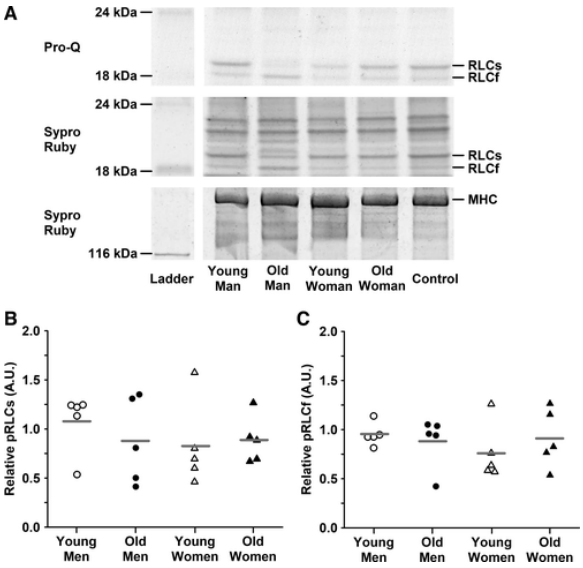

Fig. 8. Relative phosphorylation of myosin regulatory light chain (RLC). *A*: representative ProQ Diamond (phosphorylated protein) and Sypro Ruby-stained (total protein) gels are shown with the corresponding molecular weight markers to the *left*. Phosphorylation signals were normalized to total protein and then to a control homogenate comprising pooled samples from all four cohorts. *B*: phosphorylation of slow RLC (RLCs). *C*: phosphorylation of fast RLC (RLCf). Individual subjects are shown as symbols with group means indicated by gray horizontal lines. There were no age or sex differences. Significance level $P < 0.05$. Data were analyzed with a two-way ANOVA. $N = 5$ for each cohort.

DISCUSSION

The primary purpose of this study was to test the effects of age and sex on single muscle fiber Ca^{2+} sensitivity (pCa-force relationship), rate of force development (k_{tr}), and maximal shortening velocity (V_o) at short and long displacements. Importantly, we investigated these contractile properties in both maximal and submaximal Ca^{2+}. Contrary to our hypotheses, we found no effect of age on Ca^{2+} sensitivity, k_{tr}, or V_o for men or women, suggesting that contractile function is preserved with age in both sexes. Consistent with our previous work in men (69), we found marked atrophy and lower absolute P_o in MHC IIa fibers with aging, but no change in size-specific P_o, indicating that the age-related differences in P_o for both men and women were explained by fiber size. Because fiber CSA and P_o are lower in young women compared with young men, the selective atrophy of fast fibers with age may be more detrimental to older women. These data, along with those of Sundberg et al. (69), suggest that the age-associated reductions in contractile function for both men and women are due primarily to the selective atrophy of fast fibers rather than changes in muscle quality.

Age and sex have little to no effect on the force-pCa relationship.

We observed no differences in Ca^{2+} sensitivity (pCa_{50}) with age, which is in line with some studies (32, 33) but in contrast to others (37, 68). The explanation for the discrepancies between studies is unclear; however, one possibility is that three of the four previous studies did not control the temperature of the experiments or measure sarcomere spacing (32, 33, 37), both factors known to affect Ca^{2+} sensitivity (65). It is also notable that our study is *1*) the largest human aging data set on the force-pCa relationship, examining 68 and 73 MHC I and 61 and 67 MHC IIa fibers from young and older adults, respectively, and *2*) includes older adults that were on average 6–10 yr older than previous studies (32, 33, 37, 68). Similar to our findings on pCa_{50}, we found no effect of age on the slopes (n_1 and n_2) or activation threshold of the force-pCa relationship, which agrees with a majority of studies (32, 33, 37) but not all (68). When the Hill coefficient is calculated, forces less than 50%

P_o (n_2) show greater cooperative activation of thin filaments, increasing the likelihood of myosin strong binding to actin and increasing force at low [Ca^{2+}] (44, 53). Our n_2 data suggest that cooperative activation of thin filaments is preserved with aging.

