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Short report

Mouse forepaw lumbrical muscles are resistant to age-related declines in force production

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ABSTRACT

A progressive loss of skeletal muscle mass and force generating capacity occurs with aging. Mice are commonly used in the study of aging-associated changes in muscle size and strength, with most models of aging demonstrating 15–35% reductions in muscle mass, cross-sectional area (CSA), maximum isometric force production (P_o) and specific force (sP_o), which is P_o/CSA. The lumbrical muscle of the mouse forepaw is exceptionally small, with corresponding short diffusion distances that make it ideal for in vitro pharmacological studies and measurements of contractile properties. However, the aging-associated changes in lumbrical function have not previously been reported. To address this, we tested the hypothesis that compared to adult (12 month old) mice, the forepaw lumbrical muscles of old (30 month old) mice exhibit aging-related declines in size and force production similar to those observed in larger limb muscles. We found that the forepaw lumbricals were composed exclusively of fibers with type II myosin heavy chain isoforms, and that the muscles accumulated connective tissue with aging. There were no differences in the number of fibers per whole-muscle cross-section or in muscle fiber CSA. The whole muscle CSA in old mice was increased by 17%, but the total CSA of all muscle fibers in a whole-muscle cross-section was not different. No difference in P_o was observed, and while sP_o normalized to total muscle CSA was decreased in old mice by 22%, normalizing P_o by the total muscle fiber CSA resulted in no difference in sP_o. Combined, these results indicate that forepaw lumbrical muscles from 30 month old mice are largely protected from the aging-associated declines in size and force production that are typically observed in larger limb muscles.

1. Introduction

Skeletal muscle size and strength decline with age. In humans, the number and size of fibers within muscles remain relatively stable from puberty until the fifth decade in life, at which point a marked decline in fiber size and abundance begins (Lexell et al., 1988). Between the ages of 50 and 80 years, there is approximately a 50% reduction in the size and number of fibers in the quadriceps muscle group (Lexell et al., 1988), and the resulting decrease in muscle size and strength can impair the ability of individuals to perform activities of daily living (Gumucio and Mendias, 2013). Mice are a useful model for the study of aging-related changes in muscle size and strength, as their muscles undergo relative changes similar to those of humans throughout their lifespan (Brooks and Faulkner, 1988; Liu et al., 2013). In mice, there is a reduction in force production as animals advance from adulthood (9–10 months of age) to old age (22–24 months of age), with a sharp additional decrease in muscle size and force production between old mice and oldest-old mice (>26 months of age) (Brooks and Faulkner, 1988; Graber et al., 2013). Mice also undergo an aging-associated increase in connective tissue accumulation which can contribute to diminished whole muscle force production (Ramaswamy et al., 2011).

Several large limb muscles from mice, such as the extensor digitorum longus (EDL), tibialis anterior, soleus and gastrocnemius, have been used to study aging-associated changes in muscle function (Gumucio and Mendias, 2013). The lumbrical is a long, spindle-shaped muscle that flexes the metacarpophalangeal joint in the forepaw, and is up to two orders of magnitude smaller than these other limb muscles. The small size and the relatively high surface area to volume ratio make the lumbrical an ideal muscle to study direct changes to fibers during contractions, as well as studies that require rapid diffusion of compounds between the muscle and its environment (Bergantin et al., 2011; Claflin and Brooks, 2008; Ng et al., 2008; Sloboda and Brooks, 2013; Smith et al., 2013). To our knowledge, aging-associated changes in lumbrical muscle contractility have not previously been reported.

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Our objective was to characterize the structure and function of the forepaw lumbrical muscles in adult (12 month old) and oldest-old (30 month old) mice. We hypothesized that the lumbrical muscles of old mice exhibit aging-related declines in size and force production similar to those observed in larger limb muscles.

2. Materials and methods

2.1. Animals and contractility measurements

Experiments were approved by the University of Michigan IACUC. Male C57Bl/6 mice aged 12 months (adult, N = 7) or 30 months (old, N = 7) were obtained from the National Institute on Aging Aged Rodent Colony. Mice were anesthetized with isoflurane, the forepaws were surgically removed and animals were humanely euthanized by cervical dislocation. Lumbrical muscles were isolated from the 4th digit, trimmed, placed in Tyrode's solution, and contractility was measured based on previously described techniques (Claflin and Brooks, 2008; Ng et al., 2008; Sloboda and Brooks, 2013). The small size of the lumbrical muscle permitted visualization of sarcomere-based striations using standard bright field microscopy, which allowed real-time monitoring of sarcomere length using a video analysis system (900B, Aurora Scientific, Aurora, ON). Fiber lengths were inferred from a series of sarcomere length measurements as follows. The muscle length was first adjusted to be "just taut" using the servomotor micrometer drive, and the micrometer setting and sarcomere length were noted. The muscle was then lengthened by 100 μm using the micrometer and the resulting sarcomere length was noted. This procedure was repeated three additional times, resulting in a total of five micrometer drive settings and corresponding sarcomere lengths. Sarcomere length (ordinate) was then plotted as a function of micrometer setting (abscissa) and a least-squares line was fitted. The inverse of the slope of the fitted line was taken as the number of sarcomeres in series in the fibers. Fiber length (L_f) was defined as the number of series sarcomeres multiplied by 2.5 μm/sarcomere. Muscle length (L_o) was measured as the distance between the origins of the proximal-most fibers to the insertions of the distal-most fibers after setting sarcomere length to 2.5 μm. Initial muscle length was maintained at L_o for all subsequent measurements.

