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RESEARCH ARTICLE

Stuck in gear: age-related loss of variable gearing in skeletal muscle

Natalie C. Holt^{1,*}, Nicole Danos¹, Thomas J. Roberts² and Emanuel Azizi¹

ABSTRACT

Skeletal muscles power a broad diversity of animal movements, despite only being able to produce high forces over a limited range of velocities. Pennate muscles use a range of gear ratios, the ratio of muscle shortening velocity to fiber shortening velocity, to partially circumvent these force–velocity constraints. Muscles operate with a high gear ratio at low forces; fibers rotate to greater angles of pennation, enhancing velocity but compromising force. At higher forces, muscles operate with a lower gear ratio; fibers rotate little so limiting muscle shortening velocity, but helping to preserve force. This ability to shift gears is thought to be due to the interplay of contractile force and connective tissue constraints. In order to test this hypothesis, gear ratios were determined in the medial gastrocnemius muscles of both healthy young rats, and old rats where the interaction between contractile and connective tissue properties was assumed to be disrupted. Muscle fiber and aponeurosis stiffness increased with age ($P < 0.05$) from 19.1 ± 5.0 kPa and 188.5 ± 24.2 MPa, respectively, in young rats to 39.1 ± 4.2 kPa and 328.0 ± 48.3 MPa in old rats, indicating a mechanical change in the interaction between contractile and connective tissues. Gear ratio decreased with increasing force in young ($P < 0.001$) but not old ($P = 0.72$) muscles, indicating that variable gearing is lost in old muscle. These findings support the hypothesis that variable gearing results from the interaction between contractile and connective tissues and suggest novel explanations for the decline in muscle performance with age.

KEY WORDS: Muscle shape change, Gear ratio, Aging, Connective tissue, Fibrosis, AGR, Pennate, Bulging

INTRODUCTION

Skeletal muscle performance is determined, to a large extent, by the cross-bridge interactions that occur between the contractile proteins actin and myosin. This process of cyclical formation and detachment of cross-bridges means that the force a muscle cell (fiber) can produce declines with increasing shortening velocity (Hill, 1938; Caiozzo, 2002). Hence, there are constraints to the force muscle fibers can produce, and the velocity at which they shorten. Despite these apparent constraints, skeletal muscles exhibit the broad functional range required to produce the diversity of animal movement observed in nature. This broad functional range of the musculoskeletal system is often attributed to features at higher levels of organization, such as changing moment arms (Carrier et al., 1998), the presence of elastic tendons (Roberts and Azizi, 2011),

and the architectural arrangement of fibers within muscles (Gans, 1982; Otten, 1988; Brainerd and Azizi, 2005; Azizi et al., 2008; Wakeling et al., 2011; Randhawa et al., 2013; Azizi and Roberts, 2014).

In pennate muscles, dynamic changes in architecture during contraction can allow muscles to partially circumvent force–velocity constraints to fiber performance. During a contraction, fibers can rotate and pennation angle can increase. As a result, the shortening velocity of the muscle is not necessarily equal to that of the fibers. This relationship between muscle and fiber velocity is characterized using the gear ratio (muscle velocity/fiber velocity). High gear ratios, where fibers undergo significant rotation, enable muscle shortening velocity to exceed fiber shortening velocity. However, this rotation results in high pennation angles, decreasing the component of fiber force acting in the line of action of the muscle and therefore compromising muscle force production. Low gear ratios, where fibers undergo less rotation, limit muscle shortening velocity to that of the fibers. However, less rotation results in lower pennation angles and so, to some extent, preserves force production. In order to enhance shortening velocity, and so circumvent force–velocity constraints, without compromising force production, muscles vary gear ratio over their functional range. High gear ratios enhance velocity at low forces, whilst low gear ratios preserve force at high forces (Azizi et al., 2008).