Similar to the effects of age, we found minimal effects of sex on the pCa-force relationship. We did, however, observe that women had a greater n_1 in fast MHC IIa fibers and a greater activation threshold in slow MHC I fibers compared with men. An activation threshold at lower [Ca^{2+}] (higher pCa) indicates that more Ca^{2+} is needed to initiate force in fibers from men compared with women. To our knowledge, the only other study to investigate pCa-force parameters separately for men and women is Straight et al. (68), and they found no sex difference in either young or old participants. Overall, the data show little, if any, effect of age or sex on the force-pCa relationship, suggesting that myofilament Ca^{2+} sensitivity is not a primary contributor to declines in force with aging in either men or women.

Low- to high-force kinetics of the cross-bridge cycle are preserved with aging.

Similar to our previous work in saturating Ca^{2+} conditions (69), we found no age differences in k_{tr} and, additionally, no sex differences. To our knowledge, this is the first study on the effects of aging and sex on k_{tr}, and our data show that k_{tr} of fibers from older women are equivalent to the other cohorts in saturating Ca^{2+}. This finding indicates that the net movement of cross-bridges from the low- to high-force state (47) is not affected by sex or age. The only other study on the effect of aging on k_{tr} reported a reduced k_{tr} in slow fibers from older compared with young men (61). Factors that could explain the discrepancy between studies, as mentioned in Sundberg et al. (69), include methods of fiber typing based on V_o rather than MHC composition, experimental temperature (10 vs. 15°C in the current study), fiber compliance due to attachment method, or sarcomere spacing (2.8 vs. 2.5 µm in the current study). Given the robustness of our data (i.e., number of subjects and fibers analyzed), it seems unlikely that aging affects the low- to high-force transition of the cross-bridge cycle in either men or women.

To further test whether aging affects the low- to high-force transition, we also compared k_{tr} of fibers from young and old men and women in submaximal Ca^{2+}. It is known from animal studies that k_{tr} is depressed by submaximal Ca^{2+}, an effect that seems primarily dependent on Ca^{2+} binding to troponin C (TnC) (10). Contrary to our hypothesis, we observed no age differences in k_{tr} at pCa_{50}, suggesting that any condition that lowers [Ca^{2+}], such as muscle fatigue, would not exacerbate the depression of k_{tr} in older adults. This finding in combination with our observation that aging did not alter the pCa-force relationship, which is also affected by Ca^{2+} binding to TnC (53, 55), suggests that aging does not alter the ability of Ca^{2+} to bind to TnC.

Maximal shortening velocity is unaffected by age and sex.

Our finding that V_o is preserved with aging in saturating Ca^{2+} is in agreement with several previous publications (20, 36, 69, 74), but in contrast to others reporting an age-induced decline in fiber V_o (8, 13, 38, 58, 61). The discrepancies between studies are unclear but may involve the number of subjects, fiber storage conditions, or the statistical analyses (nested vs. nonnested). Similar to the findings on aging, we also observed no sex differences in V_o. Nearly all previous studies on V_o and aging either collected the data only in men or did not indicate whether women were included. To our knowledge, the only study that analyzed men and women is Trappe et al. (74), and in agreement with our data, they did not observe an age- or sex-related decline in V_o. Together, these data suggest that in saturating Ca^{2+} V_o is preserved with age in both men and women.

Previous studies have focused on V_o in maximal Ca^{2+} (pCa 4.5), whereas in vivo Ca^{2+} conditions may at times be subsaturating (e.g., muscle fatigue) (4). Contrary to our hypothesis, we did not observe an age difference in V_o in men or women in submaximal Ca^{2+}, nor with shortening below or above 10% of fiber length. Similar to other studies at pCa 4.5 (52, 54), the V_o determined from the slope of the time to redevelop force and the slack

