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Age-related reduction in single muscle fiber calcium sensitivity is associated with decreased muscle power in men and women

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Abstract

Age-related declines in human skeletal muscle performance may be caused, in part, by decreased responsiveness of muscle fibers to calcium (Ca^{2+}). This study examined the contractile properties of single vastus lateralis muscle fibers with various myosin heavy chain (MHC) isoforms (I, I/IIA, IIA and IIAX) across a range of Ca^{2+} concentrations in 11 young (24.1 ± 1.1 years) and 10 older (68.8 ± 0.8 years) men and women. The normalized pCa-force curve shifted rightward with age, leading to decreased activation threshold (pCa_{10}) and/or Ca^{2+} sensitivity (pCa_{50}) for all MHC isoforms examined. In older adults, the slope of the pCa-force curve was unchanged in MHC I-containing fibers (I, I/IIA), but was steeper in MHC II-containing fibers (IIA, IIAX), indicating greater cooperativity compared to young adults. At sub-maximal $[\text{Ca}^{2+}]$, specific force was reduced in MHC I-containing fibers, but was minimally decreased in MHC IIA fibers as older adults produced greater specific forces at high $[\text{Ca}^{2+}]$ in these fibers. Lesser pCa_{50} in MHC I fibers independently predicted reduced isokinetic knee extensor power across a range of contractile velocities, suggesting that the Ca^{2+} response of slow-twitch fibers contributes to whole muscle dysfunction. Our findings show that aging attenuates Ca^{2+} responsiveness across fiber types and that these cellular alterations may lead to age-related reductions in whole muscle power output.

Keywords

Myosin heavy chain isoforms; Human; Specific force; Muscle function

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1. Introduction

Physical function declines with age in humans (Fielding et al., 2011). Whole muscle contractile performance is a critical determinant of physical function (Bean et al., 2002; Foldvari et al., 2000; Suzuki et al., 2001; Skelton et al., 1994), with both muscle strength and power declining precipitously with advancing age (Delmonico et al., 2009; Clark et al., 2013; Bouchard et al., 2011). Muscle size decreases with age, but atrophy alone does not explain decrements in whole muscle strength and power (Delmonico et al., 2009; Hughes et al., 2001). Whole muscle function is determined, in part, by the intrinsic contractile properties of the underlying muscle fibers (Miller et al., 2014; Bottinelli and Reggiani, 2000; Cormie et al., 2011). Thus, understanding age-related changes at the cellular level is crucial for developing targeted countermeasures to age-related muscle weakness that seek to improve physical function.

The role of calcium (Ca^{2+}) in regulating skeletal muscle contraction is well-known (Gordon et al., 2000; MacIntosh, 2003). However, only a small number of studies have examined the influence of age on the Ca^{2+} response of single skeletal muscle fibers in humans. A recent study found that older adults had a reduction of pCa_{50} in all myosin heavy chain (MHC) II fibers combined (e.g., MHC IIA and IIAX), but did not observe age-related differences in the Ca^{2+} response of MHC I fibers (Lamboley et al., 2015). Two other studies from a different research group showed no significant age-related differences in pCa_{10} , pCa_{50} or slope of the pCa -force curve (Hill coefficient; h) in either MHC I or IIA fibers (Hvid et al., 2011; Hvid et al., 2013). While these studies provide valuable insights, several factors that modify age-related single fiber function need to be considered. First, experimental control for physical activity level is important, as physical inactivity and exercise can modulate Ca^{2+} sensitivity (Widrick et al., 1985; Mounier et al., 2009; Yamashita-Goto et al., 2001; Malisoux et al., 2006; Gejl et al., 2016; Godard et al., 2002). Previous studies did match the young and older populations using questionnaires, but self-reporting of physical activity has limitations (Sallis and Saelens, 2000). Second, previous work evaluated fibers at 120% of slack length (Lamboley et al., 2015; Hvid et al., 2011; Hvid et al., 2013), so the sarcomere length was unknown. This is important as Ca^{2+} sensitivity is altered with sarcomere length, due to changes in the proximity of actin to the myosin binding site (MacIntosh, 2003; Endo, 1972; Wang and Fuchs, 2000). Finally, no study has examined the relationship between the Ca^{2+} responsivity of single muscle fibers and *in vivo* contractile function to determine whether alterations in cellular function scale to the whole muscle level to contribute to age-related functional deficits.

The aim of the present study was to examine the effects of age on the Ca^{2+} response of chemically-skinned, single vastus lateralis muscle fibers (MHC I, I/IIA, IIA and IIAX) from young and older men and women. Notably, sarcomere length under relaxing conditions (pCa 8) was set at 2.65 μm and physical activity of the young and older groups was matched using accelerometry. Furthermore, we aimed to examine the relationship between single fiber Ca^{2+} sensitivity and whole muscle isokinetic power output of the knee extensors. As an exploratory analysis, we examined whether sex interacted with age to alter Ca^{2+} sensitivity in MHC I and IIA fibers, as we have previously observed sex specific changes in molecular,

cellular and whole muscle contractile function among older adults (Miller et al., 2013; Callahan et al., 2014a).

2. Methods

2.1. Participants

Eleven young (5 men, 6 women) and 10 older (3 men, 7 women) adults participated in this study. Young and older adults had habitual physical activity levels measured over 7 days using accelerometry, as previously described (Toth et al., 2010). In order to match young and older adults for physical activity level, we recruited young adults who were minimally active (not engaged in a structured exercise program) and older adults who were moderately active (~30 min of walking on 3–5 days per week). All volunteers had no symptoms or signs of heart disease, hypertension, or diabetes (fasting blood glucose > 112 mg/dl); normal resting electrocardiogram; normal electrocardiogram response to an exercise stress test; normal thyroid function; and normal blood cell counts and blood biochemistry values. Volunteers were excluded if they currently or had participated in a weight loss or exercise training program in the past year, a history of smoking (within 1 yr), unintentional weight loss of > 2.5 kg during the last 3 mo, a body mass index > 30.0 kg/m², a hospitalization longer than 3 days in the past 5 yr, an active neoplasm or history of one within the past 5 yr, or were taking/had taken hormone replacement therapy (combined estrogen/progestin for older women and testosterone for older men). Participants taking statins or oral corticosteroids were excluded. Young women taking oral contraceptives were included (n = 4), and all older women were postmenopausal (cessation of menses > 1 yr). Data on clinical characteristics as well as whole muscle and single fiber structure and function in MHC I and IIA fibers from young and older volunteers have been reported previously (Miller et al., 2013; Callahan et al., 2014b), as have relationships between molecular and cellular function in MHC I, I/IIA, IIA and IIAX fibers from young adults (Miller et al., 2015). The present study examines a subset of the entire cohort, and was limited to those volunteers that had pCa-force measurements performed. All participants provided written informed consent prior to enrollment in this study. The protocol was approved by the Committees on Human Research at the University of Vermont.

