Integrative and Comparative Biology

Integrative and Comparative Biology, volume 58, number 2, pp. 207–218
doi:10.1093/icb/icy054

Society for Integrative and Comparative Biology

SYMPOSIUM

Structural Determinants of Muscle Gearing During Dynamic Contractions

Carolyn M. Eng,[1,*] Emanuel Azizi[†] and Thomas J. Roberts[*]

*Department of Ecology and Evolutionary Biology, Brown University, 171 Meeting St, Box GB204, Providence, RI 02912, USA; [†]Department of Ecology and Evolutionary Biology, University of California, Irvine, 321 Steinhaus Hall, Irvine, CA 92697, USA

From the symposium "Spatial Scale and Structural Heterogeneity in Skeletal Muscle Performance" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2018 at San Francisco, California.

[1]E-mail: carolyn_eng@brown.edu

Synopsis In skeletal muscle, interactions between contractile and connective tissue elements at multiple scales result in emergent properties that determine mechanical performance. One of these phenomena is architectural gearing, which is quantified as the ratio of muscle velocity to muscle fiber velocity. Many pennate muscles operate with a gear ratio greater than one because muscles shorten through a combination of muscle fiber shortening and fiber rotation. Within a muscle, gearing is variable across contractions. During low force contractions, muscles operate at high gear while muscles operate at low gear during high force contractions. This variable gearing has a significant impact on muscle performance as muscle architectural changes favor muscle speed during fast contractions and muscle force during slow, high force contractions. We hypothesize that gearing in any given contraction is determined by the dynamic interaction of fiber-generated forces, fluid force transmission, and the elastic behavior of intramuscular connective tissues. Because muscle is isovolumetric, muscle fibers must bulge radially when they shorten. Radial bulging and fiber-generated forces off-axis from the muscle line of action exert forces that load connective tissues that ensheath fibers, fascicles, and the whole muscle. The way in which fluid pressures and fiber forces interact to load connective tissues in three-dimensions remains poorly understood because of the complex and multiscale nature of these interactions. Here we review evidence for variable gearing in pennate muscles, present a conceptual model that describes the fundamental interactions that determine gearing, and discuss where gaps remain in our understanding of the determinants and consequences of muscle shape change and variable gearing.

Muscle fiber architecture under static and dynamic conditions

In many skeletal muscles, fibers are oriented at an angle to the muscle's axis of force transmission, or line of action (Gans 1982). Fiber orientation defines a muscle's architecture, which is typically categorized as either parallel-fibered or pennate (Fig. 1). In parallel-fibered muscles, the fibers extend nearly the entire length of the muscle and are parallel to the muscle's axis of force transmission (Fig. 1, images 1, 2, and 9). The fibers in pennate muscles are arranged at an angle relative to the muscle's axis of force transmission, the pennation angle (Fig. 1, images 3–7, 10, and 11). Most muscles have some degree of pennation. For example, in the human lower extremity, 23 of the 28 measured muscles had fibers that extended less than 60% of the total muscle length (Ward et al. 2009). Within the broad category of pennate muscles, there are a wide range of anatomical configurations including unipennate, bipennate, and multipennate as well as a range of possible pennation angles (Fig. 1).

The architecture of a muscle has important functional consequences. For a given muscle volume, parallel-fibered muscles can generate larger excursions and achieve faster contractile speeds (Bodine et al. 1982; Gans 1982) because they have more sarcomeres in series (assuming equivalent fiber properties, e.g., intrinsic maximal shortening velocities). Pennate muscles allow for more sarcomeres in

Advance Access publication June 7, 2018

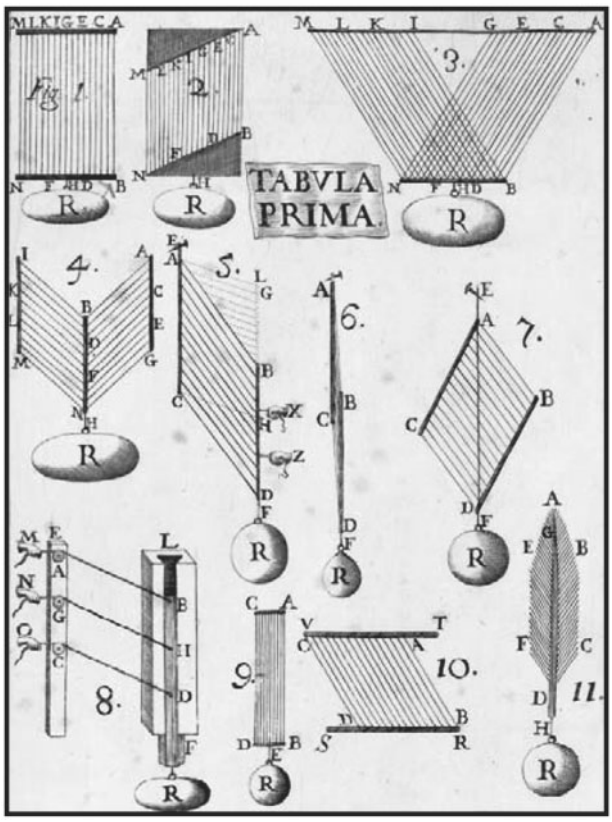

Fig. 1 Borelli's (1680) analysis of muscle architecture highlights many of the features of pennate muscles that are prominent in biomechanical analyses today. Shown are different possible arrangements of parallel (images 1, 2, and 9) and pennate (images 3-7, 10, and 11) muscles. Fiber rotation in pennate muscles is depicted in image 5, and the mechanical analog depicted in image 8 embodies the common assumption that fiber origin and insertion distances remain constant during fiber shortening in unipennate muscles.

parallel (i.e., larger total fiber cross-sectional area or physiological cross-sectional area) and can therefore generate higher maximum forces for a given muscle volume (Powell et al. 1984). There is, however, a cost to force production in pennate muscles because only the component of fiber force that is in line with the muscle axis of force transmission will contribute to muscle force, but this effect is generally small relative to the benefit of a greater physiological cross-sectional area. Thus, the architecture of a muscle defines a trade-off between force and speed; a parallel arrangement favors speed while a pennate arrangement favors force.

It is straightforward to define the influence of muscle architecture on mechanical output when we consider only static conditions, but more difficult when the changes that occur dynamically during contraction are considered. Dynamic changes in muscle architecture in pennate muscles occur across a range of conditions and are the focus of this review.

