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SHORT COMMUNICATION

Age-related reductions in the number of serial sarcomeres contribute to shorter fascicle lengths but not elevated passive tension

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ABSTRACT

We investigated age-related changes to fascicle length, sarcomere length and serial sarcomere number (SSN), and how this affects passive force. Following mechanical testing to determine passive force, the medial gastrocnemius muscle of young (n=9) and old (n=8) Fisher 344BN hybrid rats was chemically fixed at the optimal muscle length for force production; individual fascicles were dissected for length measurement, and laser diffraction was used to assess sarcomere length. Old rats had ∼14% shorter fascicle lengths than young rats, which was driven by a ∼10% reduction in SSN, with no difference in sarcomere length (∼4%). Passive force was greater in the old than in the young rats at long muscle lengths. Shorter fascicle lengths and reduced SSN in the old rats could not entirely explain increased passive forces for absolute length changes, owing to a slight reduction in sarcomere length in old rats, resulting in similar sarcomere length at long muscle lengths.

KEY WORDS: Passive force, Skeletal muscle, Rat, Laser diffraction, Medial gastrocnemius, Sarcomerogenesis, Ageing, Elderly, Muscle architecture

INTRODUCTION

In old age, there is a loss of muscle mass and alterations to the structural components of the neuromuscular system, impairing contractile function and performance (Power et al., 2013), and there appears to be elevated passive tension for a given muscle length (Marcucci and Reggiani, 2020). Ageing muscles of rodents and humans undergo structural remodelling, whereby muscle fascicle lengths become shorter than in the young (Hooper, 1981; Narici et al., 2003). An underappreciated factor for increased passive tension in old age could be decreased muscle fascicle lengths owing to either a decrease in the number of sarcomeres in series and/or shorter sarcomere lengths. Thus, the fascicles and sarcomeres from the old may experience greater relative length changes for a given displacement or joint angular rotation, resulting in increased passive tension as compared with that in the young.

Across structural levels, passive mechanical properties of skeletal muscle are altered with ageing. Investigations at the whole-muscle and single-fibre level in rodents and humans have demonstrated an age-related increase in passive tension (Alnaqeeb et al., 1984; Kovanen and Suominen, 1988; Blanpied and Smidt, 1993; Gosselin et al., 1998; Brown et al., 1999; Valour and Pousson, 2003; Ochala et al., 2004; Holt et al., 2016; Lim et al., 2019; Noonan et al., 2020). Holt et al. (2016) reported an age-related increase in rodent muscle fibre and aponeurosis stiffness, indicating a mechanical change in the relationship between contractile and non-contractile tissue. However, others have demonstrated no age-related differences in whole muscle (Brown et al., 1999), or single muscle fibre passive tension (Wood et al., 2014). Two recent studies reported elevated passive tension in single muscle fibres of old humans (Lim et al., 2019; Noonan et al., 2020). Lim et al. (2019) reported greater passive tension and increased viscoelastic function in single muscle fibres of old compared with young humans. More recently, Noonan et al. (2020) reported that, at shorter sarcomere lengths (1.9–2.65 µm), single muscle fibres from old humans demonstrated a greater passive stiffness compared with those of young humans, which led to a greater passive tension in the older group at sarcomere lengths between 2.1 and 3.55 µm; meanwhile, at longer sarcomere lengths, there was no difference in the passive elastic modulus or passive tension between young and old. In contrast, Pavan et al. (2020) noted no age-related differences at the single-fibre level, but the passive tension of fibre bundles from old humans was greater than that of young humans across all sarcomere lengths, as a result of collagen-induced stiffening of the extracellular matrix. It is important to note that, at the joint level, older adults have greater musculotendinous stiffness (Ochala et al., 2004; Marcucci and Reggiani, 2020), and experience an earlier increase in passive force for a given joint rotation (Gajdosik et al., 2004) than that experienced by young. At the whole-muscle and joint level, muscle architecture contributes relatively more to understanding contractile function than do single-fibre properties (Narici et al., 2016). Thus, previously reported age-related increases in passive tension at the whole-muscle level may be caused by shorter fascicles, owing to reductions in the number of sarcomeres, or shorter sarcomere length, resulting in sarcomeric structures (i.e. titin) experiencing greater stretch.