2.2. Experimental protocol

Eligibility for participation was determined during screening visits, which also included whole muscle strength testing, a treadmill test, and resting and exercising electrocardiograms. At least one week following the screening visit, a percutaneous needle biopsy of the vastus lateralis muscle was performed under lidocaine anesthesia. The majority of tissue was immediately placed into cold (4 °C) dissecting solution and processed for single fiber mechanical assessment. The remaining tissue was frozen in liquid nitrogen and stored at –80 °C or prepared for electron microscopy or immunohistochemical assessment, the results of which are presented elsewhere (Callahan et al., 2014b).

2.3. Whole body measurements

Body mass was measured using a digital scale (ScaleTronix, Wheaton, IL) and total and regional body composition was assessed via dual-energy X-ray absorptiometry, or DEXA

(GE Lunar, Madison, WI). Leg fat-free mass was determined (Heymsfield et al., 1990) and used as a proxy for muscle mass. Maximum isokinetic knee extensor torque of the right leg was measured, after establishing range of motion and gravity correction, using a dynamometer (Humac Norm, CSMi, Stoughton, MA) at five different isokinetic testing velocities (60, 120, 180, 240 and 300°/s) in a randomized fashion. For each testing speed, participants performed 4 repetitions, and each trial was separated by a 2-min rest period. Following each trial, data were reviewed to ensure that participants attained the target isokinetic velocity in at least 3 of the 4 repetitions. The peak power output was calculated during the isovelocity phase of the contraction for each velocity where the target was achieved.

2.4. Muscle tissue processing

Biopsy tissue was immediately placed into cold (4 °C) dissecting solution (in mM: 20 N,N-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid (BES), 5 ethylene glycol-bis(2-amino-ethylether)-N,N,N',N'-tetraacetic acid (EGTA), 5 MgATP, 1 free Mg²⁺, 1 dithiothreitol and 0.25 phosphate (P_i)) with an ionic strength of 175 mEq, pH 7.0, and at pCa 8.0 for isolation of single fiber bundles for mechanical measurements. Muscle bundles of approximately 50 fibers were dissected and tied to glass rods at slightly stretched lengths and placed in skinning solution (in mM: 170 potassium propionate, 10 imidazole, 5 EGTA, 2.5 MgCl₂, 2.5 Na₂H₂ATP, 0.05 leupeptin and 0.05 antipain at pH 7.0) for 24 h at 4 °C. After skinning, fibers were placed in storage solution (identical to skinning solution, but with 1 mM sodium azide and without leupeptin and antipain) with increasing concentrations of glycerol (10% v/v glycerol for 2 h, 25% glycerol v/v for 2 h) until reaching the final storage solution (50% v/v glycerol), in which they were incubated at 4 °C for 18–20 h. Thereafter, fibers were stored at –20 °C until isolation of single fibers for mechanical measurements, which occurred within 4 weeks of the biopsy.

2.5. Experimental solutions

Constituents of all solutions used during mechanical measurements were calculated using the equations and stability constants according to Godt and Lindley (Godt and Lindley, 1982). Relaxing solution was dissecting solution with 15 mM creatine phosphate and 300 units/ml of creatine phosphokinase. Pre-activating solution was the same as relaxing solution, except at an EGTA concentration of 0.5 mM. Activating solution was the same as relaxing solution, except at pCa 4.5. All solutions were adjusted to proper ionic strength (175 mEq) using sodium methane sulfate.

2.6. Single fiber pCa-force measurements

The Ca²⁺ response of single muscle fibers is routinely determined by exposing a chemically- or mechanically-skinned fiber to a range of [Ca²⁺] that produce minimum to maximum force and plotting the results as a pCa-force curve, where pCa = –log₁₀[Ca²⁺] and force is normalized to maximal force. The [Ca²⁺] at which 10 and 50% of maximum force production occur are defined as the activation threshold (pCa₁₀) and calcium sensitivity (pCa₅₀), respectively. The slope of the pCa-force curve, *h* (Hill coefficient), represents the degree of cross-bridge cooperativity, and primarily occurs because the binding

of one myosin molecule to the actin filament will increase the affinity for nearby myosins to bind actin (Gordon et al., 2000).

Muscle bundles were incubated in dissection solution containing 1% Triton X-100 (*v/v*) for 20 min at 4 °C. Segments (~2.5–3.0 mm) of single chemically-skinned fibers were isolated from muscle bundles and their ends fixed with glutaraldehyde, as described (Miller et al., 2010). Aluminum T-clips were placed on the fixed regions and the fiber was mounted in relaxing solution onto hooks to perform top and side diameter measurements at three positions along the length of the fiber using a filar eyepiece micrometer (Lasico, Los Angeles, CA, USA) and a right-angled, mirrored prism to calculate average cross-sectional area. The experimental apparatus specifications as well as fiber mounting and preparation procedures have been previously described (Miller et al., 2010). Briefly, the T-clipped fiber ends were attached to a piezoelectric motor (Physik Instrumente, Auburn, MA, USA) and a strain gauge (SensorNor, Horten, Norway) in relaxing solution, sarcomere length was set to 2.65 μm (IonOptix, Milton, MA, USA) and the length of the fiber's unfixed portion was determined. Starting in relaxing solution, the fiber was slackened completely, the force gauge zeroed, the fiber pulled back to its original position, allowed to equilibrate for 1 min and relaxed isometric specific force (force per cross-sectional area) measured. The fiber was transferred to pre-activating solution for 30 s, to improve fiber stability by allowing for the rapid influx of Ca^{2+} into the fiber (Larsson and Moss, 1993; Moisescu, 1976), and then to activating solution at pCa 4.5, with specific force recorded at its plateau. This initial maximal Ca^{2+} activation was performed to verify fiber integrity (maintenance of normal sarcomere register). Afterwards, fibers were placed in relaxing solution, sarcomere length set to 2.65 μm , slackened completely, the force gauge zeroed, the fiber pulled back to its original position, allowed to equilibrate for 1 min and relaxed isometric specific force measured. The fiber was transferred to pre-activating solution for 30 s and subsequently activated in solutions containing progressively greater Ca^{2+} levels (pCa 8.0, 7.0, 6.5, 6.0, 5.75, 5.5, 5.25, 5.0, 4.5), with specific force measured at each plateau. Normalized specific force for most individual fibers was calculated using $(x - \text{specific force at pCa 8.0}) / (\text{specific force at pCa 4.5} - \text{specific force at pCa 8.0})$, where x is the tension at a given Ca^{2+} level. However, MHC IIAX fibers on average had their maximum specific force occur at pCa 5.0 (2% higher than values at pCa 4.5), so their normalized specific force was calculated using $(x - \text{specific force at pCa 8.0}) / (\text{specific force at pCa 5.0} - \text{specific force at pCa 8.0})$. Individual recordings of normalized force were fit to the Hill equation $[\text{Ca}^{2+}]^h / ([\text{Ca}^{2+}]_{50}^h + [\text{Ca}^{2+}]^h)$, where $[\text{Ca}^{2+}]_{50}$ = calcium concentration at half activation, $\text{pCa}_{50} = -\log [\text{Ca}^{2+}]_{50}$, and h = Hill coefficient. To permit comparisons with other studies, activation threshold (pCa_{10}) was calculated by setting the normalized force to 0.1 (10% of maximal force), solving the Hill equation for $[\text{Ca}^{2+}]_{10}$ and calculating $\text{pCa}_{10} = -\log [\text{Ca}^{2+}]_{10}$. Experiments were performed at 15 °C.