Muscle architecture is typically measured in muscles dissected from preserved specimens (Sacks and Roy 1982; Lieber et al. 1990; Lieber and Fridén 2000; Ward et al. 2009). Values for fiber length, muscle length, and pennation angle are almost always taken from one muscle conformation (e.g., at a "resting" muscle length) and do not capture how muscle architecture changes during a contraction. In a few cases, video of exposed muscles *in situ* has shown changes in key architectural features, like pennation angle, during contraction. For example, light video of a longitudinal section of the contracting rat medial gastrocnemius (MG) muscle demonstrated that there are large changes in pennation angle during contractions (Huijing and Woittiez 1985; Zuurbier and Huijing 1993). Newer imaging techniques have allowed for significant improvements in the ability to directly visualize changes in muscle architecture *in vivo*. Ultrasound imaging provided some of the first measurements of human muscle architecture *in vivo* and showed that pennation angle can increase more than two-fold during fixed-end contractions [i.e., isometric at the level of the muscle–tendon unit (MTU)] in the human MG (Narici et al. 1996) and brachialis muscles (Herbert and Gandevia 1995). Using phase-contrast MRI of the human MG, Shin et al. (2009) demonstrated that pennation angle decreased by about 35% during eccentric contractions. Subsequent ultrasound studies have confirmed that substantial changes in architecture occur across a normal range of movement in human muscles *in vivo* including during walking and running (Lichtwark et al. 2007), cycling (Wakeling et al. 2011; Dick and Wakeling 2017), and jumping (Kurokawa et al. 2001).

While technology has only recently enabled measurements of pennation angle changes during contraction, theory predicting that a pennate fiber architecture naturally results in a change in pennation angle during muscle contraction dates back centuries. It is apparent in Borelli's drawings from *de Motu Animalium* (1680), where rotation of fibers occurs as a result of shortening in a unipennate muscle (Fig. 1, image 5). Borelli's drawings also indicate the very early origin of an assumption that is still common in muscle models today, namely that the distance between the aponeuroses, or the surfaces upon which the fibers originate, remains constant (i.e., a constant muscle thickness by our terminology). This assumption is implicit in a mechanical analog where the trajectory of the plane of fiber insertion is constrained by a slot mechanism to move

vertically as the muscle lifts a load vertically (Fig. 1, image 8). Steno (1667), as translated in Stensen et al. (1994), formalized the geometric analysis to calculate fiber angle as a function of shortening using a simple parallelogram model, also assuming that the thickness of the muscle remains constant during shortening.

The assumption that the thickness (distance between fiber origin and insertion) of a muscle remains constant during contraction has dominated modern theoretical analyses of the significance of dynamic muscle architecture (Pfuhl 1937; Benninghoff and Rollhäuser 1952; Gans and Bock 1965; Alexander 1968; Hatze 1978; Otten 1988). The constant-thickness model is rooted in a planar parallelogram configuration that shows that the constraint of constant muscle volume will be met if both muscle thickness and muscle width remain constant as the parallelogram shears to shorter fiber lengths and greater angles of pennation (e.g., Fig. 1, image 5). Otten (1988) presents a mathematical proof of this concept, but in his influential review may have overstated the case, concluding the proof with the statement "the distances between the planes of insertion needs to remain constant if the volume of the muscle is to remain constant." A more accurate statement for this model is that a constant area model of a shearing parallelogram is one solution to maintaining a constant muscle volume. Shape change in some muscles is constrained by hard tissues, such as muscles that insert on an exoskeleton, like the bipennate muscles of crab claws, where fiber origins are fixed. For muscles without such constraints, a wide range of shape changes is possible.

It is only relatively recently that the long history of theoretical treatments of dynamic muscle architecture has been tested with empirical measurements of dimensional changes in active contractions. These measurements suggest that a wide range of shape changes is possible, and in particular they challenge the constant thickness assumption. *In situ* measurements of rat MG muscle showed thickness increase during contraction (Zuurbier and Huijing 1992), and in the turkey lateral gastrocnemius muscle both an increase and decrease in thickness has been observed (Azizi et al. 2008). Ultrasound studies provide measurements for a variety of activities and contractile conditions in human muscles. These measurements show variability in the pattern of shape change, with observations of virtually all possible outcomes, including an increase in thickness (Herbert and Gandevia 1995; Maganaris et al. 1998; Wakeling et al. 2011; Randhawa et al. 2013; Raiteri et al. 2016; Dick and Wakeling 2017), constant thickness (Narici et al. 1996; Maganaris et al. 1998), or a decrease in thickness (Randhawa et al. 2013). While it is not yet possible to discern a consistent pattern in the variation in shape changes that have been observed under all conditions, these measurements provide convincing evidence that shape change is variable, and that the constant thickness model does not accurately predict vertebrate limb muscle shape changes *in vivo*.

The dynamic architectural changes that occur during contraction are important because they directly affect the force and speed of contraction. The change in pennation angle contributes to shortening and can result in muscle speed exceeding fiber shortening speed. Classic models of pennate muscle have shown that when fiber shortening is combined with a change in pennation angle, the shortening of the muscle belly exceeds fiber shortening (Benninghoff and Rollhäuser 1952). These predictions have been confirmed experimentally showing that dynamic changes in architecture tend to amplify muscle fiber shortening (Azizi et al. 2002). The effect of changes in pennation angle can be characterized by measuring the ratio of muscle velocity to fiber velocity, a measure that has been termed the architectural gear ratio (AGR; Brainerd and Azizi 2005). Fiber rotation during contractions in pennate muscles commonly results in gear ratios greater than one (Azizi et al. 2008; Wakeling et al. 2011; Randhawa et al. 2013; Azizi and Roberts 2014; Holt et al. 2016; Dick and Wakeling 2017).

Variable gearing in pennate muscles

The relationship between changes in pennation angle and changes in fiber length is related to muscle shape change during a contraction. Muscle is isovolumetric and therefore may bulge in directions orthogonal to its axis of force transmission when it shortens (Baskin and Paolini 1967). Muscles can bulge in thickness, width, or both, and the direction of shape change mediates the change in pennation angle during a contraction and therefore determines AGR in pennate muscles (Brainerd and Azizi 2005). Since there is variation in how a muscle changes shape and this determines AGR, there is no single relationship between the length changes of a fiber and that of the muscle (Fig. 2). A simple geometric schematic shows how for a given fiber length change, a muscle operates with a low AGR (lower displacement and speed) when the thickness of the muscle is allowed to decrease (Fig. 2A, schematic 3), a moderate AGR when the thickness of the muscle is constant (Fig. 2A, schematic 1), and a high AGR (higher