Therefore, the purpose of this study was to compare the medial gastrocnemius of young and old rats to determine differences in fascicle length, serial sarcomere number (SSN) and the sarcomere length at which peak force is obtained (apparent optimal reference length, RL), and record passive tension from short to long muscle lengths. It was hypothesized that fascicle length in old rats would be shorter than that in young rats owing to fewer sarcomeres in series, and the sarcomere length at RL would not differ between groups. Furthermore, passive tension was hypothesized to be greater in old than in young rats at long muscle lengths, as a result of the greater relative stretch an absolute length change will impose on shorter fascicles.

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MATERIALS AND METHODS

Animals

Barrier-reared male Fisher 344×Brown Norway (FBN) hybrid rats were obtained from the National Institutes of Aging facilities at Harlan Sprague–Dawley Inc. (Indianapolis, IN, USA). Animals were housed at the University of Calgary in conventional housing, one per cage for a maximum of 30 days on average. Housing conditions consisted of a 12 h:12 h dark:light cycle while the temperature was maintained at 22±2°C. Animals were provided with food and water *ad libitum* and allowed to recover from shipment for at least 2 weeks before experimentation began. During this time, the animals were carefully observed and weighed weekly to ensure none exhibited signs of failure to thrive, such as precipitous weight loss, disinterest in the environment, or unexpected gait alterations. All procedures were approved by an animal care committee at the University of Calgary (AC13-0252).

Surgical preparation

Nine young (8.9±0.6 months old, 504±48 g, equivalent human age ∼20 years) and 8 old (32.3±0.8 months old, 517±50 g, equivalent human age ∼75–80 years) rats were anaesthetized, the medial gastrocnemius muscle was surgically isolated and the Achilles tendon was tied in series with a force transducer for measurement of contractile properties *in situ* at 37±1°C as described previously in great detail by our group (https://www.jove.com/video/3167; MacIntosh et al., 2011).

Electrical stimulation procedures

The distal stump of the sciatic nerve was stimulated with 50 µs pulses of supramaximal intensity. The stimulating voltage was set at 1.5× the maximum voltage (the lowest voltage that activated all motor units) to ensure maximal activation of all motor units with each stimulus. A series of double-pulse contractions (5–10 ms delay) at differing muscle lengths (described below) with a 20 s rest between contractions was then used to determine the muscle length that elicited the greatest active force. This length is referred to as the reference length (RL). These contractions have been shown to yield an optimal reference length, which is similar to that obtained with longer duration tetanic contractions, while preventing fatigue (MacIntosh et al., 2011).

Passive force measurements

Passive force was recorded at nine muscle lengths relative to the length producing optimal active force: RL−4 mm, RL−3 mm, RL−2 mm, RL−1 mm, RL, RL+1 mm, RL+2 mm, RL+3 mm and RL+4 mm. The 4 mm shortened and stretched positions used in the present study represent the normal range of motion for the rat ankle joint, with the knee at 155 deg, +4 mm would represent a maximal terminal ankle joint angle in the rat of ∼26 deg plantar flexion and −4 mm would represent ∼150 deg of plantar flexion (Woittiez et al., 1985). Additionally, excursion of the gastrocnemius in rodents well approximates relative length changes of the human plantar flexors (Hu et al., 2017). To allow for dissipation of force transients owing to stress–relaxation, passive force was recorded 120 s following each of the length steps. The force transducer (Entran strain gauges bonded to a stainless steel lever; Entran Devices Inc., Fairfield, NJ, USA) was mounted on a translation table that could be precisely positioned by a computer-controlled stepper motor (model MD2, Arrick Robotics Systems, Hurst, TX, USA). The output of the force transducer was amplified (model PM-1000, CWE) and then analog-to-digital converted at 4000 Hz (analog-to-digital board, PCI-MIO-16E-4, National Instruments, Austin, TX, USA).