2.7. Gel electrophoresis

Following pCa-force measurements, single fibers were placed in 30 μl loading buffer, heated for 2 min at 65 °C and stored at -80 °C until determination of MHC isoform composition by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to identify fiber type, as described (Miller et al., 2010).

2.8. Statistical analysis

Data are expressed as means \pm SE. A one-way analysis of variance (ANOVA) was used to examine the main effect of age on all outcomes, and a two-way ANOVA to determine the interaction between age and sex on the Hill fit parameters. For variables with multiple observations within the same individual (e.g., single fiber specific force measurements), a linear mixed model was used, as described (Callahan et al., 2014a). To account for clustering of observations within individuals, we included a repeated effect in the model, as described (Toth et al., 2012). Partial correlations adjusted for leg fat-free mass were conducted to examine the association between single fiber Ca^{2+} response and whole muscle isokinetic power. Finally, linear regression models adjusted for leg fat-free mass were created to examine the relative contributions of MHC I and IIA Ca^{2+} sensitivity to whole muscle power at each iso-kinetic testing velocity. All statistical analyses were conducted using SPSS for Windows version 22.0 (IBM, Armonk, NY) and SAS version 9.4 (SAS Institute, Cary, NC).

3. Results

3.1. Clinical characteristics

There were no differences between young and older adults in height, weight, body mass index or measures of body composition (Table 1, $p = 0.07$ – 0.75). Young and older adults did not differ with respect to habitual physical activity level (Table 1, $p = 1.00$).

3.2. Single muscle fiber contractile properties

We present data from 198 single muscle fibers from 21 men and women (11 young and 10 older adults). Pure (I and IIA) and hybrid (I/IIA and IIAX) MHC fiber types were compared, as the number of MHC IIX and I/IIA/IIX fibers were insufficient to permit statistical analysis (0 IIX and 1 I/IIA/IIX fiber or 0.5% of all fibers examined). Maximum specific force (maximal Ca^{2+} -activated isometric force divided by CSA), maximum force and CSA were not different between young and older adults in MHC I, I/IIA or IIAX fibers (Fig. 1). However, the specific force of MHC IIA fibers was 17% greater in older fibers (young: 118 ± 7 vs. older: 138 ± 7 mN/mm², $p = 0.05$), due to their maintenance of force production (0.60 ± 0.04 vs. 0.54 ± 0.05 mN, $p = 0.33$) despite a 22% lower CSA relative to the young (5216 ± 384 vs. 4053 ± 402 μm^2 , $p = 0.05$).

3.3. Single muscle fiber pCa-normalized specific force relationships

Normalized specific force was plotted versus pCa ($-\log_{10}[\text{Ca}^{2+}]$) and fit using the Hill equation for each MHC isoform in young and older adults (Fig. 2). Although specific force was measured across a range of $[\text{Ca}^{2+}]$ (pCa 8.0 to 4.5), the specific force values at pCa 8.0 and 7.0 were nearly identical. Thus, all pCa-force curves are plotted showing pCa 7.0 as the lowest $[\text{Ca}^{2+}]$. In the normalized pCa-force curve, a rightward shift indicates that more Ca^{2+} is needed to achieve a given fraction of the maximum Ca^{2+} -activated force and is measured by an increase in the activation threshold (pCa_{10}) and/or the Ca^{2+} sensitivity (pCa_{50}). A rightward shift in the pCa-force curve with age was observed for all fiber types, thus causing a decline in pCa_{10} and/or pCa_{50} (Table 2). The pCa_{10} and pCa_{50} were

decreased with age in the pure fiber types (MHC I and IIA), while hybrids showed either a lesser pCa_{50} (MHC I/IIA) or pCa_{10} (MHC IIAX). The significant age effects on pCa_{50} were largest for MHC I ($pCa_{50} = -0.14$) and smallest for MHC IIA ($pCa_{50} = -0.07$), while the changes in pCa_{10} were the same for MHC I, IIA and IIAX ($pCa_{10} = -0.11$). The steepness of the pCa -force curve (h) was unchanged with age in MHC I-containing fibers (I, I/IIA; Table 2). However, slope of the pCa -force curve was greater with age in MHC II-containing fibers (IIA, IIAX; Table 2).

In older adults, MHC I-containing fibers had a greater Ca^{2+} sensitivity (higher pCa_{50}) than MHC II-containing fibers; however, fibers that expressed the MHC II isoform had a larger h , indicating a greater degree of cooperative binding (Table 2). For the young group, pCa_{50} had the same pattern as the older group; lesser in MHC I- versus MHC II-containing fibers, except that the pCa_{50} was similar between MHC I/IIA and IIA fibers. The young group had a greater h for MHC IIA fibers compared to MHC I, similar to the older group, although hybrid slopes were similar to both MHC I and IIA fibers. There were no differences between fiber types with respect to pCa_{10} within the young or older groups.

3.4. Single muscle fiber pCa -specific force relationships

We also plotted pCa -force relationships for all fibers using specific force (Fig. 3). Similar to the results shown in Fig. 2, MHC I-containing fibers of older adults exhibited a rightward shift in the curve, indicating lower specific force production at submaximal pCa values (MHC I: pCa 6.0–5.5, MHC I/IIA: pCa 5.75–5.5) compared to young adults. However, in MHC IIA fibers, older adults generated greater specific force than young at pCa values where maximum specific force is produced (pCa 5.25–4.5) and lower specific force at pCa 6.0. MHC IIAX fibers showed no age-related differences in the pCa -specific force curve.

3.5. Associations with whole muscle power

Absolute and adjusted isokinetic knee extension power was lower for older adults compared to young (Table 3). Relationships between absolute knee extension power and single muscle fiber Ca^{2+} responsiveness were examined using bivariate correlations. Absolute power at all velocities was related to pCa_{10} ($r = 0.47$ – 0.51 , $p < 0.05$) and pCa_{50} ($r = 0.45$ – 0.49 , $p < 0.05$), except $120^\circ/s$ ($r = 0.43$, $p = 0.06$), in MHC I fibers. Isokinetic power was not related to the Hill coefficient (h) in MHC I fibers. In MHC IIA fibers, there were no significant relationships between pCa_{10} ($r = 0.16$ – 0.25), pCa_{50} ($r = -0.03$ – 0.00) or h ($r = -0.42$ to -0.32) and absolute power (all $p > 0.05$).