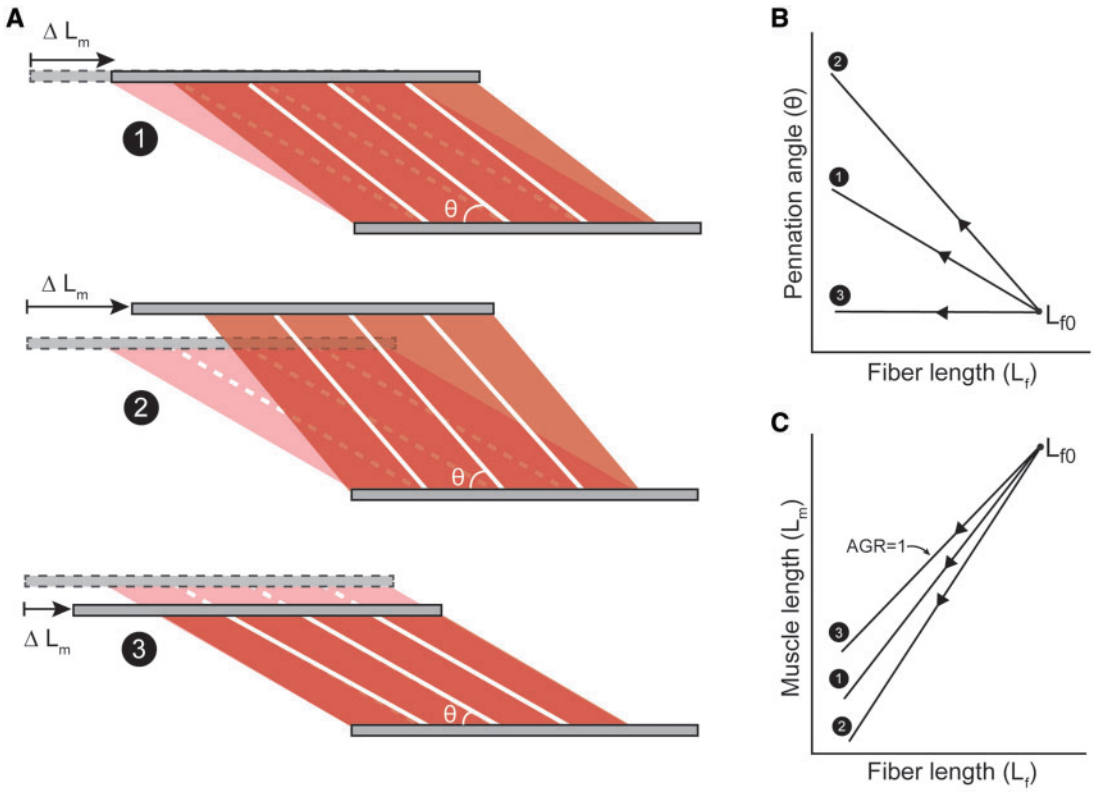

Fig. 2 A simple geometric model shows that the relationship between pennation angle and fiber shortening is variable across contractions and is mediated by muscle shape change. Compared with the contraction in which muscle thickness decreases (**A**, 3), for the same magnitude of fiber shortening, fibers rotate to greater angles of pennation when thickness is constant (A, 1) and undergo increased rotation when the thickness increases (A, 2). Plots of pennation angle as a function of fiber length (**B**) and muscle length as a function of fiber length (**C**) as the fiber shortens from its initial length (L_{f0}) demonstrate how variability in fiber rotation results in variable gearing with the muscle operating at a low (A, 3), intermediate (A, 1), or high gear (A, 2).

displacement and speed) when the thickness of the muscle increases during the contraction (Fig. 2A, schematic 2). Given this potential variation, models predicting the mechanical output of a pennate muscle are constrained to a limited range of results based on assumptions of how the muscle changes shape. It is likely that making the wrong assumption in a pennate muscle model may have minimal effects on the predicted force and speed of muscles with relatively small starting pennation angles ($<10°$), whereas such errors can be substantial in muscles with larger pennation angles. This is due to the fact that the amount of muscle length change resulting from a given fiber rotation will be greater for muscles with higher initial pennation angles, resulting in higher gear ratios (Brainerd and Azizi 2005).

Empirical studies have quantified the AGR of pennate muscle over a range of conditions and they suggest that for a given muscle, no single prediction of gear ratio is accurate across contractions. Instead, a muscle's AGR varies from contraction to contraction. This was first demonstrated using isolated muscle experiments on the turkey lateral gastrocnemius muscle (Azizi et al. 2008). Variable gearing has been replicated during shortening contractions in rat MG (Holt et al. 2016), frog plantaris (Azizi and Roberts 2014), and human MG muscles (Dick and Wakeling 2017) and during lengthening contractions in frog plantaris muscle (Azizi and Roberts 2014). Across muscles, these experiments have consistently shown that gearing decreases with increasing contractile force during shortening contractions (Fig. 3).

Muscle force and velocity covary according to the force–velocity property of muscle and studies decoupling the effects of force and velocity on gearing have demonstrated that contractile force, and not velocity, drives variable gearing. Azizi et al. (2008) used a fatigue protocol in an *in situ* preparation of the turkey lateral gastrocnemius muscle to vary muscle force while keeping muscle shortening velocity constant. Trains of contractions of varying duration used to fatigue the muscle resulted in decreased and variable force output for a series of contractions at the same velocity. Gearing decreased with

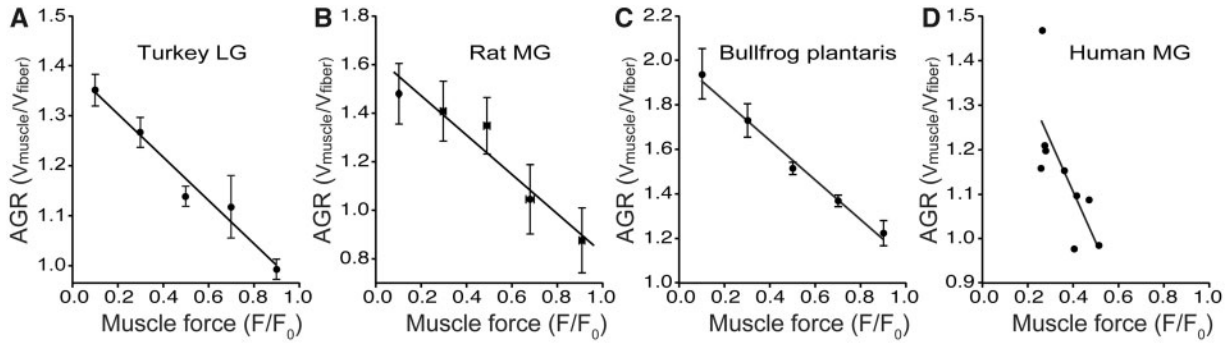

Fig. 3 Measures of architectural gear ratio (AGR) as a function of load demonstrate that gearing decreases with increasing load in the lateral gastrocnemius (LG) of the turkey (**A**; Azizi et al. 2008), medial gastrocnemius (MG) of the rat (**B**; Holt et al. 2016), bullfrog plantaris (**C**; Azizi and Roberts 2014), and human MG (**D**; Dick and Wakeling 2017).

