

REVIEW

Contribution of elastic tissues to the mechanics and energetics of muscle function during movement

Thomas J. Roberts*

ABSTRACT

Muscle force production occurs within an environment of tissues that exhibit spring-like behavior, and this elasticity is a critical determinant of muscle performance during locomotion. Muscle force and power output both depend on the speed of contraction, as described by the isotonic force-velocity curve. By influencing the speed of contractile elements, elastic structures can have a profound effect on muscle force, power and work. In very rapid movements, elastic mechanisms can amplify muscle power by storing the work of muscle contraction slowly and releasing it rapidly. When energy must be dissipated rapidly, such as in landing from a jump, energy stored rapidly in elastic elements can be released more slowly to stretch muscle contractile elements, reducing the power input to muscle and possibly protecting it from damage. Elastic mechanisms identified so far rely primarily on in-series tendons, but many structures within muscles exhibit spring-like properties. Actomyosin cross-bridges, actin and myosin filaments, titin, and the connective tissue scaffolding of the extracellular matrix all have the potential to store and recover elastic energy during muscle contraction. The potential contribution of these elements can be assessed from their stiffness and estimates of the strain they undergo during muscle function. Such calculations provide boundaries for the possible roles these springs might play in locomotion, and may help to direct future studies of the uses of elastic elements in muscle.

KEY WORDS: Metabolic economy, Tendon, Elastic energy, Locomotion

Introduction

It is difficult, if not impossible, to isolate muscle performance from the influence of elastic structures. This truth holds significance for how muscles operate *in vivo*, but in many studies of isolated muscle mechanics, it is an issue of practical importance. Early muscle physiologists went to lengths to develop apparatus that could eliminate the contribution of elastic elements by maintaining contractions at a constant force. As A. V. Hill put it, in 1938, ‘The great virtue of the constant load is simply that it eliminates one variable, it avoids change of length of the undamped elastic component’ (Hill, 1938). Then, in graceful prose that deserves arrangement as verse, Hill explains that:

If load be not constant,

Then the elastic element will vary in length

As the load varies:

To it we can then apply the ordinary rules for elastic bodies.

Since Hill, the instrumentation has evolved from clever mechanical systems to sophisticated servomotors, but the importance of characterizing muscle performance under constant force remains.

But for muscles operating *in vivo*, it is safe to say that under almost all conditions of movement, the load ‘be not constant’. The stretch and recoil of elastic elements in series with muscles may present an artifact to be avoided in some muscle mechanics studies, but in the body it plays an essential role in allowing muscles to perform a wide range of tasks. Springs exchange energy, both with the muscle and with the external environment, and influence muscle speed, force and power. We also know that within muscles, the elastic behavior of many structures plays a fundamental role in force production. Elastic behavior from the cross-bridges to the whole muscle and associated tendon influences everything from the energetics to the mechanics to the neuromotor control of muscle.

The goal here is to review some of our current understanding of the contribution of elastic elements to muscle function during locomotion. Among vertebrates, the elastic functions of in-series tendons provide the most familiar and best-described elastic mechanisms, and some of these are reviewed briefly. Elastic structures within muscles, including myofilaments and the extracellular matrix (ECM), are receiving increasing attention for their possible roles in locomotion, but our understanding of how and where these springs might contribute is still developing. I will review what we know about the mechanical properties of these springs, and use the known elastic properties to explore how these springs might contribute to movement.

Familiar springs – the cooperative function of muscles and tendons

Power amplification and attenuation – altering the timing of muscle work

Some activities, such as jumping, require the rapid production of mechanical energy by muscle contraction, while other activities, like landing, require the rapid absorption of energy by active muscle lengthening. Tendon springs cannot act as a source or a significant sink for energy for such activities, because they can only return energy put into them, and they return almost all of it (Ker, 1981; Matson et al., 2012). The benefits of the spring-like action of tendon during rapid, high-power activities ultimately result from the contractile characteristics of skeletal muscle. The force–velocity properties in particular describe limits to muscle speed and power (Fig. 1). For a single-shot activity, like jumping, the performance will be directly related to the work done in the contraction, or the force produced multiplied by the distance shortened. When a muscle shortens very rapidly, it develops little force. Some specialized jumpers circumvent this constraint by storing muscle work in series elastic elements prior to motion, then releasing this energy rapidly during the jump. Such mechanisms spread out the time over which the contraction occurs (Fig. 2), which allows muscles to shorten more slowly. Because of force–velocity properties, slower contractions produce higher forces and do more work (given the same strain). The release of elastic energy in such systems can produce power outputs that exceed the capacity of the

Department of Ecology and Evolutionary Biology, Brown University, Providence, RI 02912, USA.

*Author for correspondence (Thomas_Roberts@Brown.edu)

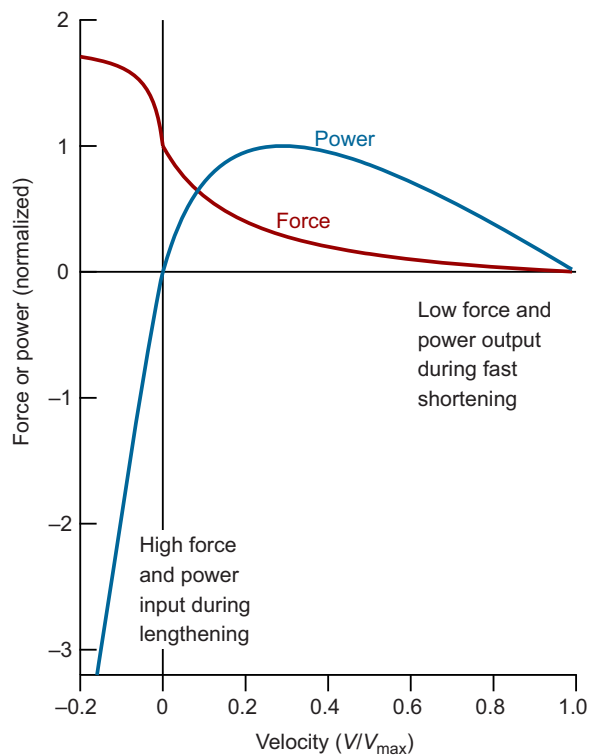


Fig. 1. Typical force–velocity relationship for skeletal muscle. For shortening muscle (positive velocities), force declines as a function of shortening velocity. Power is maximum at intermediate shortening velocities, typically $\sim 0.3 V_{\max}$ (where V_{\max} is the unloaded maximal shortening velocity). Only very low rates of lengthening (negative velocities) are required to develop very high forces and power inputs to the muscle.

muscle for power production; thus, this process is often called ‘power amplification’. In vertebrates, this mechanism has been demonstrated in specialized jumpers like bushbabies (Aerts, 1998) and frogs (Marsh and John-Alder, 1994), but it has also been observed during ordinary running accelerations (Roberts and Scales, 2002). The general principle, that the storage and release of elastic energy alters the timing of muscle work relative to the timing of motion, may be an important feature in many rapid movements.

Tendon springs play an important role in energy absorption as well. Any controlled decrease in the energy of the body requires the dissipation of mechanical energy by muscles that generate tension as they are lengthened by external forces. Such eccentric contractions can occur rapidly for a quick deceleration, such as in landing from a jump. Recent work suggests that tendon action significantly alters the timing of energy dissipation in muscles during these events. In the lateral gastrocnemius muscle of wild turkeys executing a landing, energy is initially absorbed by the tendon during the first phase of landing (Konow et al., 2012; Konow and Roberts, 2015), when there is joint motion but little to no muscle fiber lengthening (Fig. 2). This energy absorption is associated with very high power inputs to the tendon, during a period when the muscle produces force but does not lengthen or absorb energy. This is followed by a longer period, largely after joint motion has ended, when the tendon recoils to stretch active muscle, doing work on it. As a result, the maximum rate of power input to the muscle is much lower than that experienced by the tendon. This action has been called ‘power attenuation’ (Konow et al., 2012; Roberts and Konow, 2013).