Muscle fixing

After all passive force and length measurements had been obtained, the animals were killed and the hindlimb was immediately placed in a 10% formalin (fixative) solution (VWR) at the muscle length corresponding to the RL. After a 1 h period of fixation, the medial gastrocnemius muscle was dissected from the hindlimb and firmly secured to a wooden applicator stick at RL and allowed to fix for 2 weeks in the 10% formalin solution. The muscles were then dissected into four lengthwise sections medial and lateral of the centre of each medial gastrocnemius muscle belly. After a 4 h 30% nitric acid digestion process, five individual fascicles from each muscle section were isolated and placed on slides for sarcomere length measurement at five locations along the fascicle by laser diffraction (Lieber et al., 1984), with an accuracy of 2% (Koh and Herzog, 1998). Fascicle length measurements were taken using a digital camera and Matrox imaging software. Serial sarcomere number was calculated by dividing the fascicle length by the average sarcomere length. In total, 20 fascicle length and 100 sarcomere length measurements were obtained from each muscle, roughly equivalent to 10% of available sarcomeres for measurement.

Statistical analyses

Unpaired two-tailed *t*-tests were used to assess age-related differences in muscle architecture measurements (muscle mass, fascicle length, sarcomere length and serial sarcomere number). Differences in passive force between young and old rats across muscle length was assessed using a two-way ANOVA. For all tests, α was set to $P<0.05$.

RESULTS AND DISCUSSION

Muscle architecture

Despite a similar body mass for young and old rats, the old rats had ∼34% less medial gastrocnemius muscle mass (690±40 mg) as compared with young rats (1040±80 mg; $P<0.05$), indicating significant age-related muscle atrophy. A reduction in muscle fascicle length of ∼14% was observed in old rats when compared with young rats ($P<0.05$; Fig. 1A). There was no difference in average sarcomere length at RL between groups ($P>0.05$; Fig. 1B). The medial gastrocnemius of old rats had ∼10% fewer sarcomeres in series when compared with that of young rats ($P<0.05$; Fig. 1C). Therefore, the shorter fascicle length in old muscle is driven by a reduction in serial sarcomere numbers; while sarcomere length at RL was ∼4% shorter in old rats, this difference was not statistically significant (Fig. 1D).

Passive tension

There was an interaction of age and muscle length for passive tension, whereby old rats experienced up to ∼125% greater passive tension at long muscle lengths (>RL+3 mm) as compared with young rats ($P<0.05$; Fig. 1E). There was no difference in passive tension at short muscle lengths between groups.

We found that the age-associated reduction in muscle fascicle length is primarily due to fewer sarcomeres arranged in series, without a significant difference in resting sarcomere length. Additionally, passive force was greater in muscles from old rats than in those from young rats at long muscle lengths. It was expected that the shorter fascicles and fewer serial sarcomeres in muscles from old rats would experience greater relative length changes for a given displacement, resulting in increased passive tension at long muscle lengths compared with that in young rats. However, the ∼4% shorter sarcomere lengths in muscles from old as compared with young rats may have dampened the effects of shorter fascicles in old