Partial correlations between single muscle fiber Ca^{2+} responsiveness and isokinetic muscle power were conducted by adjusting for leg fat-free mass (measured via DEXA) to account for variation among subjects in muscle size. Isokinetic power output at most contractile velocities (60 to $240^\circ/s$) was positively related ($p < 0.01$) to both pCa_{10} (MHC I: $r = 0.61$ – 0.67 , MHC IIA: $r = 0.50$ – 0.56) and pCa_{50} (MHC I: $r = 0.65$ – 0.67 , MHC IIA: $r = 0.47$ – 0.50) in MHC I and IIA fibers. Isokinetic power output at $300^\circ/s$ was positively related ($p < 0.05$) to pCa_{10} ($r = 0.50$) and pCa_{50} ($r = 0.56$) in MHC I fibers, but no significant correlation was found for MHC IIA fibers. In contrast, and similar to bivariate correlations, isokinetic power output was not significantly correlated to h in MHC I ($r = -0.25$ to -0.01) or IIA ($r = -0.38$

to -0.33) fibers. The partial correlation between two variables is shown by plotting the residuals (observed minus predicted values) of the independent variables (i.e., isokinetic power and Ca^{2+} sensitivity), which are obtained by regressing each on the covariate (leg fat-free mass) (Fig. 4). We also conducted partial correlations between single fiber Ca^{2+} responsiveness and whole muscle power with additional adjustment for age. However, controlling for age attenuated all observed associations ($p > 0.10$ for MHC I and IIA fibers).

As skeletal muscle is heterogeneous and multiple fiber types contribute to whole muscle function (Bottinelli and Reggiani, 2000), we examined the relative contributions of MHC I and IIA single fiber Ca^{2+} sensitivity to isokinetic knee extension power using multiple linear regression models (Table 4). After adjusting for thigh fat-free mass, our analyses revealed that Ca^{2+} sensitivity (pCa_{50}) of MHC I fibers was a significant independent predictor of whole muscle power output at multiple testing velocities (60–240°/s). In contrast, no relationship was evident between Ca^{2+} sensitivity of MHC IIA fibers and whole muscle power.

3.6. Sex differences

Because we have previously observed sex-based differences in molecular and cellular contractile function among older adults (Miller et al., 2013; Callahan et al., 2014a), we examined whether sex interacted with age to determine Ca^{2+} sensitivity of MHC I and IIA fibers. However, we did not observe an age \times sex interaction for any of the Hill fit parameters in MHC I ($p = 0.38$ – 0.74) or MHC IIA ($p = 0.11$ – 0.92) fibers. Overall, these results indicate sex did not affect the Ca^{2+} response of either fiber type.

4. Discussion

This study investigated the effects of aging on the Ca^{2+} response of single vastus lateralis muscle fibers from young and older men and women. The major finding was that the Ca^{2+} response was attenuated in muscle fibers from older adults in both pure (MHC I and IIA) and hybrid (I/IIA and IIAX) fibers, showing that older adults need more Ca^{2+} to activate their fibers to a similar percentage of maximum force compared to young. In addition, the slope of the pCa -force curve was steeper in MHC II-containing fibers from older adults, indicating a greater degree of cooperative binding in fast-twitch fibers with age, suggesting an enhanced ability to facilitate additional myosin binding (Gordon et al., 2000). Using linear regression, we found that reduced Ca^{2+} sensitivity (pCa_{50}) of MHC I fibers independently predicted lower isokinetic knee extension power across a range of velocities, indicating that age-related alterations in single fiber function may help explain the lower whole muscle performance in older adults. Importantly, young and older adults in this study were matched for physical activity level, as verified by accelerometry, improving the likelihood that our results reflect the effects of aging.

4.1. How can single fiber Ca^{2+} responsivity impact whole muscle function?

As whole muscle specific force or power (force or power per whole muscle size) should be directly influenced by single fiber specific force (force per fiber CSA) (Harridge et al., 1996), understanding the Ca^{2+} response in terms of absolute specific force is relevant for

predicting alterations in whole muscle function that are independent of age-related atrophy. Older adults had reduced specific forces at sub-maximal $[Ca^{2+}]$ in MHC I fibers, while MHC IIA fibers generated higher specific forces at maximal $[Ca^{2+}]$ and similar specific forces at sub-maximal $[Ca^{2+}]$, except at the lowest activation level (pCa 6.0) where specific force was reduced. These alterations in force production in MHC I and IIA fibers both lead to a reduced Ca^{2+} response, but may have different effects on whole muscle function.

How can decreased single fiber Ca^{2+} responsiveness lead to reduced knee extensor power output? Power is the product of contractile force and velocity, and reducing $[Ca^{2+}]$ decreases both single fiber force (Lamboley et al., 2015; Hvid et al., 2011; Hvid et al., 2013; McDonald, 2000) (Figs. 2–3) and shortening velocity (Gilliver et al., 2011; Morris et al., 2003; Moss, 1986) in a sigmoidal manner. Thus, if Ca^{2+} sensitivity of the myofilaments or sarcoplasmic $[Ca^{2+}]$ is significantly reduced, power output at the single fiber level and, in turn, the whole muscle would be reduced. Both of these phenomena may occur, as other human studies have reported an age-related decrease in the amount of Ca^{2+} in the sarcoplasmic reticulum (SR) (Lamboley et al., 2015) and SR Ca^{2+} release (Delbono et al., 1995). That single fiber Ca^{2+} sensitivity influences whole muscle power is suggested by studies showing pharmacologic agents that improve myofilament Ca^{2+} sensitivity increase diaphragm single fiber (van Hees et al., 2009) and whole muscle (Doorduyn et al., 2012) function. Accordingly, the decrease in Ca^{2+} sensitivity we observed should lead to reduced whole muscle function through the same mechanism. While others have suggested that age-related changes in Ca^{2+} handling and sensitivity could have functional implications (Gordon et al., 2000; MacIntosh, 2003; Lamboley et al., 2015), to our knowledge, the present study is the first to demonstrate a relationship between single fiber Ca^{2+} sensitivity and in vivo contractile function. Whole muscle power is an important determinant of daily activities, such as walking speed, climbing stairs and rising from a chair (Bassegy et al., 1992; Bean et al., 2003). Thus, cellular changes that reduce whole muscle power may impair physical function, in keeping with models of the disablement process (Verbrugge and Jette, 1994).