Power amplification and power attenuation can be viewed as the same process operating in opposite directions (Fig. 2). In power amplification, muscles perform work slowly on tendons, and this energy is then released rapidly to power movement. In power attenuation, the environment performs work rapidly on the tendon, then this energy is released slowly to do work on active muscles as they lengthen. In both cases, tendon action changes the time course of muscle work. However, the benefits of tendon action are likely quite different for the two activities. During activities that require energy production, such as jumping, tendon springs allow for an increase in muscle work output and better performance. During energy dissipation, such as in landing, power attenuation by tendons may protect muscle from damage. The temporary storage of energy in tendons reduces the power input to muscle and the maximum rate of lengthening, both of which may be associated with muscle damage during active lengthening (McHugh et al., 1999; Proske and Morgan, 2001). Because tendon stretch limits muscle lengthening, it also tends to prevent muscle from reaching the very high forces that would be developed were the muscle to operate eccentrically (i.e. at negative velocities) during the period of force development (Fig. 1) (Roberts and Azizi, 2010; Roberts and Konow, 2013). A limit to maximum muscle forces during rapid energy-absorbing events may be important for reducing the risk of damage to muscle as well as associated musculoskeletal structures.

Recycling energy in running – reducing muscle work

Perhaps the most familiar elastic mechanism in vertebrate locomotion occurs in running, the ‘bouncing’ gait. The idea that tendons store and recover significant energy during running is now widely supported by evidence ranging from analyses of whole-body mechanics and energetics (Cavagna et al., 1977), to direct measurements of the behavior of individual muscles and tendons in running animals (Roberts et al., 1997; Lichtwark et al., 2007), to modeling, which shows that many of the key mechanical features of running emerge from the behavior of simple spring–mass systems (Blickhan, 1989; McMahon and Cheng, 1990; Geyer et al., 2006). It is clear that tendon elasticity provides a significant portion of the cyclic work as the kinetic and potential energy of a runner’s center of mass fluctuates with each step. The springiness of tendons lets runners move like bouncing balls.

Is there a benefit to elastic energy storage and recovery during running? The answer to this question might seem obvious: work done by tendons does not have to be performed by muscles; thus, tendons reduce muscle work, and therefore metabolic cost, during running. Such inference is supported by measurements of work, metabolic cost and efficiency during running that show that runners perform the work of running with an efficiency that exceeds that of isolated muscle (Cavagna et al., 1964; Heglund et al., 1982; Minetti et al., 1999). These observations support the idea that tendons do much of the work ‘for free’, thus increasing the measured efficiency.

However, a recent study challenges the idea that tendons reduce the cost of running by reducing muscle work (Holt et al., 2014). Measurements of heat production in isolated muscles showed that the cost of force production in isometric contractions was not significantly different from the cost of force production in muscles that produced force while undergoing a cycle of lengthening followed by shortening. The isometric contractions were meant to mimic the conditions of ‘ideal’ elastic energy storage, where muscle must only produce force, while the lengthening–shortening contraction mimicked the muscle action required to effectively replace the role of tendon in cyclic energy absorption and

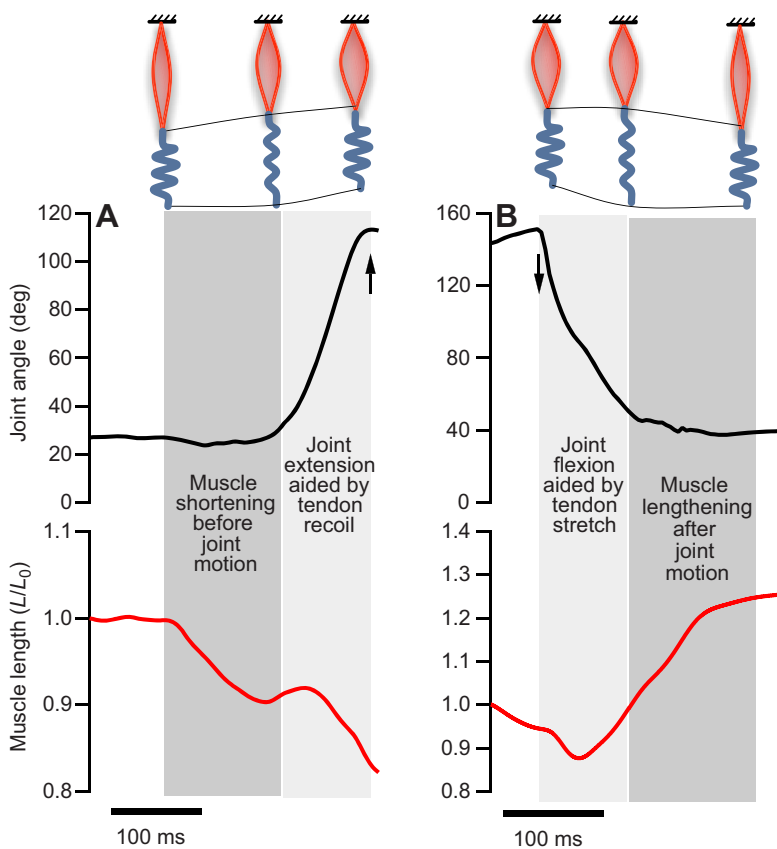


Fig. 2. The influence of tendon elasticity on the timing of muscle length change and work. Examples are shown of (A) a bullfrog jumping and (B) a turkey landing. In both instances, much of the muscle fiber length change occurs as a result of tendon elasticity, rather than joint motion. In the frog plantaris (A), muscle fibers shorten before the ankle extends, so that muscle shortening occurs over a time period that is about twice the duration of the period of ankle extension. In the turkey gastrocnemius (B), ankle flexion immediately following toe-down (arrow) is accompanied by relatively little change in muscle fiber length, indicating that this motion is accommodated by tendon stretch. Most of the muscle fiber lengthening occurs when joint flexion is complete, indicating that active fibers are lengthened by tendon recoil. Tendon action in A allows for high power outputs during the jump; in B, tendon action enables high power inputs to the muscle–tendon system while limiting power input to the muscle. In both cases, the time course of muscle action is altered by tendon action. Arrows indicate toe-off (bullfrog) and toe-down (turkey). Data in A are from Astley and Roberts (2012); data in B are from Konow and Roberts (2015).

production. All runners must produce the same force against the ground – one body weight – over an entire stride, and thus the cost of force production in muscles is a good currency for evaluating muscle energy use during locomotion. The muscles in Holt's study also showed high efficiencies when undergoing lengthening–shortening behaviors, which is consistent with the observation that muscle shortening work may be less metabolically expensive when it follows lengthening (Trinh and Syme, 2007), or when it occurs during muscle relaxation (Lou et al., 1999). These studies are examples of only a few that have examined muscle energetics during the kind of dynamic contractions that occur *in vivo*. If we are to understand the energetics of muscle function during running, and the role of tendon springs, more studies of the energetics of muscle contraction under the kind of dynamic conditions that occur *in vivo* are needed.

Other lines of evidence support the idea of energy savings from the reduction of muscle work by elastic mechanisms. In human locomotion, interventions that would be expected to disrupt or reduce elastic energy storage tend to increase metabolic cost. Running at lower than preferred stride frequencies increases metabolic cost and may disrupt the normal behavior of a resonant elastic system (Snyder and Farley, 2011). Reducing muscle work by means of a powered orthosis reduces metabolic cost, but not by as much as predicted from typical models of muscle efficiency, and it has been suggested that the orthosis reduces elastic energy storage and return (Sawicki and Ferris, 2008). Evidence from comparative studies also supports a role for tendons in energy savings. Wallabies and kangaroos can increase hopping speed with little increase in metabolic cost, and it has been shown that the amount of energy stored elastically increases with increasing speed in these species (Biewener et al., 1998). Isolated muscle studies also suggest an

energetic benefit of elastic energy storage and recovery; the efficiency of cyclic work in an isolated muscle increases when an artificial tendon is added in series (Lichtwark and Barclay, 2010). Yet, the proposed role for elastic energy storage and recovery is the reduction of muscle work, and at least for one study of frog muscles, it does not appear that replacing muscle work with tendon work reduces cost (Holt et al., 2014). We have more to learn about the energetic significance of elastic energy storage and recovery in cyclic motions.