Variable gearing significantly influences muscle performance, yet the underlying mechanism is unknown. The observation that changes in gear ratio occur without changes to the neural command has led to the hypothesis that variable gearing is due to the interaction between contractile and connective tissues (Azizi et al., 2008). It has been suggested that this interaction results in the force–dependent variation in muscle shape change during contraction thought to be responsible for variable gearing (Azizi et al., 2008). As muscles have been shown to be isovolumetric (Baskin and Paolini, 1967), active muscle shortening must be accompanied by an increase in width, thickness or both (Fig. 1). Resistance to increases in muscle thickness is thought to be due to the force generated by contractile tissue (the fibers), whereas resistance to increases in muscle width is thought to be due to connective tissue constraints to muscle bulging (Fig. 1). Hence, it has been hypothesized that when the force produced by contractile tissue is low, muscle thickness increases, fibers rotate, pennation angle increases (Fig. 1) and the muscle operates with a high gear ratio. As the force produced by fibers increases, contractile tissues provide greater resistance to increasing thickness, and shortening must be accommodated by greater increases in width. Increasing width rather than thickness allows fibers to shorten with less rotation (Fig. 1), and so allows muscles to operate with a lower gear ratio. This proposed mechanism suggests that the interplay of fiber forces and connective tissue resistance to shape change underlies variable gearing in skeletal muscle (Azizi et al., 2008).

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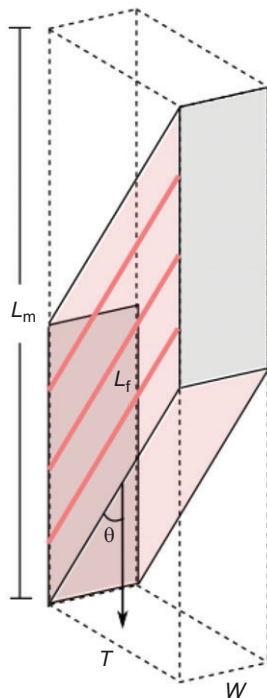


Fig. 1. Schematic representation of a pennate muscle. A unipennate muscle is depicted with fibers (red) running at an angle (pennation angle, θ) to the line of action of the muscle (indicated by the arrow). Muscle fibers are attached to two aponeuroses (gray). Fiber length (L_f), and muscle length (L_m), width (W) and thickness (T) are indicated. The force produced by the fibers acts to pull the aponeuroses together and so resists increases in thickness. The stiffness of connective tissues wrapping fibers and the muscle resists increases in width. Increases in thickness during muscle and fiber shortening require fibers to rotate and pennation angle to increase, thereby resulting in high gear ratios. Increases in muscle width can allow fibers to shorten without rotating or increasing pennation angle, thereby resulting in low gear ratios.

Here, we used the age-related changes in connective tissue properties to test the hypothesis that the interaction between contractile and connective tissues is responsible for variable gearing. The increase in connective tissue stiffness that occurs with age will disrupt the interaction of contractile and connective tissues. Hence, if the hypothesis that variable gearing is due to the interaction of contractile and connective tissues is correct, the ability to shift gears will be compromised in old muscles.

MATERIALS AND METHODS

Experiments were conducted on the medial gastrocnemius muscles of young ($N=12$, age 5–9 months) and old ($N=13$, age 33–34 months) male Fischer 344-Brown Norway rats (F344BN; National Institute on Aging, Bethesda, MD, USA). This work was carried out at UC Irvine under Institutional Animal Care and Use committee protocol no. 2012-3043. Animals were housed and handled in accordance with the US Public Health Service Policy for the humane care and use of laboratory animals and all protocols were approved by the UC Irvine Institutional Animal Care and Use Committee. Contractile properties and gear ratios were determined *in situ*, and connective tissue properties were determined *in vitro* post-mortem. This allowed us to determine whether connective tissue properties change with age, so disrupting the relationship between contractile and connective tissues, and to assess the effects of this disruption on variable gearing.

Contractile tissue properties and gear ratios

Rats were anesthetized and maintained at 1.5–2.5% isoflurane anesthesia using closed system anesthesia (Parkland Scientific, Coral Springs, FL, USA); they were placed, prone, on a heat pad. The sciatic nerve was exposed, a nerve cuff was placed around the nerve, and the nerve was cut proximally. A pocket of skin was formed around the nerve site, filled with warm mineral oil and sutured closed. An incision was made in the skin overlying the medial gastrocnemius muscle and Achilles tendon. Sonomicrometry transducers (1 mm diameter; Sonometrics Inc., London, ON, Canada) were implanted along a fascicle of the medial gastrocnemius. This allowed measurement of muscle fiber length and velocity. All tendons except that connecting to the medial gastrocnemius were severed and the calcaneus cut. The tendon was clamped as close to the end of the muscle as possible and the clamp connected to the lever arm of a servomotor (310 B-LR, Aurora Scientific Inc., ON, Canada). This allowed measurement of muscle force, length and velocity. The femur was exposed and clamped into a stereotaxic frame. The muscle was kept moist and temperature was maintained at 37°C.