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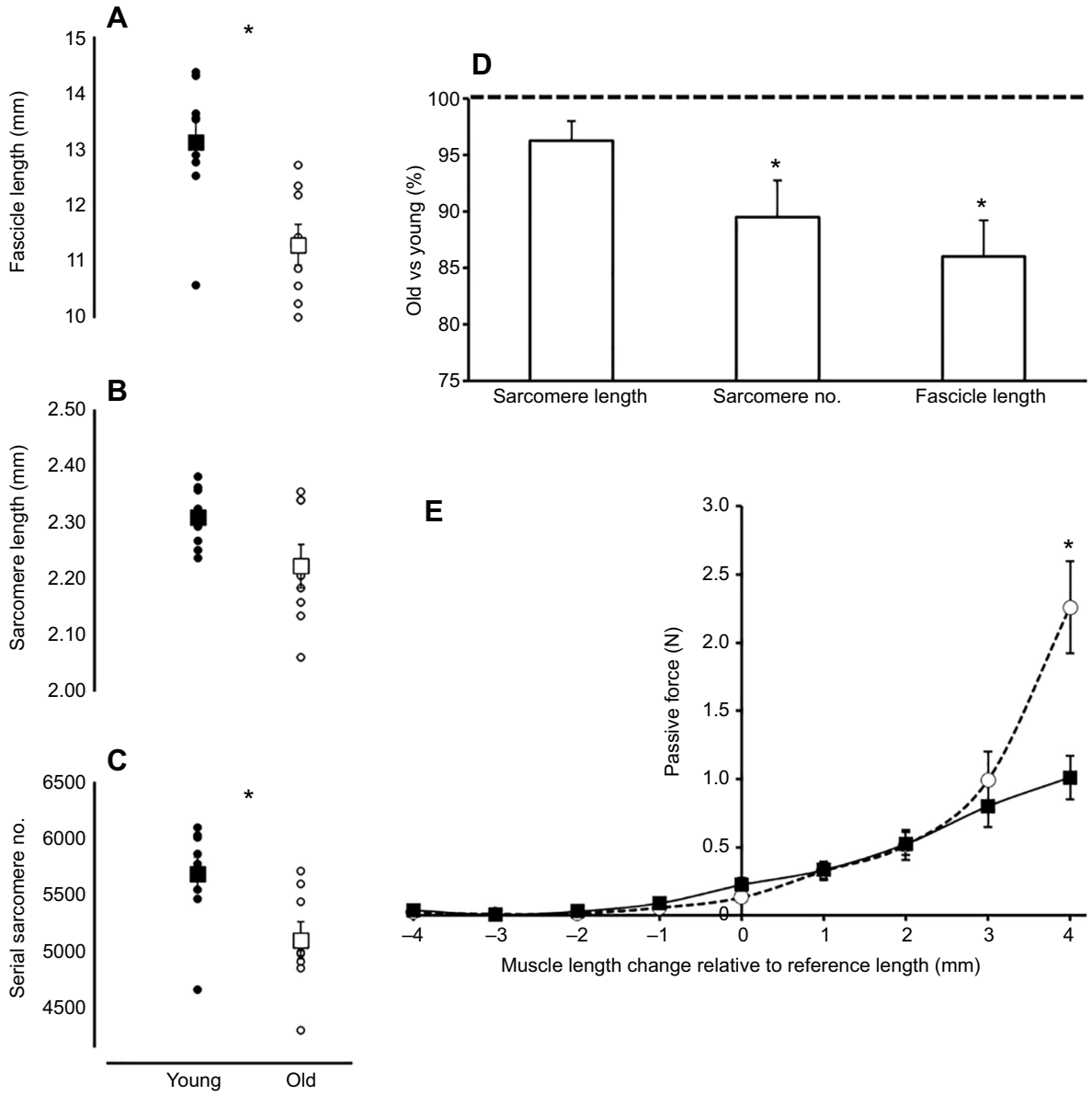

Fig. 1. Muscle architecture and passive force. (A) Fascicle length at the optimal muscle length for force production was determined using twitches. The muscle was fixed and fascicle length measured. Fascicles from old rats were shorter than those from young rats (*P<0.05). (B) Sarcomere length of the fascicles was measured using laser diffraction. There was no age-associated difference in sarcomere length (P>0.05). (C) Serial sarcomere number was determined as the quotient of fascicle and sarcomere lengths. Old rats had fewer sarcomeres in series than young rats (*P<0.05). (D) Percentage difference in sarcomere length, sarcomere number and fascicle length across age (*P<0.05). (E) Passive force, at muscle length steps beyond 3 mm, was greater in old rats (white circles) than in young rats (black squares) (*P<0.05). All data are reported as means±s.e.m.

rats, minimizing any ‘overstretching’ at long lengths. For the 4 mm absolute length change, on average, fascicles from young rats were stretched ∼30% while those from old rats were stretched ∼36%, meaning muscles from both young and old rats were stretched to an average sarcomere length of ∼3.01 µm. Therefore, in the present study, differences in sarcomere length at the long muscle length are likely not the explanation for the age-related differences in passive force. This would indicate that titin and other load-bearing sarcomeric proteins are not stretched more in old than in young rats, and the elevated passive tension is likely collagen based. The collagen matrix likely remodelled its slack length in the muscle from the old rats, resulting in this non-contractile tissue experiencing greater strain than in young rats when stretched to long muscle lengths.

