Muscle strength and size are associated with motor unit connectivity in aged mice

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Abstract

In older adults, the loss of muscle strength (dynapenia) and the loss of muscle mass (sarcopenia) are important contributors to the loss of physical function. We sought to investigate dynapenia, sarcopenia, and the loss of motor unit function in aging mice. C57BL/6J mice were analyzed with cross-sectional (males: 3 vs. 27 months; males and females: 8 vs. 12 vs. 20 months) and longitudinal studies (males: 10–25 months) using in vivo electrophysiological measures of motor unit connectivity (triceps surae compound muscle action potential and motor unit number estimation), in vivo measures of plantar flexion torque, magnetic resonance imaging of hind limb muscle volume, and grip strength. Compound muscle action potential amplitude, motor unit number estimation, and plantar flexion torque were decreased at 20 months. In contrast, grip strength was reduced at 24 months. Motor unit number estimates correlated with muscle torque and hind limb muscle volume. Our results demonstrate that the loss of motor unit connectivity is an early finding in aging male and female mice and that muscle size and contractility are both associated with motor unit number.

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1. Introduction

Forty-two percent of the 37.3 million adults aged greater than 65 years report having 1 or more physical limitations of performing daily tasks that are essential for maintaining independence in the community (Seeman et al., 2010). Preserving physical function has become a major public health priority as it would drastically reduce health-care costs and improve quality of life for many older Americans (Hoffman et al., 1996). The etiologies of age-related reductions in physical function are not completely understood. Both the loss of muscle mass (sarcopenia) and strength (dynapenia) are important contributors to impaired physical function in older adults, as both are associated with mobility limitations, frailty, obesity, osteoporosis, and a high risk for falls and future mortality (Clark and Manini, 2010; Denison et al., 2015). All these factors combined lead to a low quality of life for elders, with no direct cure for the loss of muscle function. Current therapies for elderly adults with loss of muscle strength and function (sarcopenia) rely heavily on exercise focused on increasing muscle mass, yet these methods are indirect and unable to fully treat this muscular decline (Denison et al., 2015; Francis et al., 2017; Sayer et al., 2013). To tackle this problem, there must be a more holistic examination beyond just the muscular level changes in patients, taking into account the neurological contributions as well. Age-related changes in motor units (a motor neuron and the myofibers it innervate) have been suggested to contribute to both sarcopenia and dynapenia (Hepple and Rice, 2016; Kaya et al., 2013), and in recent years, there has been a significant push to understand the aging motor unit.

More than 50 years ago, it was reported that aged-rodents exhibited deteriorated neuromuscular junction morphology (Gutmann and Hanzlikova, 1966), and today, there is similar but mixed evidence in humans (Jones et al., 2017; Oda, 1984; Wokke et al., 1990). There are also data from prior studies suggesting that aging results in motor neuron death causing motor unit loss along with cycles of denervation-reinnervation (Tomlinson and Irving, 1977) (Lexell and Downham, 1991). Interestingly, there is growing speculation that degeneration of the peripheral nervous system, including the losses of motor neurons, axons, and synapses may be important factors contributing to the loss of physical function with advancing age (Hepple and Rice, 2016; Kwon and Yoon, 2017; Lexell, 1997; Mosole et al., 2014). However, it is widely recognized that the evidence base for these assertions are limited, as they are based on studies that are cross-sectional in design (Hepple and Rice, 2016). As such, the interrelationship between the loss of motor unit connectivity and sarcopenia and dynapenia across the life span of an organism is not well understood, and longitudinal studies are clearly needed. Animal experiments in aging have the advantage of allowing longitudinal studies across the life span of the organism.

In the series of mouse experiments described herein (Table 1), we used a noninvasive motor unit number estimation (MUNE) technique to obtain indices of the number of motor neurons that are functionally connected with a given muscle group (Arnold et al., 2015; McComas et

al., 1971). Mouse age demographics were operationally defined according to prior comparisons between human and mouse ages: young adult (2–8 months), middle age (10–15 months), old (18–24), and very old (27+ months) (Dutta and Sengupta, 2016). In experiment 1, as a pilot, proof-of-concept study, we sought to confirm that in our model (C57BL/6J mice), very old mice (27-month old) exhibited reduced triceps surae MUNE (i.e., less numbers of functional motor units) as well as reduced grip strength when compared with young adult mice (3 months old). In experiment 2, we obtained serial measures of triceps surae MUNE and grip strength from middle to old age, along with magnetic resonance imaging (MRI)–derived measures of triceps surae muscle size timed at 19 and 25 months of age. Finally, in experiment 3, we obtained measures of triceps surae MUNE, grip strength, and electrically stimulated muscle contractile properties from 3 groups of male and female mice of varying ages (young adult vs. middle aged vs. old). Collectively, these experiments were designed to determine the interrelationship between motor unit loss and muscle size and function. We hypothesized in experiment 1 that motor unit losses would be evident in aged mice based on prior findings in human studies. We next hypothesized in experiment 2 that reduced motor unit numbers would be an early finding in aging mice in relation to when muscle atrophy had previously been reported. Finally in experiment 3, we hypothesized that losses of muscle size, contractility, and function would be associated with greater reductions in MUNE.

2. Materials and methods

2.1. Animal studies

This protocol was approved by and adhered to the animal care and ethics guidelines of The Ohio State University Wexner Medical Center. All studies were approved by the Animal Institutional Care and Use Committee of The Ohio State University. C57BL/6J mice were used for all studies. Animals for experiments 1 and 2 were obtained from Taconic Biosciences, NY, USA, whereas the animals for experiment 3 were obtained from the National Institute on Aging mouse colony.