Elastic energy storage and recovery in tendons during running may provide several benefits beyond the reduction of muscle work. The long slender tendons of distal limb muscles reduce the moment of inertia of the limbs, which may contribute to a reduction in the cost of swinging the limbs (Marsh et al., 2004; Ellerby and Marsh, 2006). Long tendons also allow for short muscle fibers. Because force is proportional to cross-sectional area independent of fiber length, shorter muscles produce force more economically than long muscles because the volume of active muscle per unit force is smaller (Roberts et al., 1998; Biewener and Roberts, 2000). The protective effect of tendons observed during rapid decelerations may apply as well during the impact phase of running. The compliant gait provided by elastic structures may also aid in stability and a robust response to perturbations (Ernst et al., 2012). In addition, it has been proposed that a compliant gait reduces energy loss to sloshing viscera (Daley and Usherwood, 2010). It is worth exploring these questions further, because knowing the selective factors that have shaped the elastic properties of tetrapod limbs will inform our understanding of musculoskeletal evolution. Such analysis may also be of value to engineers and roboticists, as it may suggest which features of a compliant gait are worth mimicking, and which are not.

Capacity for elastic energy storage in muscle and tendon springs

Muscles are full of springs. The elastic properties of cross-bridges, and actin, myosin and titin filaments, as well as collagen fibers within the ECM, are central to their function. The elastic behavior of these structures has been characterized, and we can predict their mechanical action as springs. The idea that the elasticity of these structures is essential to their function is supported by observations that variation in elastic properties correlates with variation in function. For example, a point mutation in the head domain of myosin that alters cross-bridge stiffness is also associated with a change in muscle force output (Kohler et al., 2002). What is less clear is whether the elastic properties of the springs within muscles provide useful elastic storage and recovery for locomotion. The goal of the analysis below is to use the well-defined properties of elastic elements within muscles to explore their potential to act as locomotor springs. Here, it is important to clarify what is meant by ‘locomotor spring’, which I define as an elastic element that exchanges (i.e. stores and recovers) energy with the environment. Elastic energy storage and recovery in running provides a useful example. A locomotor spring during running would store mechanical energy lost by the body in the first half of the step and then return that energy to help lift and reaccelerate the body in the second half of the step.

Cross-bridge and myofilament elasticity

The elastic properties of cross-bridges and myofilaments rest at the very heart of force production in skeletal muscle. Evidence from biophysical models and experiments indicates that compliance in both the cross-bridges and the myofilaments is essential to the process of force production by actomyosin cross-bridges. The majority of cross-bridge compliance appears to rest in the head (S1 sub-fragment) and not the tail (S2 sub-fragment) of the myosin molecule (Kohler et al., 2002; Brunello et al., 2014). This compliance is functionally significant because it allows independence between the working stroke of cross-bridges and force production (Offer and Ranatunga, 2010), and it allows for small cross-bridge strokes during isometric force production (Brunello et al., 2014). Actin and myosin filaments also undergo measurable strain during force production, and compliance in these elements is central to models of force production by actomyosin cross-bridges (Offer and Ranatunga, 2013; Piazzesi et al., 2014).

The primary limitation to the potential for cross-bridges to act as useful locomotor springs is that cross-bridges cycle, and any strain energy within a cross-bridge is lost once the myosin head detaches from actin. For cross-bridges to store and recover external energy over the course of a locomotor cycle, the event must (1) occur within the duration of a cross-bridge cycle and (2) involve muscle strains that do not exceed that which can be accommodated by elastic deformation of the cross-bridge. Both of these factors likely significantly limit both the total energy that can be stored and the kind of movement that can benefit from the elastic behavior of cross-bridges.

Energetic measurements provide one source for estimating the rate of cross-bridge turnover. Estimates of the rate of ATP hydrolysis taken from heat measurements, combined with estimates of the density of cross-bridges in muscle, can be used to calculate the rate of cross-bridge turnover, assuming one cross-bridge cycle per ATP molecule consumed (Barclay et al., 2010). Such calculations yield an estimate of a rate of approximately 1.5 s^{-1} for isometric force production in frog muscle at 0°C (Barclay et al., 2010), or a duration

of about 670 ms for each cycle. Assuming a duty cycle of ~ 0.5 (Kaya and Higuchi, 2010), this translates to a duration of about 335 ms for the bound life of a cross-bridge in frog muscle contracting isometrically at 0°C . Studies of rabbit muscle fibers suggest that Q_{10} values for cross-bridge detachment rates are quite high, between 3 and 4 (Wang and Kawai, 2001). For a Q_{10} of 3, we would expect the process of detachment and reattachment to increase by a factor of 27 from 0 to 30°C ; thus, the duration of binding for a cross-bridge would be less than 10 ms at typical avian or mammalian body temperatures. This likely represents an overestimate, because the rate of cross-bridge detachment increases in shortening (Piazzesi et al., 2007) and lengthening (Lombardi and Piazzesi, 1990) muscle, and a muscle would have to shorten and lengthen for cross-bridges to cycle elastic energy. The duration of the stance phase of running for a human sprinting at maximum speed is of the order of 100 ms (Weyand et al., 2010), and the powerstroke of a 3.4 g rufous hummingbird’s wingbeat lasts approximately 25 ms (Tobalske et al., 2007). These values suggest that the time course of cross-bridge cycling is not favorable for energy storage over the course of a locomotor cycle in vertebrates.

The small strains that can be accommodated by cross-bridge elasticity alone also make it unlikely that cross-bridges can contribute to significant elastic energy storage and recovery in locomotion. Alexander and Bennet-Clark (1977) used estimates of maximum extension of an attached cross-bridge to calculate that the capacity for energy storage in tendons in typical vertebrate skeletal muscles is 35–70 times the energy that can be stored and recovered in stretched cross-bridges. Energy storage is low because the size of the elastic deformation is low. The size of the working stroke of a cross-bridge, estimated from x-ray diffraction and contractile measurements on single fibers (Reconditi et al., 2004; Piazzesi et al., 2007), can be as high as 10–13 nm. For a typical vertebrate half-sarcomere length of $1.1 \mu\text{m}$, this means a maximum possible strain of 0.9–1.2%. This likely represents an upper limit, because the stroke size at high loads may be smaller than the upper limit value of 10–13 nm (Reconditi et al., 2004; Piazzesi et al., 2007).

It is straightforward to calculate the potential for energy storage in a spring of known properties and dimensions, and we can apply such calculations to elastic elements within muscles. For a purely elastic spring with a single value of stiffness (i.e. a linear force–length relationship):

$$W = \frac{f \cdot x}{2}, \quad (1)$$

where W is the work done on the spring (i.e. the energy stored, in J), f is the force (in N) and x is the displacement (in m). The energy stored per unit spring mass can be calculated by dividing both sides by mass, M :

$$\frac{W}{M} = \frac{f \cdot x}{2} \cdot \frac{1}{M}. \quad (2)$$

For a material with known density and cylindrical shape, we can substitute for mass:

$$\frac{W}{M} = \frac{f \cdot x}{2} \cdot \frac{1}{A \cdot L \cdot \rho}, \quad (3)$$

where A is the cross-sectional area (in m^2), L is the length (in m) and ρ is the material density (in kg m^{-3}). Stress (σ) in the material is given by f/A . Substituting $\sigma \cdot A$ for f , we get:

$$\frac{W}{M} = \frac{\sigma \cdot A \cdot x}{2} \cdot \frac{1}{A \cdot L \cdot \rho}. \quad (4)$$

Simplifying and rearranging:

$$\frac{W}{M} = \frac{\sigma \cdot (x/L)}{2 \cdot \rho} = \frac{\sigma \cdot \epsilon}{2 \cdot \rho}, \quad (5)$$

where ϵ is the strain. Eqn 5 can be used to calculate spring energy storage, and it also can be used to calculate muscle work. Because vertebrate muscles are all capable of about the same maximum stress and strain during active contraction, this equation is the basis for the idea that all vertebrate skeletal muscles should be capable of producing the same work per unit mass, regardless of architecture.