Because younger and older volunteers had similar physical activity levels by design, our results suggest that otherwise healthy older adults can maintain daily activity levels on par with younger individuals despite impaired single fiber function. Considered through the lens of interventions to improve overall activity in older adults, this result is heartening, as it suggests that impaired myofilament function does not limit the capacity of older individuals to achieve youthful daily activity levels and the associated health benefits. However, our isokinetic knee extensor data would suggest that deficits in myofilament function limit the capacity to perform tasks requiring high power output in older adults. Accordingly, activities or physical movements that require high power output, such as preventing a fall (McKinnon et al., 2017), may still be impaired. In this context, exercise and rehabilitation strategies that target these cellular and molecular deficits may help restore power output and, in turn, more effectively mitigate disability. Following this logic, knowledge of the fundamental molecular and cellular deficits contributing to impaired function in older adults across the disability spectrum would allow for more targeted, and hopefully effective, interventions to counter disability.

4.2. Correlation between age-related Ca^{2+} response and whole muscle function

We found associations between single fiber Ca^{2+} response parameters (pCa_{10} and pCa_{50}) and in vivo contractile function, specifically isokinetic knee extensor power output. A substantial proportion (25–45%) of the individual variability in whole muscle power output was accounted for by pCa_{10} and pCa_{50} from MHC I fibers. Likewise, our multivariate regression analyses revealed that Ca^{2+} sensitivity of MHC I fibers was an independent predictor of isokinetic power output across a range of contractile velocities, indicating that participants with greater single fiber Ca^{2+} responsiveness had greater whole muscle power. Importantly, because all participants reached the target iso-kinetic velocity during testing at each speed (60°/s, 120°/s, etc.), the age-related deficits in muscle power were likely attributable to reduced torque production. Collectively, these findings suggest that the decline in knee extensor power output is commensurate with reductions in the Ca^{2+} response of the underlying MHC I fibers in vastus lateral is muscle.

Our multivariate analysis revealed MHC I fiber Ca^{2+} responsiveness as the best predictor of whole muscle power, which begs the question: how can function in the relatively low-power MHC I fibers dictate whole muscle power production? We have previously shown that slower MHC I fiber velocity is associated with a reduced whole muscle rate of torque development in older adults with knee osteoarthritis (Callahan et al., 2015), indicating that slower contracting fibers play an important role in dictating whole muscle function. Slower contracting myosins have a more dominant effect on contractile velocity than faster contracting myosins in pre-clinical models (Cuda et al., 1997) and human tissue (Larsson and Moss, 1993), potentially due to their longer myosin attachment times. In this context, MHC I fibers may be acting as a molecular brake for overall muscle power output. This has been shown using the in vitro motility assay (Cuda et al., 1997), where small amounts of MHC I can disproportionately reduce contractility. In an analogous manner, and considering the substantial proportion of MHC I fibers in human muscle, a reduction in power output from MHC I fibers secondary to decreased Ca^{2+} responsiveness could disproportionately reduce whole muscle power.

While our results suggest that single fiber Ca^{2+} sensitivity, primarily of MHC I fibers, partially explains decrements in isokinetic knee extension power, skeletal muscle function is influenced by a multitude of physiologic factors. In the present study, we statistically controlled for the contribution of muscle mass to isokinetic power. However, while early research suggested muscle mass was a major determinant of strength (Frontera et al., 1991), subsequent prospective studies showed that atrophy accounts for a relatively small proportion of the decrements in muscle strength (Delmonico et al., 2009; Hughes et al., 2001). These results agree with studies showing that factors other than muscle mass contribute to muscle function in older adults (Beliaeff et al., 2008; Landers et al., 2001; Jubrias et al., 1997). Thus, changes in other physiologic regulators of whole muscle function contribute to reductions with age, including single fiber contractile properties (Callahan et al., 2015), neuromuscular activation (Clark et al., 2013; Kent-Braun and Ng, 1999; Moore et al., 2014), excitation-contraction coupling (Delbono et al., 1995), slowing of myosin-actin cross-bridge kinetics (Miller et al., 2013), and fibrosis (Cadore et al., 2012), which are potentially driven by sex hormone levels (Schaap et al., 2005), systemic inflammation

(Visser et al., 2002; Cesari et al., 2004), skeletal muscle lipid infiltration (Goodpaster et al., 2001) and obesity (Choi et al., 2016).

4.3. Comparison to previous Ca^{2+} response studies in young and older adults

The impact of aging on the Ca^{2+} response of human skeletal muscle fibers has received little attention, with studies generally showing no age-related changes in pCa_{10} , pCa_{50} or h in MHC I (Lamboley et al., 2015; Hvid et al., 2011; Hvid et al., 2013) and IIA (Hvid et al., 2011; Hvid et al., 2013) fibers, except for one study that found a decrease in pCa_{50} for MHC II fibers (Lamboley et al., 2015). In this latter study, however, MHC IIA and IIA isoforms, the two most predominant MHC II fiber types, were examined together. In contrast, our results indicate MHC I and IIA fibers show age-related Ca^{2+} response changes, as pCa_{10} and pCa_{50} were reduced in older adults. Variability in results may be due to methodological differences between studies or our matching for weight-bearing activity using accelerometers instead of questionnaires (Widrick et al., 1985; Mounier et al., 2009; Yamashita-Goto et al., 2001; Malisoux et al., 2006; Gejl et al., 2016; Godard et al., 2002). Regarding the latter, as inactivity decreases Ca^{2+} sensitivity (e.g., shifts the pCa -force curve to the right), studying young and older adults with similar activity levels increases confidence that our results are due to aging, and not age-related disuse.

There are potential differences among existing studies in the number and age of participants, as well as the number of fibers for each MHC isoform examined. The number of participants in our study ($N = 21$) was similar to others ($N = 15-22$) (Lamboley et al., 2015; Hvid et al., 2011; Hvid et al., 2013), as were the mean ages (young = 24 y and older = 69 y in current study vs. young = 22-24 y and older = 67-70 y) (Lamboley et al., 2015; Hvid et al., 2011; Hvid et al., 2013). With regard to number of fibers per fiber type, we examined 45-50 MHC I fibers for each age group, which was similar to other studies ($n = 40-62$), except for young adults ($n = 26$) in one study (Lamboley et al., 2015). The number of MHC IIA fibers examined for young and older adults was lowest in the two studies that showed no alterations in Ca^{2+} response with age; 16 and 11 for young and older adults, respectively, in one study (Hvid et al., 2013) and 40 and 15 in the other (Hvid et al., 2011). In contrast, our study in MHC IIA fibers ($n = 42$ young; $n = 38$ older) and another (Lamboley et al., 2015) in MHC II fibers ($n = 21$ young; $n = 25$ older) observed decreases in pCa_{50} of MHC IIA fibers with age.

