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Review

Do muscles function as adaptable locomotor springs?

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Summary

During normal animal movements, the forces produced by the locomotor muscles may be greater than, equal to or less than the forces acting on those muscles, the consequences of which significantly affect both the maximum force produced and the energy consumed by the muscles. Lengthening (eccentric) contractions result in the greatest muscle forces at the lowest relative energetic costs. Eccentric contractions play a key role in storing elastic strain energy which, when recovered in subsequent contractions, has been shown to result in enhanced force, work or power outputs. We present data that support the concept that this ability of muscle to store and recover elastic strain energy is an adaptable property of skeletal muscle. Further, we speculate that a crucial element in that muscle spring may be the protein titin. It too seems to adapt to muscle use, and its stiffness seems to be 'tuned' to the frequency of normal muscle use.

Key words: elastic recoil, strain energy, eccentric, hopping, stride frequency, titin, muscle.

Usual view of muscle

During ballistic movement (e.g. a jumping frog), normal locomotion (e.g. a walking human) or even sound production (e.g. by a rattlesnake), when the force produced by the muscle exceeds the opposing forces of gravity, tissue inertia, pressure, etc., acting on the muscle, the muscle shortens and does work (force×distance). When the force generated by active muscle equals the opposing force, the muscle will contract isometrically, without doing work (e.g. postural muscles). Historically, since the days of A. V. Hill, these modes of muscle contractions (shortening 'isotonic' or constant-length 'isometric') have been the primary foci of muscle experimentation and, hence, they comprise the standard 'textbook' view of skeletal muscle function. The typical presentation of force/velocity curves and length/force relationships implies that these encompass the normal domain of skeletal muscle function.

However, whenever the opposing force acting on a muscle exceeds the force produced by the muscle, the muscle will lengthen while being activated, absorbing mechanical work. The past few decades have brought an increased understanding of the importance of these lengthening (eccentric) contractions in normal animal movement. While the obvious examples include hiking downhill or cushioning a fall, recent evidence implicates eccentric contractions as an integral part of most cyclic movements, especially in terrestrial locomotion. For example, many locomotor muscles are actively stretched prior to shortening, described as the stretch–shorten cycle (Komi and Bosco, 1978; Hof et al., 1983; Ettema, 1996b). Whenever work is done on a muscle, or muscle/tendon element, energy is absorbed. This absorbed energy can either be lost as heat (as it is when hiking downhill) or stored as elastic strain energy (elastic recoil potential energy), a portion of which may subsequently be recovered (Asmussen and Bonde-Petersen 1974; Komi and Bosco, 1978; Biewener and Roberts, 2000; Ettema, 1996b; Dickinson et al., 2000; Lindstedt et al., 2001). The storage and recovery of elastic strain energy may be of greatest 'energetic value' when locomotor muscles perform a stretch–shorten cycle because the energy stored during a lengthening cycle can amplify force production in a subsequent shortening cycle (Komi and Bosco, 1978; Biewener and Roberts, 2000; Ettema et al., 1990; Prilutsky et al., 1996; Olson and Marsh, 1998; Seyfarth et al., 2000). The pervasive role of eccentric muscular force may be most substantial during high-force locomotor movements such as running (Cavagna et al., 1971; Cavagna, 1977), sprinting (Mero and Komi, 1986; Farley, 1997; Chelly and Denis, 2001), hopping (Chelly and Denis, 2001; Lindstedt et al., 2001) and jumping (Seyfarth et al., 2000). Here, we examine how these kinds of muscle uses can be quantified and ask if there is evidence that muscle may adapt to eccentric contractions.

Lengthening contractions in normal locomotion

During terrestrial locomotion, there is a cyclic transfer

between kinetic and potential energy. At low speeds, these shifts are largely between kinetic and gravitational potential energy. However, as locomotor speed increases, more energy is stored as elastic strain (potential) energy and less as gravitational potential energy (McMahon, 1984). For strain energy to be stored, there must be a stretch of the muscle/tendon system, usually including stretching the activated muscle (an eccentric muscle contraction). For example, typically during (fast) terrestrial locomotion, when the foot comes into contact with the ground, leg extensors are activated and they lengthen until the foot is directly beneath the hip or shoulder, following which they shorten, producing work. Much of the energy stored during the initial lengthening phase of the step cycle is recovered during the final shortening phase. This cyclic 'stretch–shorten' use of muscles during locomotion occurs at a predictable body-size-dependent frequency such that a running animal behaves like a simple spring mass system (Farley et al., 1993) with stride frequency selected to maximize elastic strain energy recovery. As a consequence, locomotion, at least in some animals, may be accompanied by little muscle shortening and hence minimal muscle work. For example, Roberts et al. (1997) have elegantly demonstrated that it is the stretch of the tendon and subsequent recoil that provide the force necessary for locomotion in turkeys.