To calculate an upper limit for cross-bridge elastic energy storage, we can substitute into Eqn 5 the maximum muscle strain that can be accommodated by bound cross-bridges, calculated above to be 1.2%. A maximum isometric stress of 250 kPa is a typical value for vertebrate skeletal muscle (Marsh, 1994). This value increases nearly 2-fold during active muscle lengthening, so an assumption of 500 kPa for maximum stress during loading of cross-bridge springs is reasonable. Muscle has a density of about 1060 kg m^{-3} (Mendez and Keys, 1960). Substituting these values into Eqn 5 yields a maximum energy storage value of 2.8 J kg^{-1} muscle (Fig. 3). The small strains and rapid cycling rates of cross-bridges may represent a limit to their ability to act as locomotor springs, but the energy that can be stored in cross-bridges is not insignificant.

Beyond cross-bridges, actin and myosin filaments also have measurable compliance and undergo elastic deformation during muscle force production. In practice, the elastic behavior of these filaments is determined by the force response to rapid length changes applied to active muscle fibers (Piazzesi et al., 2014; Brunello et al., 2014), which results in estimates of the compliance of the whole filament backbone (actin and myosin together, though it is thought that most of the compliance rests in the actin filament; Offer and Ranatunga, 2010). Recent estimates of actin and myosin filament compliance in isolated frog fibers yield a value of about 13 nm MPa^{-1} per half sarcomere (Brunello et al., 2014). For a half-sarcomere length of $1.1 \text{ }\mu\text{m}$, this corresponds to an elastic modulus of approximately 85 MPa. Strain is stress divided by elastic modulus, so for a maximum muscle stress during lengthening of 500 kPa the filaments should strain by $0.50/85=0.006$, or a length change of 0.6% of resting length. The energy that can be stored in the filaments under maximum load, calculated by Eqn 5, is about 1.4 J kg^{-1} .

Titin- and single-fiber-based passive forces

Titin is the myofilament with the greatest potential to act as a locomotor spring. Isolated titin filaments exhibit spring-like properties and can undergo elastic deformations that are orders of magnitude greater than the small strain in actin and myosin filaments (Kellermayer et al., 1997). Although it is difficult to assess the exact limit to elastic strain of titin within muscle, studies of passive deformation of muscles, fibers and isolated filaments all indicate that titin accommodates large strains. A study of split muscle fibers from rabbit psoas shows that these fibers deform elastically for approximately $1 \text{ }\mu\text{m}$ beyond resting length, after which they yield to extend without further increases in force (Wang et al., 1993). Taking the strain over this elastic region as the region over which titin can act as a useful locomotor spring, and assuming a sarcomere length of $2.4 \text{ }\mu\text{m}$, this gives a total elastic extension of about 30–40% of muscle length. The negligible displacement that limits the potential contribution of actin and myosin as locomotor springs does not apply to titin.

The capacity for elastic energy storage in structures such as titin and other elastic structures that contribute to passive force in muscle is most conveniently calculated by considering that:

$$E = \frac{\sigma}{\epsilon}, \quad (6)$$

where E is the elastic modulus. Thus:

$$\sigma = E \cdot \epsilon, \quad (7)$$

and substituting into Eqn 5 gives:

$$\frac{W}{M} = \frac{E \cdot \epsilon^2}{2 \cdot \rho}. \quad (8)$$

This equation can be used to calculate the energy storage capacity of muscle springs for a given strain, using an estimate of tangent elastic modulus (use of a tangent modulus will tend to produce overestimates of energy storage for elastic elements with J-shaped or exponential force–length curves). In estimating the modulus of the titin-based forces in muscle, the challenge is in isolating the

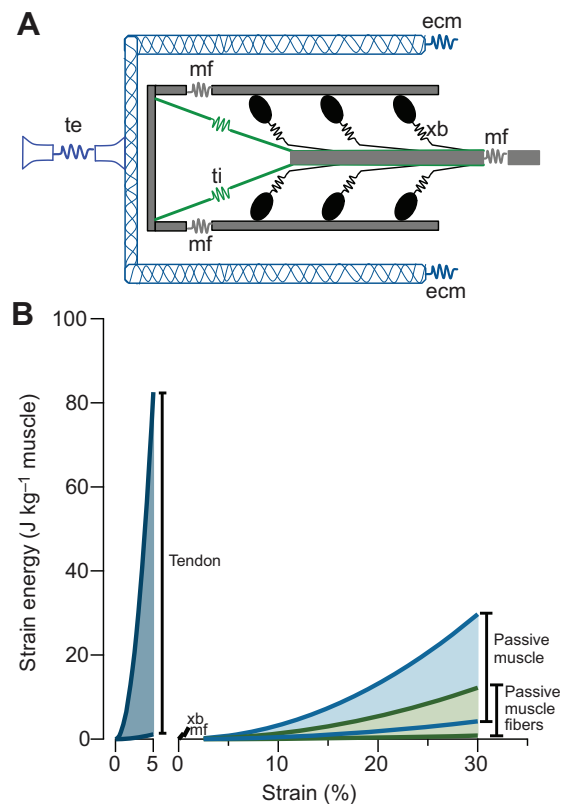


Fig. 3. Elastic energy storage potential for several muscle springs. (A) A diagrammatic representation of some spring elements associated with skeletal muscles. Elastic behavior can be characterized for the myofilaments (mf, which is a lumped spring behavior for myosin and actin), cross-bridges (xb), titin (ti), extracellular matrix (ecm) and tendon (te). (B) Estimates of muscle mass-specific capacity for elastic energy storage in muscle and tendon spring elements. Ranges are given for tendon (dark blue), passive muscle (light blue) and passive isolated muscle fibers (green). Maximum elastic strains within cross-bridges (black) and myofilaments (gray) are small. Note that for all structures, energy storage is calculated per unit muscle mass. Strain is calculated with respect to tendon length for tendon, and relative to muscle length for all other structures. See text for a description of the methods used to calculate the values for B.

contributions of titin versus other contributors to force during passive stretch, such as the ECM and various intermediate proteins. Here, I take the approach of using estimates of stiffness from isolated titin molecules, isolated myofibrils and passive single fibers to estimate titin-based forces. Single muscle fibers include several elastic elements that may contribute to force development in passive muscle, including intermediate filaments that connect myofibrils to each other and to the sarcolemma transversely (e.g. desmin; Wang and Ramirez-Mitchell, 1983; Gajdosik, 2001). Single-fiber preparations also include some associated collagenous ECM. The force–length behavior of skinned rabbit psoas muscle fibers is predicted remarkably well from scaled values from single titin molecules, suggesting that passive single fibers provide a good estimate of titin-based force (Kellermayer et al., 1997). Modulus measured in skinned fibers for a number of different mammalian muscles ranges from just below 10 kPa to nearly 300 kPa (Kellermayer et al., 1997; Prado et al., 2005; Gillies and Lieber, 2011). A recent measurement of stiffness in single myofibrils falls within this range, with a modulus of approximately 50 kPa (Powers et al., 2014; estimated from the slope of passive stress versus length in their fig. 3).

Estimates of a range of capacities for energy storage based on modulus values for single fibers, myofibrils and titin molecules are given as a function of muscle strain in Fig. 3, based on a high value estimate of 288 kPa (Kellermayer et al., 1997) and a low one of 10 kPa (Gillies and Lieber, 2011). Because passive fibers are relatively compliant, their potential for energy storage at low strains is relatively small. When muscle fibers undergo relatively large strains, there is potential for significant energy storage in the structures that bear elastic loads in passive muscle fibers, at least for some muscles.

There is increasing evidence that muscle activation increases the non-cross-bridge stiffness in muscles, and it is thought that this results from an influence of activation on titin stiffness, possibly via Ca^{2+} -mediated binding of the N2A region of titin to actin (Nishikawa et al., 2012; Powers et al., 2014). Force measured in stretched single myofibrils is higher in the presence of calcium, even beyond the region of overlap of actin and myosin filaments, supporting the idea that stiffness of titin or some other intrafibrillar element may be higher than that measured in passive muscle fibers (Powers et al., 2014). In one study, stiffness in activated fibers measured beyond the region of actin–myosin overlap increased by 3-fold (Powers et al., 2014). The higher stiffness observed in calcium-activated myofibrils suggests a significantly higher capacity for energy storage than values for purely passive muscle would suggest. Such changes could elevate significantly the values for energy storage potential in fibers and whole muscles shown in Fig. 3.