Another important experimental consideration is the sarcomere length of the fibers examined. In our study, sarcomere length was set at 2.65 μm , while others stretched to 120% of slack length (Lamboley et al., 2015; Hvid et al., 2011; Hvid et al., 2013). A sarcomere length of 2.65 μm should occur at or near knee angles where whole muscle iso-kinetic peak power occurs (40-70°), based on sarcomere lengths measured in vivo in resting vastus lateralis muscle of young adults (Chen et al., 2016). In addition, a sarcomere length of 2.65 μm places our measurements in the middle of the force plateau on the force-sarcomere length curve for MHC I fibers from humans, meaning that our fibers should produce maximal force (Gollapudi and Lin, 2009) and be removed from the ascending or descending portion of this curve. Using a set stretch distance based on slack length, as prior studies have done, may, in contrast, increase variability in sarcomere length and, in turn, variability in

single fiber force and Ca^{2+} sensitivity (MacIntosh, 2003). Notably, a recent study determined slack length in MHC I fibers to be $\sim 2.22 \mu\text{m}$ (Gollapudi and Lin, 2009), such that stretching a fiber to 120% of its slack length would result in a sarcomere length of $\sim 2.66 \mu\text{m}$. However, the relationship between slack length and sarcomere length may change with myofilament stiffness, which is altered with fiber type (Miller et al., 2015) and age (Miller et al., 2013), leading to variability in sarcomere length and possibly functional parameters.

In addition, our single fiber measurements were performed at a lower temperature than the other studies (15 vs. 22–23 °C). Animal studies using skinned skeletal and cardiac fibers have shown that Ca^{2+} sensitivity is temperature-dependent, with increased sensitivity at higher temperatures (Debold et al., 2006; Harrison and Bers, 1989; Maughan et al., 1995; Sweitzer and Moss, 1990). However, temperature affects Ca^{2+} sensitivity of slow- and fast-twitch mammalian skeletal muscle fibers differentially, such that slow-twitch fibers are insensitive to temperature, while fast-twitch fibers become less sensitive to Ca^{2+} as temperature increases from 5 to 25 °C (Stephenson and Williams, 1981). In this context, our observation of an age-related reduction in Ca^{2+} sensitivity of MHC IIA fibers may be influenced by the temperature at which mechanical experiments were performed.

Finally, there were also differences between studies in the approach used to remove the sarcolemma of single fibers. In the present study, we used chemically-skinned single fibers, which is the method used in studies by Hvid et al. (Hvid et al., 2011; Hvid et al., 2013), but different than Lamboley et al. (Lamboley et al., 2015), who evaluated mechanically-skinned fibers. However, this is unlikely to account for discrepant results between studies as previous research shows that the pCa-force relationship does not vary between chemically- and mechanically-skinned fibers (Endo, 1972).

4.4. Age-related Ca^{2+} response of hybrid fibers

Hybrid skeletal muscle fibers (MHC I/IIA and IIAX), which are expressed more frequently in older adults (D'Antona et al., 2003; Klitgaard et al., 1990; Andersen et al., 1999), similarly showed an attenuated Ca^{2+} response, decreasing pCa_{50} in MHC I/IIA and pCa_{10} in MHC IIAX, but a greater cooperativity in the fast-contracting MHC IIAX. Although we recognize that only a small number of hybrid fibers were examined in each age group ($n = 4-7$), these patterns are consistent with our observations in the pure MHC I and MHC IIA fibers. MHC isoform is the primary determinant of single fiber contractile performance (Harridge et al., 1996) and the relative expression of each MHC isoform in these hybrid fiber types may account for the similarity of these observations to pure MHC I and IIA fibers. Additionally, MHC expression also affects the isoforms of regulatory proteins on the actin filament (e.g., troponin) (O'Connell et al., 2004), and this could impact Ca^{2+} sensitivity (Geiger et al., 1999). Due to the limited number of MHC I/IIA and IIAX fibers examined in the present analysis, future human studies are needed to confirm the effect of age on hybrid muscle fibers.

5. Conclusion

In conclusion, our results indicate an attenuated Ca^{2+} response from muscle fibers of older adults, and suggest that these changes may contribute to reduced whole muscle power

output. As the loss of muscle power is a determinant of age-related physical limitations, exercise and/or pharmacologic interventions that increase Ca^{2+} sensitivity in muscle fibers of older adults may improve whole muscle performance and reduce the risk of future physical disability.

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Abbreviations:

ANOVA	analysis of variance
Ca^{2+}	calcium
CSA	cross-sectional area
DEXA	dual-energy x-ray absorptiometry
EGTA	ethylene glycol-bis(2-amino-ethylether)-N,N,N',N'-tetraacetic acid
h	Hill coefficient
MHC	myosin heavy chain
n	number of fibers
N	number of subjects
pCa	$-\log_{10}[\text{Ca}^{2+}]$
pCa₁₀	$[\text{Ca}^{2+}]$ at which 10% of maximal force is produced (activation threshold)
pCa₅₀	$[\text{Ca}^{2+}]$ at which 50% of maximal force is produced (Ca^{2+} sensitivity)
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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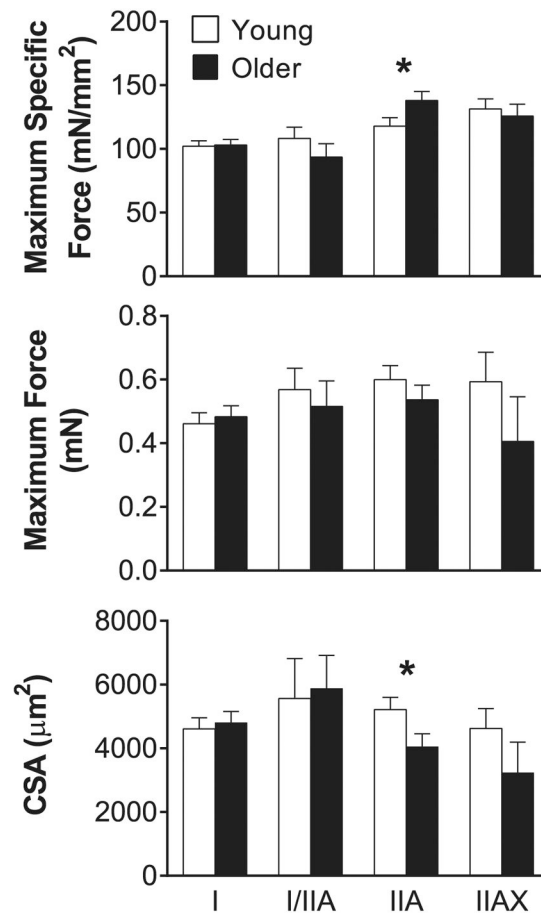


Fig. 1. Single skeletal muscle fiber maximum specific force, maximum force and cross-sectional area (CSA).