However, while tendons certainly function to store strain energy, their relative contribution is apparently greatest in animals with long tendons, such as turkeys, and in large animals (Pollock and Shadwick, 1994; Ettema, 1996a). The tendons of small animals are not proportioned to optimize this function (Bennett and Taylor, 1995), although small animals can still recover some elastic strain energy during locomotion (Ettema, 1996a). This ability to recover elastic strain energy is apparently energetically so advantageous that stride frequency in running mammals may be set by this key property alone (Taylor, 1985; Farley et al., 1993). In addition, ample evidence shows that storage and recovery of elastic strain energy can occur in the absence of tendons (Cavagna et al., 1994). Thus, apart from the role of tendons and collagen in energy storage, the muscle itself is certainly contributing to the storage and recovery of elastic strain energy. In a sense, because the muscle is composed of both muscle fibers and tendinous materials, all these structures must be collectively 'tuned' to the spring properties for the muscle/tendon system to store and recover elastic strain energy during locomotion.

Elastic strain energy storage in muscle itself

During the stretch–shorten cycle, the stretching of an activated muscle fiber results in a significant enhancement of the force produced by the fiber in a subsequent shortening (Komi and Bosco, 1978; Biewener and Blickhan, 1988; Ettema et al., 1990; Prilutsky et al., 1996; Olson and Marsh, 1998; Seyfarth et al., 2000). This power enhancement is strongly time-dependent (Cavagna et al., 1985, 1994; Lensel-Corbeil and Goubel, 1989; Lindstedt et al., 2001), and the magnitude of recovery of a muscle's elastic recoil may be fiber-type-dependent (Bosco et al., 1982). This added force is most dramatic in powering athletic-like performance tasks. Cavagna et al. (1971) first proposed that the elastic-stretch component of the stretch–shorten cycle provided the additional power required to sustain high running speeds. That is, the power generated from the contractile component of the leg muscles increased in parallel at submaximal speeds, but the additional power during running at maximal speeds was provided by the muscle's elastic component. Others have noted increases in the 'apparent' spring constant of the eccentric-stretching phase during sprinting (Luthanen and Komi, 1980; Mero and Komi, 1986) and that increasing leg stiffness is coupled with high running speeds (Chelly and Denis, 2001).

How does the muscle spring adapt to eccentric contractions?

There is evidence that both the contractile and metabolic properties of muscle respond to changes in both the nature and intensity of muscle use (Staron et al., 1991; Booth and Baldwin, 1996). Is the nature of the muscle spring also responsive to changes in eccentric demand during an animal's lifetime, i.e. is this also a phenotypically plastic trait of muscle?

One dramatic anecdotal example suggests that the answer is yes. If naïve to hiking downhill (eccentric lengthening contractions), one can experience devastating delayed onset muscle soreness (DOMS) after an initial hike (Armstrong, 1984). There is strong evidence linking DOMS with muscle damage, suggesting that the muscle cells themselves have been injured. For example, levels of the myoplasm enzyme creatine kinase may increase to concentrations two orders of magnitude higher in the blood after acute eccentric exercise (Bar et al., 1997), indicative of serious muscle damage. Likewise, structural damage to the contractile elements of the muscle fiber can be seen directly by electron microscopy. The most frequent observation is Z-band streaming (Friden et al., 1983; Bar et al., 1997). However, if one hikes downhill repeatedly, after relatively few hikes there is no soreness or muscle damage whatsoever. Hence, the chronic use of eccentric contractions, in this case downhill hiking, results in a pronounced protective adaptation termed the 'repeated bout effect' (Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Nosaka et al., 1991) within the muscle. In fact, this protective adaptation occurs between 48 and 72 h after the initial exercise (Nosaka and Clarkson, 1996; Smith et al., 1994) and may be evident as soon as 24 h after a first damaging eccentric bout (Chen and Hsieh, 2001). Consequently, the identical eccentric activity that caused serious damage no longer has any harmful effect.

