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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.

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

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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

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.