Muscle fascicle length is reduced in old age owing to a loss of serial sarcomeres

The reported fascicle length at optimal muscle length in the present study is similar to that reported previously (13 mm) for the medial gastrocnemius in the rat (Woittiez et al., 1985). Additionally, the age-related reduction in fascicle length of ∼14% observed in our study was consistent with previously published findings of a 10–17% reduction in fascicle length in old humans (Narici et al., 2003; Power et al., 2013) and mice (Hooper, 1981). As sarcomeres have an optimal length for force production based on the overlap of thick and thin filaments (Burkholder and Lieber, 2001), and vertebrates exhibit very little range for this optimal sarcomere length, no age-related change in resting sarcomere length was expected. Accordingly, we did not observe a significant difference

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in resting sarcomere length between young and old rats. Therefore, the ∼14% shorter fascicle length in old age (Fig. 1A) was expected to be primarily due to a loss of sarcomeres in series. Specifically, we observed a ∼10% reduction in the number of serial sarcomeres (Fig. 1C). One possible functional role of sarcomerogenesis is to re-position the muscle, based on environmental constraints, to maintain optimal overlap of actin and myosin filaments to maximize cross-bridge formation and force output (Koh and Herzog, 1998; Williams and Goldspink, 1978). For rat skeletal muscle, assuming an actin length of 1.09 µm (Herzog et al., 1992; Ter Keurs et al., 1984), one would presume maximal force would be obtained at a sarcomere length between 2.28 and 2.45 µm, which corresponds to the sarcomere length observed in the present study (Fig. 1B). As the plateau of the force–length curve contains a range of sarcomere lengths (2.28–2.45 µm), the ∼4% sarcomere length difference between young and old rats falls within that range, and likely does not affect optimal force production.

Passive tension is elevated at long muscle lengths in old as compared with young rats

Passive tension was greater in the muscles of old versus young rats, but only at long muscle lengths. A variety of factors contribute to a muscle's passive mechanical properties, including the structure and composition of the giant protein titin (Brynnel et al., 2019), and the collagen-based extracellular matrix (Prado et al., 2005; Gillies and Lieber, 2011; Meyer and Lieber, 2018). The epimysium of muscles and aponeurosis have been reported to be stiffer in old compared with young rats (Gao et al., 2008; Holt et al., 2016) and, similarly, Alnaqeeb et al. (1984) showed that rat extensor digitorum longus and soleus passive stiffness increased with old age and was related to an increased total collagen concentration. Despite these age-related changes in connective tissue, muscle architecture is thought to play an important role in dictating mechanical properties at the whole-muscle/joint level (Narici et al., 2016; Holt et al., 2016), especially when considered in terms of absolute excursions. For example, pennation angle is reduced in aged rats as compared with young rats (Holt et al., 2016), and is also reduced in older humans (Narici et al., 2016), which likely contributes to reductions in whole-muscle stress. Everything else being equal, the shorter fascicles in older animals are stretched to a relatively greater magnitude compared with the long fascicles of young animals, which, if sarcomere length remains proportional, translates into greater average sarcomere length and apparently greater passive tension at long muscle lengths. In the present study, owing to a non-significant age-related reduction in sarcomere length, this effect was buffered.

Functional implications of fascicle length

SSN is important when considering the sarcomere force–length relationship (Gordon et al., 1966). For example, if the joint angles of young and old rats were matched, such as occurs *in vivo*, assuming a given joint angular displacement produces a similar sarcomere excursion across age, the shorter fascicles in the old rats would mean that optimal length could be at the same or smaller joint angles (i.e. shorter overall muscle length), and the sarcomeres would be stretched more, to longer lengths. Based on the sarcomere force–length relationship, this would mean that, at the sarcomere level, during maximal activation at longer muscle lengths, older adults may be operating further on the descending limb of the force–length relationship, while during sub-maximal contractions, they may be less impaired, as a result of an activation-dependent shift to longer optimal operating lengths (Rack and Westbury, 1969; Holt and Azizi., 2014, 2016; MacDougall et al., 2020). Thus, muscle from old rats would be generating less active force but more passive force, ultimately contributing to muscle weakness throughout a functional range of motion. Additionally, during stretch, aged muscle sarcomeres are being pulled to relatively longer lengths than young sarcomeres, and passive force-producing elements such as titin may play a greater role in increased passive tension in older adults (Kellermayer et al., 1997). Moreover, it has been proposed that alterations to tendon compliance with old age may have a compensatory effect, whereby the shorter fascicles and fewer sarcomeres of aged muscle are stretched less, or shorten more during a fixed-end contraction (Thom et al., 2007). This age-related increase in tendon compliance in old age would assist in maintaining the sarcomere filament overlap to be closer to the optimal force production length (Onambele et al., 2006; Narici and Maganaris, 2007; Thom et al., 2007; Stenroth et al., 2012; Svensson et al., 2016). Additionally, in the same strain of rats used in the present study, Danos et al. (2016) noted that the age-related increases in passive stiffness coupled with a reduced active force capacity in the muscle can lead to shifts in the force–length operating range, impairing mechanical performance. However, there is little consensus on whether ageing affects tendon properties. This indicates that the effect of ageing on tendon material properties may vary on the basis of preparation, species and age group being compared (Danos et al., 2016).