What is the nature of this adaptation? The changes within the muscle responsible for this adaptation are largely unknown. There are, however, suggestions that groups of the more fragile, stress-susceptible fibers are reduced in number after the first bout while stronger fibers survive and provide a protective effect (Armstrong, 1984). Even light eccentric training

protocols, however, that do not lead to an increase in creatine kinase levels and do little or no muscle damage, are still sufficient to bring about protection (Clarkson and Tremblay, 1988). It is also possible that the protective effect may lie outside the muscle and is neurologically mediated, i.e. that muscle fibers specifically adapted to repeated eccentric contractions may be preferentially recruited (Golden and Dudley, 1992; Hortobagyi et al., 1996). Hence, while the exact nature of this adaptation is as yet unknown, one possibility remains that the muscle/tendon structure may become functionally 'stiffer', allowing the muscle to absorb mechanical work without damage. Some have reported that training produces a less compliant locomotor muscle (Benn et al., 1998; Pousson et al., 1990; Reich et al., 2000), while others note greater elastic recoil in trained compared with untrained subjects (Kubo et al., 2000).

To investigate further the hypothesis of muscle/tendon stiffness plasticity, we used two model systems which both suggest that muscle 'spring stiffness' can indeed change acutely in response to chronic eccentric muscle use. Young, healthy human subjects rode a high-force eccentric ergometer for 8 weeks (30 min three times per week). The eccentric load was gradually increased in these subjects until, during the final 3 weeks, subjects were working at nearly –500 W (i.e. absorbed power) (see LaStayo et al., 2000). While this training resulted in increased muscle strength and size (LaStayo et al., 2000), we were also interested in how it affected the apparent muscle spring stiffness. To investigate the impact of this high-force eccentric training on muscle/tendon stiffness, we performed two experiments. In the first, the subjects hopped 'in place' vertically with instructions to select the frequency that felt the most comfortable. The subjects performed successive counter-measure hops to a height equal to 107 % of subject height. We regularly use this as a physiology laboratory exercise because it demonstrates how the 'comfortable frequency' is the most economical; the cost per hop doubles when the subjects are forced to hop at half this frequency (see Lindstedt et al., 2001). Following eccentric training, all the subjects selected a higher hopping frequency than they did prior to training; the 12 % mean overall increase was highly significant, while none of the control subjects (those exercising on a traditional, concentric bicycle) changed their hopping frequency (Fig. 1).

Another set of subjects (local high school basketball players) were trained with the same protocol. In this case, we recorded their maximum (vertical) jump height before and after 6 weeks of high-force eccentric cycle ergometry training. A weight-training control group was drawn from the same high school basketball players. Maximum jump height was taken as the peak of three jumps. While both groups of subjects had identical initial maximum jump heights at the start of the study, all the eccentric-trained subjects increased their jump height, with the overall mean increase being approximately 8 % (approximately 5 cm, Fig. 2). Thus, not only does high-force eccentric training evoke gains in muscle strength (Hortobagyi et al., 1996; LaStayo et al., 1999, 2000) and size (Hortobagyi et al., 1996; LaStayo et al., 2000), it apparently also results in a significant increase in the muscle spring stiffness (Lindstedt et al., 2001). In response to high-force eccentric training, hopping frequency increased and subjects were able to jump significantly higher, suggesting an enhanced strain energy storage and recovery when performing single or repeated counter-measure jumps (see also Seyfarth et al., 2000).

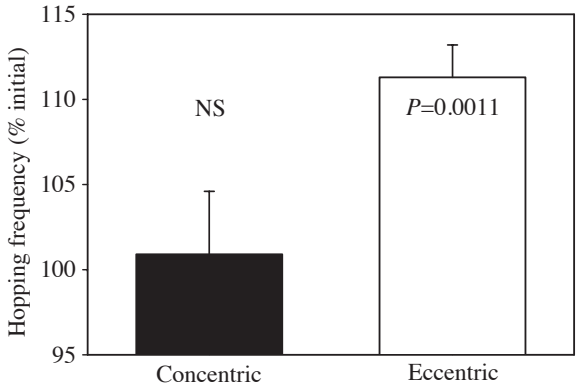

Fig. 1. When subjects were asked to hop in place at a 'comfortable frequency', they chose a highly reproducible body-size-dependent frequency. After 8 weeks of high-force eccentric training, all experimental subjects (N=7) hopped at a higher frequency while the concentrically trained controls (N=6) showed no change in frequency. Values are means + S.E.M. NS, not significant.