distance was well fit by a simple linear regression. However, further analysis revealed that even in maximal Ca^{2+} the reduction in V_o at slack distances above compared with below 10% fiber length can be >1 fl/s for fast fibers (Table 3). We also observed a larger reduction in V_o with greater slack distances in pCa_{50} compared with pCa 4.5, and this reduction was greater for MHC IIa compared with MHC I fibers. Specifically, the percent decrease in V_o with decreased Ca^{2+} at >10% shortening was 61% for MHC I and 71% for MHC IIa fibers (Table 3). These findings agree qualitatively with data from rabbit muscle (52, 54); however, unlike in rabbit muscle where V_o below 10% shortening was unaltered in pCa 4.5 compared with pCa_{50}, we observed a decreased V_o in both fast and slow fibers from humans (Table 3). Consequently, in pCa_{50} the decreased slope from below to above 10% shortening was less than observed in rabbit fibers (52, 54). The explanation for the discrepancy between studies is unknown but could be due to species and/or muscle fiber type differences. For example, fibers from the psoas muscle of rabbit are almost exclusively MHC IIb (5), whereas fibers from the vastus lateralis of humans are primarily MHC I and IIa.

The reduction in V_o under low [Ca^{2+}] and high shortening lengths has been attributed to increased internal drag from slower cross-bridge turnover due to MyBP-C binding to actin, and/or the cooperative inactivation of thin filaments decreasing the number of strongly bound cross-bridges (31, 34, 44, 52, 54, 71). The latter is thought to be related to reduced thick filament cooperativity in low [Ca^{2+}] such that the reduced force also slows velocity (25). Consequently, our finding of no age differences in the effects of low [Ca^{2+}] or the degree of shortening on V_o provides further evidence that Ca^{2+} sensitivity is unaltered by aging. Additionally, it suggests that processes altering the speed of cross-bridge turnover and internal drag, such as MyBP-C tethering the myosin head to actin, are also unaltered.

Protein content and phosphorylation of a few key regulatory proteins are preserved with age.

One focus of this study was to determine if aging induces changes in key regulatory proteins known to affect Ca^{2+} sensitivity and contractile function in submaximal Ca^{2+}. We found no differences in the protein content of RLC, TnI, or MyBP-C between young and older adults (Fig. 7), which is consistent with our findings of preserved Ca^{2+} sensitivity and contractile function in both maximal and submaximal Ca^{2+} with age. These findings, however, are in contrast to other studies that observed an increase in the slow RLC isoform with aging in both rodents (23) and humans (24). The reported increase in slow RLC occurred in conjunction with an age-related increase in the slow MHC isoform. We observed no age difference in the distribution of MHC isoforms, which may explain the discrepancies between studies and is consistent with previous observations from our laboratory (69). The discrepancies may also be due to species differences and/or genetic diversity between individuals, as reflected in the large variation in protein isoform content (Fig. 7). Additionally, physical inactivity and/or denervation, which often occurs in older adults, can increase the expression and coexpression of the MHC IIx isoform (6, 22, 67). In support of this possibility, our single fiber studies tested multiple hybrid IIa/IIx fibers in four of the six older women that were also assessed for protein content, but none of the young women. Given most rodent colonies are inbred, less variation between individual animals would also be expected compared with humans. This genetic diversity, or lack thereof, may in part explain why cross species results do not always agree (40, 66).

In addition to protein content, establishing whether aging alters the phosphorylation levels of key regulatory proteins is important. For example, increased phosphorylation of cardiac TnI is known to decrease Ca^{2+} sensitivity and increase cross-bridge kinetics (35, 79), while increased phosphorylation of MyBP-C is thought to untether the myosin heads, increasing the probability and kinetics of myosin strong binding to actin (64). Previous studies from mouse skeletal muscle have shown decreased phosphorylation of slow MyBP-C with age (1, 2). However, we were unable to detect phosphorylation of either MyBP-C or TnI from our human samples. The lack of detection could be due to the methods (i.e., ProQ Diamond stain is not sensitive enough) or because these proteins are not phosphorylated in the human vastus lateralis when extracted from quiescent muscle. In

agreement with our findings, but in contrast to cardiac TnI (28, 35, 78), proteomic studies have also observed little to no phosphorylation of TnI in either human or rodent skeletal muscle (11, 76). Together, these data suggest that age and sex do not alter the phosphorylation levels of TnI and MyBP-C in human skeletal muscle.

