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Effects of aging on the lateral transmission of force in rat skeletal muscle

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ABSTRACT

The age-related reduction in muscle force cannot be fully explained by the loss of muscle fiber mass or degeneration of myofibers. Our previous study showed that changes in lateral transmission of force could affect the total force transmitted to the tendon. The extracellular matrix (ECM) of skeletal muscle plays an important role in lateral transmission of force. The objective of this study was to define the effects of aging on lateral transmission of force in skeletal muscles, and explore possible underlying mechanisms. *In vitro* contractile tests were performed on extensor digitorum longus (EDL) muscle of young and old rats with series of tenotomy and myotomy. We concluded that lateral transmission of force was impaired in the old rats, and this deficit could be partly due to increased thickness of the ECM induced by aging.

1. Introduction

By the year 2030, 20% of the population of the United States will be over 65 years old (U.S. Census Bureau, 2008). Disabilities associated with muscle weakness, known as sarcopenia, are common, and significantly influence the quality of their daily life (Morley et al., 2001; Roubenoff, 2001; Janssen et al., 2002). The age-related reduction in muscle force has been commonly attributed to the loss of muscle mass. However, muscle force is lost to a greater extent than the loss of muscle mass, suggesting that other factors are involved (Nair, 2005).

The loss of muscle force in aged muscles is not simply caused by degeneration of myofibers. Changes in ATPase activity, metabolite levels, or myosin isoforms have been proposed to be the possible mechanisms; however, none of them can fully explain the loss of force in aged muscles (Phillips et al., 1993; Lowe et al., 2001; 2002). Previous studies showed that although the specific force (force per area) of the whole muscles decreases with aging (~13–20%), there is no significant deficit in specific force of single skinned muscle fibers between the young and old groups (Eddinger et al., 1986; Brooks and Faulkner, 1988, 1994; Phillips et al., 1991). The deficit in specific force of whole muscles cannot be explained by unimpaired intrinsic force-generating capacity of cross bridges in aged muscles, suggesting that the force transmission from muscle fibers to the tendon in aged muscles could be impaired, leading to the loss of skeletal muscle strength.

Two pathways are involved in transmitting force from muscle fibers to tendon: (1) longitudinal transmission, i.e., transmission along the muscle fibers via the myotendinous junctions (MTJ) to the tendon; and (2) lateral transmission, i.e., transmission laterally across one muscle fiber to the adjacent connective tissue network, the extracellular matrix (ECM), and finally to the tendon (Huijing, 1999). The myotendinous junction has been thought to be the main site of force transmission. However, muscle fibers frequently terminate within the fascicles without reaching the MTJ for muscles across species (Gaunt and Gans, 1992; Trotter, 1993; Trotter and Purslow, 1992; Huijing, 1999). This anatomic structure suggests that the force generated in these muscle fibers has to be transmitted laterally via the ECM, and then to the tendon. Therefore, the force transmitted to the end of muscle could be significantly affected by the ECM (Zhang and Gao, 2012; Gao et al., 2009). As the stiffness and the thickness of ECM in skeletal muscles increase with aging (Nishimura et al., 1997; Kjaer, 2004; Gao et al., 2008), we believe that lateral transmission of force could be affected due to these changes. However, previous studies by Ramaswamy et al. (2011) found that significant difference on lateral transmission only exists between the young (3 months) and very old rats (36–38 months), but not between the young and old (30–33 months). The conflicting findings motivated us to re-investigate the effects of aging on lateral transmission.

The objective of this study was to determine the effects of aging on the lateral transmission of force in skeletal muscle, and explore the potential underlying mechanisms. We hypothesized that lateral transmission pathway is impaired in aged skeletal muscle, and the impaired lateral transmission could be partly due to increased thickness of the ECM. To test our hypothesis, *in vitro* isometric contractile tests were performed on the extensor digitorum longus (EDL) muscle of young and

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old rats with series of tenotomy and myotomy between adjacent heads. Proportions of forces transmitted laterally and longitudinally were then calculated and compared between young and old rats.

