The influence on sarcopenia of muscle quality and quantity derived from magnetic resonance imaging and neuromuscular properties

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Abstract The relative contributions of intrinsic and extrinsic neuromuscular factors on sarcopenia are poorly understood. The associations among age-related declines of strength, muscle mass, and muscle quality in response to motor unit (MU) loss have not been systematically investigated in the same groups of subjects. The purpose was to assess MU loss, MRI-derived muscle cross-sectional area (CSA), muscle protein quantity (MPQ), and normalized strength of the dorsiflexors in one group of young (~25 years) adult males compared with two groups of healthy men aged 60–85 years. Muscle strength was assessed on a dynamometer and was ~25 % lower in both older groups, but CSA was less only in the older (>75 years) men, with no differences

between the young and old (60–73 years). Normalized strength tended to be lower in both groups of aged men compared to young. For MPQ, only the older men showed ~8 % lower values than the young and old men. Older men had fewer functioning MUs than old, and both groups of aged men had fewer MUs than young men. Muscle quality appears to be maintained in the old likely due to compensatory MU remodeling, but in the older group (>75 years), MU loss was higher and MPQ was lower.

Keywords Aging · Atrophy · Motor unit · EMG · Weakness

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Introduction

Sarcopenia is a gradual, nonpathological process of adult aging that is characterized by a decline in skeletal muscle mass. This age-related deterioration of muscle mass leads to a loss of muscle strength into the seventh decade of life and rapidly accelerates thereafter (Berger and Doherty 2010). The loss of strength is not always related directly to the loss of muscle mass and therefore when strength is normalized to the amount of contractile tissue, strength deficits often remain (Doherty 2003). Thus, factors related to the quality of the contractile tissue or extrinsic influences may contribute to a decline in contractile capacity (strength), but the interaction of these various features has not been assessed fully.

From mainly cross-sectional studies, the average decline in skeletal muscle mass is 10–20 % by the seventh

decade of life, with a further ~20 % reduction thereafter (Power et al. 2013). Many reports focus on alterations to the intrinsic properties of single muscle fibers to explain changes in muscle quality (MQ) (Canepari et al. 2010; Russ et al. 2011) and others recognize that much of this change is secondary to declines in the number of functioning motor units (MUs), one factor leading to fiber atrophy (Larsson 1995; Lexell et al. 1988). The relationship between intrinsic contractile changes and extrinsic neuroanatomical factors is not fully appreciated and few studies have attempted to assess both aspects in concert (Campbell et al. 1973; Lexell et al. 1988). Functioning MUs undergo remodeling throughout the lifespan, but it is not until the process of denervation outpaces reinnervation in old age that a loss of MU integrity and the ensuing substantive loss of muscle fibers occur (Gordon et al. 2004). This process could be one of the extrinsic changes to whole muscle affecting MQ (Doherty 2003), but the possible relationship between loss of MUs and MQ has not been explored systematically. In separate studies, MU loss was related to strength loss for only a very old (~80 years) group of subjects (McNeil et al. 2005b), but muscle cross-sectional area quantified from magnetic resonance imaging (MRI) showed no relationship between amount of muscle tissue and strength when comparing young with older subjects (McNeil et al. 2007). This supports that age-related alterations in MQ rather than only muscle quantity are related to functional declines.

Although MRI and other imaging techniques have been used extensively in studies on human aging and limb muscles, these anatomical images have been mostly limited to assessments of muscle cross-sectional areas (CSAs) or volumes. Many studies have attempted to improve basic CSA measurements by eliminating apparent noncontractile tissue (Kent-Braun et al. 2000; Klein et al. 2001; Rice et al. 1989). Muscle CSA or volume allows for the calculation of normalized (specific) strength (i.e., force or torque per amount of tissue), but it cannot assess intrinsic MQ. Single fiber MQ assessed by estimating specific strength has been accomplished through invasive muscle biopsy methods (Frontera and Larsson 1997; Lexell et al. 1988; Trappe et al. 2003), but the findings are equivocal (Canepari et al. 2010; D'Antona et al. 2007; Frontera et al. 2001, 2000) and may relate to limitations of the method and sample size. Furthermore, alterations in muscle architecture and quantity and quality changes to noncontractile tissue have important consequences on the expression of

contractile function of whole muscle (Morse et al. 2005; Narici et al. 2003; Thom et al. 2007).

A specialized MRI protocol, magnetization transfer (MT) has been used which can assess muscle protein quantity (MPQ) noninvasively at the whole muscle level [for technical details, see (Henkelman et al. 2001; Sinclair et al. 2010)]. A lower MT ratio (MTR) reflects a decreased quantity and quality of the muscle protein content (McDaniel et al. 1999; Schwenzer et al. 2009; Sinclair et al. 2010). To date, one study assessed MPQ in older subjects (~65 years) and found a decreased MTR, indicating reduced MPQ in old compared to young men in the tibialis anterior (Schwenzer et al. 2009), although that study did not assess other structural or functional measures.

To better understand the sarcopenic process, MTR in combination with structural MRI and standard neuromuscular measures offers a noninvasive means to evaluate whole muscle quantity and quality as related to structural integrity of contractile and noncontractile proteins. Thus, the purpose of this study was to evaluate age-related declines in the number of functioning MUs with measures of muscle quantity and quality obtained by electrophysiological and MRI techniques in the dorsiflexors for a cross-sectional comparison of young (22–30 years), old (60–73 years), and older (76–85 years) men. We expected that the apparent dissociation between the age-related loss of MUs and strength with increased age will be explained by MTR-assessed muscle protein quantity.