Experiment 1 (young vs. very old): for this proof-of-concept, pilot experiment, we obtained data from a cohort of very young adult male mice (3 months, n = 10) and a cohort of very old adult male mice (27 months, n = 5). Outcome measures, obtained by an evaluator blinded to mouse age, included electrophysiology variables (described in Section 2.4), body mass, and grip strength.

Experiment 2 (longitudinal measures): for this experiment longitudinal data were obtained across mid age to old age from 10 male mice. Electrophysiological variables were obtained starting at 10 months of age, and repeat assessments continued at 13, 15, 17, 20, 22, and 24 months of age. The starting point of 10 months of age was chosen on the basis of prior findings that hind limb muscle size of C57BL/6J mice is maintained through midlife and is in fact larger in 15-month-old mice as compared with 3-month-old young adult mice (Shavlakadze et al., 2010), and hind limb muscles appear to show preserved mass until closer to 24 months (Hamrick et al., 2006; Shavlakadze et al., 2010). Grip strength and body mass assessments were obtained at 15 months of age, and repeat assessments continued at 17, 18, 20, 22, and 24 months of age. MRI-derived measures of muscle volume were

obtained at age 19 and 25 months in 6 mice to assess muscle atrophy and to assess the association between muscle volume and motor unit connectivity. MRI measures were obtained at these ages based on prior work that showed that muscle atrophy appears to occur in C57BL/6J mice between 18–24 months (Hamrick et al., 2006).

Experiment 3 (young vs. midlife vs. old): for this experiment, we obtained data from a cohort of young adult mice (8 months, n = 5 males and n = 5 females), a cohort of middle-age mice (12 months, n = 5 males and n = 5 females), and a cohort of old mice (20 months, n = 5 males and n = 5 females). Outcome measures included electrophysiology variables, grip strength, in vivo muscle contractile properties (described in the following), and body mass.

2.2. Anesthesia and animal preparation

For electrophysiological MRI muscle volume assessment and muscle contractility procedures, mice were anesthetized using inhaled isoflurane adjusted for adequate sedation (induction 3%– 5% and maintenance 1%–3%), taking care to avoid oversedation. During the electrophysiological procedures, body temperature was maintained using a low noise, thermostatically controlled warming plate (World Precision Instruments, Sarasota, FL, USA) set to maintain temperature at 37 °C. During the muscle contractility procedures, a warm water bath HTP-1500 heat therapy pump (Androit Medical Systems, Loudon, TN) was set to maintain temperature at 37 °C. During MRI, physiological parameters including respiration and temperature were monitored throughout the imaging procedure using small animal monitoring and gating system (SA Instrument Inc, NY, USA). During all procedures performed under anesthesia, veterinarian petroleum-based ointment was applied to the eyes to prevent dryness and corneal irritation.

2.3. Muscle strength testing

All limb and forelimb grip strength were assessed using a standard grip device (DEFII-002, Chatillon, Largo, FL, USA) with a range of 0–1000 g with an accuracy of at least 0.25% of the full scale. During grip testing, mice were grasped by the proximal tail and placed on the grip device and then pulled until the grip was lost. Three trials were performed for the all limb grip strength measure and the forelimb grip strength measure. The maximum value (in grams) recorded for the respective tests was considered the muscle grip strength and was used in all analyses.

2.4. In vivo electrophysiology

Compound muscle action potential (CMAP) and single motor unit potential (SMUP) were recorded from the sciatic nerve-innervated, triceps surae muscles of the right hind limb as previously described. (Arnold et al., 2015, 2016). Two insulated 28-gauge monopolar needle electrodes (Teca, Oxford Instruments Medical, NY, USA) were inserted subcutaneously at the proximal hind limb in the region of the sciatic nerve for stimulation. Two fine wire ring electrodes (Alpine Biomed, Skovlunde, Denmark) were used for recording, with the active electrode placed over the knee joint and the reference electrode placed over the metatarsal region of the foot. One disposable strip ground electrode (Carefusion, Middleton, WI, USA) was placed on the tail (Arnold et al., 2016). CMAP responses were recorded following

supramaximal stimulation (constant current intensity of <10 mA with a pulse duration of 0.1 ms) of the sciatic nerve in the proximal thigh. Baseline-to-peak and peak-to-peak CMAP amplitudes were recorded. Baseline-to-peak CMAP amplitude was used for comparison between groups, and peak-to-peak CMAP amplitude was used for calculation of MUNE. The average SMUP amplitude was determined using the incremental stimulation technique (Arnold et al., 2015; McComas et al., 1971). Briefly, submaximal stimulations were delivered until a stable, minimal all-or-none response was obtained. Ten increments were recorded following the criteria of the incremental stimulation technique, and these increments were averaged to obtain the average SMUP amplitude. MUNE was calculated using the following equation: MUNE = CMAP amplitude (peak-to-peak)/average SMUP (peak-to-peak).

2.5. MRI-derived muscle volume

MRI procedures were performed on a 94/30 BioSpec MRI system (Bruker BioSpin, Germany) with the ParaVision 5.1 software. Images were acquired using 35 mm Quadrature coil. Gd-based contrast agent was administrated before image acquisition by intraperitoneal injection (100 mL/20 g of 0.1M Magnevist). Three-dimensional T1 weighted images were acquired using fast low angle. Fast low angle shot (FLASH) sequence with following parameters: repetition time = 7.4 ms, echo time = 2.3 ms, FA = 20°, NA = 3 and isotropic resolution of 100 microns in each direction.