Alterations in the stiffness of titin upon calcium activation would also change the muscle lengths at which titin can contribute as a locomotor spring. Passive forces are not developed in muscle typically until after the length at which peak active muscle force is produced. In cases where we have measurements of muscle operating length during locomotion, most force production appears to occur at lengths less than that at which passive force is developed (Azizi and Roberts, 2010; Rubenson et al., 2012). If non-cross-bridge elements are stiffened in the presence of Ca^{2+} , the fiber lengths at which they can contribute force may be shorter than suggested by the passive force–length curve. It has also been suggested that titin may wind on the long axis of actin during muscle contraction (Nishikawa et al., 2012), a mechanism that would also serve to shift the range of fiber lengths over which titin might contribute to force.

The spring function of titin within muscle has generated significant interest recently. Uncertainty remains as to the magnitude of the effect of energy storage and recovery in this myofilament. Many estimates of passive fiber stiffness fall near the lower limit values shown in Fig. 3, and for these muscles even a substantial increase in stiffness upon Ca^{2+} activation would result in a spring that is capable of storing relatively little energy even when experiencing relatively large strains. Titin clearly plays an important role in muscle function. Future work should determine how significant its elastic behavior is for the flow of mechanical energy in muscle.

Whole muscles and the ECM

The other ‘parallel’ spring in muscle is the connective tissue scaffolding of the ECM (Gajdosik, 2001; Gillies and Lieber, 2011). A challenge in calculating the stiffness of the portion of passive force associated with the ECM is that it is difficult to determine the relative contribution of connective tissue versus intrafibrillar elements, such as titin, to the force produced during a passive muscle stretch. The relative importance of titin versus other structures to passive muscle stiffness remains under debate (Magid and Law, 1985; Prado et al., 2005; Gillies and Lieber, 2011). A comparison of the stiffness of isolated muscle fibers, which include relatively little connective tissue, and bundles of fibers, suggests that the contribution of the ECM can be significant, and in many muscles appears to provide the majority of the passive force (reviewed in Gillies and Lieber, 2011). The ratio of passive muscle modulus to single-fiber modulus varies widely, from close to one in some muscles to greater than 10 in muscles of the human upper extremity (Gillies and Lieber, 2011).

An upper limit estimate of the capacity for elastic energy storage in the ECM can be obtained by using modulus estimates for whole muscles measured during a passive stretch. It should be emphasized that this represents an upper limit for ECM-based stiffness, because the stiffness of a whole muscle will include contributions from all of the components discussed above. Passive stiffness varies considerably between individual muscles (Brown et al., 1996; Azizi and Roberts, 2010; Azizi, 2014), with modulus values ranging from a low of about 100 kPa to a high above 700 kPa (calculated from fig. 2 of Brown et al., 1996, and fig. 5 and supplementary table S1 of Azizi, 2014). The potential for energy storage per unit muscle mass is high in the structures that develop force in passive muscle, if they are strained sufficiently (Fig. 3).

Energy storage capacity of tendon

The capacity for energy storage in tendon is very high, because it has a high modulus and can undergo relatively large strains. The modulus of elasticity in tendon is somewhat variable, with reported values from mechanical testing that range from about 500 MPa to nearly 2000 MPa for tendons from adult animals (Matson et al., 2012). If we assume a typical modulus of 1000 MPa and a density for tendon of 1120 kg m^{-3} (Ker, 1981), we can calculate the energy storage capacity per unit tendon mass from Eqn 8. For a tendon strain of 5%, the strain energy stored in 1 kg of tendon is over 1000 J. Such calculations remind us why tendon provided the power for many medieval siege weapons. To put the energy value above in perspective, 1000 J is sufficient energy to launch a 10 kg catapult projectile over 20 m.

To compare the capacity for energy storage in tendon with elastic structures within muscle, we need to calculate tendon energy storage on a per-unit muscle mass basis. This estimate can be made with the following calculation and assumptions. Rewriting Eqn 8 with the

subscript ‘t’ to indicate tendon, we have

$$\frac{W_t}{M_t} = \frac{E_t \cdot \epsilon_t^2}{2 \cdot \rho_t} \quad (9)$$

We can multiply both sides of this equation by the ratio of tendon mass to muscle mass, M_t/M_m , and substitute volume-based mass estimates (using a subscript ‘m’ to indicate muscle), to get:

$$\frac{W_t}{M_m} = \frac{E_t \cdot \epsilon_t^2}{2 \cdot \rho_t} \cdot \frac{A_t \cdot L_t \cdot \rho_t}{A_m \cdot L_m \cdot \rho_m} \quad (10)$$

Rearranging and simplifying:

$$\frac{W_t}{M_m} = \frac{E_t \cdot \epsilon_t^2}{2 \cdot \rho_m} \cdot \frac{A_t}{A_m} \cdot \frac{L_t}{L_m} \quad (11)$$

Tendons come in a variety of shapes and sizes; some muscles have little to no tendon while others, like most distal muscles of tetrapod limbs, have long, slender tendons. A reasonable estimate for the ratio of tendon area to muscle area, A_t/A_m , can be obtained as follows. First, we assume that the strain a tendon will undergo at loads corresponding to the maximum isometric force a muscle can produce is 5%. This assumption keeps the maximum tendon strain below that associated with tendon failure, which occurs within the range 7–12% strain (Shadwick, 1990; Matson et al., 2012). A maximum strain of 5% is comparable to tendon strains that have been measured *in vivo*, though it should be noted that strains up to ~9% have been measured for high-speed running and hopping (Dimery et al., 1986; Biewener et al., 1998; Lichtwark et al., 2007). From Eqn 6, we know that a tendon with an elastic modulus of 1000 MPa will experience a stress of 50 MPa at 5% strain. If this strain develops at the maximum estimated muscle stress of 500 kPa, then:

$$\frac{\sigma_t}{\sigma_m} = \frac{50 \text{ MPa}}{0.50 \text{ MPa}} = 100. \quad (12)$$

Substituting for muscle and tendon stress:

$$\frac{F_t/A_t}{F_m/A_m} = 100. \quad (13)$$

Tendons and muscle fibers are in series, thus $F_t=F_m$, and Eqn 13 can be rewritten:

$$\frac{A_m}{A_t} = 100. \quad (14)$$

Measurements of the dimensions of muscles and tendons for a wide range of limb muscles in mammals show that this ratio is actually quite variable. Values of 50–100 are common among muscles with significant tendons (Ker et al., 1988; Shadwick, 1990), suggesting the value calculated here is reasonable but may be lower for some tendons (i.e. some tendons may be relatively thicker than suggested by a muscle to tendon area ratio of 100). Substituting into Eqn 11:

$$\frac{W_t}{M_m} = \frac{E_t \cdot \epsilon_t^2}{2 \cdot \rho_m} \cdot 0.01 \cdot \frac{L_t}{L_m} \quad (15)$$

The length of the tendon relative to the length of muscle fibers is quite variable among limb muscles. A value of 0.1 for L_t/L_m would represent a stiff muscle–tendon unit, while the tendons of some distal limb muscles, particularly for animals specialized for running, can be several times the length of their associated muscle fibers. To bracket at least some of this variation, tendon energy storage is

calculated for a muscle with a very short tendon, $(L_t/L_m)=0.1$, and a relatively long tendon, $(L_t/L_m)=7$. The latter value corresponds approximately to the ratio of tendon length to muscle length in the human gastrocnemius, a muscle for which elastic energy storage has been shown to have a significant impact on muscle length change and mechanical energetics during locomotion (Lichtwark et al., 2007; Farris and Sawicki, 2012). Values for muscle mass-specific energy storage in tendon are shown in Fig. 3.

Interpreting values for energy storage capacity in muscle and tendon springs

The values calculated in Fig. 3 include significant uncertainties. They should be considered starting points, rather than a reference for exact values of energy storage capacity of each muscle spring. One goal of such calculations is to illustrate that because the rules for the mechanical behavior of elastic bodies are so simple, their potential to contribute to locomotor mechanics and energetics can be calculated. Whether these components actually contribute as locomotor springs will depend on a number of factors beyond their capacity for energy storage. Some of these are discussed below.