Methods

Participants

Twelve young (22–30 years), nonsystematically trained, recreationally active men were recruited from the university population, and six old (60–73 years) and six older (76–85 years) men were recruited from a local university senior's activity group which includes walking and light calisthenics thrice weekly. Inclusion criteria included those under the age of 30 and over 60 years (Table 1). Participant activity characteristics were determined via self-reported exercise levels during a pre-testing interview. All participants were living independently and with no known neurological, metabolic, or cardiovascular diseases. Additionally, all participants were free from medications that could impact

Table 1 Participant characteristics

Group	Age (years)	Height (cm)	Weight (kg)
Young	25±3	179.0±5.3	82.8±9.5
Old	68±5*	175.0±3.9	95.1±15.1*
Older	79±3*†	174.2±3.8	80.3±10.8†

Values are means ± standard deviations. * significantly different than young, † significantly different than old ($P<0.05$)

neuromuscular or musculoskeletal function and normal health (e.g., statins, corticosteroids). The study protocol was approved by the local university's ethics board and conformed to the Declaration of Helsinki. Informed, oral and written consent were obtained from each participant prior to testing.

Magnetic resonance imaging

During a single visit to the research imaging unit of a local hospital, MRIs were acquired via serial axial planes in a 3.0 Telsa magnet (Magnetom Verio, Siemens Healthcare, Erlangen, Germany). Participants were inserted into the magnet bore feet first, in the supine position, with the motor point of the tibialis anterior (TA) of their right leg isocentered to the bore of the magnet. To ensure no movement between scans, the feet and knees were strapped together using inelastic Velcro straps. The entire musculature of the right leg from the tibial plateau to the malleoli was imaged using the body coil for transmission and body and spine coil elements for receiving. Proton density images for anatomical measures were acquired using a 2D FLASH sequence with the following parameters: 1,500 ms repetition time (TR), 14 ms echo time (TE), 256×192 matrix, 243×325 mm field of view, 30 slices, 5-mm slice thickness with slice separation of 2 mm.

Magnetization transfer ratio

Using a second series of scans, immediately following the sequence above, MPQ was assessed using MT. Magnetization transfer is a specialized MRI protocol which has been initially used in the characterization of white matter disease (e.g., multiple sclerosis) in the brain of clinical populations but has garnered some usage for assessing skeletal MPQ (Henkelman et al. 2001). For a detailed description of the MTR technique,

please see McDaniel et al. (1999). Skeletal muscle normally has a prominent MT effect because of the high concentration of protein bound to water (Dixon et al. 1990). A lower MT ratio indicates reduced capacity of the macromolecular protons to exchange magnetization with free protons and therefore reflects decreased structural integrity and quality of macromolecules (i.e., protein) in muscle tissue (Michaelis et al. 2008; Schwenzer et al. 2009). For the MT protocol, the MT pre-saturation, off-resonance frequency scans were taken first, followed by a second scanning without pre-saturation of the same anatomical locations. The MT pulse was Gaussian, 10.2 ms long, 500° flip angle, and 1,200 Hz offset from water resonance. Each scan series required about 7–8 min and the time between the two scans was 5–10 s during which time the participant remained motionless.

Magnetization transfer is based on longitudinal magnetization interaction between free protons and macromolecular (bound) protons. Normally, the protons associated with macromolecules such as muscle protein are not visible in MRI due to their short T2 relaxation time. By saturating the protons associated with macromolecules via an off-resonance pulse, the excitation is transferred to the free protons decreasing the net magnetization signal (Henkelman et al. 2001). Using the acquired MR images, M_0 values and M_1 values (where M_0 denotes images without off-resonance pre-saturation and M_1 denotes images with off-resonance pre-saturation) were measured for the same region of interest (ROI) used for anatomical (T1) area and volume determinations. In pilot testing (results not shown), single slice analysis was found to be not significantly different than whole muscle volume MTR; therefore, to make relative comparisons to muscle CSA, the same slice ROI was used for MTR and CSA. The interaction of macromolecular protons and free protons of a specific tissue is quantitatively described by the magnetization transfer ratio (Schwenzer et al. 2009; Sinclair et al. 2010).

$$\text{MTR} = [(M_0 - M_1) / M_0]$$

Magnetization transfer ratio was calculated in percentage units (p.u.) as a change from the baseline scan (M_0). It should be noted that the MTR-derived values depend on the parameters of the sequence and the MT pre-pulse, and cannot be directly compared with values from other studies (Sinclair et al. 2010) and therefore represents a relative change expressed as a ratio.

Muscle composition area and volume

Off-line from the acquired images, TA CSAs, CSA composition, and whole TA volumes were calculated pixel-wise using a combination of manual and semiautomated techniques with open-source OsiriX image processing software (version 4.1, Geneva, Switzerland). Tibialis anterior CSAs were calculated from the slice with the largest TA CSA. Analysis began proximally from the first slice in which the TA appeared, to the most distal slice containing identifiable muscle tissue of the TA. In total, 20 slices were taken; each slice was 5 mm thick, and separated by 2 mm from the subsequent slice. The image processing software accounted for the 2 mm of separation between slices when performing the muscle volume calculation. The ROI was manually outlined on the most proximal TA slice, with the brush tool and the outline repeated on every fifth slice; missing ROIs on the intervening slices were automatically interpolated. With the TA outlined, all pixels outside the ROIs were set to zero. To quantify the contractile-only tissue, a three-dimensional threshold-growing tool was used to ensure only muscular tissue was included in the ROIs (excluding noncontractile tissue and septal spaces). Any errors produced by the automatic generation were corrected manually by the same investigator. The software calculated muscle CSA and volume for the ROIs. Previous research has shown a high degree of intra- (ICC=0.99) and inter-rater reliability (ICC=0.99) with this analysis technique (Berger et al. 2012).