Images were analyzed using ImageJ software (NIH, Bethesda). All images were scaled by factor 0.5 in each direction, and then the region of interest, the distal hind limb muscles, has been manually traced on each projection (Supplemental Fig. 1). The regions with hyperintense and hypointense signal corresponding to subcutaneous fat and bone were excluded for a volume calculation. The region of interest areas for each projection were summated and multiplied by the depth of the projections to calculate hind limb muscle volume.

2.6. Muscle contractility

In vivo plantar flexion torque assessment was performed using an in vivo contractility apparatus (Model 1300A, Aurora Scientific Inc, Canada) similar to methods previously described by Iyer et al. with modifications for noninvasive limb fixation (Iyer et al., 2016). The data output from the contractility apparatus was interfaced to a desktop computer using a PXIe-8135 quadcore processor-based embedded controller and a 2-channel acquisition board PXI-4461 from National Instruments (Austin, TX, USA). The right hind paw was taped to the force plate and positioned so that the foot and tibia were aligned at 90°. As per Iyer et al's prior description of plantar flexion torque assessment in vivo, the knee joint was secured using a 27-gauge needle (Iyer et al., 2016). In contrast, for the plantar flexion torque experiments described herein, the knee joint was securely clamped at the femoral condyles while avoiding compression of the fibular nerve at the fibular head. Two insulated monopolar electrodes (F-E2M-48, Grass Technologies, Warwick, RI, USA) were inserted subcutaneously over the tibial nerve, just posterior/posterior-medial to the knee. Peak twitch torque (millinewtons) was measured following single 200 ms square wave stimuli delivered at a frequency of 0.5 Hz to determine maximal stimulus intensity. A supramaximal stimulus

(120% of stimulus intensity required for maximal twitch torque) was delivered to record the maximal twitch torque. After obtaining the peak twitch torque, maximum plantar flexion tetanic torque was recorded following a train of supramaximal 200 ms square wave stimuli delivered at 125 Hz.

2.7. Statistics

Statistical analyses were performed using GraphPad Prism (GraphPad Software, Inc La Jolla, CA, USA). All electrophysiology, muscle torque, and grip strength data are reported as median values with upper and lower 95% confidence intervals (CIs). Because of tendency for nerve conduction parameters to follow a non-Gaussian distribution, nonparametric tests were used for statistical comparisons (Robinson et al., 1991). For comparisons of 2 groups (experiment #1), the Mann-Whitney rank-sum test was used. For comparison of multiple groups (experiments #2 and 3), the Kruskal-Wallis and Dunn's multiple comparison tests were used. Pearson correlation coefficients were calculated for correlation analyses between motor unit connectivity, muscle area, and muscle torque. Results with p values less than 0.05 were considered significant.

3. Results

3.1. Experiment 1: cross-sectional comparison of young versus very old male mice

A total of 5 male mice at 27 months (very old) and 10 male mice at 3 months (very young) were studied. Very old mice demonstrated significant reductions in all limb grip strength normalized to body mass (7.4 grams/grams body mass, 95% confidence interval [CI]: 6.9–8.9, male, n = 5) when compared with young mice (13.3 grams per grams body mass, 95% CI: 10.4–15.2, male, n = 10; $p < 0.001$). Similarly, very old mice demonstrated reduced forelimb grip strength normalized to body mass (3.3 grams per grams body mass, 95% CI: 2.9–4.2) when compared with young mice (5.8 grams per grams of body mass, 95% CI 5.3–6.3; $p < 0.001$). As hypothesized, the estimated numbers of functional motor units were reduced in very old mice compared with young adult mice (Fig. 1C). The average SMUP was increased in the very old male mice (Fig. 1B), but this was insufficient to maintain CMAP amplitudes (Fig. 1A) in the very old male mice.

3.2. Experiment 2: longitudinal analysis of middle-aged male mice through old age

A total of 10 male mice were studied from 10 months through 25 months. Absolute forelimb grip strength decreased significantly as mice aged from 15 to 24 months (male, n = 10), whereas forelimb grip normalized to body weight, absolute all limb, and normalized all limb strength were unchanged (Fig. 2). MUNE was reduced at 20 and 24 months when compared with baseline at 10 months (Fig. 3). This reduction in MUNE was accompanied by a decrease in CMAP amplitude beginning at 20 months through 24 months (compared with baseline at 10 months). The average SMUP size was unchanged (Fig. 3).

Distal hind limb MRI muscle volumes (absolute and normalized to body mass) were significantly reduced at 25 months compared with 19 months ($n = 6$, $p = 0.03$; Fig. 4A and B). At 25 months, normalized distal hind limb MRI muscle volumes were significantly

correlated with MUNE (Fig. 4C), but absolute distal hind limb MRI muscle volumes at 25 months did not show correlation with MUNE ($r = -0.494$, $p = 0.32$, $n = 6$; data not shown).