Muscles were stimulated (701C, Aurora Scientific) through two platinum plate electrodes located on either side of the muscle. To determine maximum isometric force (P_o), the muscle was stimulated for 300 ms using 0.2 ms current pulses delivered at a rate of 125 Hz. Cross-sectional area (CSA) measurements were determined by histology, and specific force (sP_o) was calculated by dividing P_o by CSA. Susceptibility to contraction-induced injury was then assessed by subjecting the muscle to a protocol of 10 lengthening contractions. For each lengthening contraction, a stretch of 0.40 L_f was applied at a velocity of 1.5 L_f/s. The stretch was initiated after P_o had been attained, 300 ms after the onset of stimulation. The stimulation was continued until the lengthening of the muscle by the servomotor was complete. Lengthening contractions were separated by 1 min periods of rest. To determine force deficits, force was measured during the isometric portion of each contraction, immediately preceding each stretch. A final isometric contraction was performed 1 min after the last lengthening contraction to obtain the final force deficit. Muscle mass was estimated by first multiplying L_f by CSA to approximate muscle volume, and then multiplying volume by the density of skeletal muscle, 1.06 g/cm^3.

2.2. Histology

Histology was performed as described (Mendias et al., 2012). After testing, muscles were snap frozen in Tissue-Tek (Sakura, Torrance, CA), sectioned through the mid-belly at a thickness of 10 μm, and incubated with primary antibodies against type II myosin heavy chain (My32, ThermoFisher, Waltham, MA) and type I collagen (biotinylated, AbCam, Cambridge, MA). AlexaFluor conjugated secondary antibodies and streptavidin (Life Technologies, Grand Island, NY) were used to detect primary antibodies. Sections were mounted and imaged using a Zeiss Axiovert 200 M microscope (Carl Zeiss, Thornwood, NY). ImageJ software (NIH, Bethesda, MD) was used to perform quantitative measurements.

2.3. Immunoblot

Immunoblots were performed as described (Mendias et al., 2012). Muscles were homogenized in LSB (Bio-Rad, Hercules, CA) and then placed in boiling water for 5 min. Protein concentration was determined using an RC DC Assay (Bio-Rad). A total of 0.1 μg of protein was loaded into 4%/7.5% polyacrylamide gels. Following electrophoresis, proteins were transferred to nitrocellulose membranes, blocked with casein and incubated with primary antibodies against type II myosin heavy chain (My32) or type I myosin heavy chain (A4.840, Developmental Studies Hybridoma Bank, Iowa City, IA), and HRPO conjugated secondary antibodies (ThermoFisher). Membranes were developed with SuperSignal West Dura (ThermoFisher) in a chemiluminescent cabinet (Alpha Innotech, San Leandro, CA).

2.4. Statistics

Data are presented as mean ± SD. Differences between adult and old groups were tested using Student's t-tests ($\alpha = 0.05$) using Prism 6.0 software (GraphPad Software, San Diego, CA). For lengthening contractions, Holm–Sidak corrections were used to account for multiple observations.

3. Results and discussion

Rapid declines in muscle mass and strength are frequently observed during the transition from adulthood to old age (Gumucio and Mendias, 2013). The loss of muscle fibers is largely considered to be the primary contributor to aging-associated skeletal muscle atrophy, but a decrease in muscle fiber CSA is also often observed in the remaining fibers (Dupont-Versteegden, 2005; Lexell et al., 1988). The reduction in whole muscle P_o is thought to occur because of a decrease in muscle fiber CSA, a loss of muscle fibers and fast-to-slow fiber type switching. These changes as well as an accumulation of connective tissue are thought to be chiefly responsible for the aging-related reduction in sP_o (Ramaswamy et al., 2011; Wood et al., 2014). Rader and Faulkner observed a 35% reduction in P_o, and a 20% reduction in fiber number, CSA and sP_o in the gastrocnemius muscles of old mice compared to adults (Rader and Faulkner, 2006). Brooks and Faulkner reported an approximately 15% decrease in CSA, a 25% decrease in P_o and a 20% decrease in sP_o for the EDL and soleus muscles in old mice compared to adults (Brooks and Faulkner, 1988), and similar findings were observed by Graber and colleagues (Graber et al., 2013). When EDL muscles are exposed to injury-inducing lengthening contractions, the acute magnitude in force reduction is similar for adult and old animals, although the secondary injury response and long-term deficits in force production are much greater in old mice (Lockhart and Brooks, 2006).

Based on these and numerous other findings reported in the literature, we expected similar declines in the contractility of old lumbrical muscles. Unexpectedly, lumbrical muscles were largely protected from the aging-associated changes in structure and function observed in other muscles. No differences in body mass were observed between adult and old mice, but lumbrical muscles from old mice had a 6% increase in L_o, a 14% increase in L_f and a 21% increase in mass (Table 1). No differences in twitch parameters were observed (Table 1). The myosin heavy chain composition of both adult and old lumbricals was entirely type II, and there was a grossly apparent accumulation of connective tissue in muscles from old mice (Fig. 1A–B). No differences in the number of fibers per whole-muscle cross section or muscle fiber CSA were observed (Fig. 1C–D), although the fiber CSA values are