

Muscle performance during frog jumping: influence of elasticity on muscle operating lengths

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A fundamental feature of vertebrate muscle is that maximal force can be generated only over a limited range of lengths. It has been proposed that locomotor muscles operate over this range of lengths in order to maximize force production during movement. However, locomotor behaviours like jumping may require muscles to shorten substantially in order to generate the mechanical work necessary to propel the body. Thus, the muscles that power jumping may need to shorten to lengths where force production is submaximal. Here we use direct measurements of muscle length *in vivo* and muscle force–length relationships *in vitro* to determine the operating lengths of the plantaris muscle in bullfrogs (*Rana catesbeiana*) during jumping. We find that the plantaris muscle operates primarily on the descending limb of the force–length curve, resting at long initial lengths ($1.3 \pm 0.06 L_o$) before shortening to muscle's optimal length ($1.03 \pm 0.05 L_o$). We also compare passive force–length curves from frogs with literature values for mammalian muscle, and demonstrate that frog muscles must be stretched to much longer lengths before generating passive force. The relatively compliant passive properties of frog muscles may be a critical feature of the system, because it allows muscles to operate at long lengths and improves muscles' capacity for force production during a jump.

Keywords: length-tension; stiffness; passive tension; locomotion; biomechanics

1. INTRODUCTION

The anuran body plan is highly specialized for jumping. Features such as long hindlimbs, a stout vertebral column and a relatively small body size are considered specializations associated with enhanced jumping performance (Zug 1972; Emerson 1978; Shubin & Jenkins 1995). Although these morphological features are undoubtedly important, the frog jump is ultimately reliant on the mechanical force and power supplied by the hindlimb musculature.

The muscles that power frog jumping, like all vertebrate skeletal muscles, are governed in their mechanical function by well-known contractile properties. Gordon and coworkers described one such property more than 40 years ago when they showed that force output in a contracting muscle varied with muscle length (Gordon *et al.* 1966). The familiar force–length relationship shows that muscle force reaches a plateau at intermediate lengths, the midpoint of which defines the muscle's optimal length for force production (L_o). The plateau of the force–length relationship is relatively narrow and as a result vertebrate skeletal muscles can generate maximal force only within about ± 5 per cent of L_o . At lengths longer and shorter than this range, the force output of the muscle declines. Because high force output is probably desirable in muscle contractions, this region is generally considered physiologically optimal and previous investigators have suggested that muscles'

operating lengths *in vivo* should be limited to this region (e.g. Lieber *et al.* 1992; Rome 1998).

Do frog muscles function optimally during jumping by operating only at lengths where force is maximized? A consideration of the mechanics of jumping suggests that a restricted range of muscle operating lengths may not maximize performance. Jump distance is ultimately determined by the muscle work done during takeoff (Marsh 1994), and work is the product of muscle force and shortening distance. Thus, to maximize work output and power in a single contraction, muscles should shorten substantially during the takeoff phase of a jump.

Recent measurements of muscle fascicle length changes in jumping frogs support this prediction. Some muscles have been shown to shorten by as much as 30 per cent of their initial length during a jump (Olson & Marsh 1998; Roberts & Marsh 2003). Based on a typical force–length relationship, a muscle shortening by 30 per cent cannot avoid the significant influence of length on force output. A muscle starting at L_o and shortening by 30 per cent would end the contraction at a length where force output approaches only about 50 per cent of the muscles' maximum force. Thus, the predicted optimal function for frog muscles during jumping faces the problem that the substantial shortening associated with increased muscle work would seemingly require a tradeoff in force.

Here we used direct measurements of muscle length *in vivo* and muscle force–length relationship *in vitro* to determine the operating lengths of the plantaris muscle in bullfrogs (*Rana catesbeiana*) during jumping. We tested the hypothesis that, owing to large shortening strains associated with the high work requirements of a

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jump, the plantaris muscle would shorten to lengths well below the plateau of the force–length curve, where force output is submaximal.

2. MATERIAL AND METHODS

(a) *Animals*

Four adult bullfrogs (*R. catesbeiana*) ranging in body mass from 135–163 g were purchased from a herpetological vendor. Animals were housed in the Brown University Animal Care facility in large aquaria, which included both aquatic and terrestrial regions. Frogs were kept on a diet of large crickets provided ad libitum.