To examine whether this apparent increased stiffness was a result of changes in the muscle contractile properties, we used a model of rats walking down a steep (36 %) decline. To ensure eccentric loading, we added an additional weight equal to 15 % of body mass to small Velcro backpacks. After 8 weeks of running (30 min five times per week), the triceps muscles of the eccentrically trained animals were significantly stiffer than those of the inactive controls (Reich et al., 2000). These *in vitro* measurements of active muscle stiffness excluded that portion of tendon outside the muscle belly and, hence, reflect muscle

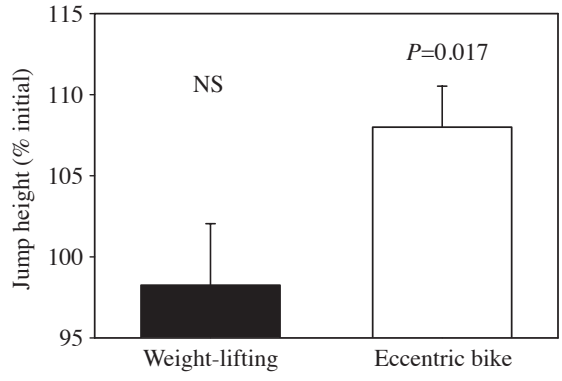

Fig. 2. A group of high school basketball players engaged in either high-force eccentric training or traditional weight-lifting for 6 weeks. Following the training period, all the eccentrically trained subjects (N=6) increased their maximum jump height but none of the control subjects (N=6) did so. Jump height is defined as the maximum height achieved in three jump attempts. Values are means + S.E.M. NS, not significant.

belly stiffness. The point is that, after just 8 weeks of training, the muscle had indeed demonstrated a structural adaptation by becoming approximately 40 % 'stiffer', without an increase in either muscle mass or isometric force production capabilities, when subjected to chronic eccentric use. Thus, we conclude that the apparent increases in muscle/tendon stiffness in the human subjects were also probably attributable primarily to shifts in muscle stiffness.

Where and what is the muscle spring?

Our results would seem to confirm those of others (Benn et al., 1998; Pousson et al., 1990; Kubo et al., 2000) in demonstrating that muscle stiffness changes in response to chronic eccentric muscle use; but they do little to suggest where the muscle spring may be located. Both tendon and collagen are structures capable of storing and releasing elastic strain energy. No doubt, these contribute to the overall muscle spring in a meaningful way (Alexander and Bennet-Clark, 1977; Ettema, 1996a,b; Han et al., 1999). However, it is quite clear that there is an active muscle spring as well. Hence, muscle itself is acting to store and recover elastic strain energy (Cavagna et al., 1994) because the amount stored may exceed what can be stored in the tendon (Biewener and Blickhan, 1988). Further, evidence suggests that, within the fiber, the spring is not the heavy meromyosin (Tidball and Daniel, 1986). What are the best candidates for the location of this spring? Perhaps one component of the spring within the muscle is the gigantic cytoskeletal protein titin.

Titin, the third filament system within muscles, is thought to be responsible for the elastic properties of vertebrate myofibrils. Discovered over two decades ago (Maruyama et al., 1977), titin is a huge protein (2.5–3.5 MDa), and is the only known protein to span an entire half-sarcomere from Z-disc to M-line with cross-links from titin molecules of adjacent sarcomeres in both regions (Obermann et al., 1997) (for a review, see Gregorio et al., 1999). The I-band region of titin functions as a molecular spring that develops tension when sarcomeres are stretched (Linke et al., 1996, 1999; Linke and Granzier, 1998). This force is responsible for restoring the muscle to slack length after being stretched beyond or shortened below resting length (Helmes et al., 1996) and for maintaining the structural integrity of the sarcomere in actively contracting muscle (Horowits and Podolsky, 1987). Because titin has numerous binding sites for other proteins within the sarcomere, it is likely that it provides a blueprint for precise sarcomere assembly (Gregorio et al., 1999; van der Ven et al., 2000).

Although there is only one titin gene, there are multiple titin isoforms which vary in I-band region stiffness. These isoforms vary in their lengths of serially linked immunoglobulin-like domains (Ig domains) and lengths of a region rich in proline (P), glutamate (E), valine (V) and lysine (K) residues (PEVK region) because of an uncharacterized, complicated method of differential splicing (Labeit and Kolmerer, 1995; Centner et al., 2000). Passive tension/stiffness properties of skeletal muscle tissues differ; for example, cardiac cells are much stiffer than skeletal muscle cells. These differences in stiffness/passive tension properties correspond to differences in titin isoform expression because titins from different tissues have different electrophoretic mobilities (Wang et al., 1991; Frieburg et al., 2000).