increasing force and constant velocity, demonstrating that contractile force drives variable gearing. This result was confirmed by Dick and Wakeling (2017) who measured gearing in the human MG muscle of subjects cycling under a variety of conditions where cycling cadence and crank torque were independently varied to examine the effects of muscle force and velocity on gearing. They used ultrasound along with a lower limb musculoskeletal model scaled to each subject to measure muscle fascicle velocity and tendon strain and calculate muscle belly and MTU velocities. MG tendon force was calculated from tendon strain and tendon stiffness. The authors found that muscle belly gearing decreased with increasing tendon force but was independent of MTU velocity. While these studies confirm that variable gearing is determined by variation in contractile force, the mechanism underlying variable gearing remains poorly understood.

The exact determinants of shape change and gearing in a given contraction are unclear but our current hypothesis highlights the central importance of the multiscale structural organization of skeletal muscle. *In situ* experiments where muscles are isolated from the nervous system show variable gearing, demonstrating that gearing is mediated by interactions occurring at the level of the muscle. We hypothesize that muscle gearing in a given contraction is determined by the dynamic interaction of fiber-generated forces, fluid force transmission, and the elastic behavior of intramuscular (IM) connective tissues. This prediction is difficult to test directly because the multiscale nature and structural complexity of these interactions does not allow for practical experimental manipulations that can isolate each component. In the following section, we discuss our current understanding of the determinants of variable gearing as well as gaps in our knowledge.

The determinants of variable gearing

Experiments with a simple array of pneumatic actuators provide insight into the mechanism underlying variable gearing in muscle (Azizi and Roberts 2013). McKibben actuators shorten in tension when filled with compressed gas (Schulte 1961). Like muscles, McKibben actuators expand radially when they shorten. When these actuators are arranged in a pennate array so that their axes of shortening are at an angle to the line of action of the array (Fig. 4A), the actuators rotate as the array shortens against a load. Actuator rotation occurs because of the radial expansion associated with shortening. This radial bulging of individual actuators loads neighboring actuators at angles to their direction of shortening, causing the actuators to rotate and the entire array to bulge in plane with the array. While actuator shortening is constant, actuator rotation varies with load so that the array displays variable gearing (Fig. 4C). Gearing is high at low loads and decreases as the load lifted by the array increases. Thus, similar to muscle, a pennate array of McKibben actuators shows load-dependent variable gearing and fiber rotation.

Variable gearing in a McKibben array results from variation in the direction of actuator radial expansion as a function of force. Specifically, closely packing the actuators in a planar array causes resistance to radial expansion in the actuator's height (parallel to plane of array), but not width (perpendicular to array), dimension by neighboring actuators (Fig. 4B). When the actuators shorten against no load, radial expansion occurs equally in all directions. When the actuators lift a load against gravity, forces from the expanding neighboring actuators tend to compress the actuators in height and cause greater expansion in actuator width. This leads to deformation of the actuators such that they are not round in cross-section but are bulging more in width than height

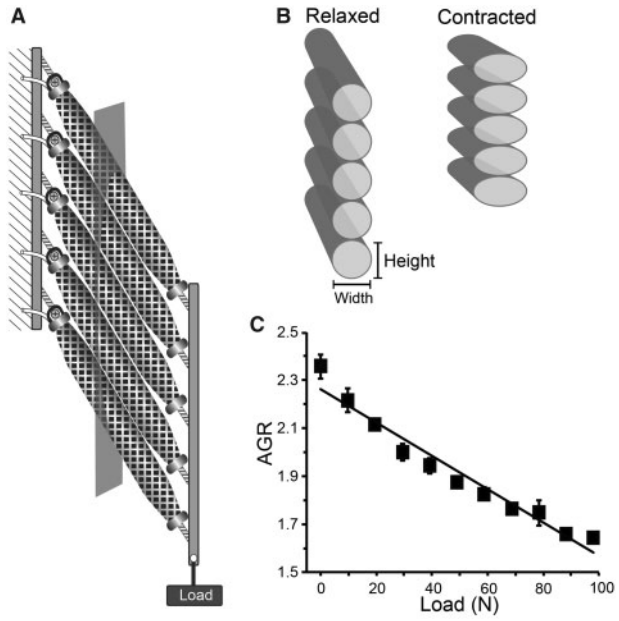

Fig. 4 In the McKibben actuator array schematized in (**A**), actuators are arranged at an angle relative to the line of action of the array. The actuators are attached to two plates and are allowed to rotate on the leftmost plate and are rigidly fixed to the rightmost plate that is attached to the load. When they are filled with air and contract against a load the actuators radially expand and this causes bulging of the array and rotation of the actuators. Neighboring actuators cause compression in actuator height so that the actuators expand more in width than height (**B**). This height-wise compression increases with load, resulting in decreased array bulging, actuator rotation, and AGR as a function of load (**C**). Modified from Azizi and Roberts (2013).

(Fig. 4B). As the load on the array increases, the load tending to cause compression in cross-sectional height and expansion in cross-sectional width increases. Because bulging in cross-sectional height drives actuator rotation, rotation decreases as the actuators bulge more in width and less in height. Variable actuator shape changes across force levels results in changes in actuator rotation and gearing.

There are important commonalities between pennate muscle and the McKibben array that we hypothesize determine variable gearing. Both systems undergo an increase in the radial dimension with linear shortening, although it should be noted that the McKibben actuators are not isovolumetric but increase in volume during contraction. This radial expansion of force-generating components (actuators or fibers), along with their pennate architecture, creates off-axis forces at both the fiber and muscle level. Interaction of off-axis forces with constraints on expansion (i.e., neighboring actuators or an IM spring) determines the direction of shape change, which leads to variable rotation of actuators or fibers and variable gearing.

A key feature of the hypothesized mechanism for variable gearing in muscle is the generation of forces that are off-axis from the linear axis of fibers. These off-axis forces load the IM connective tissues that determine how a muscle changes shape during contraction. There are two potential sources of off-axis forces in pennate muscle that occur at different scales of organization. During muscle contraction, shortening of muscle fibers is accompanied by fiber expansion in the radial dimension that has the potential to generate forces off-axis to the fiber linear axis (F_{radial} in Fig. 5). These off-axis forces result from the incompressible nature of fluid within muscle that requires muscle fibers to expand radially when they shorten, to maintain a constant volume. The second source of off-axis forces is the thickness force, which is the component of fiber force in pennate muscles that tends to compress the muscle in the thickness direction ($F_{thickness}$ in Fig. 5).