3.3. Experiment 3: cross-sectional comparison of young versus middle-aged versus old mice

We studied 3 groups of mice ($n = 5$ males and $n = 5$ females in each group) at young (8 months), middle (12 months), and old (20 months) ages to investigate the relationship of strength, muscle contractility, and electrophysiological assessment of motor unit number and connectivity. In this experiment, similar to the longitudinal group, all limb grip and forelimb grip demonstrated no significant decrease in old mice (Fig. 5). The electrophysiological assessment of the triceps surae muscle demonstrated reduced MUNE and increased average SMUP in 20-month old mice (Fig. 6). Similarly, plantar flexion twitch and tetanic torque were reduced in 20-month old mice (Fig. 7). Furthermore, there was a significant correlation between triceps surae motor unit number and both twitch and tetanic plantar flexion torque (Fig. 8).

To explore the effect of sex on strength, muscle contractility, and electrophysiological assessment of motor unit number and connectivity during aging, we performed a secondary, separate statistical comparison between the 3 cohorts of male mice (n = 5 per group, shown in Table 2) and between the 3 cohorts of female mice (n = 5 per group, shown in Table 3). When analyzed separately, both the male and female mice show reductions at 20 months compared with 8 months in absolute and normalized muscle twitch and tetanic isometric contraction torque and in MUNE. In addition, the female mice also show significant changes in absolute and normalized all limb grip strength, normalized forelimb grip strength, and SMUP across 8, 12, and 20 months, but only normalize forelimb grip strength is significantly changed (reduced) at 20 months compared with 8 months on multiple comparison testing.

4. Discussion

In this study, we explored the loss of motor unit connectivity, muscle size, grip strength, and muscle contractility in aging mice. Our results demonstrate that electrophysiological loss of motor unit number in hind limb muscles was a relatively early finding in mice during aging. We demonstrated that the loss of motor unit number occurred before overt losses of muscle function as assessed by grip strength. Furthermore, we showed that losses in both muscle contractility and size were correlated with reductions in motor unit number in aged mice. Together our findings, particularly those showing early losses of motor unit connectivity in our longitudinal studies, provide strong evidence that motor unit connectivity is an important factor in dynapenia and sarcopenia.

There are conflicting findings in the literature regarding morphological motor neuron and ventral root axon loss in aging mice (Chai et al., 2011; Chung et al., 2017; Valdez et al., 2010). In contrast, denervation at the neuromuscular junction (NMJ) has been a consistently described phenotype in older mice (Chai et al., 2011; Chung et al., 2017; Fahim, 1993, 1997; Hepple and Rice, 2016; Li, et al., 2011; Valdez et al., 2010). A recent study investigated ventral root axon counts, neuromuscular junction innervation, and single fiber

electromyography recordings for neuromuscular transmission in aging mice (Chung et al., 2017). In this work, Chung et al. suggested that denervation at the NMJ is related to a dying back axonal degeneration due to finding similar ventral root counts in very young (2–4 months) and old (22–25 months) C57BL/6J mice despite significant denervation at the NMJ in the older mice (Chung et al., 2017). It is important to keep in mind that reduction in MUNE values does not necessarily suggest anatomical loss of motor neurons but only reflect the loss of motor neuron connectivity with the particular muscle being tested. Thus, our electrophysiological studies could be compatible with motor neuron dysfunction (without frank death and loss of motor neuron soma) and associated motor unit loss occurring as a dying back phenomenon. However, it is important to note that prior work has also suggested that disruption at the NMJ may be explained by primary degeneration in muscle fibers as supported by findings of muscle fiber degeneration before the loss of NMJ innervation in neck muscles of aging mice (Li et al., 2011). In addition, a recent study showed that the loss of satellite cells may be an important driver of NMJ degeneration in aging (Liu et al., 2017). Yet, a primary process of NMJ degeneration would not be expected to result in reduction of MUNE.

Our findings are consistent with prior nerve conduction study data that demonstrated relatively early reduction in CMAP amplitudes in aging rodents while recording from hind limb but not forelimb muscles (McNeil et al., 2005; Pannérec et al., 2016; Piasecki et al., 2016). When interpreting CMAP amplitude changes, it is important to note that CMAP amplitude loss can be related to the loss of muscle, nerve, NMJ, or MN function. Therefore reduction of CMAP amplitude during aging is a relatively nonspecific finding. We recently showed that triceps surae CMAP amplitudes and in situ gastrocnemius muscle force were reduced in 25-month-old mice compared with 3-month-old C57BL/6J male mice, and these findings were not associated with reduced cross-sectional muscle fiber area in the 25-month-old mice (Arnold et al., 2017). The findings of our present study support the notion that CMAP amplitude loss in aging is due, at least in part, to the loss of motor unit connectivity rather than solely muscle or primary NMJ loss.

A limitation of our study includes the fact that morphologic correlates of motor neuron counts, peripheral motor axonal counts, or neuromuscular junction innervation status were not performed. A previous study investigated triceps surae muscle mass and showed decline between 18 and 24 months, which is aligned with the onset of CMAP and MUNE decline in our longitudinal studies (Hamrick et al., 2006). An another recent study demonstrated similar declines in soleus and gastrocnemius mass by 24 months, but interestingly, the quadriceps muscle, which was not examined in our experiments, showed declines between 4 and 15 months (White et al., 2016b). In addition, our findings are well aligned with those recently reported by Krishnan et al. regarding peripheral axonal counts in aging C57BL/6J mice (Krishnan et al., 2016). In this report, the authors demonstrated significant defects between the ages of 18 and 22 months, which are remarkably well aligned with CMAP, MUNE, and SMUP changes in our study (Krishnan et al., 2016). Interestingly, subsequent studies demonstrated that axonal defects were not corrected with resistance wheel running beginning in midlife (15 months) despite the fact that muscle mass was maintained (Krishnan et al., 2017; White et al., 2016a). Similarly, a prior study demonstrated significant loss of NMJ innervation in older mice that was corrected with both calorie restriction and

exercise, but in these mice, motor neuron losses during aging were not corrected (Valdez et al., 2010). Future studies are needed to understand the relationships between muscle size, motor unit connectivity, and morphological changes in aging and to investigate the effect of exercise or other preclinical age-related therapeutics on electrophysiological measures such as CMAP and MUNE. Furthermore, our studies only included 2 time points for MRI correlation with MUNE, and future longitudinal MRI assessments across the life span could help better delineate the associations of when motor unit connectivity and muscle size are reduced in aging.