The potential contribution of a spring to locomotor mechanics will depend not only on how much energy the spring can store (as shown in Fig. 3) but also on how much of the stored energy is returned. Measurements of stretching–shortening cycles in muscles and tendons indicate that the fraction of energy returned is variable among different components. Tendons return almost all (~85–95%) of the energy stored (Shadwick, 1990; Matson et al., 2012), but passive muscle exhibits viscoelastic behavior and is much less resilient (Fig. 4). The muscle shown in Fig. 4, for example, returns only about 40% of the energy on recoil that was loaded into it when it was stretched. The structural basis for this behavior is unclear. Single-molecule measurements of titin mechanics suggest that viscoelastic behavior (and thus energy loss) is associated with folding and unfolding of titin immunoglobulin (Ig) domains, and may be strain dependent (Kellermayer et al., 1997). Collagen is very resilient; thus, the collagen fibers responsible for passive force production in the ECM would be expected to be efficiently elastic. Viscoelastic behavior in whole muscle may result from the complex interaction of collagen fibers and muscle fluid pressure that occurs as fibers reorient and generate force (Gindre et al., 2013). At the level of both whole muscles and single fibers, the viscoelastic behavior of passive muscle is strain rate dependent (Rehorn et al.,

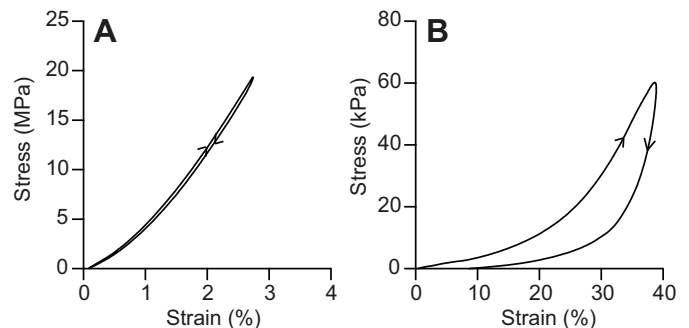


Fig. 4. Stress–strain work loops. Single stress–strain work loops are shown for tendon (A) and passive muscle (B) from wild turkey muscles. Both are cycled with a sine wave at 4 Hz, a value close to the stride frequency for running for this animal. The hysteresis represents the energy lost in the cycle, which is negligible for the tendon and significant (approximately 60%) for the passive muscle. Scales for both stress and strain differ in the two panels. The arrows indicate the direction of length change.

2014); thus, the potential spring-like function of these elements may be variable depending upon the speed of movement.

An important difference between the elastic behavior of spring elements within muscles versus those in tendons is that energy storage is coupled to muscle length change for intramuscular springs, while it is independent of muscle length change for tendon. Thus, it would seem that for the springs represented by the elastic behavior of passive whole muscles and single fibers, significant elastic contributions would require (1) that the muscle is stretched, either when active or when passive, by an outside force and (2) that it undergoes significantly large excursions. By comparison, energy storage in tendon depends only on the force developed by the muscle. Tendon can, and does, store energy during muscle shortening, lengthening and isometric force production. In-series tendon can store energy from muscles during contraction of the muscles, as in frog jumping, but energy storage within intramuscular springs requires muscle lengthening, as in a countermovement prior to a jump.

The capacity for energy storage, measured in terms of total energy, is only one measure of the potential for elastic elements to contribute as locomotor springs. For activities where performance is determined by the total work done by muscle, this measure is important. However, all of the elastic components considered in Fig. 3 are capable of producing brief bursts of power that exceed the isotonic power capacity of muscle. In such instances they might make an essential contribution to the high power required for movement even if they do not perform much work. The springs represented by single-fiber and whole-muscle passive stiffness will also produce force when stretched to long lengths, and this force production might be put to useful ends even if significant energy is not stored.

Even though there are some uncertainties in the exact values in Fig. 3, we can draw some broad conclusions. First, for muscles with significant tendons, the capacity for energy storage in tendon far exceeds the capacity for energy storage within muscle. Furthermore, large amounts of elastic energy storage and recovery can occur in tendons even when muscle fiber strains are negligible. Second, with the exception of titin, the capacity for energy storage in myofilaments and cross-bridges is small. Third, both titin and other parallel elastic structures within muscle have the potential to contribute significant amounts of elastic energy storage, if the muscle is stretched to relatively large values of strain.

Evidence for energy storage and recovery in muscle springs

There are a few existing examples of the use of intramuscular springs. George and co-workers used X-ray diffraction measurements to show that in hawkmoth muscles, cross-bridges may remain bound as the muscle transitions from lengthening to shortening at the limits of the wingbeat (George et al., 2013). They suggested that the storage and recovery of elastic energy in bound cross-bridges could assist with some of the inertial work of decelerating and accelerating the wings. In this system, the cool temperatures maintained in a portion of the muscle may be critical for allowing cross-bridges to remain bound for long enough to contribute elastically to a portion of the locomotor cycle, as the mechanism does not occur in warmer muscle (George et al., 2013). This study demonstrates that while cross-bridges may be very limited in their overall strain and energy contribution, under just the right circumstances (e.g. cool muscles, fast cycles and a benefit resulting from a small energy contribution) they may act as locomotor springs.

Toads use a power-amplifying system to produce rapid mouth opening to propel the tongue forward ballistically during feeding

(Lappin et al., 2006). In a comprehensive study of muscle function *in vivo* and *in vitro*, Lappin and co-workers produced evidence that much of the elastic recoil that powers the rapid jaw movement results from elastic elements within the muscle. Jaw opening appears to be a very high-power activity that does not necessarily involve a very large amount of muscle work, on a muscle mass-specific basis (Lappin et al., 2006). Such movements may be particularly well suited for contributions from intramuscular springs to power amplification.

There is also evidence that the springs involved in force production in stretched passive muscle may also provide useful function in locomotion. In hopping toads, measurements of limb kinematics and electromyographic activity suggest that passive muscle elasticity helps to reposition the hindlimb to a flexed position during aerial phase of a jump, in preparation for landing (Schnyer et al., 2014). A systematic study of joint torques in passive rat hindlimbs showed that passive muscle forces establish a joint neutral position, from which the joint deviates as individual muscle units are removed by dissection (Wu et al., 2012). Passive muscle restoring torques were measured within the range of movement for ordinary locomotion, suggesting some passive force is developed during normal gait (Wu et al., 2012). In humans, measurements of passive joint torques combined with walking kinematics have been used to demonstrate that passive muscle forces provide small contributions to moments developed at the ankle and relatively large contributions at the hip (Whittington et al., 2008). Correlative evidence points to a possibly significant effect of passive muscle spring properties on locomotor performance. In human sprinters, performance was decreased after bouts of stretching immediately prior to a timed run trial (Nelson et al., 2005). Other studies have found a correlation between inflexibility and running economy, with runners with greater muscle stiffness using less energy to move (Gleim et al., 1990; Craib et al., 1996).

Conclusions

The actomyosin cross-bridges that generate force in skeletal muscle operate in series and in parallel with spring-like elements that have the potential to store and recover energy. For some of these springs, like tendons, we know that the storage and recovery of elastic energy can have a profound effect on the force, power and speed of movement. Our understanding of the contributions of the springs within muscle is more limited. Because the behavior of elastic elements follows ordinary rules, we can apply simple calculations to illuminate boundaries of function for these springs, which can help to guide exploration of their role in movement. While the range of functions provided by muscle elasticity is not yet established, we can be certain that these springs have as yet undiscovered roles. The spring-like function of structures within muscle may be simple but it presents opportunities, and nature exploits opportunities.

Acknowledgements

I am grateful to members of the Brown Morphology group for critical reading of the manuscript, and for productive discussions that contributed to ideas presented here. I thank Hans Hoppeler and Stan Lindstedt for organizing the very stimulating and timely symposium that provided the motivation for this manuscript.

Competing interests

The authors declare no competing or financial interests.

Funding

The support of National Institutes of Health (NIH) grant AR055295 and National Science Foundation (NSF) grant 526256 is gratefully acknowledged. Deposited in PMC for release after 12 months.