Strength

Within 7 days, participants attended the neuromuscular lab to test dorsiflexor contractile properties. To determine maximal dorsiflexion torque, participants were seated in a custom-built isometric dynamometer with the hip and knee angles positioned at 90° and the ankle at 30° plantar flexion. To minimize confounding hip and knee joint movement during dorsiflexion contractions, an adjustable C-shaped brace was secured firmly on the distal portion of the thigh. Velcro strapping across the toes and dorsum secured the foot to the dynamometer.

All testing was performed on the right (dominant) leg. Twiches were evoked electrically using single pulses (100 µs) of progressively greater current intensity (Model DS7AH, Digitimer Welwyn Garden City, Hertfordshire, UK) were applied every 5 s until the

dorsiflexion twitch torque did not increase further. Participants performed three maximum voluntary isometric contractions (MVC), with at least 2-min rest between attempts and were provided with real time visual feedback of their torque on a computer monitor, and verbally exhorted. Voluntary activation was assessed using the interpolated twitch technique (Belanger and McComas 1981). Single supramaximal pulses were applied during and after each MVC in order to determine the percentage of voluntary activation. The investigator triggered the stimulator manually ~2–4 s after MVC onset, when the torque trace plateaued and again ~1 s after the contraction when the muscles were relaxed fully. The amplitude of the interpolated torque evoked during the plateau of the MVC was compared with a single resting twitch torque evoked ~1 s following the MVC. Percent voluntary activation was calculated as voluntary activation (%) = $[1 - (\text{interpolated twitch} / \text{resting twitch})] \times 100 \%$.

The peak torque amplitude of the three MVC attempts was taken as the maximal torque for the participant. This torque value was then normalized to the contractile CSA and volume so that normalized strength equaled MVC/CSA (N m/cm²) and MVC/volume (N m/cm³). Inherent changes in the contractile function of the dorsiflexors were assessed by measures of electrically evoked twitch properties. Peak twitch torque (N m), time to peak twitch torque (TPT; ms), the time required for torque to reach peak twitch torque amplitude as measured from baseline, and half relaxation time (HRT; ms), the time required for peak twitch torque amplitude to reach half of the peak twitch torque amplitude, were reported.

Electrophysiological properties

Using the same experimental setup as above during the MVCs (three attempts), surface EMG data were collected from the TA using self-adhering Ag-AgCl electrodes (1.5 cm × 1.0 cm; Marquette Medical Systems, Jupiter, Florida). The skin over the muscle was cleaned with alcohol (70 % isopropyl alcohol solution) prior to application of the electrodes. An active electrode was positioned on the proximal portion of the TA over the motor point [7 cm distal to the tibial tuberosity and 2 cm lateral to the tibial anterior border], a reference electrode was placed over the distal tendon at the malleoli, and a ground electrode was placed on the patella. For decomposition-enhanced spike-triggered averaging

(DE-STA) measures that include an estimate of the number of functional motor units (MUNE), intramuscular EMG signals were recorded via a disposable concentric needle electrode with a recording surface of 0.03 mm² (Model N53153; Teca, Hawthorne, New York) inserted into the TA, 5–10 mm proximal to the active surface electrode. The MUNE technique was performed similarly to what we have reported previously (Dalton et al. 2008; McNeil et al. 2005b; Power et al. 2012a, 2010). Electromyography data were acquired using customized software on a Neuroscan Comperio system (Neurosoft, El Paso, Texas). The DE-STA and its associated algorithms have been described previously (Doherty and Stashuk 2003; Stashuk 1999) and proven reliable (Boe et al. 2004). The surface and intramuscular EMG signals were bandpass filtered at 5 Hz to 5 kHz and 10 Hz to 10 kHz, respectively.

Data collection began by determining the compound muscle action potential (CMAP). The CMAP was evoked via supramaximal stimulation of the common fibular nerve distal to the fibular head. The electrical current intensity was progressively increased until a plateau in CMAP amplitude was reached. At this point, the stimulation intensity was increased 20 % to ensure complete activation of all motor axons. During CMAP determination, the active electrode was repositioned manually to maximize the negative-peak amplitude and minimize negative-peak rise time.

Following the MVCs (described above), participants were given 5 min of rest to ensure no residual fatigue. The investigator then inserted and manipulated the concentric needle to minimize rise times of the negative-peak amplitudes of the first two to three detected motor unit potentials (MUPs) during a low level voluntary contraction. Once the investigator was satisfied with the needle position, the participants were asked to slowly increase their dorsiflexion torque to match a target line of 25 % MVC within 1–2 s and hold the contraction steady for ~30s, during which both the intramuscular and surface EMG were sampled and stored for future analysis. This contraction intensity was found to be the most effective for obtaining a representative MUNE in the TA (McNeil et al. 2005a). Participants were given at least 1 min of rest between these contractions, and the needle was repositioned by either adjusting the depth of insertion or sampling from a new insertion area. These procedures were repeated until at least 20 suitable needle-detected trains of MUPs and their respective surface MUPs (SMUPs) were collected.