Contractions were elicited by applying supra-maximal square-wave pulses (duration 0.2 ms, frequency 100 Hz) to the sciatic nerve (Holt and Azizi, 2014). A twitch force–length curve was produced and all subsequent contractions were performed at a starting length corresponding to the right-hand edge of the plateau (Holt and Azizi, 2014). An isometric tetanus was performed to determine peak isometric force (F_0) (Holt and Azizi, 2014). After-loaded isotonic tetanic contractions (Askew and Marsh, 1997; Holt and Askew, 2012) (Fig. 2) were performed at a range of forces (0.1 to 0.9 F_0) to determine the relationships between force and muscle velocity, force and fiber velocity, and force and gear ratio (muscle velocity/fiber velocity) (Azizi et al., 2008). Isometric contractions were performed at regular intervals to assess muscle performance; F_0 never fell below 90% of its original value. Following contractions, the rat was killed using an overdose of sodium pentobarbital. The muscle was fixed *in situ* at the length at which contractions were

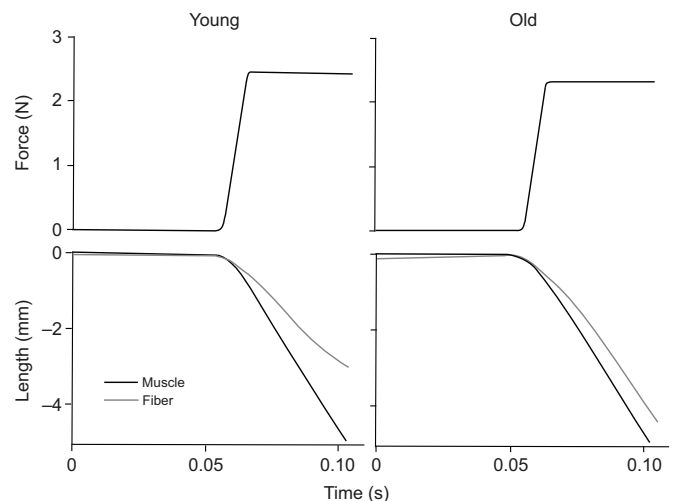


Fig. 2. Time-series traces of muscle force, muscle length and fiber length in a young and an old rat. Representative force and muscle and fiber lengths are shown during an isotonic contraction from a young and an old individual at a relative force of 0.1 F_0 , where F_0 is peak isometric force. Muscles develop force until they reach a pre-determined level at which the servomotor regulates force and the muscle shortens. The ratio of muscle to fiber shortening velocity characterizes the gear ratio. In this example, we observe a lower gear ratio in the old muscle compared with the young muscle.

performed, and later used to measure muscle length, fiber length and pennation angle. The contralateral medial gastrocnemius muscle was dissected out, weighed and fiber bundles and aponeuroses extracted to be used for determination of connective tissue properties.

Peak isometric stress was calculated from F_0 , physiological cross-sectional area and pennation angle (Mendez and Keys, 1960; Sacks and Roy, 1982). Relative force (F/F_0), and muscle and fiber velocities were determined during isotonic contractions (Fig. 2). Gear ratio was calculated as the ratio of muscle velocity to fiber velocity during a period of constant force production as the muscle shortened over the plateau of the force–length relationship. We assume, based on previous work, that higher gear ratios reflect greater increases in pennation angle during contractions (Azizi et al., 2008; Randhawa et al., 2013; Brainerd and Azizi, 2005). *t*-Tests were used to assess the effect of age on muscle morphological parameters and peak isometric stress. Mixed effect models, with individual included as a random factor, were used to assess the effect of age on the relationships between force and velocity, and force and gear ratio (R; v3.1.3, The R Foundation for Statistical Computing).

Connective tissue properties

Materials testing was performed on muscle fiber bundles immediately after extraction, and on aponeuroses within 12 months of extraction (stored at -20°C in 0.9% saline). Fiber bundles were clamped between a fixed point and a servomotor (model 360C [1 N], Aurora Scientific Inc.). This allowed length changes to be imposed, and passive force and fiber bundle length to be measured. Sarcomere length was measured using laser (650 nm) diffraction (Lieber et al., 1984; Lieber and Brown, 1992). Fiber bundles were cyclically stretched by 20% of their initial length (length at a passive force of 0.01 N) at 5 Hz. Fiber bundle mass and length, at a sarcomere length of 2.2 μm , were determined and cross-sectional area calculated (Mendez and Keys, 1960). Stress and strain were calculated, and the elastic modulus determined as the slope of the linear region (final 40%) of the stress–strain curve. It is assumed that the stiffness of these passive fiber bundles reflects the stiffness of the connective tissues surrounding fibers.