Establishing whether aging alters RLC phosphorylation is also important, as phosphorylation of RLC is known to increase fiber Ca^{2+} sensitivity and the rate of force development (46). RLC phosphorylation was detectable on the ProQ Diamond-stained gel; however, we found no age or sex differences in the phosphorylation levels of the slow or fast isoforms (Fig. 8). Interestingly, and like our protein content results, we observed large variability between individuals in all four cohorts, suggesting that factors other than age or sex may influence RLC phosphorylation. In partial agreement with our results, Miller et al. (49) reported no age differences in the phosphorylation levels of either RLC isoform in men but found lower phosphorylation of the fast isoform in old women. In contrast, Brocca et al. (8) observed no differences in the fast RLC isoform but an increased phosphorylation of the slow isoform in physically fit older men. Collectively, the data from humans suggest that age-induced decline in RLC phosphorylation either does not occur or is restricted to the fast isoform in women.

In contrast to human studies, studies on aging rodent models report differences in both the content and phosphorylation level of RLC (26, 76). For example, proteomics on the rat gastrocnemius muscle report increases in fast RLC content, but decreased phosphorylation (26, 76), as well as increased phosphorylation levels of the slow isoform with age (23). Gregorich et al. (26) reported the age-induced decline in phosphorylation of the fast RLC isoform was correlated with the loss of MHC IIb fiber function and suggested that interventions designed to maintain phosphorylation of this protein might be protective. Unfortunately, the correlation was not tested in MHC I or IIa fibers, the primary fiber types in humans. In addition, the findings from aging rodent models do not appear to agree with those from humans, suggesting caution is warranted when extending results from rodents to aging humans.

Concluding Remarks

We previously demonstrated that the primary event responsible for declines in skeletal muscle force and power in older men was atrophy of fast MHC II fibers (69). The present study similarly suggests that the age-associated reductions in fiber force in both men and women are primarily due to fast fiber atrophy. While age-induced decreases in limb muscle fiber Ca^{2+} sensitivity have been reported in humans, we found no effect of age on the pCa-force relationship or k_{tr} and V_o in either maximal or submaximal Ca^{2+}. The older adults in this study represent the healthy physically active population, and thus these findings may not extend to mobility-limited older adults. Similar to rodent skeletal muscle, we observed V_o to be depressed with fiber shortening >10% of fiber length; however, the effect was unaltered with age in men or women. Rodent studies suggest that age-induced losses of muscle protein content or phosphorylation status, particularly reduced RLC phosphorylation, may contribute to the loss in contractile function with age. This is unlikely in humans, as we found RLC content and phosphorylation levels to be unaltered with age in men or women. We conclude that other than the severe age-related atrophy of fast fibers, Ca^{2+} sensitivity and contractile function of skeletal muscle is preserved in older men and women.

GRANTS

This work was supported by National Institute on Aging Grant R01AG-048262 to R. H. Fitts and S. K. Hunter and American Heart Association postdoctoral fellowship 19POST34380411 to C. W. Sundberg.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.H.F. conceived and designed research; L.E.T., C.W.S., and L.J.K. performed experiments; L.E.T., C.W.S., and L.J.K. analyzed data; L.E.T., C.W.S., L.J.K., and R.H.F. interpreted results of experiments; L.E.T., C.W.S., and L.J.K. prepared figures; L.E.T., C.W.S., L.J.K., and R.H.F. drafted manuscript; L.E.T., C.W.S., L.J.K., S.K.H., and R.H.F. edited and revised manuscript; L.E.T., C.W.S., L.J.K., S.K.H., and R.H.F. approved final version of manuscript.

ACKNOWLEDGMENTS

We thank Dr. Mehdi Maadooliat for assistance with the statistical analysis and figure preparation. We also thank Dr. Scott Trappe for comments on an earlier version of this manuscript.

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