Decomposed EMG signals were reviewed off-line to determine the acceptability of the needle-detected MUP trains and their corresponding SMUPs. First, an acceptable MUP train required greater than 50 detected discharges which acted as triggers for spike-triggered averaging. Then, the MU discharge pattern was inspected visually for a constant rate (i.e., coefficient of variation ≤ 30 %) and a physiological mean discharge rate. Lastly, the interdischarge interval histogram was examined to confirm that it followed a Gaussian distribution. The SMUP trains which did not meet these criteria were excluded from further analysis (Boe et al. 2004). The SMUPs were inspected visually to identify a distinct waveform which was temporally linked to the needle potential. The computer-generated negative-peak onset and negative-peak amplitude markers of the acceptable SMUPs were manually inspected to ensure they were accurate. Any markers not correctly set were repositioned manually. A computer algorithm then aligned the negative onset markers for all accepted SMUPs and created a mean SMUP template based upon their data-point by data-point average. Finally, a MUNE was derived by dividing the negative-peak amplitude of the CMAP by the negative-peak amplitude of the mean SMUP.

Voluntary and electrically evoked torque data were sampled at a rate of 500 Hz and converted to digital format using a 12-bit analog-to-digital converter (model 1401 Plus, Cambridge Electronic Design, Cambridge, UK) to be stored on the computer. Spike2 software (Cambridge Electronic Design) was used during off-line analysis to determine voluntary and evoked isometric torques, and contraction duration (time to peak twitch+half relaxation time) of the electrically evoked twitch.

Statistics

All data were analyzed using SPSS software (version 18, SPSS Inc. Chicago, IL). One-way ANOVA were performed to identify the differences among groups. When a significant main effect was present, a Tukey HSD post hoc test was performed to identify where significant differences existed. Because voluntary activation values are not normally distributed, a Mann-Whitney *U* test was employed to test for statistical significance of this variable. The level of significance was set at $P \leq 0.05$. All data are presented as means $\pm$ SDs.

Results

CSA, CSA composition and volume

The largest total CSA measure was not significantly different across all three age groups ($P>0.05$); however, when adjusted for noncontractile tissue, the contractile CSA was ~20 % lower in the older men versus the young ($P<0.05$) men, but not different between the young and old ($P>0.05$). As a relative percentage of total muscle CSA, noncontractile tissue accounted for a significantly higher percentage in the old (20.5 ± 7.2 %) ($P<0.05$) and older men (24.2 ± 10.6 %) ($P<0.05$) compared to the young (12.0 ± 3.3 %) (Fig. 1). Muscle volume corrected for noncontractile tissue showed significant differences among the three age groups (Table 2) and was ~17 % lower in the old ($P<0.05$) and ~19 % lower in the older men ($P<0.05$) compared with the young men. However, muscle volume was not different between the two higher age groups ($P>0.05$).

Neuromuscular properties

Maximal voluntary isometric torque was ~25 % lower in both the old ($P<0.05$) and older men ($P<0.05$) compared to the young men (Table 3). Despite lower strength in both old groups, voluntary activation, as assessed by the interpolated twitch technique, was similar and near maximal (≥ 95 %) in all three groups during MVCs (Table 3)

Table 2 Cross-sectional area, cross-sectional area composition and volume

Group	Contractile Area (cm ²)	Noncontractile Area (cm ²)	Maximal Total CSA (cm ²)	Contractile Volume (cm ³)
Young	13.4±1.9	1.8±0.5	15.2±2.2	235.2±33.8
Old	11.7±1.4	3.0±1.1	14.7±1.3	194.4±26.5*
Older	11.2±1.5*	3.6±1.8*	14.8±1.0	191.5±25.6*

Values are means ± standard deviations

* $P<0.05$ (significantly different than young)

indicating both old groups were capable of activating their muscles fully, thus ruling out any confounding limitations of the central nervous system contributing to weakness (Power et al. 2012b, c). When strength was related to total CSA, both the old ($P<0.05$) and older ($P<0.05$) men showed ~25 % lower normalized strength than the young men (Fig. 2). However, when strength was expressed relative to contractile CSA, the age-related differences were no longer present between the young, old, and older men, although there was a trend for normalized strength to be lower in both old groups ($P=0.07$) (Fig. 2). Similar to CSA, although significant differences existed among the groups in volume measures (Table 2) when strength was expressed relative to contractile volume, no significant differences existed for normalized strength among the groups. The lack of differences in normalized strength

Fig. 1 Noncontractile tissue composition as a percent of total CSA in the young, old, and very old men. Although the total CSAs were similar among the three groups, the percent of noncontractile tissue was significantly greater in both old groups compared to the young. *significantly greater than young ($P<0.05$). Values are means ± standard deviations

Table 3 Neuromuscular properties of the tibialis anterior

Group	MVC (N m)	VA (%)	Pt (N m)	TPT (ms)	HRT (ms)	CD (ms)
Young	53.9±7.8	96.2±2.4	6.6±2.2	78.5±14.5	94.2±8.5	172.7±19.1
Old	39.8±6.5*	95.8±2.1	5.8±1.3	97.8±12.9*	119.5±10.8*	217.3±10.9*
Older	40.9±6.4*	94.6±1.9	6.2±1.4	102.6±5.7*	121.6±13.7*	223.7±15.0*