Aponeuroses were dissected out and cut into small strips oriented perpendicular to the muscle's line of action (transverse). Custom-made clamps were hooked through either end of the tissue and each clamp was connected to a separate servomotor (model 305C-LR, Aurora Scientific Inc.). These two servomotors, positioned opposite one another, then each applied the same length change to the sample and measured force. This loaded the sample symmetrically along its transverse axis and minimized strain heterogeneity. Aponeuroses were stretched by 10% of their resting length. Samples were filmed at 1000 Hz and digitized videos were used to calculate the strain in a central region of the tissue, in order to avoid any effects of clamping. Aponeurosis mass and length were measured and cross-sectional area determined (Shadwick, 1990). Stress and strain were calculated, and the elastic modulus was determined as the slope of the linear region (final 20%) of the stress–strain curve. Mixed-effect models, with individual included as a random factor, were used to assess the effect of age on fiber bundle and transverse aponeuroses modulus (R; v3.1.3, The R Foundation for Statistical Computing).

RESULTS

Connective tissue properties and gear ratios were determined in the medial gastrocnemius muscle of young ($N=12$, age 5–9 months) and old ($N=13$, age 33–34 months) rats. Details of the animals used,

Table 1. Animal, muscle and connective properties in young and old rats

	Young	Old
Animal mass (g)*	383±23 ($N=12$)	508±15 ($N=13$)
Muscle mass (g)*	0.9±0.05 ($N=12$)	0.7±0.02 ($N=13$)
Fiber length (mm)	15.8±0.3 ($N=12$)	15.8±0.5 ($N=13$)
Pennation angle (deg)*	26.1±2.4 ($N=8$)	18.2±2.3 ($N=8$)
Peak isometric force (N)*	24.4±0.9 ($N=12$)	14.3±1.2 ($N=13$)
Peak isometric stress (N cm $^{-2}$)*	47.9±2.9 ($N=12$)	33.0±3.0 ($N=13$)
Fiber bundle modulus (kPa)*	19.1±5.0 ($N=5$)	39.1±4.2 ($N=5$)
Transverse aponeurosis modulus (MPa)*	188.5±24.2 ($N=5$)	328.0±48.3 ($N=5$)

*Significant ($P<0.05$) difference between young and old rats.

and muscle and connective tissue properties, are given in Table 1. All data are presented as means±s.e.m.

Connective tissue properties

There was a significant effect of age on the passive stiffness of muscle fiber bundles ($P<0.05$). Fiber bundle modulus increased from 19.1±5.0 kPa ($N=5$) in young rats to 39.1±4.2 kPa ($N=5$) in old rats (Table 1). This increase in longitudinal fiber stiffness is likely to be the result of the increases in endomysium stiffness that occur with age (Kragstrup et al., 2011). There was also a significant effect of age on the transverse stiffness of the aponeuroses ($P<0.05$). Transverse aponeurosis modulus increased from 188.5±24.2 MPa ($N=5$) in young rats to 328.0±48.3 MPa ($N=5$) in old rats (Table 1). The observed change in transverse aponeurosis stiffness is likely to be a result of increases in the amount and cross-linking of collagen with age (Alnaqeeb et al., 1984; Bailey, 2001; Haus et al., 2007).

Contractile tissue properties and gear ratios

Muscle mass, pennation angle, peak isometric force, and peak isometric stress all decreased with age (Table 1), as has been demonstrated previously (Brooks and Faulkner, 1994; Narici et al., 2003; Morse et al., 2005; Kubo et al., 2007). Some of the decrease in force is likely to be due to reduced muscle mass and pennation