References

- Aerts, P.** (1997). Vertical jumping in *Galago senegalensis*: the quest for an obligate mechanical power amplifier. *Philos. Trans. R. Soc. B Biol. Sci.* **353**, 1607-1620.
- Alexander, R. McN. and Bennet-Clark, H. C.** (1977). Storage of elastic strain energy in muscle and other tissues. *Nature* **265**, 114-117.
- Astley, H. C. and Roberts, T. J.** (2012). Evidence for a vertebrate catapult: elastic energy storage in the plantaris tendon during frog jumping. *Biol. Lett.* **8**, 386-389.
- Azizi, E.** (2014). Locomotor function shapes the passive mechanical properties and operating lengths of muscle. *Proc. R. Soc. B Biol. Sci.* **281**, 20132914.
- Azizi, E. and Roberts, T. J.** (2010). Muscle performance during frog jumping: influence of elasticity on muscle operating lengths. *Proc. R. Soc. B Biol. Sci.* **277**, 1523-1530.
- Barclay, C. J., Woledge, R. C. and Curtin, N. A.** (2010). Inferring crossbridge properties from skeletal muscle energetics. *Prog. Biophys. Mol. Biol.* **102**, 53-71.
- Biewener, A. A. and Roberts, T. J.** (2000). Muscle and tendon contributions to force, work, and elastic energy savings: a comparative perspective. *Exerc. Sport Sci. Rev.* **28**, 99-107.
- Biewener, A. A., Konieczynski, D. D. and Baudinette, R. V.** (1998). *In vivo* muscle force-length behavior during steady-speed hopping in tammar wallabies. *J. Exp. Biol.* **201**, 1681-1694.
- Blickhan, R.** (1989). The spring-mass model for running and hopping. *J. Biomech.* **2**, 1217-1227.
- Brown, I. E., Liinamaa, T. L. and Loeb, G. E.** (1996). Relationships between range of motion, Lo, and passive force in five strap-like muscles of the feline hind limb. *J. Morph.* **230**, 69-77.
- Brunello, E., Caremani, M., Melli, L., Linari, M., Fernandez-Martinez, M., Narayanan, T., Irving, M., Piazzesi, G., Lombardi, V. and Reconditi, M.** (2014). The contributions of filaments and cross-bridges to sarcomere compliance in skeletal muscle. *J. Physiol.* **592**, 3881-3899.
- Cavagna, G. A., Saibene, F. P. and Margaria, R.** (1964). Mechanical work in running. *J. Appl. Physiol.* **19**, 249-256.
- Cavagna, G. A., Heglund, N. C. and Taylor, C. R.** (1977). Mechanical work in terrestrial locomotion: two basic mechanisms for minimizing energy expenditure. *Am. J. Physiol.* **233**, R243-R261.
- Craib, M. W., Mitchell, V. A., Fields, K. B., Cooper, T. R., Hopewell, R. and Morgan, D. W.** (1996). The association between flexibility and running economy in sub-elite male distance runners. *Med. Sci. Sports Exerc.* **28**, 737-743.
- Daley, M. A. and Usherwood, J. R.** (2010). Two explanations for the compliant running paradox: reduced work of bouncing viscera and increased stability in uneven terrain. *Biol. Lett.* **6**, 418-421.
- Dimery, N. J., Alexander, R. McN. and Ker, R. F.** (1986). Elastic extension of leg tendons in the locomotion of horses (*Equus caballus*). *J. Zool. Lond.* **210**, 415-425.
- Ellerby, D. J. and Marsh, R. L.** (2006). The energetic costs of trunk and distal-limb loading during walking and running in guinea fowl *Numida meleagris*: II. Muscle energy use as indicated by blood flow. *J. Exp. Biol.* **209**, 2064-2075.
- Ernst, M., Geyer, H. and Blickhan, R.** (2012). Extension and customization of self-stability control in compliant legged systems. *Bioinspir. Biomim.* **7**, 046002.
- Farris, D. J. and Sawicki, G. S.** (2012). Human medial gastrocnemius force-velocity behavior shifts with locomotion speed and gait. *Proc. Natl. Acad. Sci. USA* **109**, 977-982.
- Gajdosik, R. L.** (2001). Passive extensibility of skeletal muscle: review of the literature with clinical implications. *Clin. Biomech.* **16**, 87-101.
- George, N. T., Irving, T. C., Williams, C. D. and Daniel, T. L.** (2013). The cross-bridge spring: can cool muscles store elastic energy? *Science* **340**, 1217-1220.
- Geyer, H., Seyfarth, A. and Blickhan, R.** (2006). Compliant leg behaviour explains basic dynamics of walking and running. *Proc. R. Soc. B Biol. Sci.* **273**, 2861-2867.
- Gillies, A. R. and Lieber, R. L.** (2011). Structure and function of the skeletal muscle extracellular matrix. *Muscle Nerve* **44**, 318-331.
- Gindre, J., Takaza, M., Moerman, K. M. and Simms, C. K.** (2013). A structural model of passive skeletal muscle shows two reinforcement processes in resisting deformation. *J. Mech. Behav. Biomed. Mater.* **22**, 84-94.
- Gleim, G. W., Stachenfeld, N. S. and Nicholas, J. A.** (1990). The influence of flexibility on the economy of walking and jogging. *J. Orthop. Res.* **8**, 814-823.
- Heglund, N. C., Fedak, M. A., Taylor, C. R. and Cavagna, G. A.** (1982). Energetics and mechanics of terrestrial locomotion. IV. Total mechanical energy changes as a function of speed and body size in birds and mammals. *J. Exp. Biol.* **97**, 57-66.
- 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., Roberts, T. J. and Askew, G. N.** (2014). The energetic benefits of tendon springs in running: is the reduction of muscle work important? *J. Exp. Biol.* **217**, 4365-4371.
- Kaya, M. and Higuchi, H.** (2010). Nonlinear elasticity and an 8-nm working stroke of single myosin molecules in myofilaments. *Science* **329**, 686-689.
- Kellermayer, M. S. Z., Smith, S. B., Granzier, H. L. and Bustamante, C.** (1997). Folding-unfolding transitions in single titin molecules characterized with laser tweezers. *Science* **276**, 1112-1116.
- Ker, R. F.** (1981). Dynamic tensile properties of the plantaris tendon of sheep (*Ovis aries*). *J. Exp. Biol.* **93**, 283-302.
- Ker, R. F., Alexander, R. McN. and Bennett, M. B.** (1988). Why are mammalian tendons so thick? *J. Zool. Lond.* **216**, 309-324.
- Kohler, J., Winkler, G., Schulte, I., Scholz, T., McKenna, W., Brenner, B. and Kraft, T.** (2002). Mutation of the myosin converter domain alters cross-bridge elasticity. *Proc. Natl. Acad. Sci. USA* **99**, 3557-3562.
- Konow, N. and Roberts, T. J.** (2015). The series elastic shock absorber: tendon elasticity modulates energy dissipation by muscle during burst deceleration. *Proc. R. Soc. B Biol. Sci.* **282**, 20142800.
- Konow, N., Azizi, E. and Roberts, T. J.** (2012). Muscle power attenuation by tendon during energy dissipation. *Proc. R. Soc. B Biol. Sci.* **279**, 1108-1113.
- Lappin, A. K., Monroy, J. A., Pilarski, J. Q., Zepnewski, E. D., Pierotti, D. J. and Nishikawa, K. C.** (2006). Storage and recovery of elastic potential energy powers ballistic prey capture in toads. *J. Exp. Biol.* **209**, 2535-2553.
- Lichtwark, G. A. and Barclay, C. J.** (2010). The influence of tendon compliance on muscle power output and efficiency during cyclic contractions. *J. Exp. Biol.* **213**, 707-714.
- Lichtwark, G. A., Bougoulas, K. and Wilson, A. M.** (2007). Muscle fascicle and series elastic element length changes along the length of the human gastrocnemius during walking and running. *J. Biomech.* **40**, 157-164.
- Lombardi, V. and Piazzesi, G.** (1990). The contractile response during steady lengthening of stimulated frog muscle fibres. *J. Physiol.* **431**, 141-171.
- Lou, F., Curtin, N. A. and Woledge, R. C.** (1999). Elastic energy storage and release in white muscle from dogfish *Scyliorhinus canicula*. *J. Exp. Biol.* **202**, 135-142.
- Magid, A. and Law, D. J.** (1985). Myofibrils bear most of the resting tension in frog skeletal muscle. *Science* **230**, 1280-1282.
- Marsh, R. L.** (1994). Jumping ability of anuran amphibians. In *Advances in Veterinary Science and Comparative Medicine*, Vol. 38B (ed. J. H. Jones), pp. 51-111. New York: Academic Press.
- Marsh, R. L. and John-Alder, H. B.** (1994). Jumping performance of hylid frogs measured with high-speed cine film. *J. Exp. Biol.* **188**, 131-141.
- Marsh, R. L., Ellerby, D. J., Carr, J. A., Henry, H. T. and Buchanan, C. I.** (2004). Partitioning the energetics of walking and running: swinging the limbs is expensive. *Science* **303**, 80-83.
- Matson, A., Konow, N., Miller, S., Konow, P. P. and Roberts, T. J.** (2012). Tendon material properties vary and are interdependent among turkey hindlimb muscles. *J. Exp. Biol.* **215**, 3552-3558.
- McHugh, M. P., Connolly, D. A. J., Eston, R. G. and Gleim, G. W.** (1999). Exercise-induced muscle damage and potential mechanisms for the repeated bout effect. *Sports Med.* **27**, 157-170.
- McMahon, T. A. and Cheng, G. C.** (1990). The mechanics of running: how does stiffness couple with speed? *J. Biomech.* **23** Suppl. 1, 65-78.
- Mendez, J. and Keys, A.** (1960). Density and composition of mammalian muscle. *Metabolism* **9**, 184-188.
- Minetti, A. E., Ardig, O. L., Reinach, E. and Saibene, F.** (1999). The relationship between mechanical work and energy expenditure of locomotion in horses. *J. Exp. Biol.* **202**, 2329-2338.
- Nelson, A. G., Driscoll, N. M., Landin, D. K., Young, M. A. and Schexnayder, I. C.** (2005). Acute effects of passive muscle stretching on sprint performance. *J. Sports Sci.* **23**, 449-454.
- Nishikawa, K. C., Monroy, J. A., Uyeno, T. E., Yeo, S. H., Pai, D. K. and Lindstedt, S. L.** (2012). Is titin a 'winding filament'? A new twist on muscle contraction. *Proc. R. Soc. B Biol. Sci.* **279**, 981-990.
- Offer, G. and Ranatunga, K. W.** (2010). Crossbridge and filament compliance in muscle: implications for tension generation and lever arm swing. *J. Muscle Res. Cell Motil.* **31**, 245-265.
- Offer, G. and Ranatunga, K. W.** (2013). A cross-bridge cycle with two tension-generating steps simulates skeletal muscle mechanics. *Biophys. J.* **105**, 928-940.
- Piazzesi, G., Reconditi, M., Linari, M., Lucii, L., Bianco, P., Brunello, E., Decostre, V., Stewart, A., Gore, D. B., Irving, T. C. et al.** (2007). Skeletal muscle performance determined by modulation of number of myosin motors rather than motor force or stroke size. *Cell* **131**, 784-795.
- Piazzesi, G., Dolfi, M., Brunello, E., Fusi, L., Reconditi, M., Bianco, P., Linari, M. and Lombardi, V.** (2014). The myofilament elasticity and its effect on kinetics of force generation by the myosin motor. *Arch. Biochem. Biophys.* **552-553**, 108-116.
- Powers, K., Schappacher-Tilp, G., Jinha, A., Leonard, T., Nishikawa, K. and Herzog, W.** (2014). Titin force is enhanced in actively stretched skeletal muscle. *J. Exp. Biol.* **217**, 3629-3636.
- Prado, L. G., Makarenko, I., Andresen, C., Kruger, M., Opitz, C. A. and Linke, W. A.** (2005). Isoform diversity of giant proteins in relation to passive and active contractile properties of rabbit skeletal muscles. *J. Gen. Physiol.* **126**, 461-480.
- Proskov, U. and Morgan, D. L.** (2001). Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J. Physiol.* **537**, 333-345.
- Reconditi, M., Linari, M., Lucii, L., Stewart, A., Sun, Y.-B., Boesecke, P., Narayanan, T., Fischetti, R. F., Irving, T., Piazzesi, G. et al.** (2004). The myosin motor in muscle generates a smaller and slower working stroke at higher load. *Nature* **428**, 578-581.