Values are means ± standard deviations

MVC absolute maximal isometric contraction torque, VA voluntary activation, Pt twitch torque, TPT time to peak torque, HRT half relaxation time, CD contraction duration (which is TPT+HRT)

* $P<0.05$ (significantly different than young)

values were due to a concurrent decrease of strength and CSA or strength and volume, suggesting a maintenance of intrinsic contractile capacity for the ankle dorsiflexors to produce force. Consistent with similarities in normalized strength, there were no significant differences among groups for twitch torque amplitude ($P>0.05$). However, there was a slowing of evoked contractile properties; time to peak torque and half relaxation times were longer in the old [~25 % ($P<0.05$) and ~27 % ($P<0.05$), respectively] and older [~31 % ($P<0.05$) and ~30 % ($P<0.05$), respectively] men compared to the young men. As a consequence, contraction duration (time to peak torque+half relaxation time) was ~26 % longer in the old and ~30 % longer in the older men compared with young (Table 3).

Magnetization transfer ratio

Qualitatively, the MTR images appear darker in the older men than both the young and old men, indicating less magnetization transfer (Fig. 3). Indeed, quantitatively, MTRs were ~8 % greater between the young (0.262 ± 0.01 p.u.) and older men (0.242 ± 0.02 p.u.) ($P<0.05$) as

well as ~9 % between the old (0.266 ± 0.01 p.u.) and older men ($P<0.05$) but not significantly different between the young and old ($P=0.844$) (Fig. 4).

Motor unit properties

The maximum negative-peak amplitude of the CMAP was not significantly different between the young and old ($P>0.05$), whereas the CMAP was 19 % smaller in the older men compared with the young men ($P<0.05$), whereas the two old groups did not differ ($P>0.05$) (Fig. 5a). The negative-peak amplitude of the mean SMUP was only significantly larger (53 %) in the older men compared to the young ($P<0.05$) men. The SMUP of the old men was not significantly different from the young or older men ($P>0.05$) (Fig. 5b). An ~19 % decrease in mean CMAPs with an increase, albeit not statistically significant, for SMUPs in the old men resulted in significantly lower MUNE compared to the young men ($P<0.05$). The combination of a smaller mean CMAP with significantly greater mean SMUP of the older men resulted in significantly lower MUNE

Fig. 2 Strength normalized to total CSA and muscle CSA. *significantly weaker than young ($P<0.05$). Values are means ± standard deviations

Fig. 3 Sample magnetic resonance imaging (MRI) images of the leg, without off-resonance pre-saturation (*a1*, *b1*, *c1*) and with off-resonance pre-saturation (*a2*, *b2*, *c2*) of (A) young (22 years old),

(B) old (67 years old), and (C) Older (81 years old) participants. The tibialis anterior (TA) muscle is outlined in white in each image

than both the young ($P<0.05$) and old ($P<0.05$) men. It was estimated that the young men had 146 ± 31 MUs, the old men had 109 ± 25 MUs, and the older men had 80 ± 21 MUs (Fig. 6).

Discussion

The multidimensional nature of sarcopenia highlights the importance of examining both quantitative and qualitative changes occurring in various portions of the neuromuscular system with adult aging. The purpose of the current study was to compare young adult men with two groups of aged men to characterize sarcopenia (albeit from cross-sectional comparisons) by assessing

and comparing changes in strength and muscle quality by using a novel combination of anatomical and electrophysiologic measures. Prior studies have compared young adults on some of these measures, but none have included a comprehensive assessment, and with the addition of muscle quality using the MTR technique, with two groups of aged men. By using this design, the structural and electrophysiological measures from this investigation show discordant changes potentially explaining changes in MQ.

Our results show that contractile muscle area was well maintained in the old group for this particular muscle group, likely due to compensatory MU collateral reinnervation maintaining excitable muscle mass (i.e., approximated by M-wave amplitude), whereas the older

Fig. 4 MTR for young, old, and older men. *significantly lower than young, † significantly lower than old ($P<0.05$). Values are means $\pm$ standard deviations

Fig. 5 **a** The negative-peak amplitude of the CMAP was significantly lower in the older men compared to the young. **b** The mean SMUP was significantly higher in the older men compared to the old and young. *significantly different than young ($P<0.05$). Values are means $\pm$ standard deviations

men showed a loss of excitable muscle mass as compared to young. The older men featured a greater absolute amount and relative proportion of noncontractile intramuscular tissue than both the old or young groups (Table 2 and Fig. 1). Although we did not directly assess the composition of this noncontractile tissue, previous studies indicate it is likely composed of fat and connective tissue and that it is unlikely to be viable muscle tissue (Lexell 1995; Rice et al. 1990). Absolute strength was indeed lower in the old and older men, but when strength

Fig. 6 MUNE were significantly lower in the old and older men compared to the young. *significantly different than young ($P<0.05$). †significantly lower than old ($P<0.05$). Values are means $\pm$ standard deviations

was normalized to the amount of contractile muscle tissue, the age-related differences no longer existed (Fig. 2). Therefore, based on the present results, it could be assumed that the integrity of the contractile machinery (force per unit of muscle) is well maintained in the old and older men for this particular muscle. These results are similar to previous findings in older men (68 years) in which the CSA of the TA was assessed using MRI (Kent-Braun and Ng 1999). As well, the current results corroborate the findings of previous research which showed contractile properties of human single muscle fibers were well maintained with aging (Frontera et al. 2008, 2000) most likely owing to a compensatory overload mechanism of viable MUs when de-innervated units were lost. Thus, at the whole muscle level, in the present study, both old groups were weaker but the intrinsic contractile properties of the contractile muscle mass, at least for torque generation, appears to be essentially intact. In contrast, McNeil et al. (2007) found a decrease in normalized strength in their very old age group of men who were older (~5 years) than the upper age group of men in our study. In that prior study, the very old men were found to have decreased strength values, but not significantly different muscle CSA which accounted for the difference

in normalized strength. Perhaps at these later decades, the rate of change is steeper and an average of 5 years older becomes functionally important.