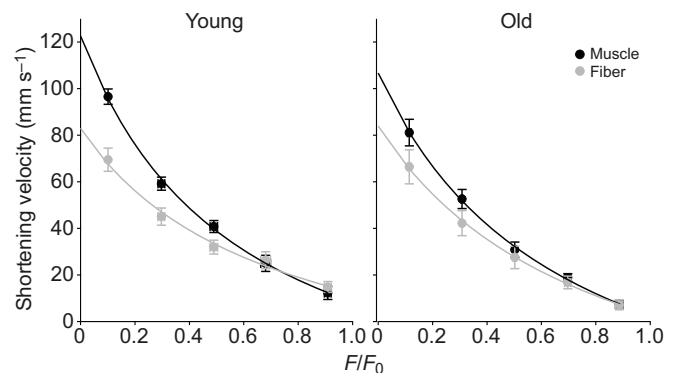


Fig. 3. The effect of relative force and age on muscle and fiber shortening velocity. Muscle and fiber shortening velocities during isotonic contractions are shown at different relative forces (F/F_0) in young and old rats. Curves show force–velocity relationships fitted using the Hill rectangular-hyperbola equation, from which predicted maximum shortening velocity was determined. Note that forces refer only to the force of the whole muscles but velocities are measured independently in the muscle and the fiber. $N=12$, 12, 10, 8 and 6 in young rats and $N=12$, 13, 11 and 10 in old rats for relative forces of 0.1, 0.3, 0.5, 0.7 and 0.9 F/F_0 .

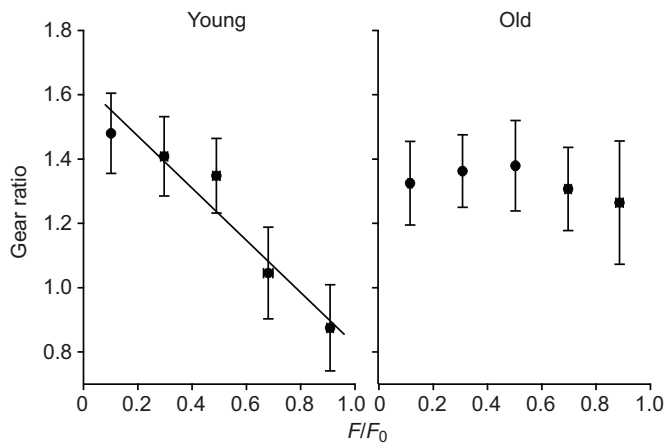


Fig. 4. The effect of relative force and age on gear ratio. Gear ratio, the ratio between muscle and fiber shortening velocities, is shown for different relative forces (F/F_0) in young and old rats. $N=12, 12, 10, 8$ and 6 in young rats and $12, 12, 13, 11$ and 10 in old rats for relative forces of $0.1, 0.3, 0.5, 0.7$ and $0.9 F/F_0$.

angle leading to a decrease in physiological cross-sectional area. However, the decrease in peak isometric stress indicates that factors other than sarcopenia and changes in muscle morphology contribute to the age-related decrease in the force production capacity of muscle (Brooks and Faulkner, 1994; Morse et al., 2005; Kubo et al., 2007).

In order to determine the effect of age on the relationship between force and gear ratio, muscle and fiber force–velocity relationships were determined. There was a significant effect of age on the relationship between muscle force and muscle velocity ($P<0.005$), and the relationship between muscle force and fiber velocity ($P<0.01$) (Fig. 3). However, the differences in the muscle force–velocity relationship with age appear to be much larger than differences in the fiber force–velocity relationship, particularly at low forces (Fig. 3). This is reflected in the large difference in maximum muscle shortening velocity (122.6 mm s^{-1} in young rats compared with 106.7 mm s^{-1} in old rats), despite the similarity in maximum fiber shortening velocity (83.0 mm s^{-1} in young rats compared with 84.0 mm s^{-1} in old rats).

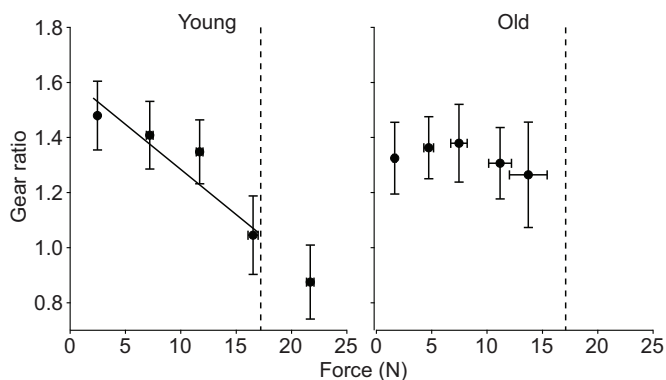


Fig. 5. The effect of absolute force on gear ratio. Gear ratios are shown at different absolute forces in young and old muscle. The range of absolute forces produced by old muscle is smaller than that for young muscle. Over the range of forces produced by old rats (cut-off calculated as mean+2 s.d. force at $0.9 F/F_0$ in old muscle, denoted by the dashed lines) there is a significant relationship between gear ratio and force in young ($P<0.005$) but not old ($P=0.1$) muscle.