Radial expansion of muscle fibers and its importance for force generation has been demonstrated at multiple hierarchical levels. X-ray diffraction studies of relaxed muscle have shown that lattice spacing, or the radial distance between actin and myosin filaments, decreases as the sarcomere is stretched in order to maintain a constant sarcomere lattice volume (lattice cross-sectional area × sarcomere length; Huxley 1951; Elliott et al. 1963). Force production

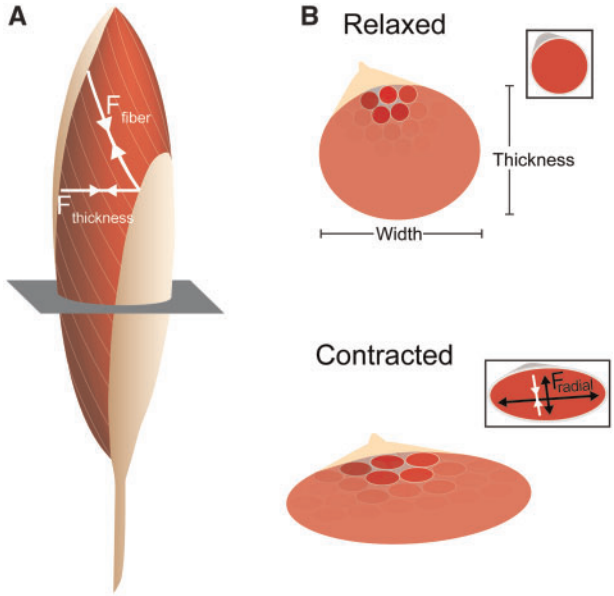

Fig. 5 A schematic of pennate muscle shows the component of the fiber contractile force (F_{fiber}) that tends to compress the muscle in the thickness direction (**A**; $F_{thickness}$). A cross-section of the whole muscle and fiber (inset) demonstrates how off-axis forces including the radial force (F_{radial}; black arrows) and thickness force ($F_{thickness}$; white arrows) interact to determine shape change in a contracted muscle (**B**).

is reduced when the lattice spacing of skinned muscle fibers is osmotically compressed via a high-concentration solution (reviewed in Millman 1998; Williams et al. 2013). At the whole muscle level, a physical constraint limiting the muscle's ability to expand radially reduced muscle shortening and muscle work suggesting that radial expansion is necessary for muscle shortening (Azizi et al. 2017). Interaction between radial and longitudinal force transmission is apparent from the observation that a transverse load on the muscle belly influenced muscle force along the axis of force transmission (Siebert et al. 2014, 2016).

Radial expansion of fibers will tend to cause expansion of the muscle in directions orthogonal to the muscle fiber (i.e., both thickness and width). Assuming transverse isotropy, parallel-fibered muscles would be expected to radially expand equally in all directions but fiber pennation sets up an additional force that drives asymmetric shape change. In a parallel-fibered muscle, the fiber contractile force is, by definition, aligned with the direction of muscle shortening so all its force is directed to shortening of the whole muscle. In a unipennate muscle there is a component of force from the contracting fibers, perpendicular to the aponeuroses, tending to decrease the distance between aponeuroses and compress the muscle in the thickness direction ($F_{\text{thickness}}$ in Fig. 5). This thickness force is off-axis to the muscle axis of force transmission and because it is a component of the contractile force, it increases with the force of contraction. Interaction of these off-axis forces, including the radial force and thickness force, with IM connective tissues determines how a muscle changes shape according to our current hypothesis.

Off-axis fiber forces create asymmetric loading, which tends to compress a muscle more in thickness than width. The thickness force tending to compress a muscle in the thickness direction must be balanced by structures that resist deformation to prevent the muscle from flattening during contraction.

We propose that an IM spring resists thickness compression and is loaded by both off-axis forces. Candidates for this spring include the IM connective tissues that constrain the direction of muscle shape change and these connective tissues likely experience complex three-dimensional loading from tensile forces along the fiber, shearing forces between fibers, and radial forces off-axis to the fiber. Connective tissues surround muscle fibers (endomysium), muscle fascicles (perimysium), and whole muscles (epimysium and aponeurosis) and likely several or all of these elements contribute to IM elasticity. These connective tissues are primarily composed of collagen and are therefore effective at resisting tensile but not compressive forces. How does the compressive thickness force load these tissues in tension? We propose that the thickness force acting to compress a pennate muscle ultimately applies a tensile load to collagen fibers (or other IM springs) via the radial off-axis force.

The fluid in muscle is incompressible and has the potential to transmit forces during muscle contraction. We hypothesize that fluid pressure plays a central role in loading the IM spring and driving muscle shape change. There is evidence that the interaction between IM pressure (IMP) and connective tissue constraints plays a role in muscle force transmission. Using a physical model of muscle with a fluid-filled tube constrained by a helically wound sleeve, Sleboda and Roberts (2017) demonstrated that the interaction between the sleeve and fluid influences force transmission. Increasing the fluid volume in the physical model as well as in a frog semimembranosus muscle resulted in increased passive force in both systems, suggesting that fluid plays an important mechanical role in muscle force transmission. Gindre et al. (2013) used a model of a single muscle fiber surrounded by helically wound collagen fibers to show that stress developed in the collagen fibers during fiber compression contributed to pressure inside the muscle fiber that opposed fiber compression, suggesting that IM connective tissues influence muscle force through their influence on IMP.

When the incompressible muscle fibers shorten, they expand and can potentially generate radial forces that load the IM spring. The thickness force creates asymmetric resistance to the radial force to oppose expansion in the muscle thickness direction, which in turn causes more expansion in the width direction. It is this width-wise expansion of the fiber and muscle that stretches the IM spring that resists increases in muscle width. Therefore, it is the fluid pressure within the muscle fiber that acts as a conduit to redirect the compression caused by the thickness force to stretch the IM spring in the width direction. In other words, the fluid pressure in muscle couples expansion in muscle width to force oriented in the thickness direction so that resistance to muscle width expansion results in resistance to muscle thickness compression. We hypothesize that the IM spring dictates muscle shape change by resisting decreases in muscle thickness and increases in muscle width.

We hypothesize that the elastic behavior of the IM spring governs fiber rotation by resisting muscle thickness compression, resulting in variable gearing.

Because this IM spring is primarily loaded by contractile forces, which in turn influence the fluid pressure that develops in muscle, the magnitude of loading and deformation of the IM spring will vary with the force of contraction. Variable deformation of the IM spring results in variable shape change of the muscle, which influences fiber rotation.