Despite similar normalized strength values for the two old groups, there was a slowing of whole muscle twitch contractile properties that was significant for both the old and older men compared to the young. This indicates intrinsic contractile properties perhaps related to crossbridge kinetics (D'Antona et al. 2003, 2007; Ochala et al. 2007; Prochniewicz et al. 2005, 2007) are also affecting the progression of sarcopenia which may have important consequences related to dynamic muscle performance and functional activities in old age (Power et al. 2013). The impaired electrically evoked twitch contractile properties in old age, reflected in a slower contracting muscle, may in part be explained by reduced MPQ as indicated by differences in MTR values between the older men compared with the young and old (Fig. 4). The lower MTR ratio indicates reduced protein content and therefore an age-associated loss of viable contractile material relative to CSA. This, presumably results in a decreased number of actin (thin filament) myosin (thick filament) crossbridge interactions (D'Antona et al. 2003) contributing to impaired force production in the older men. Additionally, as shown in *in vitro* motility assay experiments, decreased actin filament sliding speed (D'Antona et al. 2003), independent of muscle fiber type (Hook et al. 1999, 2001), is associated with reductions in maximal shortening velocity in old age. These findings point to a slowing of the kinetic steps within crossbridge cycling contributing to impaired muscle function.

In the present study, for the same subjects, neither proxy of neuromuscular function occurs independently; rather all factors occur simultaneously but each likely at different rates of progression. For the older men, MU death outpaces remodeling resulting in muscle fiber loss, atrophy, and decreases in MQ, leading to an accelerated decline of neuromuscular function. Hence, at the whole muscle level, the subsequent larger decline of neuromuscular function in older men is at least in part due to MU death (Fig. 6) and importantly by a decrease in MPQ. Similar to our finding of a 8 and 9 % lower MTR in older men relative to the young and old men, respectively (Fig. 4), Schwenzer et al. (2009) reported ~6 % lower MTR values for healthy old (~65 years) versus young participants in the TA. Thus, MTR may be a sensitive measure of MQ and the functional declines to follow, as MTR depicts a decrease in MPQ in the eighth decade of life, but a decrease in normalized strength was

not found in this muscle until the ninth decade of life (McNeil et al. 2007). Results from the present study also highlight that sarcopenia cannot be explained by a single process but is the result of a number of contributing factors that perhaps converge due to reduced compensatory abilities in each factor by the eighth decade.

The decline in MPQ we observed for the older men indicates that impaired intrinsic contractile properties of muscle may occur and dominate contractile function more than reduced CSA in the later decades of life (Sinclair et al. 2010). Hence, the functional effects of muscle structure decline may not become apparent until later in the aging process as shown by our results of decreased absolute strength concomitant with a loss of functional MUs owing to deficient collateral reinnervation in older men. Our MUNE results are consistent with previous research on the TA showing an age-related loss of functioning MUs (McNeil et al. 2005b; Power et al. 2010). In the present study, an age difference of 45 years between the young and old group showed a ~25 % lower value in mean MUNE, whereas with a ~10-year difference between two old groups, the MUNE was lower by ~27 %. Presumably, the compensatory strategy of collateral reinnervation maintains most of the excitable muscle mass through recapturing orphaned muscle fibers until at some point during the aging process this proposed compensatory strategy is unable to compensate for further motor neuron death, resulting in larger remodeled MUs dying with what appears to be an accelerated loss of muscle tissue. This process is supported in the older men by greater negative-peak amplitude of the SMUP and smaller CMAP (Fig. 5). When remodeled MUs with a large number of muscle fibers die (decreased MUNE), a larger amount of excitable muscle mass (decreased CMAP and CSA) is lost, resulting in greater loss of muscle mass and consequently, reduced absolute strength as observed here in the older men. However, high levels of physical activity in old age have been shown to mitigate the loss of MUs, excitable muscle mass (Power et al. 2010) and maintain muscle fiber quality (D'Antona et al. 2007).

Magnetization transfer ratio has been used previously to assess MPQ, and ~17 % decrease of MTR in leg muscles of middle-aged men and women (~45 years) was found in patients with a clinical neuropathy compared with controls (Sinclair et al. 2010). This indicates MTR is sensitive to, and reflective of myogenic changes due to neurogenic processes. However, this technique has not been validated against muscle biopsies or models of animal aging, but physiologically, reflects the amount

of water-bound protein in the tissues. Therefore, a lower MTR reflects a decreased quantity and quality of the muscle protein content of the tissues (McDaniel et al. 1999; Schwenzer et al. 2009; Sinclair et al. 2010) that is not apparent in T1 weighted MR imaging.