Gear ratio was calculated as the ratio between muscle velocity and fiber velocity at all force levels (Fig. 2), and the relationship between force and gear ratio was determined. There was a significant relationship between relative force and gear ratio in young muscle ($P<0.001$): gear ratio decreased from 1.5 ± 0.12 at a relative force of $0.10\pm 0.0026 F/F_0$ to 0.88 ± 0.13 at a relative force of $0.91\pm 0.012 F/F_0$ (Fig. 4). This supports previous findings showing a decrease in gear ratio with increasing relative force (Azizi et al., 2008; Wakeling et al., 2011; Azizi and Roberts, 2014), and provides evidence of variable gearing in the rat medial gastrocnemius muscle. In contrast, there was no significant relationship between gear ratio and relative force in old muscle ($P=0.72$): gear ratio was relatively constant around 1.3 at all relative forces (Fig. 4). This demonstrates that variable gearing is lost in old muscle. These findings persist when we consider the relationship between gear ratio and absolute force over only the range of forces produced by old muscle; there was a significant relationship between gear ratio and absolute force in young ($P<0.005$) but not old muscle ($P=0.1$) (Fig. 5). Hence, there is a loss of variable gearing in old muscle that is not simply due to its reduced force-producing capacity.

DISCUSSION

Muscle mechanical performance is determined by cellular-level properties (Hill, 1938), but also by the properties and behavior of structures at higher levels of organization (Gans, 1982; Otten, 1988; Carrier et al., 1998; Brainerd and Azizi, 2005; Azizi et al., 2008; Roberts and Azizi, 2011; Wakeling et al., 2011; Randhawa et al., 2013; Azizi and Roberts, 2014). The use of gearing enables pennate muscle to partially circumvent cellular-level force–velocity constraints (Gans, 1982; Otten, 1988; Brainerd and Azizi, 2005; Azizi et al., 2008; Wakeling et al., 2011; Randhawa et al., 2013; Azizi and Roberts, 2014). The ability to vary gearing broadens a muscle's functional range by increasing velocity during rapid movements while preserving force during slow, forceful movements (Azizi et al., 2008). Here, we used age-related changes in connective tissue properties to test the hypothesis that the interaction between contractile and connective tissues is responsible for variable gearing. The increase in connective tissue stiffness, and therefore the disruption of the interaction between contractile and connective tissues, with age (Table 1) demonstrated here makes aging a suitable model in which to test this hypothesis.

The results from this study show that whilst variable gearing occurs in young muscle, it is absent in old muscle under the conditions studied here (Fig. 4). When combined with the observed changes in connective tissue properties with age (Table 1), this provides support for the hypothesis that the interaction between contractile and connective tissues is responsible for variable gearing. In this muscle, the ability to vary gear ratio is not just reduced with age, it is completely absent (Fig. 4). This suggests that a finely tuned relationship between contractile and connective tissue properties is essential for dynamically varying muscle gear ratio. Hence, the results of this study suggest that, in addition to well-established structural (Kjaer, 2004) and elastic functions (Roberts and Azizi, 2011), connective tissues play a significant role in modulating the mechanical output of skeletal muscle.

It has previously been hypothesized that contractile and connective tissue interactions result in variable gearing by enabling force-dependent variation in the way muscle changes shape during contraction (Azizi et al., 2008). The changes in fiber bundle and transverse aponeurosis stiffness observed here, and the concurrent loss of variable gearing, may support this mechanism. It

is thought that the decrease in gear ratio that occurs with increasing force results from muscle transitioning from accommodating shortening by increasing thickness (resisted by the force produced by the fibers), to accommodating shortening by increasing width (resisted by connective tissues). The increases in fiber bundle stiffness, and therefore endomysium stiffness (Kragstrup et al., 2011), and transverse aponeurosis stiffness with age are likely to have increased the resistance of the muscle to bulging and increasing width. This increased resistance may have prevented the normal transition from increasing thickness to increasing width and resulted in gear ratio remaining high in old muscle.