2. Methods

Two groups of male Brown Norway rats were used in our experiments: young (3–4 months old, $n=6$) from Harlan Laboratories (Indianapolis, IN), and old (32 months old, $n=5$) from National Institutes of Aging (Baltimore, MD). All procedures used in this study were approved by Cornell University's Institutional Animal Care and Use Committee (IACUC).

2.1. Experimental procedures

After anesthesia, the left *extensor digitorum longus* (EDL) muscle of each rat was isolated. The EDL is a multi-tendon muscle with distal insertions on digits II–V of the foot, a well-established model for characterizing the force transmission through ECM between the four muscle heads (Huijing et al., 1998; Maas et al., 2003). Silk suture was tied to the distal tendon of the muscle as proximally as possible without damaging the muscle. The suture stayed intact throughout the experiments.

After dissected free from the body, the EDL was fixed to an *in vitro* contractile testing system (1205A, Aurora Scientific, Toronto, ON) with the proximal end fixed by a clamp, and the suture on the distal end attached to force transducer (resolution 1.0 mN). The EDL was placed in mammalian ringer's solution, and stimulated by a 100 Hz, 30 V stimulation signal for maximum force generation for 600 ms (Brooks and Faulkner, 1988). Three minutes rest was applied between each contraction. The optimal length was then determined, and maximum isometric tetanic force generated was measured and recorded as F_0.

The distal end of the EDL was then detached from the force transducer. The tendon of head II was cut (tenotomy) without touching the suture on the tendon, and the distal end was reattached to force transducer. The same electrical simulation was then applied to the whole muscle at the optimal length. The force measured was recorded as F_2', which is the force measured after longitudinal transmission pathway is cut off. After measuring force generated with tenotomy to head II, the distal end was detached again, and the ECM between heads II and III was cut to separate heads II and III. Force generated by the EDL with this process was measured as F_2, which is the force measured after both longitudinal and lateral transmissions were cut off for head II. We assume the difference between F_0 and F_2, i.e., F_0-F_2, was the force generated by head II; the difference between F_0 and F_2', i.e., F_0-F_2' was the force generated by head II and transmitted through tendon II; and the difference between F_2' and F_2, i.e., $F_2'-F_2$, was the force generated by head II and transmitted laterally through the ECM between head II and head III. Repeated tenotomy and myotomy were then applied to heads III, IV and the interface between corresponding heads to measure F_3', F_3, F_4', and F_4.

A schematic illustration of the procedure is shown in Fig. 1. For each force measurement, three contractile tests were taken and an average value was calculated. To reduce the geometric variances between EDLs, we normalized each measurement to the maximum force generated by the whole EDL muscle, F_0, i.e., $\alpha_i=F_i/F_0$, $\alpha_i'=F_i'/F_0$ (i=2, 3, and 4). The contribution of each head to the total force of the whole muscle was calculated as $F_{i-1}-F_i/F_0$ (i=2, 3, and 4, $F_1=F_0$). The force generated in each head $F_{i-1}-F_i$ (i=2, 3, and 4, $F_1=F_0$) was divided into two parts, the force transmitted laterally ($F_i'-F_i$) and the force transmitted longitudinally ($F_{i-1}-F_i'$), and two respective ratios $\beta_i=F_i'-F_i/F_{i-1}-F_i$ and $\gamma_i=F_{i-1}-F_i'/F_{i-1}-F_i$ (i=2, 3, and 4, $F_1=F_0$) were calculated.

The right EDL of each rat was dissected from rats and fixed in 10% neutral buffered formalin solution. The tissue was then fixed and cut into 3 μm sections for hematoxylin and eosin (H&E) staining. ImageJ (National Institutes of Health, Bethesda, MD) was used to measure the thickness of the perimysium.

2.2. Statistics analysis

The variance equality and the normality of measurements were checked, and student's *t*-test between two groups of small samples was conducted to compare forces transmitted through the ECM between young and old groups. Differences in proportion of force transmitted laterally between young and old groups were assessed using analysis of variance (ANOVA). Difference was considered statistically significant at $p<0.05$.