Maximum specific force (maximum force/CSA), maximum force and CSA of myosin heavy chain (MHC) I, I/IIA, IIA and IIAX single muscle fibers from young and older adults. The number of fibers analyzed in each group for each fiber type is provided by *n* in Table 2. Data were acquired under maximal Ca²⁺-activated conditions (pCa 4.5) at 15 °C and represent means ± SE. Asterisks indicate significant difference (**p* < 0.05) between young and older groups.

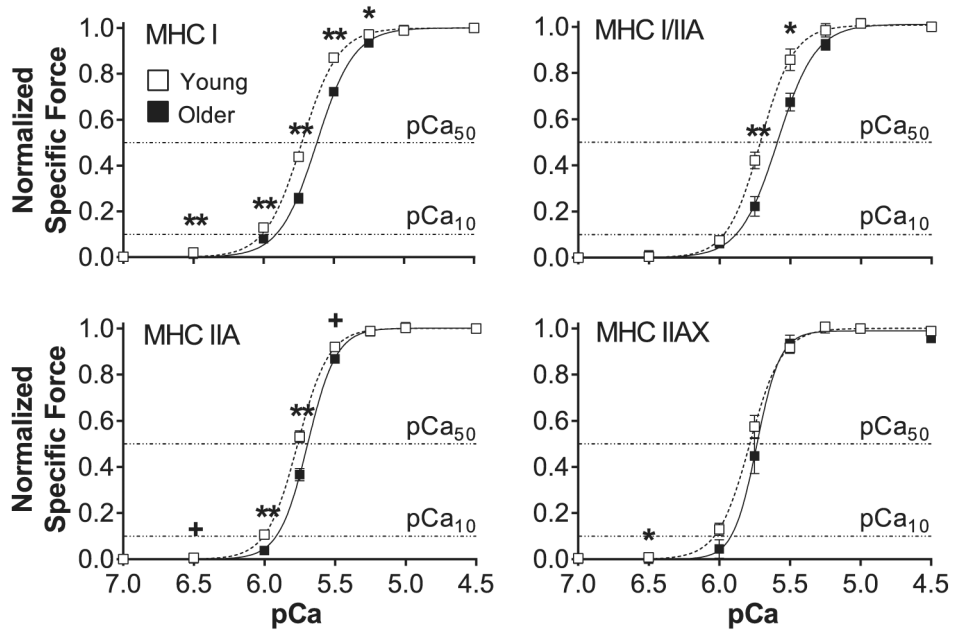


Fig. 2. Normalized pCa-force relationships for young and older adults. Normalized pCa-force relationships of myosin heavy chain (MHC) I, I/IIA, IIA and IIAX single muscle fibers from young and older adults. The horizontal dashed lines represent the activation threshold (pCa₁₀) and Ca²⁺ sensitivity (pCa₅₀). The number of fibers analyzed in each group for each fiber type is provided by n in Table 2. Data were acquired at 15 °C and represent means ± SE. Asterisks and plus sign indicate significant difference (**p* < 0.05 or ***p* < 0.01) and trends (+*p* < 0.10) between young and older groups.

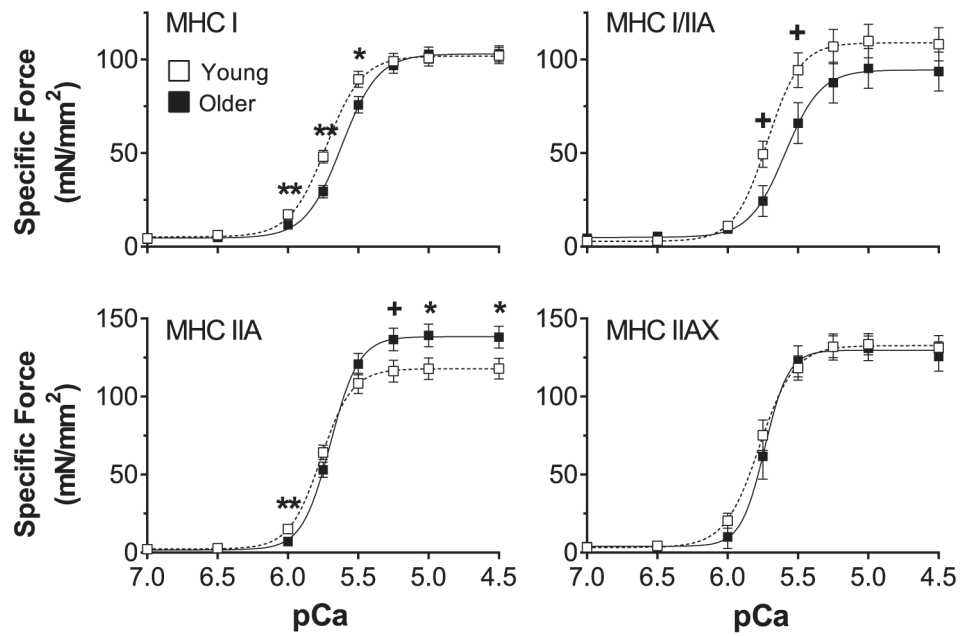


Fig. 3. Absolute pCa-force relationships for young and older adults.

Absolute pCa-force relationship of myosin heavy chain (MHC) I, I/IIA, IIA and IIAX single muscle fibers from young and older adults. The number of fibers analyzed in each group for each fiber type is provided by *n* in Table 2. Data were acquired at 15 °C and represent means \pm SE. Asterisks and plus sign indicate significant difference ($*p < 0.05$ or $**p < 0.01$) and trends ($+p < 0.10$) between young and older groups.