Conclusion

In the old men, MTR was relatively maintained compared to the older men due to the ability of the compensatory mechanisms of MU remodeling to maintain excitable muscle mass and function. In the older men, however, MTR was lower due to the inability of the compensatory mechanisms of MU remodeling to maintain muscle mass as a precursor to impaired contractile properties and a decline in whole muscle function. Thus, although the current study was cross-sectional in design, it appears that first the number of MUs is decreased (consistent with loss of contractile muscle mass), followed by a decrease in MPQ as determined by a lower MTR thus leading to impaired intrinsic contractile properties and lower normalized strength. The reduced MPQ and presumed impaired crossbridge kinetics in the older men may be reflected by slowed muscle contractile properties and thus may be more evident during everyday tasks which require dynamic action and relatively fast contractile velocities to obtain adequate power.

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Conflict of interest The authors have no conflicts of interest to disclose.

References

- Belanger AY, McComas AJ (1981) Extent of motor unit activation during effort. *J Appl Physiol* 51:1131–1135
- Berger MJ, Doherty TJ (2010) Sarcopenia: prevalence, mechanisms, and functional consequences. *Interdiscip Top Gerontol* 37:94–114. doi:10.1159/000319997
- Berger MJ, McKenzie CA, Chess DG, Goela A, Doherty TJ (2012) Sex differences in quadriceps strength in OA. *Int J Sports Med* 33:926–933. doi:10.1055/s-0032-1311587
- Boe SG, Stashuk DW, Doherty TJ (2004) Motor unit number estimation by decomposition-enhanced spike-triggered averaging: control data, test-retest reliability, and contractile level effects. *Muscle Nerve* 29:693–699
- Campbell MJ, McComas AJ, Petito F (1973) Physiological changes in ageing muscles. *J Neurol Neurosurg Psychiatry* 36:174–182
- Canepari M, Pellegrino MA, D'Antona G, Bottinelli R (2010) Single muscle fiber properties in aging and disuse. *Scand J Med Sci Sports* 20:10–19. doi:10.1111/j.1600-0838.2009.00965.x
- D'Antona G, Pellegrino MA, Adami R, Rossi R, Carlizzi CN, Canepari M, Saltin B, Bottinelli R (2003) The effect of ageing and immobilization on structure and function of human skeletal muscle fibres. *J Physiol* 552:499–511
- D'Antona G, Pellegrino MA, Carlizzi CN, Bottinelli R (2007) Deterioration of contractile properties of muscle fibres in elderly subjects is modulated by the level of physical activity. *Eur J Appl Physiol* 100:603–611. doi:10.1007/s00421-007-0402-2
- Dalton BH, McNeil CJ, Doherty TJ, Rice CL (2008) Age-related reductions in the estimated numbers of motor units are minimal in the human soleus. *Muscle Nerve* 38:1108–1115
- Dixon WT, Engels H, Castillo M, Sardashti M (1990) Incidental magnetization transfer contrast in standard multislice imaging. *Magn Reson Imaging* 8:417–422
- Doherty TJ (2003) Invited review: aging and sarcopenia. *J Appl Physiol* 95:1717–1727
- Doherty TJ, Stashuk DW (2003) Decomposition-based quantitative electromyography: methods and initial normative data in five muscles. *Muscle Nerve* 28:204–211
- Frontera WR, Larsson L (1997) Contractile studies of single human skeletal muscle fibers: a comparison of different muscles, permeabilization procedures, and storage techniques. *Muscle Nerve* 20:948–952
- Frontera WR, Suh D, Krivickas LS, Hughes VA, Goldstein R, Roubenoff R (2000) Skeletal muscle fiber quality in older men and women. *Am J Physiol Cell Physiol* 279:C611–C618
- Frontera WR, Hughes VA, Krivickas LS, Roubenoff R (2001) Contractile properties of aging skeletal muscle. *Int J Sport Nutr Exerc Metab* 11(Suppl):S16–S20
- Frontera WR, Reid KF, Phillips EM, Krivickas LS, Hughes VA, Roubenoff R, Fielding RA (2008) Muscle fiber size and function in elderly humans: a longitudinal study. *J Appl Physiol* 105:637–642. doi:10.1152/jappphysiol.90332.2008
- Gordon T, Hegedus J, Tam SL (2004) Adaptive and maladaptive motor axonal sprouting in aging and motoneuron disease. *Neurol Res* 26:174–185
- Henkelman RM, Stanisz GJ, Graham SJ (2001) Magnetization transfer in MRI: a review. *NMR Biomed* 14:57–64
- Hook P, Li X, Sleep J, Hughes S, Larsson L (1999) The effect of age on in vitro motility speed of slow myosin extracted from single rat soleus fibres. *Acta Physiol Scand* 167:325–326
- Hook P, Sriramou V, Larsson L (2001) Effects of aging on actin sliding speed on myosin from single skeletal muscle cells of mice, rats, and humans. *Am J Physiol Cell Physiol* 280:C782–C788
- Kent-Braun JA, Ng AV (1999) Specific strength and voluntary muscle activation in young and elderly women and men. *J Appl Physiol* 87:22–29