For a given amount of fiber shortening, a larger decrease in muscle thickness is associated with decreased fiber rotation. An increase in fiber force (and the component of the force tending to decrease thickness, $F_{thickness}$) will result in greater thickness compression and width expansion and therefore reduce fiber rotation, resulting in the muscle operating with a low gear ratio. In contrast, at low contractile forces, decreased thickness compression results in fibers rotating to greater angles of pennation to achieve high gear ratios. Thus, the interaction between IM springs and fiber contractile forces via fluid forces determine how the muscle changes shape and ultimately, how gearing changes under variable conditions.

There is growing evidence that the IM connective tissues that make up the IM spring play an important role in modulating muscle shape change and gearing. Holt et al. (2016) measured increased stiffness in MG muscle fiber bundles and aponeurosis from aged rats compared with young rats. Using isotonic contractions at varying force levels, they found an altered relationship between AGR and contractile force in the aged rat muscle where gearing did not decrease with increasing force, as seen in young rat muscle. It was shown that AGR remained relatively high (~1.3) across a range of forces in aged rat muscle (Holt et al. 2016). In other words, the aged rat muscle lost the ability to vary gear ratio with muscle force. We hypothesize that low gearing at high forces in young rat muscle resulted from the inability of the IM spring to resist thickness compression and width expansion when thickness force was high. Increased stiffness of the endomysium and aponeurosis in the aged rat muscle may have resulted in increased resistance to thickness compression and width expansion compared with the young muscle. This increased resistance to compression may have been responsible for the reduced thickness compression at high contractile forces in the aged compared with young muscle so that the aged rat muscle underwent increased fiber rotation, resulting in relatively higher gear ratios compared with the young muscle. These findings suggest that the interaction between contractile and connective tissue properties is vital for achieving variable gearing.

Eng and Roberts (2018) found that reducing the radial integrity of the turkey lateral gastrocnemius aponeurosis resulted in altered gearing. The investigators explored whether the aponeurosis is an important component of the IM spring resisting increases in muscle width by incising the aponeurosis before and after eliciting isotonic contractions. In high force, but not low force contractions, gearing was reduced after multiple longitudinal aponeurosis incisions. Reduced gearing in the incised aponeurosis compared to the intact aponeurosis implies increased thickness compression and width expansion, suggesting that aponeuroses play a central role in resisting width expansion and determining muscle shape change and gearing during high force contractions.

Gaps in our understanding of variable gearing

To date, most experiments showing variable gearing have been conducted *in situ* and under a narrow set of very controlled conditions. Muscles are maximally activated, and measurements of gearing are taken during the constant force region of an isotonic contraction. Within a muscle, gearing is measured at approximately the same fiber length for all contractions (Azizi et al. 2008; Azizi and Roberts 2014; Holt et al. 2016; Eng and Roberts 2018). Such controlled conditions help isolate the influence of variable muscle shape change on gearing. Taking measurements when muscle force is constant is particularly important when gearing is measured using external measurements of muscle velocity (e.g., from a muscle servomotor). Tendon elasticity can contribute significantly to muscle speed, but any elasticity in series with the muscle should remain at constant length during the period of constant force.

While measuring AGR under controlled conditions provides many practical advantages, it likely misses some important features of dynamic architectural changes that occur in muscles *in vivo*. Importantly, our current hypothesis for the factors that determine the direction of muscle shape change emphasizes the interplay of orthogonal muscle forces and the IM spring. This model predicts that rapid force change in a muscle—as occurs during most locomotor events—would have a significant influence on instantaneous architectural gearing because the change in force would tend to compress the muscle. More measurements of AGR under dynamic conditions will add to our understanding of the influence of dynamic muscle architecture *in vivo*.

Ultrasound measurements of architectural gearing provide some advantages over measurements that rely on a muscle servomotor to determine muscle speed. Using ultrasound, muscle belly shortening

can be calculated using fiber shortening and pennation angle, thus providing a measure of speed that is relatively unaffected by series elasticity (Randhawa et al. 2013). Using an alternative method of calculating muscle speed by subtracting tendon length measured from ultrasound from MTU length obtained from a subject-specific musculoskeletal model, Dick and Wakeling (2017) were able to measure architectural gearing during cycling. Under such dynamic conditions, they confirmed a relationship between gearing and force, when gearing was measured at the point of peak muscle shortening velocity.

Many ultrasound studies measure fiber rotation and length change during contraction, and thus might provide information about muscle gearing. However, it can be difficult to infer patterns in gearing from reported values. For example, a central observation of Maganaris et al. (1998) is that graded recruitment corresponding to 20%, 40%, 60%, and 80% maximum voluntary contractions in a fixed limb position show that fiber rotation increased with increasing plantarflexion moment in the human triceps surae muscles. This would seem to contradict the idea that higher forces are associated with less fiber rotation and lower gearing. However, the greater fiber rotation at higher forces in these fixed-end contractions can be explained by the increased amount of muscle shortening, as higher forces result in greater stretch of the series elastic structures. If normalized by muscle shortening, we predict that fiber rotation would decrease with increased force.

Much of our understanding of architectural gearing comes from experiments under supramaximal conditions, but muscles are rarely maximally activated during normal movement. How muscles change shape and the gear ratio they operate with during submaximal contractions is not well understood. There is some evidence that under submaximal conditions, gearing is still determined by contractile force. At muscle activations between 32% and 45% maximum activation in the human MG muscle of cycling subjects (Dick and Wakeling 2017) gearing decreased with increased muscle force. However, there are limited data comparing shape change and gearing across activation levels within a muscle. In submaximal contractions, the muscle will contain both force-generating active fibers and passive muscle fibers. What influence do the inactive fibers have on the ability of the active fibers to drive muscle shape change? As described previously, the thickness force component of the fiber contractile force drives muscle thickness compression, which is resisted by radial expansion of the shortening fibers. Passive muscle tissue is less stiff than active muscle (Morgan 1977) so presumably less force is needed to compress a passive muscle. Because fiber rotation decreases as thickness compression increases, a submaximally activated muscle may operate at a lower gear ratio than a maximally active muscle. Furthermore, the influence of passive muscle fibers on gearing may vary depending on the spatial pattern of activation. In a submaximally activated muscle, the activated motor units may be uniformly distributed throughout the muscle or spatially segregated (English 1984; Bodine et al. 1988; Hodson-Tole et al. 2013). Rahemi et al. (2014) used a 3D finite element model to compare the influence of submaximal contractions on dynamic changes in architecture during contractions where the muscle is activated uniformly or regionally. They found that in muscles with either regional or uniform activation that each had around 10% of fibers activated, fiber rotation and thickness compression varied between models of uniform activity compared with regionalized activity. This suggests that not only do we not understand how level of activation influences the relationship between gearing and contractile force within a muscle but that this relationship may also be affected by the spatial distribution of active fibers in the muscle.