There are important implications from our findings. First, the findings of early motor unit number losses in aging mice suggest that the functional status of the motor unit pool supplying a muscle could be an important driver of losses in physical function during the aging process. As such, development of therapeutic targets aimed at either increasing or preserving the number of motor units or improving the output of the remaining motor units may be critical to maintain and improve physical function during aging. Sensitive and relevant biomarkers are needed to track aging phenotypes and to quantify potential treatment responses over time (Cesari et al., 2012). The association between muscle strength and size with motor unit number in our study suggests that MUNE may be a potential prognostic or diagnostic biomarker to be further explored. As such, prior work has supported serum measurements of C-terminal agrin fragment, a marker of NMJ degeneration, as a putative biomarker of sarcopenia (Drey et al., 2013; Landi et al., 2016). Exercise has been shown to be an effective treatment in at least some individuals affected by sarcopenia (Denison et al., 2015; Fisher et al., 2014). Prior cross-sectional studies suggested that lifelong physical activity may preserve motor units in aging individuals (Power et al., 2010, 2012). Therefore, electrophysiological measures of motor unit connectivity may also hold potential as measures to understand treatment response (i.e., efficacy or pharmacodynamics biomarker) in sarcopenic individuals. Motor unit connectivity may be a tractable response that could be used to facilitate early-stage clinical trials.

In experiment 3, we included both male and female mice. Interestingly, we noted significant differences in average SMUP amplitude in the cohorts with combined male and female mice, but in our longitudinal studies that included only males (experiment 2), we did not see significant changes in average SMUP amplitude. When the male and female cohorts were analyzed separately, SMUP was significantly changed in the females but not the male mice (Tables 2 and 3). These findings raise the possibility that motor neurons in female mice are better able to form collateral sprouts during aging or that there is increased demand on individual motor units to form collateral sprouts in females. Interestingly, prior work in rats has suggested increased collateral sprouting in female compared with male rats (Kova i et al., 2009). This would appear to be consistent with the changes in SMUP in our cross-section cohort (experiment 3), which included female mice which were not seen in our longitudinal, male only cohort (experiment 2). Yet, another clinical study also suggested that the loss of motor units during aging may be more prominent in females (Gawel and Kostera-Pruszczyk, 2014). Prior work has demonstrated that sarcopenia is more evident in aged male C57BL/6J mice (White et al., 2016a). Therefore, future work should include investigating sex-dependent differences in parameters of motor unit connectivity.