- Rehorn, M. R., Schroer, A. K. and Blemker, S. S.** (2014). The passive properties of muscle fibers are velocity dependent. *J. Biomech.* **47**, 687-693.
- Roberts, T. J. and Azizi, E.** (2010). The series-elastic shock absorber: tendons attenuate muscle power during eccentric actions. *J. Appl. Physiol.* **109**, 396-404.
- Roberts, T. J. and Konow, N.** (2013). How tendons buffer energy dissipation by muscle. *Exerc. Sport. Sci. Rev.* **41**, 186-193.
- Roberts, T. J. and Scales, J. A.** (2002). Mechanical power output during running accelerations in wild turkeys. *J. Exp. Biol.* **205**, 1485-1494.
- Roberts, T. J., Marsh, R. L., Weyand, P. G. and Taylor, C. R.** (1997). Muscular force in running turkeys: the economy of minimizing work. *Science* **275**, 1113-1115.
- Roberts, T. J., Chen, M. S. and Taylor, C. R.** (1998). Energetics of bipedal running. II. Limb design and running mechanics. *J. Exp. Biol.* **201**, 2753-2762.
- Rubenson, J., Pires, N. J., Loi, H. O., Pinniger, G. J. and Shannon, D. G.** (2012). On the ascent: the soleus operating length is conserved to the ascending limb of the force-length curve across gait mechanics in humans. *J. Exp. Biol.* **215**, 3539-3551.
- Sawicki, G. S. and Ferris, D. P.** (2008). Mechanics and energetics of level walking with powered ankle exoskeletons. *J. Exp. Biol.* **211**, 1402-1413.
- Schnyer, A., Gallardo, M., Cox, S. and Gillis, G.** (2014). Indirect evidence for elastic energy playing a role in limb recovery during toad hopping. *Biol. Lett.* **10**, 20140418.
- Shadwick, R. E.** (1990). Elastic energy storage in tendons: mechanical differences related to function and age. *J. Appl. Physiol.* **68**, 1033-1040.
- Snyder, K. L. and Farley, C. T.** (2011). Energetically optimal stride frequency in running: the effects of incline and decline. *J. Exp. Biol.* **214**, 2089-2095.
- Tobalske, B. W., Warrick, D. R., Clark, C. J., Powers, D. R., Hedrick, T. L., Hyder, G. A. and Biewener, A. A.** (2007). Three-dimensional kinematics of hummingbird flight. *J. Exp. Biol.* **210**, 2368-2382.
- Trinh, M. and Syme, D. A.** (2007). Effects of stretch on work and efficiency of frog (*Rana pipiens*) muscle. *J. Exp. Biol.* **210**, 2843-2850.
- Wang, G. and Kawai, M.** (2001). Effect of temperature on elementary steps of the cross-bridge cycle in rabbit soleus slow-twitch muscle fibres. *J. Physiol.* **531**, 219-234.
- Wang, K. and Ramirez-Mitchell, R.** (1983). A network of transverse and longitudinal intermediate filaments is associated with sarcomeres of adult vertebrate skeletal muscle. *J. Cell Biol.* **96**, 562-570.
- Wang, K., McCarter, R., Wright, J., Beverly, J. and Ramirez-Mitchell, R.** (1993). Viscoelasticity of the sarcomere matrix of skeletal muscles. The titin-myosin composite filament is a dual-stage molecular spring. *Biophys. J.* **64**, 1161-1177.
- Weyand, P. G., Sandell, R. F., Prime, D. N. L. and Bundle, M. W.** (2010). The biological limits to running speed are imposed from the ground up. *J. Appl. Physiol.* **108**, 950-961.
- Whittington, B., Silder, A., Heiderscheit, B. and Thelen, D. G.** (2008). The contribution of passive-elastic mechanisms to lower extremity joint kinetics during human walking. *Gait Posture* **27**, 628-634.
- Wu, M. M., Pai, D. K., Tresch, M. C. and Sandercock, T. G.** (2012). Passive elastic properties of the rat ankle. *J. Biomech.* **45**, 1728-1732.