3. Results

The average maximum isometric force, represented by F_0, generated in young group is 2.04 ± 0.22 N, and that of the aged

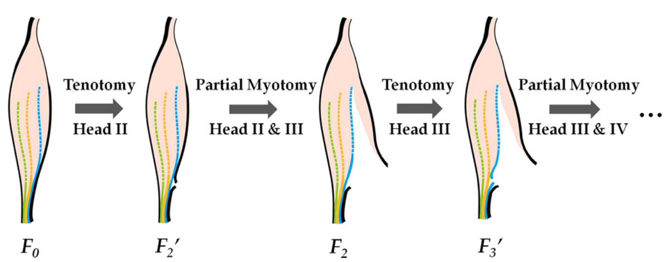

Fig. 1. A schematic representation of contractile tests with sequential tenotomy and myotomy between different heads in EDL. First of all, maximum isometric tetanic force of the intact muscle, F_0, was measured at the optimal length. F_2' is the force measured after tenotomy of tendon of head II, and F_2 is the force measured after a partial myotomy, cutting of the ECM, between heads II and head III. Same procedure was then repeated for heads III and IV.

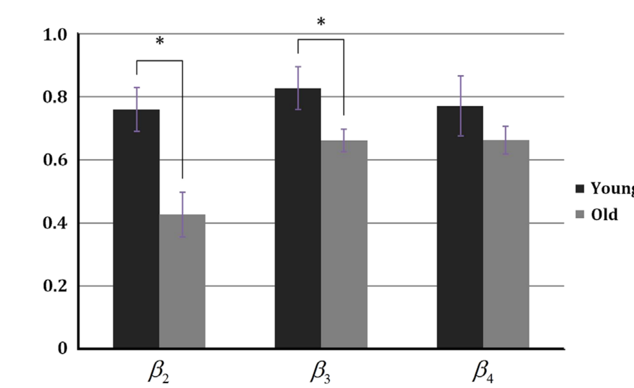

Fig. 3. Comparison in the proportion of force transmitted laterally in each head ($\beta_i=(F_i'-F_i)/(F_{i-1}-F_i)(i$=2, 3, and 4, $F=F_0$)) between young and old groups.

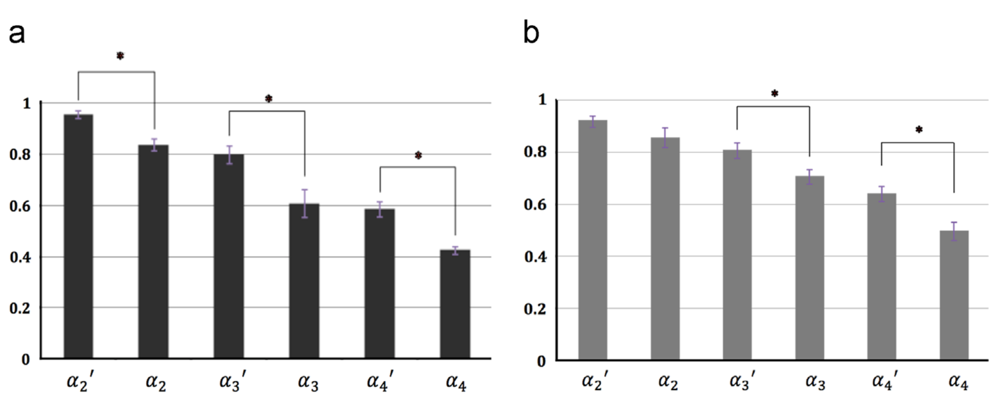

Fig. 2. (a) Forces transmitted during series of tenotomy and partial myotomy of the young group. (b) Forces transmitted during series of tenotomy and partial myotomy of the old group. ($\alpha_i=F_i/F_0$, $\alpha_i'=F_i'/F_0$ (i=2, 3, and 4)).