5. Conclusion

In this series of experiments, we examined the interrelationship between motor unit loss and muscle size and function. We hypothesized that motor unit losses would be an early finding in aging mice and that losses of muscle size, contractility, and function would be associated with greater reductions in MUNE. Our results demonstrate that the loss of motor unit connectivity is a relatively early finding in aging mice, and motor unit estimates are associated with both muscle function and muscle size. These results suggest that the loss of motor neuron connectivity is an important factor in both sarcopenia and dynapenia. Furthermore, these findings have implications for the identification of future biomarkers and development of therapeutics that target the nervous system and motor unit to maximize impact in individuals with sarcopenia and dynapenia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.neurobiolaging.2018.03.016.
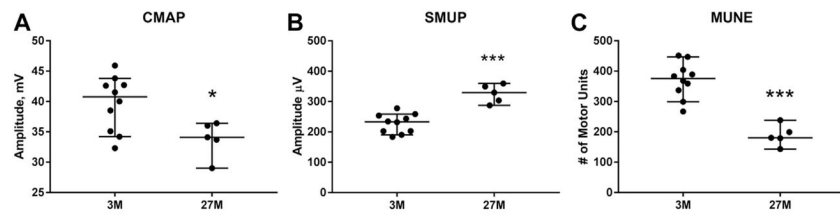
Fig. 1.
Electrophysiology in young adult (3M) versus very old adult (27M) mice. Very old mice (*n* = 5) demonstrated significant reduction in (A) compound muscle action potential (CMAP) amplitude, (B) increased average single motor unit potential (SMUP) size, and (C) reduced motor unit number estimation (MUNE) as compared with young adult mice (*n* = 10). (*p < 0.05, ***p < 0.001). Abbreviation: M, months.
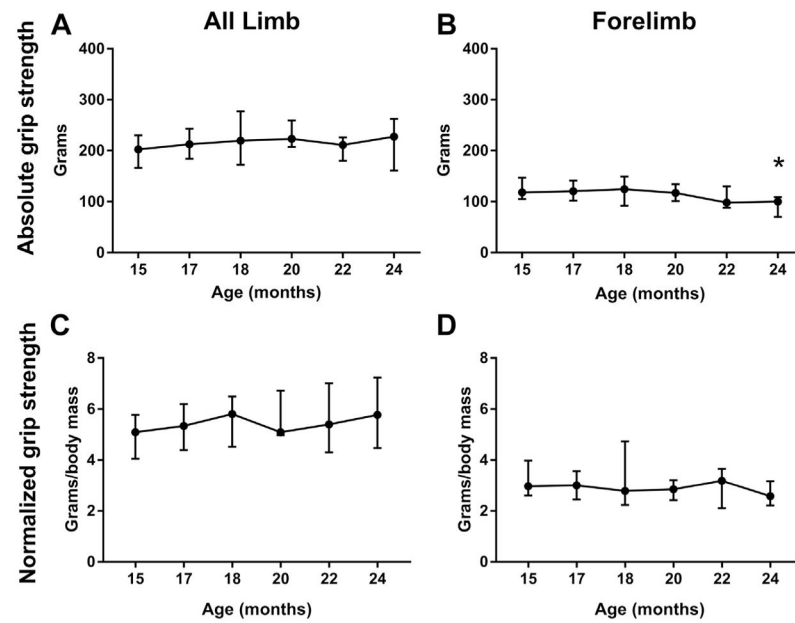
Fig. 2.
Longitudinal grip strength in aging male mice. (A) Absolute measures of all limb grip strength demonstrated no significant reduction between 15 and 24 months; however, (B) forelimb grip demonstrated a reduction at 24 months. (C, D) When normalized to body mass, all limb and forelimb strength demonstrated no significant differences. ($^{*}p < 0.05$).
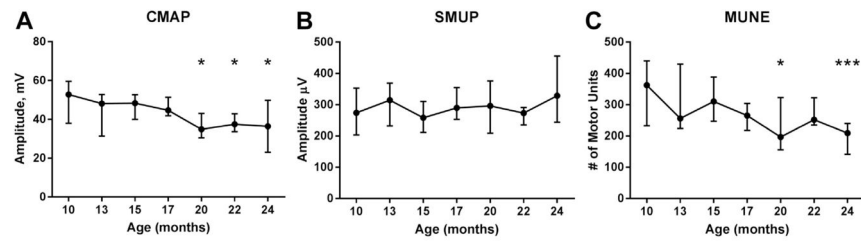
Fig. 3.
Longitudinal alterations of electrophysiology in middle-aged male mice through old age. Compared with the baseline (10 months), (A) the sciatic CMAP amplitude was reduced at 20, 22, and 24 months, (B) SMUP was unchanged, and (C) MUNE was reduced at 20 and 24 months. ($*p < 0.05$, $***p < 0.001$, $n = 10$). Abbreviations: CMAP, compound muscle action potential; MUNE, motor unit number estimation; SMUP, average single motor unit potential.
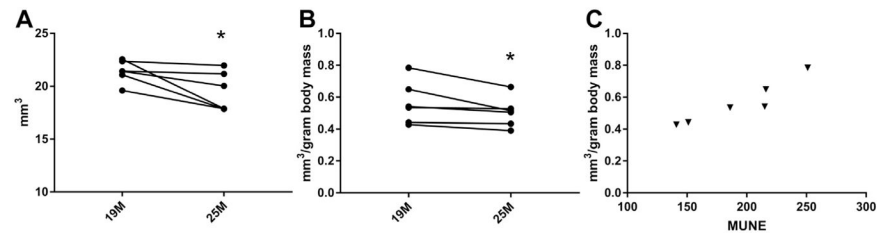
Fig. 4.