The pennation angle of fibers can vary within a muscle and this influences local fiber strains and hence, gearing, within a muscle (Azizi and Deslauriers 2014). Azizi and Deslauriers (2014) used a simple geometric model of muscle in which pennation angle varied along the length of the muscle. For a given muscle strain in an active contraction, they found that the fibers with a lower pennation angle underwent larger fiber strains than the more pennate fibers and this difference increased with the magnitude of muscle strain. This means that under most conditions, fibers with a higher pennation angle operated at a higher gear ratio than fibers with lower pennation angle. Thus, their model shows that variation in architecture can lead to variation in gearing within a muscle. While the modeling results were consistent with empirical data, the model generally predicted higher fiber strains than measured experimentally. This may be due to the model's assumption that muscle thickness does not change when the muscle contracts. As previously discussed, many studies show that a muscle's thickness changes during a contraction (Zuurbier and Huijing 1992; Herbert and Gandevia 1995; Maganaris et al. 1998; Azizi et al. 2008; Wakeling et al. 2011; Randhawa et al. 2013; Dick and

Wakeling 2017). Furthermore, changes in thickness occurring in a contraction may vary across muscle regions according to regional differences in the pennation angles of the fibers. For example, assuming similar fiber contractile forces, fibers with a larger pennation angle would exert a larger off-axis thickness force and thus may undergo a greater decrease in thickness than fibers with a smaller pennation angle. There is some experimental evidence that muscle fiber strains are not uniformly distributed in muscles with complex architectures (Böl et al. 2015), but additional empirical measures of regional heterogeneity in both fiber strain and shape change are necessary to better understand the influence of variation in architecture on variation in gearing.

We hypothesize that the IM spring not only controls shape change but can also contribute to muscle work and power production. The hypothesized mechanism of variable gearing implies that the IM spring is loaded and stretched by off-axis forces. This loading of the IM spring during contraction could potentially be stored as elastic energy that is recovered at the end of the contraction to contribute to the mechanical output of the muscle. Energy recovered from the IM spring could reduce the cost of force production in muscle fibers. For example, during a muscle stretch-shorten cycle, the muscle may store energy in the IM spring if the muscle compresses in the thickness direction as it lengthens. This thickness compression may result in fibers rotating to lower angles of pennation and the fibers may rotate to greater angles of pennation when the muscle springs back in the thickness direction to recover the stored energy to aid in muscle shortening. While the role of off-axis energy storage in the cross-bridges has been hypothesized to occur at the sarcomere level (Williams et al. 2012), the potentially significant role of off-axis energy storage in the IM spring in the cycling of elastic energy in locomotion has not been explored.

Summary

The ability of pennate muscles to undergo dynamic changes in architectural gearing during contractions is an important phenomenon that results from interactions of contractile elements, connective tissue structures, and fluid pressures occurring on multiple scales. Through architectural gearing, muscles can partially circumvent sarcomere-level constraints on force and velocity production by undergoing dynamic changes in architecture to amplify fiber speeds when needed. Across animals studied, muscles consistently display variable gearing, suggesting that force-driven dynamic and variable architectural gearing may be a feature common to most pennate muscles.

Acknowledgments

The authors thank Natalie Holt and David Williams for organizing this symposium. We are grateful to Rich Marsh, Bill Kier, Michael Rosario, and David Sleboda for insightful discussions.

Funding

This research was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under award number F32AR067564 to C.M.E. and award number AR055295 to T.J.R. and the National Science Foundation award IOS-1354289 to T.J.R. and CMMI-1436476 to E.A. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National lnstitutes of Health.

References

Alexander RM. 1968. Animal mechanics. London: Sidgwick and Jackson.

Azizi E, Brainerd EL, Roberts TJ. 2008. Variable gearing in pennate muscles. Proc Natl Acad Sci U S A 105:1745–50.

Azizi E, Deslauriers A, Holt N, Eaton C. 2017. Resistance to radial expansion limits muscle strain and work. Biomech Model Mechanobiol 16:1633–43.

Azizi E, Deslauriers AR. 2014. Regional heterogeneity in muscle fiber strain: the role of fiber architecture. Front Physiol 5:303.

Azizi E, Gillis GB, Brainerd EL. 2002. Morphology and mechanics of myosepta in a swimming salamander (*Siren lacertina*). Comp Biochem Physiol A Mol Integr Physiol 133:967–78.

Azizi E, Roberts TJ. 2013. Variable gearing in a biologically inspired pneumatic actuator array. Bioinspir Biomim 8:026002.

Azizi E, Roberts TJ. 2014. Geared up to stretch: pennate muscle behavior during active lengthening. J Exp Biol 217:376–81.

Baskin RJ, Paolini PJ. 1967. Volume change and pressure development in muscle during contraction. Am J Physiol 213:1025–30.

Benninghoff A, Rollhäuser H. 1952. Zur inneren mechanik des gefiederten muskels. Pflugers Arch Gesamte Physiol Menschen Tiere 254:527–48.

Bodine SC, Garfinkel A, Roy RR, Edgerton VR. 1988. Spatial distribution of motor unit fibers in the cat soleus and tibialis anterior muscles: local interactions. J Neurosci 8:2142–52.

Bodine SC, Roy RR, Meadows DA, Zernicke RF, Sacks RD, Fournier M, Edgerton VR. 1982. Architectural, histochemical, and contractile characteristics of a unique