The age-related changes to gearing observed here not only provide insight into the mechanism of variable gearing but also contribute to our understanding of the decline in muscle performance with age. Force per unit cross-sectional area (Table 1) and maximum muscle shortening velocity (Fig. 3) decreased with age. Decreases in active muscle stress with age have largely been attributed to an increased contribution of connective tissues (Kjaer, 2004; Gao et al., 2008; Kragstrup et al., 2011) and fat (Kent-Braun et al., 2000) to muscle cross-sectional area. However, the present results suggest that changes in pennation angle during contraction may also contribute to the decrease in stress in old muscles. The inability of old muscles to reduce gear ratio with increasing force means that they use high gear ratios, and therefore experience large changes in pennation angle, even at high forces. This reduces the component of fiber force acting in the line of action of the muscle and ultimately reduces muscle stress. The changes to muscle gearing observed here may also contribute to a reduction in maximum muscle shortening velocity. Decreases in muscle shortening velocity with age have previously been attributed to decreases in fiber length, changes in fiber type composition, and reduced shortening velocity of individual fibers (Raj et al., 2010; Miller et al., 2014). However, the present results suggest that age-related changes to gearing may have contributed to the decrease in maximum muscle shortening velocity. The tendency towards lower maximum gear ratios (Fig. 3), likely due to lower pennation angles (Table 1) (Brainerd and Azizi, 2005), seen in old muscles will have reduced the amplification of fiber shortening velocity, and therefore reduced muscle shortening velocity. This may explain the decrease in maximum shortening velocity of old muscle in the absence of a decrease in maximum fiber shortening velocity (Fig. 3).

The results presented here show that changes in the connective properties of muscles are accompanied by a loss of variable gearing in old muscles. These findings support the hypothesis that variable gearing results from the interaction of contractile and connective tissues, potentially due to its ability to effect force-dependent variation in muscle shape change. They also suggest that age-related changes in connective tissues stiffness impact muscle performance, and may therefore explain why large, consistent changes are observed in muscle performance with age (Brooks and Faulkner, 1994; Morse et al., 2005; Kubo et al., 2007; Raj et al., 2010), in the absence of such consistent changes at the cellular (fiber) level (Miller et al., 2014). This may provide novel explanations for the loss of functionality associated with aged and fibrotic muscles (Lieber and Ward, 2013).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

E.A. and T.J.R. designed the experiments. N.C.H. and N.D. conducted the experiments. N.C.H. analyzed the data and wrote the first draft of the manuscript, and all authors revised and approved the manuscript.