Distal hind limb muscle MRI volume is reduced in aging mice and is correlated with motor unit connectivity. (A) Absolute distal hind limb muscle volume is reduced at 25 months (18.95, 95% CI: 17.51–21.4 mm^3) compared with 19 months (21.42, 95% CI: 20.29–20.52 mm^3, $p = 0.03$). (B) Similarly, distal hind limb muscle volume normalized to body mass is reduced at 25 months (0.51, 95% CI: 0.41–0.60 mm^3/gram body mass) compared with 19 months (0.54, 95% CI: 0.42–0.70, mm^3/gram body mass, $p = 0.03$). (C) Normalized distal hind limb volume at 25 months was significantly correlated with motor unit number estimation (r = 0.900, $p = 0.01$, n = 6). (*$p < 0.05$). Abbreviations: M, months; MRI, magnetic resonance imaging.
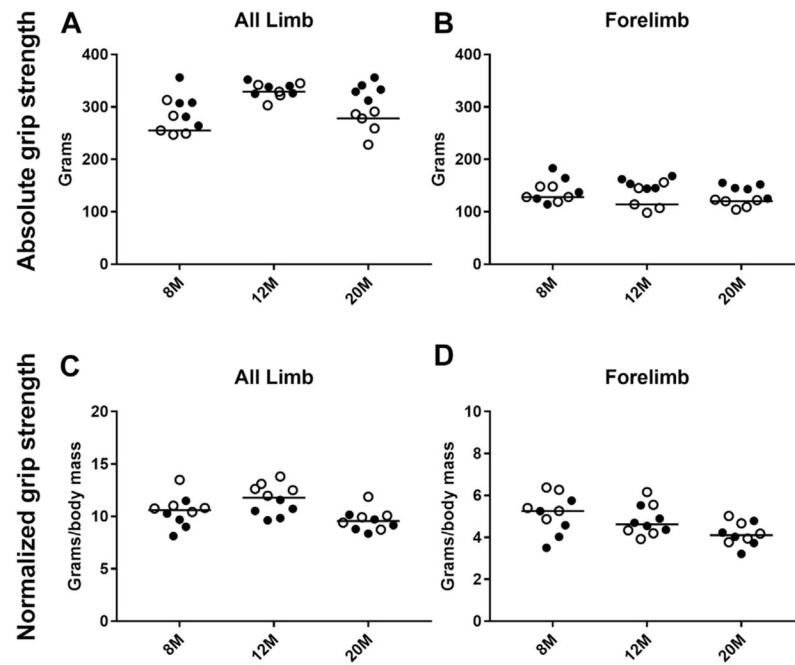
Fig. 5.
Grip strength in young (8 months), middle-aged (12 months), and old (20 months) adult mice. No significant differences were noted for (A) absolute all limb grip, (B) absolute forelimb grip, (C) normalized all limb grip, or (D) normalized forelimb grip. (n = 5 males, shown as solid circles and n = 5 females, shown as empty circles, in each group). Abbreviation: M, months.
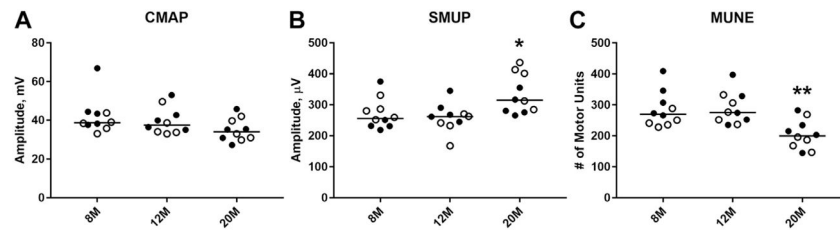
Fig. 6.
Triceps surae electrophysiology in young (8 months), middle-aged (12 months), and old (20 months) adult mice. (A) There were no significant differences in compound muscle action potential (CMAP). (B) Average single motor unit potential (SMUP) amplitude was increased in old mice compared with young mice while middle-aged mice were not significantly different. (C) Motor unit number estimation (MUNE) was decreased in old mice compared with young mice while middle-aged mice were not significantly different. ($^{*}p < 0.05$, $^{**}p < 0.01$). ($n = 5$ males, shown as solid circles and n = 5 females, shown as empty circles, in each group). Abbreviation: M, months.
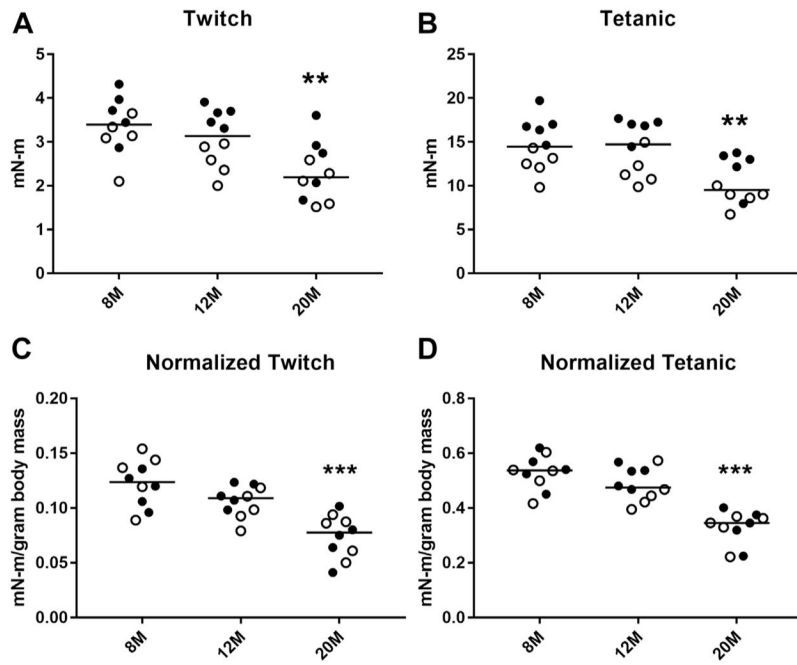
Fig. 7.
Plantar flexion muscle torque in young (8 months), middle-aged (12 months), and old (20 months) adult mice. (A, B) Absolute plantar flexion twitch and tetanic torque are reduced in old compared with young mice, but middle-aged mice are unchanged. (C, D) Similarly, plantar flexion twitch and tetanic torque normalized to body mass are reduced in old compared with young mice, but middle-aged mice are unchanged. ($^{**}p < 0.01$, $^{***}p < 0.001$) ($n = 5$ males, shown as solid circles and $n = 5$ females, shown as empty circles, in each group). Abbreviation: M, months.
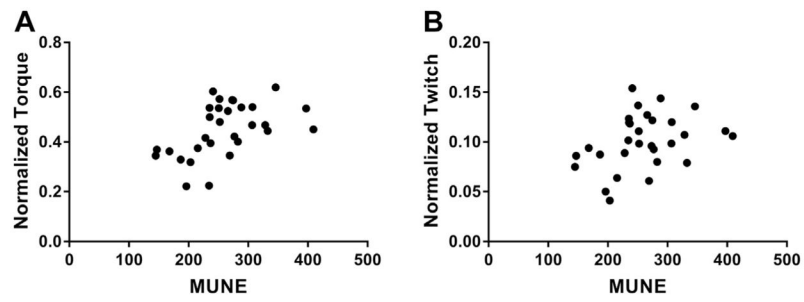
Fig. 8.
Correlations between motor unit connectivity and plantar flexion torque. Triceps surae motor unit number estimation (MUNE) demonstrates significant correlation with (A) tetanic torque normalized to body mass (Pearson: $r = 0.505$, $p < 0.01$) but not (B) plantar flexion twitch torque normalized to body mass (Pearson: $r = 0.348$, $p = 0.06$).