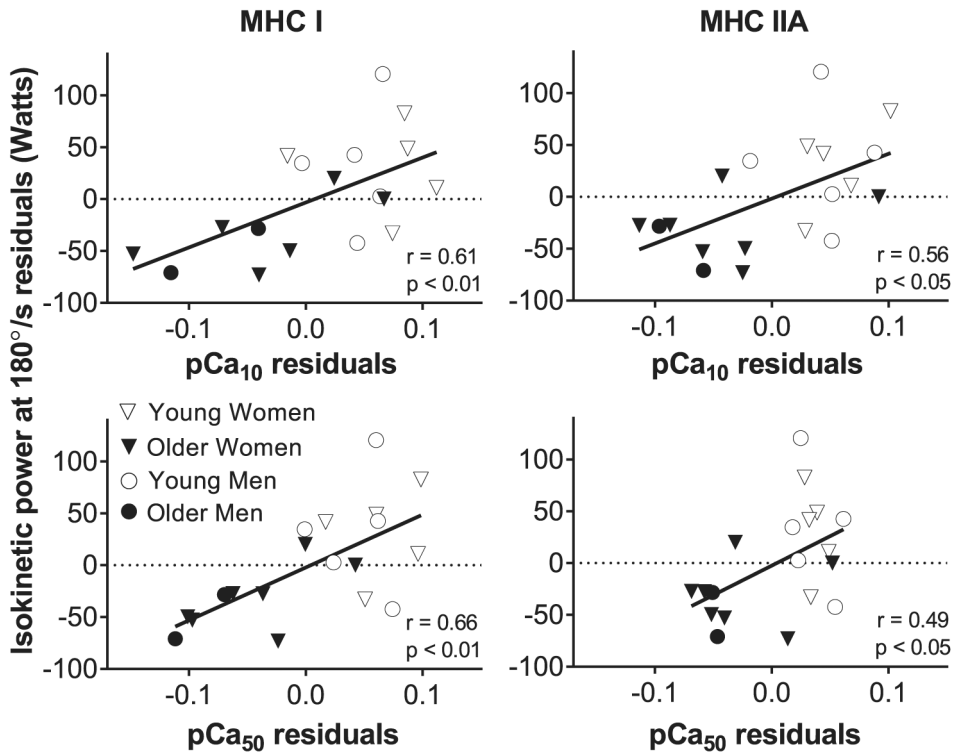


Fig. 4. Single fiber Ca^{2+} sensitivity is related to iso-kinetic power output in MHC I and IIA fibers. Relationship between whole muscle isokinetic power output at $180^\circ/\text{s}$ and single fiber activation threshold (pCa_{10}) and Ca^{2+} sensitivity (pCa_{50}) in myosin heavy chain (MHC) I and IIA fibers. Each variable was regressed on leg fat-free mass (measured via DEXA) and residuals (observed minus predicted) were calculated. Plotting the residuals for whole muscle isokinetic power and Ca^{2+} sensitivity depicts the partial correlation between these variables adjusted for leg fat-free mass. This analysis used $n = 19$ as two participants (one younger and one older adult) were missing data for isokinetic power output at $180^\circ/\text{s}$.

Table 1

Clinical characteristics of young and older adults.

	Young (<i>N</i> = 11)	Older (<i>N</i> = 10)	<i>P</i> value for age effect
N (M/F)	5/6	3/7	–
Age (y)	24.1 ± 1.1	68.8 ± 0.8	< 0.01
Height (cm)	173.2 ± 3.0	164.1 ± 3.8	0.07
Weight (kg)	67.7 ± 3.4	65.8 ± 4.8	0.75
Body mass index (kg/m ²)	22.5 ± 0.9	24.1 ± 0.9	0.21
Percent body fat (%)	28.1 ± 3.0	33.4 ± 2.8	0.23
Leg fat-free mass (kg)	16.4 ± 1.1	13.7 ± 1.3	0.13
Physical activity (kcal/d)	415 ± 34	415 ± 50	1.00

Data are presented as mean ± SE.

Bold indicates $p < 0.05$

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Activation threshold (pCa₁₀), Ca²⁺ sensitivity (pCa₅₀) and pCa-force slope (Hill coefficient, *h*) of myosin heavy chain (MHC) I, I/IIA, IIA and IIAX single muscle fibers from young and older adults.

Table 2

	<i>n</i>		pCa ₁₀		pCa ₅₀		<i>h</i>	
	Y/O	Y/O	Y	O	Y	O	Y	O
MHC	Y/O	Y/O	Y	O	Y	O	Y	O
I	45/50	11/10	6.01 ± 0.02	5.90 ± 0.02 **	5.73 ± 0.01 ^a	5.59 ± 0.02 ** ^a	3.49 ± 0.13 ^a	3.46 ± 0.13 ^a
I/IIA	7/5	3/5	5.96 ± 0.02	5.88 ± 0.03	5.71 ± 0.02 ^{ab}	5.59 ± 0.02 ** ^a	3.82 ± 0.10 ^{ab}	3.43 ± 0.27 ^a
IIA	42/38	11/10	6.01 ± 0.01	5.90 ± 0.01 **	5.76 ± 0.01 ^{bc}	5.69 ± 0.01 ** ^b	4.09 ± 0.15 ^b	4.65 ± 0.16 * ^b
IIAX	7/4	5/2	6.03 ± 0.02	5.92 ± 0.03 *	5.78 ± 0.02 ^c	5.73 ± 0.04 ^b	3.55 ± 0.12 ^{ab}	5.47 ± 0.13 ** ^b

Data are presented as mean ± SE.

Values correspond to the pCa-normalized specific force curves.

n indicates the number of fibers assessed and N indicates the number of subjects.

Y = young; O = older.

Significantly different from Young group at * = *p* < 0.05; ** = *p* < 0.01.

Where fiber type effects were observed, different letters identify pairwise differences (*p* < 0.05) between fiber types within an age group.

Table 3

Isokinetic knee extension power in young and older adults.

Isokinetic velocity ($^{\circ}/s$)	N (Y/O)	Knee extension power (Nm)		P value for age effect
		Y	O	
Absolute				
60	11/9	168.8 \pm 6.2	123.3 \pm 8.2	< 0.01
120	11/9	283.4 \pm 11.1	205.5 \pm 14.6	< 0.01
180	10/9	353.5 \pm 15.4	253.1 \pm 19.5	< 0.01
240	10/9	405.5 \pm 16.4	285.5 \pm 20.8	< 0.01
300	10/7	436.7 \pm 15.1	321.5 \pm 25.8	< 0.01
Adjusted *				
60	11/9	158.2 \pm 5.6	120.9 \pm 6.3	< 0.01
120	11/9	262.1 \pm 8.8	200.9 \pm 9.9	< 0.01
180	10/9	330.9 \pm 13.9	247.3 \pm 15.3	< 0.01
240	10/9	380.4 \pm 14.4	279.1 \pm 15.9	< 0.01
300	10/7	408.3 \pm 10.2	319.4 \pm 14.8	< 0.01

Data are presented as mean \pm SE.

N indicates the number of subjects.

Y = young; O = older.

* Adjusted for leg fat-free mass (kg) measured from DEXA.

Table 4

Linear regression analysis of Ca^{2+} sensitivity (pCa_{50}) of myosin heavy chain (MHC) I and IIA single fibers for isokinetic knee extension power (Nm) in young and older adults.

Isokinetic velocity ($^{\circ}/\text{s}$)	pCa_{50}	β	SE	<i>P</i> value
60	MHC I	333.91	122.99	0.02
	MHC IIA	-196.31	185.96	0.31
120	MHC I	545.27	195.78	0.01
	MHC IIA	-324.10	296.02	0.29
180	MHC I	790.97	303.21	0.02
	MHC IIA	-487.98	468.13	0.31
240	MHC I	901.78	347.54	0.02
	MHC IIA	-540.21	536.56	0.33
300	MHC I	604.84	428.94	0.18
	MHC IIA	-163.95	658.23	0.81

All models were adjusted for leg fat-free mass (kg) measured from DEXA. β represents the unstandardized regression coefficient.

Bold indicates $p < 0.05$