- Kent-Braun JA, Ng AV, Young K (2000) Skeletal muscle contractile and noncontractile components in young and older women and men. *J Appl Physiol* 88:662–668
- Klein CS, Rice CL, Marsh GD (2001) Normalized force, activation, and coactivation in the arm muscles of young and old men. *J Appl Physiol* 91:1341–1349
- Larsson L (1995) Motor units: remodeling in aged animals. *J Gerontol A Biol Sci Med Sci* 50:91–95
- Lexell J (1995) Human aging, muscle mass, and fiber type composition. *J Gerontol A Biol Sci Med Sci* 50:11–16
- Lexell J, Taylor CC, Sjostrom M (1988) What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. *J Neurol Sci* 84:275–294
- McDaniel JD, Ulmer JL, Prost RW, Franczak MB, Jaradeh S, Hamilton CA, Mark LP (1999) Magnetization transfer imaging of skeletal muscle in autosomal recessive limb girdle muscular dystrophy. *J Comput Assist Tomogr* 23:609–614
- McNeil CJ, Doherty TJ, Stashuk DW, Rice CL (2005a) The effect of contraction intensity on motor unit number estimates of the tibialis anterior. *Clin Neurophysiol* 116:1342–1347
- McNeil CJ, Doherty TJ, Stashuk DW, Rice CL (2005b) Motor unit number estimates in the tibialis anterior muscle of young, old, and very old men. *Muscle Nerve* 31:461–467
- McNeil CJ, Vandervoort AA, Rice CL (2007) Peripheral impairments cause a progressive age-related loss of strength and velocity-dependent power in the dorsiflexors. *J Appl Physiol* 102:1962–1968
- Michaelis I, Kwiet A, Gast U, Boshof A, Antvorskov T, Jung T, Rittweger J, Felsenberg D (2008) Decline of specific peak jumping power with age in master runners. *J Musculoskelet Neuronal Interact* 8:64–70
- Morse CI, Thom JM, Reeves ND, Birch KM, Narici MV (2005) In vivo physiological cross-sectional area and specific force are reduced in the gastrocnemius of elderly men. *J Appl Physiol* 99:1050–1055. doi:10.1152/jappphysiol.01186.2004
- Narici MV, Maganaris CN, Reeves ND, Capodaglio P (2003) Effect of aging on human muscle architecture. *J Appl Physiol* 95:2229–2234
- Ochala J, Frontera WR, Dorer DJ, Van Hoecke J, Krivickas LS (2007) Single skeletal muscle fiber elastic and contractile characteristics in young and older men. *J Gerontol A Biol Sci Med Sci* 62:375–381
- Power GA, Dalton BH, Behm DG, Vandervoort AA, Doherty TJ, Rice CL (2010) Motor unit number estimates in masters runners: use it or lose it? *Med Sci Sports Exerc* 42:1644–1650. doi:10.1249/MSS.0b013e3181d6f9e9
- Power GA, Dalton BH, Behm DG, Doherty TJ, Vandervoort AA, Rice CL (2012a) Motor unit survival in lifelong runners is muscle dependent. *Med Sci Sports Exerc* 44:1235–1242. doi:10.1249/MSS.0b013e318249953c
- Power GA, Dalton BH, Rice CL, Vandervoort AA (2012b) Power loss is greater following lengthening contractions in old versus young women. *Age* 34:737–750
- Power GA, Rice CL, Vandervoort AA (2012c) Increased residual force enhancement in older adults is associated with a maintenance of eccentric strength. *PLoS One* 7:e48044
- Power GA, Dalton BH, Rice CL (2013) Human neuromuscular structure and function in old age: a brief review. *J Sport Health Sci* 2:215–226
- Prochniewicz E, Thomas DD, Thompson LV (2005) Age-related decline in actomyosin function. *J Gerontol A Biol Sci Med Sci* 60:425–431
- Prochniewicz E, Thompson LV, Thomas DD (2007) Age-related decline in actomyosin structure and function. *Exp Gerontol* 42:931–938. doi:10.1016/j.exger.2007.06.015
- Rice CL, Cunningham DA, Paterson DH, Lefcoe MS (1989) Arm and leg composition determined by computed tomography in young and elderly men. *Clin Physiol* 9:207–220
- Rice CL, Cunningham DA, Paterson DH, Lefcoe MS (1990) A comparison of anthropometry with computed tomography in limbs of young and aged men. *J Gerontol* 45:M175–M179
- Russ DW, Grandy JS, Toma K, Ward CW (2011) Ageing, but not yet senescent, rats exhibit reduced muscle quality and sarcoplasmic reticulum function. *Acta Physiol (Oxf)* 201:391–403. doi:10.1111/j.1748-1716.2010.02191.x
- Schwenzer NF, Martirosian P, Machann J, Schraml C, Steidle G, Claussen CD, Schick F (2009) Aging effects on human calf muscle properties assessed by MRI at 3 Tesla. *J Magn Reson Imaging* 29:1346–1354. doi:10.1002/jmri.21789
- Sinclair CD, Samson RS, Thomas DL, Weiskopf N, Lutti A, Thornton JS, Golay X (2010) Quantitative magnetization transfer in in vivo healthy human skeletal muscle at 3 T. *Magn Reson Med* 64:1739–1748. doi:10.1002/mrm.22562
- Stashuk DW (1999) Decomposition and quantitative analysis of clinical electromyographic signals. *Med Eng Phys* 21:389–404
- Thom JM, Morse CI, Birch KM, Narici MV (2007) Influence of muscle architecture on the torque and power-velocity characteristics of young and elderly men. *Eur J Appl Physiol* 100:613–619. doi:10.1007/s00421-007-0481-0
- Trappe S, Gallagher P, Harber M, Carrithers J, Fluckey J, Trappe T (2003) Single muscle fibre contractile properties in young and old men and women. *J Physiol* 552:47–58