Table 1

Summary of the experiments conducted in the study

Experiment	Age groups	Gender and number	Measures	Hypotheses
1	Young (3 mo) versus very old (27 mo)	Male Young: n = 10 Old: n = 5	Grip strength, in vivo electrophysiology of the triceps surae	MUNE, similar to findings in humans, is reduced in very old mice compared with young mice.
2	Longitudinal study from midlife (10 mo) through old age (25 mo)	Male (n = 10)	Grip strength, in vivo electrophysiology of the triceps surae, MRI for muscle volume	Loss of MUNE is an early change in aging mice compared with muscle atrophy and loss of grip strength. Loss of muscle mass is associated with loss of MUNE.
3	Three groups, at young (8 mo), midlife (12 mo), and early old (20 mo) ages	Males: n = 5 in each group Females: n = 5 in each group	Grip strength, in vivo electrophysiology, and in vivo muscle contractility/torque	MUNE is reduced in male and female mice. Reduced MUNE is associated with reduction in muscle contractility.

Key: CMAP, compound muscle action potential; MUNE, motor unit number estimation; SMUP, average single motor unit potential.

Table 2

Male-only comparisons at 8, 12, and 20 months of age (5 mice at each age)

Assessment	8 Mo	12 Mo	20 Mo	*p*-value
Absolute all limb grip (grams)	307 (260, 347)	338 (322, 350)	333 (315, 354)	0.1676
Absolute forelimb grip (grams)	137 (109, 180)	153 (141, 167)	145 (130, 159)	0.5361
Normalized all limb grip (grams/grams body mass)	9.7 (8.1, 11.3)	10.5 (9.5, 11.4)	9.1 (8.3, 10.1)	0.1587
Normalized forelimb grip (grams/grams body mass)	4.6 (3.5, 5.8)	4.7 (0.42, 5.4)	4.0 (3.3, 4.7)	0.1708
Absolute twitch (mN-m)	3.7 (3.0, 4.3)	3.7 (3.3, 3.9)	2.7 (1.7, 3.5)*	0.0396
Absolute tetanic (mN-M)	16.8 (14.6, 19.2)	17.0 (15.1, 18.2)	13.0 (9.1, 15.0)*	0.0021
Normalized twitch (mN-m/grams body mass)	0.12 (0.10, 0.14)	0.11 (0.10, 0.13)	0.08 (0.04, 0.10)*	0.0105
Normalized torque (mN-m/grams body mass)	0.54 (0.46, 0.62)	0.53 (0.47, 0.57)	0.33 (0.25, 0.42)**	0.0018
CMAP (mV)	43.4 (31.2, 61.0)	39.9 (32.6, 50.3)	35.2 (26.2, 43.6)	0.1373
SMUP (µV)	232 (182, 342)	268 (234, 330)	281 (253, 345)	0.1837
MUNE	307 (247, 394)	275 (215, 379)	215 (154, 278)*	0.0333

Adjusted *p*-values for comparison of results at 12 mo and 20–8 mo (Dunn's multiple comparison test) shown as asterisks (*$p < 0.05$, and **$p < 0.01$). Data were shown as median values with upper and lower 95% confidence intervals.

Table 3

Female-only comparisons at 8, 12, and 20 months of age (5 mice at each age)

Assessment	8 Mo	12 Mo	20 Mo	*p*-value
Absolute all limb grip (grams)	255 (234, 305)	329 (307, 349)*	278 (237, 300)	0.0055
Absolute forelimb grip (grams)	128 (118, 151)	114 (93, 155)	120 (105, 126)	0.2201
Normalized all limb grip (grams/grams body mass)	10.8 (9.8, 12.8)	12.6 (12.0, 13.7)	9.9 (8.5, 11.5)	0.0047
Normalized forelimb grip (grams/grams body mass)	5.4 (4.8, 6.5)	4.3 (3.6, 6.0)	4.2 (3.7, 5.0)*	0.0458
Absolute twitch (mN-m)	3.1 (2.3, 3.8)	2.6 (2.1, 3.1)	2.1 (1.5, 2.6)*	0.0344
Absolute tetanic (mN-M)	12.5 (10.3, 14.4)	11.3 (9.4, 14.3)	9.0 (7.2, 10.2)*	0.0092
Normalized twitch (mN-m/grams body mass)	0.14 (0.10, 0.16)	0.10 (0.08, 0.12)	0.09 (0.05, 0.10)**	0.0095
Normalized torque (mN-m/grams body mass)	0.54 (0.43, 0.60)	0.44 (O.38, 0.55)	0.35 (0.25, 0.40)**	0.0009
CMAP (mV)	38.4 (33.1, 43.0)	34.1 (29.2, 46.4)	33 (28.4, 41.9)	0.5649
SMUP (μV)	281 (243, 321)	241 (185, 286)	401 (287, 453)	0.0033
MUNE	241 (219, 278)	277 (232, 330)	187 (136, 251)	0.0236

Adjusted p-values for comparison of results at 12 mo and 20–8 mo (Dunn's multiple comparison test) are shown as asterisks (*$p < 0.05$, and **$p < 0.01$). Data were shown as median values with upper and lower 95% confidence intervals.