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References

- Alnaqeeb, M. A., Alzaid, N. S. and Goldspink, G. (1984). Connective-tissue changes and physical-properties of developing and aging skeletal-muscle. *J. Anat.* **139**, 677–689.
- Askew, G. N. and Marsh, R. L. (1997). The effects of length trajectory on the mechanical power output of skeletal muscles. *J. Exp. Biol.* **200**, 3119–3131.
- Azizi, E. and Roberts, T. J. (2014). Gearing up to stretch: pennate muscle behavior during active lengthening. *J. Exp. Biol.* **217**, 376–381.
- Azizi, E., Brainerd, E. L. and Roberts, T. J. (2008). Variable gearing in pennate muscles. *Proc. Natl. Acad. Sci. USA* **105**, 1745–1750.
- Bailey, A. J. (2001). Molecular mechanisms of ageing in connective tissues. *Mech. Ageing Dev.* **122**, 735–755.
- Baskin, R. J. and Paolini, P. J. (1967). Volume change and pressure development in muscle during contraction. *Am. J. Physiol.* **213**, 1025–1030.
- Brainerd, E. L. and Azizi, E. (2005). Muscle fiber angle, segment bulging and architectural gear ratio in segmented musculature. *J. Exp. Biol.* **208**, 3249–3261.
- Brooks, S. V. and Faulkner, J. A. (1994). Skeletal muscle weakness in old age: underlying mechanisms. *Med. Sci. Sports Exerc.* **26**, 432–439.
- Caiozzo, V. J. (2002). Plasticity of skeletal muscle phenotype: mechanical consequences. *Muscle Nerve* **26**, 740–768.
- Carrier, D. R., Gregersen, C. S. and Silverton, N. A. (1998). Dynamic gearing in running dogs. *J. Exp. Biol.* **201**, 3185–3195.
- Gans, C. (1982). Movement mechanics as a determinate of muscle structure, recruitment and coordination. Fiber architecture and muscle function. *Excerc. Sport Sci. Rev.* **10**, 160–207.
- Gao, Y., Kostrominova, T. Y., Faulkner, J. A. and Wineman, A. S. (2008). Age-related changes in the mechanical properties of the epimysium in skeletal muscles of rats. *J. Biomech.* **41**, 465–469.
- Haus, J. M., Carrithers, J. A., Trappe, S. W. and Trappe, T. A. (2007). Collagen, cross-linking, and advanced glycation end products in aging human skeletal muscle. *J. Appl. Physiol.* **103**, 2068–2076.
- Hill, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. B Biol. Sci.* **126**, 136–195.
- Holt, N. C. and Askew, G. N. (2012). The effects of asymmetric length trajectories on the initial mechanical efficiency of mouse soleus muscles. *J. Exp. Biol.* **215**, 324–330.
- Holt, N. C. and Azizi, E. (2014). What drives activation-dependent shifts in the force-length curve? *Biol. Lett.* **10**, 20140651.
- Kent-Braun, J. A., Ng, A. and Young, K. (2000). Skeletal muscle contractile and non-contractile components in young and older women and men. *J. Appl. Physiol.* **88**, 662–668.
- Kjaer, M. (2004). Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. *Physiol. Rev.* **84**, 649–698.
- Kragstrup, T. W., Kjaer, M. and Mackey, A. L. (2011). Structural, biochemical, cellular, and functional changes in skeletal muscle extracellular matrix with aging. *Scand. J. Med. Sci. Sports* **21**, 749–757.
- Kubo, K., Ishida, Y., Komuro, T., Tsunoda, N., Kanehisa, H. and Fukunaga, T. (2007). Age-related differences in the force generation capabilities and tendon extensibilities of knee extensors and plantar flexors in men. *J. Gerontol. A Biol. Sci. Med. Sci.* **62**, 1252–1258.
- Lieber, R. L. and Brown, C. G. (1992). Sarcomere length-joint angle relationships of seven frog hindlimb muscles. *Acta Anat.* **145**, 289–295.
- Lieber, R. L. and Ward, S. R. (2013). Cellular mechanisms of tissue fibrosis. 4. Structural and functional consequences of skeletal muscle fibrosis. *Am. J. Physiol. Cell Physiol.* **305**, C241–C252.
- Lieber, R. L., Yeh, Y. and Baskin, R. J. (1984). Sarcomere length determination using laser diffraction. Effect of beam and fiber diameter. *Biophys. J.* **45**, 1007–1016.
- Mendez, J. and Keys, A. (1960). Density and composition of mammalian muscle. *Metab. Clin. Exp.* **9**, 184–188.
- Miller, M. S., Callahan, D. M. and Toth, M. J. (2014). Skeletal muscle myofibril adaptations to aging, disease, and disuse and their effects on whole muscle performance in older adult humans. *Front. Physiol.* **5**, 370.
- Morse, C. I., Thom, J. M., Reeves, N. D., Birch, K. M. and Narici, M. V. (2005). In vivo physiological cross-sectional area and specific force are reduced in the gastrocnemius of elderly men. *J. Appl. Physiol.* **99**, 1050–1055.
- Narici, M. V., Maganaris, C. N., Reeves, N. D. and Capodaglio, P. (2003). Effect of aging on human muscle architecture. *J. Appl. Physiol.* **95**, 2229–2234.
- Otten, E. (1988). Concepts and models of functional architecture in skeletal muscle. *Excerc. Sport Sci. Rev.* **16**, 89–138.

- Raj, I. S., Bird, S. R. and Shield, A. J.** (2010). Aging and the force–velocity relationship of muscles. *Exp. Gerontol.* **45**, 81–90.
- Randhawa, A., Jackman, M. E. and Wakeling, J. M.** (2013). Muscle gearing during isotonic and isokinetic movements in the ankle plantarflexors. *Eur. J. Appl. Physiol.* **113**, 437–447.
- Roberts, T. J. and Azizi, E.** (2011). Flexible mechanisms: the diverse roles of biological springs in vertebrate movement. *J. Exp. Biol.* **214**, 353–361.
- Sacks, R. D. and Roy, R. R.** (1982). Architecture of the hind limb muscles of cats: functional significance. *J. Morphol.* **173**, 185–195.
- Shadwick, R. E.** (1990). Elastic energy storage in tendons – mechanical differences related to function and age. *J. Appl. Physiol.* **68**, 1033–1040.
- Wakeling, J. M., Blake, O. M., Wong, I., Rana, M. and Lee, S. S. M.** (2011). Movement mechanics as a determinate of muscle structure, recruitment and coordination. *Philos. Trans. R. Soc. Biol. Sci.* **366**, 1554–1564.