biarticular muscle: the cat semitendinosus. J Neurophysiol 48:192–201.
Böl M, Leichsenring K, Ernst M, Wick C, Blickhan R, Siebert T. 2015. Novel microstructural findings in M. plantaris and their impact during active and passive loading at the macro level. J Mech Behav Biomed Mater 51:25–39.
Borelli GA. 1680. De Motu Animalium. Rome, Italy: Bernabo.
Brainerd EL, Azizi E. 2005. Muscle fiber angle, segment bulging and architectural gear ratio in segmented musculature. J Exp Biol 208:3249–61.
Dick TJM, Wakeling JM. 2017. Shifting gears: dynamic muscle shape changes and force–velocity behavior in the medial gastrocnemius. J Appl Physiol 123:1433–42.
Elliott G, Lowy J, Worthington C. 1963. An X-ray and light-diffraction study of the filament lattice of striated muscle in the living state and in rigor. J Mol Biol 6:295–305.
Eng CM, Roberts TJ. 2018. Aponeurosis influences the relationship between muscle gearing and force. J Appl Physiol published online (doi:10.1152/japplphysiol.00151.2018).
English AW. 1984. An electromyographic analysis of compartments in cat lateral gastrocnemius muscle during unrestrained locomotion. J Neurophysiol 52:114–25.
Gans C. 1982. Fiber architecture and muscle function. Exercise and Sport Science Reviews. Lexington (MA): Franklin University Press. p. 160–207.
Gans C, Bock WJ. 1965. The functional significance of muscle architecture: a theoretical analysis. Adv Anat Embryol Cell Biol 38:115–42.
Gindre J, Takaza M, Moerman KM, Simms CK. 2013. A structural model of passive skeletal muscle shows two reinforcement processes in resisting deformation. J Mech Behav Biomed Mater 22:84–94.
Hatze H. 1978. A general myocybernetic control model of skeletal muscle. Biol Cybern 28:143–57.
Herbert RD, Gandevia SC. 1995. Changes in pennation with joint angle and muscle torque: in vivo measurements in human brachialis muscle. J Physiol 484:523–32.
Hodson-Tole EF, Loram ID, Vieira TM. 2013. Myoelectric activity along human gastrocnemius medialis: different spatial distributions of postural and electrically elicited surface potentials. J Electromyogr Kinesiol 23:43–50.
Holt NC, Danos N, Roberts TJ, Azizi E. 2016. Stuck in gear: age-related loss of variable gearing in skeletal muscle. J Exp Biol 219:998–1003.
Huijing P, Woittiez R. 1985. Length range, morphology and mechanical behaviour of rat gastrocnemius muscle during isometric contraction at the level of the muscle and muscle tendon complex. Neth J Zool 35:505–16.
Huxley H. 1951. Low-angle X-ray diffraction studies on muscle. Disscus Faraday Soc 11:148–9.
Kurokawa S, Fukunaga T, Fukashiro S. 2001. Behavior of fascicles and tendinous structures of human gastrocnemius during vertical jumping. J Appl Physiol 90:1349–58.
Lichtwark GA, Bougoulias K, Wilson A. 2007. Muscle fascicle and series elastic element length changes along the length of the human gastrocnemius during walking and running. J Biomech 40:157–64.
Lieber RL, Fazeli BM, Botte MJ. 1990. Architecture of selected wrist flexor and extensor muscles. J Hand Surg 15:244–50.
Lieber RL, Fridén J. 2000. Functional and clinical significance of skeletal muscle architecture. Muscle Nerve 23: 1647–66.
Maganaris CN, Baltzopoulos V, Sargeant AJ. 1998. In vivo measurements of the triceps surae complex architecture in man: implications for muscle function. J Physiol 512:603–14.
Millman BM. 1998. The filament lattice of striated muscle. Physiol Rev 78:359–91.
Morgan D. 1977. Separation of active and passive components of short-range stiffness of muscle. Am J Physiol Cell Physiol 232:45–9.
Narici MV, Binzoni T, Hiltbrand E, Fasel J, Terrier F, Cerretelli P. 1996. In vivo human gastrocnemius architecture with changing joint angle at rest and during graded isometric contraction. J Physiol 496:287–97.
Otten E. 1988. Concept and models of functional architecture in skeletal muscle. Exerc Sport Sci Rev 16:89–137.
Pfuhl W. 1937. Die gefiederten Muskeln, ihre Form und ihre Wirkungsweise. Z Anat Entwicklungsgesch 106:749–69.
Powell PL, Roy RR, Kanim P, Bello M, Edgerton VR. 1984. Predictability of skeletal muscle tension from architectural determinations in guinea pig hindlimbs. J Appl Physiol 57:1715–21.
Rahemi H, Nigam N, Wakeling JM. 2014. Regionalizing muscle activity causes changes to the magnitude and direction of the force from whole muscles—a modeling study. Front Physiol 5:298.
Raiteri BJ, Cresswell AG, Lichtwark GA. 2016. Three-dimensional geometrical changes of the human tibialis anterior muscle and its central aponeurosis measured with three-dimensional ultrasound during isometric contractions. PeerJ 4:e2260.
Randhawa A, Jackman ME, Wakeling JM. 2013. Muscle gearing during isotonic and isokinetic movements in the ankle plantarflexors. Eur J Appl Physiol 113:437–47.
Sacks RD, Roy RR. 1982. Architecture of the hindlimb muscles of cats: functional significance. J Morphol 173:185–95.
Schulte H. 1961. The characteristics of the McKibben artificial muscle. The Application of External Power in Prosthetics and Orthotics. Washington (DC): National Academy of Sciences - National Research Council.
Shin DD, Hodgson JA, Edgerton VR, Sinha S. 2009. In vivo intramuscular fascicle–aponeuroses dynamics of the human medial gastrocnemius during plantarflexion and dorsiflexion of the foot. J Appl Physiol 107:1276–84.
Siebert T, Rode C, Till O, Stutzig N, Blickhan R. 2016. Force reduction induced by unidirectional transversal muscle loading is independent of local pressure. J Biomech 49:1156–61.
Siebert T, Till O, Stutzig N, Günther M, Blickhan R. 2014. Muscle force depends on the amount of transversal muscle loading. J Biomech 47:1822–8.
Sleboda DA, Roberts TJ. 2017. Incompressible fluid plays a mechanical role in the development of passive muscle tension. Biol Lett 13:20160630.
Steno N. 1667. Elementorum Myologiae Specimen, seu Musculi Descripto Geometrica. Florence, Italy.
Stensen N, Collins ME, Maquet P, Kardel T. 1994. Specimen of elements of myology. Trans Am Phil Soc 84:76–230.

Wakeling JM, Blake OM, Wong I, Rana M, Lee SS. 2011. Movement mechanics as a determinate of muscle structure, recruitment and coordination. Phil Trans R Soc B Biol Sci 366:1554–64.

Ward SR, Eng CM, Smallwood LH, Lieber RL. 2009. Are current measurements of lower extremity muscle architecture accurate? Clin Orthop Relat Res 467:1074–82.

Williams CD, Regnier M, Daniel TL. 2012. Elastic energy storage and radial forces in the myofilament lattice depend on sarcomere length. PLoS Comput Biol 8:e1002770.

Williams CD, Salcedo MK, Irving TC, Regnier M, Daniel TL. 2013. The length–tension curve in muscle depends on lattice spacing. Proc R Soc Lond B Biol Sci 280:20130697.

Zuurbier C, Huijing P. 1993. Changes in geometry of actively shortening unipennate rat gastrocnemius muscle. J Morphol 218:167–80.

Zuurbier CJ, Huijing PA. 1992. Influence of muscle geometry on shortening speed of fibre, aponeurosis and muscle. J Biomech 25:1017–26.