Because of these structural properties, titin could play a significant role as the muscle spring, which could explain why titin isoforms differ in skeletal tissues. For example, it is thought to play a key function in cardiac contractility, having been called the 'missing link' of diastole since it may contribute significantly to the Frank–Starling law of the heart (LeWinter, 2000). Second, as a muscle-stiffening spring, it may play a key role in the protective effect that occurs following eccentric exercise (Reich et al., 2000). Supporting this idea is the fact that high-force eccentric damage includes 'titin failure' (Thompson et al., 1999). Finally, in addition to all titin's known functions, the titin filament system may play a dynamic functional role in muscle contraction. Labeit and Kolmerer (1995) identified strong negative charges in the PEVK regions, which provide potential Ca^{2+}-binding sites. It has recently been shown that titin has an affinity for calcium in the 5′-most 400 kDa region of the PEVK segment (Tatsumi et al., 2001). With the binding of Ca^{2+}, the secondary structure of this titin fragment changes (Tatsumi et al., 2001). Thus, it has been speculated that the elasticity of titin changes in response to the flux of Ca^{2+} within the sarcomere during contraction/relaxation cycling (Tatsumi et al., 2001). It has also been suggested that the differential expression of titin isoforms mediates active force production by influencing the sensitivity of the myofilaments to activation by Ca^{2+} (Cazorla et al., 2001), by mediating changes in interfilament spacing (Cazorla et al., 2001) and by inducing conformational changes in myosin that result in a higher probability of activation at a given Ca^{2+} concentration (Fukuda et al., 2001; Granzier and Wang, 1993a). Thus, the differential expression of titin isoforms may in fact provide the means for the

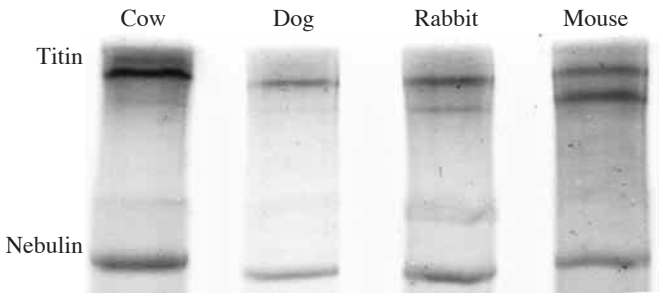

Fig. 3. SDS–PAGE analysis of titin isoform expression in quadriceps (vastus) muscles of cow, dog, rabbit and mouse. Cow vastus appears to express three titin bands, while dog, rabbit and mouse each express two. Looking at the most abundant isoform within each muscle type, there is a noticeable shift in expression from the largest, and hence most compliant, isoform (in the cow) to the smallest, stiffest isoform (in the mouse). The lowest mobility band present in each lane is nebulin (780 kDa).

dynamic regulation of active force production (Sutko et al., 2001).

If titin is functioning as a locomotor spring, then it should be 'tuned' to the frequency of muscle use. One way we can we can test this hypothesis is to examine titin isoforms in muscles that are used cyclically at different frequencies. Because stride frequency varies predictably with body size among mammals, by examining the titin expressed in differently sized animals we would detect shifts in titin isoforms as a function of body size. In particular, stride frequency at the trot/gallop transition (a physiologically equivalent speed) varies quite predictably as $4.5M^{-0.14}$ (r^2=0.98, where M is body mass) (Heglund et al., 1974). Thus, comparing the predicted stride frequency of a 25 g mouse with that of a 800 kg cow, stride frequency should decline from a predicted 7.5 to 1.8 s^{-1}. Since the muscle spring is highly time-dependent (Cavagna et al., 1994), if titin were to play a role in the storage and recovery of elastic strain energy, it should be much stiffer in a mouse than in a cow, reflecting the 4.3-fold difference in frequencies.

To examine this possibility, we identified the titin isoform present by electrophoresis using SDS–PAGE following and modifying the techniques of Granzier and Wang (1993b) and Granzier and Irving (1995). Vastus lateralis from mouse, rabbit, dog and calf were quick-frozen in liquid nitrogen. The samples were pulverized and the proteins extracted in Laemmli's sample buffer. The samples were analyzed with SDS–PAGE (4 % to 10 % acrylamide gradient gels). The gels were run at 5.5 mA and 12 °C for 22 h. After electrophoretic separation, the gels were stained with Coomassie Blue. The stained gels were scanned at 600 d.p.i. using a snap scan 1212 (AGFA) flatbed scanner. Within each lane, nebulin (780 kDa) acted as a standard. With the naked eye, it is apparent that at least two isoforms are expressed in these animals and that there is a significant and predictable shift from the most compliant (largest) isoforms in the cow to the stiffest (and smallest) isoforms in the mouse (Fig. 3). Thus, the results of these gels suggest a strong link between stride frequency and titin 'stiffness'. While this does not by itself demonstrate that titin is the muscle spring, it certainly suggests that it may be a significant and potentially 'tuned' contributor to the muscle/tendon 'tuned' spring.

In the future, we plan to investigate this final idea in detail by examining the titin isoforms present in a wide variety of mammalian muscles functioning cyclically, including the heart, the diaphragm and other locomotor muscles.

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