During growth, sarcomere length remains relatively unchanged, assuming the thick and thin filaments reach optimal overlap (Williams and Goldspink, 1971). Further increases in fascicle length are accommodated by the addition of sarcomeres in series. In the present study, fascicle length decreased in old rats by reductions in SSN, while, similar to growth, sarcomere length remained unchanged. As stated previously (Hooper, 1981), this loss of serial sarcomeres may represent a period of 'degrowth'. The question then arises – why are muscle fascicle lengths in old muscle shorter than in young? A few testable hypotheses include: (1) a systemic reduction in the animal's ankle range of motion during everyday activities, thus inhibiting sarcomerogenesis (Koh and Herzog, 1998), increasing passive muscle stiffness and reducing joint mobility; (2) a reduction in active force, minimizing tension and stretching of the fascicles during ambulation; and (3) a downregulation of mechanotransduction protein signalling, blunting environmental stimuli for growth. It is important to note that increased muscle extracellular matrix stiffness in old age impaired (re)generation of contractile tissue (Stearns-Reider et al., 2017), which may cause blunting of sarcomerogenesis. These questions are beyond the scope of this Short Communication, but may provide some insight into sarcomerogenesis and plasticity of ageing muscle.

Optimal sarcomere length in muscle from old rats

One possible explanation for the 4% shorter sarcomeres (which, although not statistically significant, influenced estimates of sarcomere length when stretched to long lengths) at optimal muscle length in old rats could be a reduced myosin concentration (D'Antona et al., 2003). Although speculative, if the old muscle has fewer myosin heads available to form cross-bridges, the sarcomere force–length relationship could be shifted to shorter lengths to possibly increase the availability of cross-bridges and thus maintain optimal overlap, at a much smaller plateau region. Additionally, the shorter sarcomere length in muscle from old rats may be related to a limitation of the study where the optimal muscle length was measured at an optimal active contraction, but the muscles were fixed in a passive state. In this same light, the passive force was taken prior to the contraction, not estimated during the active

contraction. The shorter sarcomeres in old muscle could therefore be due to factors such as the series elastic property differences in young and old muscles. Because of this limitation, it was unknown whether sarcomere length was altered as it entered the passive state or whether the difference was indeed due to an age-related alteration in the sarcomere structure.

Conclusion

The results of this study were in accordance with the hypothesis that as individuals age, sarcomeres are lost in series, resulting in shorter fascicles. Not surprisingly, average optimal sarcomere length did not differ between groups, but might therefore differ at different muscle lengths. The functional consequences of these age-related changes could be a reduced range of motion for older adults and less force production capacity throughout the range of motion encountered in activities of daily living. These results may also help to explain the increased passive tension observed for muscles of older individuals.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: G.A.P., B.R.M., W.H.; Methodology: G.A.P., S.C., J.R.F., B.R.M., W.H.; Formal analysis: G.A.P., S.C., J.R.F.; Investigation: G.A.P., S.C., J.R.F.; Resources: B.R.M., W.H.; Data curation: G.A.P.; Writing - original draft: G.A.P., S.C., J.R.F., B.R.M., W.H.; Writing - review & editing: G.A.P., S.C., J.R.F., B.R.M., W.H.; Supervision: B.R.M., W.H.; Funding acquisition: W.H.

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