(b) *Surgical procedures*

Frogs were anaesthetized using buffered tricain methanesulphonate (MS222, 0.25 g l^{-1}). Once anaesthetized, a small incision along the dorsal midline was used to feed sonomicrometry and electromyography (EMG) transducers under the skin along the leg, past the hip and knee. A second incision was made along the skin covering the plantaris muscle. Sonomicrometry transducers (1 mm, Sonometrics Inc., London, Ontario, CA, USA) were implanted along a proximal fascicle of the plantaris muscle and secured using 6-0 silk and a small drop of VetBond adhesive (figure 1*b*). Two fine-wire bipolar EMG (Medwire Corp., Mt. Vernon, NY, USA) electrodes were inserted into the muscle just medial and lateral to the fascicle implanted with sonomicrometers (figure 1*b*). EMG electrodes were implanted using a 26G hypodermic needle and were sutured in place. Once transducers were in place, all incisions were closed and the frogs were given 24 h for recovery.

(c) *In vivo data collection*

Measurements of fascicle length (sonomicrometry) and muscle electrical activity (EMG) were taken during jumps. Sonomicrometry data were collected using a Sonometrics TRX8 system (Sonometrics Inc.). The raw sonomicrometry signals were monitored using an oscilloscope in order to ensure that the transducers were consistently triggering off the first analogue peak. EMG signals were amplified ($1000\times$) with a DAM50 differential amplifier (World Precision Instruments, Sarasota, FL, USA). All data were collected at 4000 Hz using a 16-bit A/D converter (National Instruments, TX, USA). Jumps were imaged at $250 \text{ frames s}^{-1}$ (figure 1*c*) using two Fastcam 1280 high-speed video cameras (Photron Inc., CA, USA). All data were synchronized using a common external trigger. The seven longest jumps from each individual were analysed. The length of jumps used in this study ranged from 37 to 55 cm.

(d) *In vitro data collection*

Once all necessary jumps were collected from an individual, the force–length relationship of the same plantaris muscle was quantified using an *in vitro* preparation. The frogs were euthanized with a double pithing protocol. The leg previously implanted with sonomicrometry crystals for jumping trials was isolated. The distal tendon of the plantaris was severed and the muscle freed from the ankle and the tibiafibula. The proximal attachment of the muscle at the knee was kept intact. Along the femur, the sciatic nerve was freed from the surrounding tissue and a nerve cuff, constructed from silver wire was attached to stimulate the

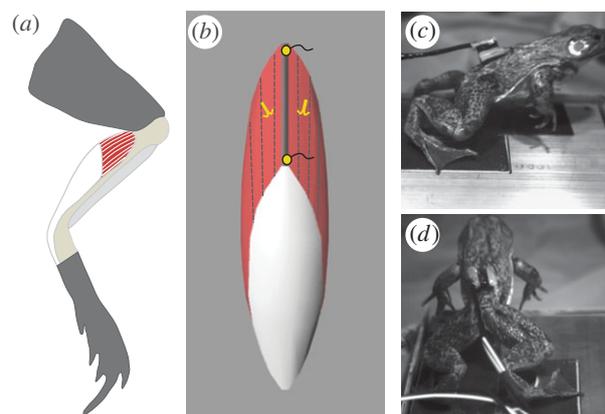


Figure 1. Methods for measuring plantaris lengths and activity during jumping. (a) The plantaris is highlighted in ventral view showing the substantial aponeurosis and tendon (white) associated with the muscle. The plantaris acts as the primary ankle extensor. (b) Sonomicrometry transducers were placed along superficial, proximal fascicles to measure length. Two bipolar EMG electrodes were also implanted in the same region to measure muscle activation patterns. (c,d) Jumps were recorded in two views with high-speed video at $250 \text{ frames s}^{-1}$.

muscle. The sonomicrometry transducers implanted prior to jumping trials were left in place and used for fascicle length measurements. Care was taken to limit any disruption of the sonomicrometry transducers during the isolation process. The femur and the tibiafibula were attached to a rigid plate and the distal end of the plantaris tendon was placed in a custom-made clamp. The preparation was placed in an amphibian Ringer's solution (100 mM NaCl, 2.5 mM KCl, 2.5 mM NaHCO_3 , 1.6 mM CaCl, 10.5 mM Dextrose), which was continuously aerated with oxygen and kept at 22°C . The tendon clamp was attached to a servomotor (310 B-LR, Aurora Scientific Inc., Ontario, CA, USA) with stiff aircraft cable. The implanted sonomicrometry transducers were used to measure muscle fascicle length, while the servomotor was used to measure muscle force.

To determine the optimum stimulation voltage, a muscle's twitch force was monitored as the voltage was increased by 1 V increments. The voltage that resulted in maximum twitch force was increased by 1 V and used to supramaximally stimulate the muscle. The muscle was then stimulated tetanically at varying lengths in order to characterize its active force–length properties. Each tetanic stimulation was performed using 0.2 ms pulses, at $100 \text{ pulses s}^{-1}$ for a duration of 300 ms. The passive force–length properties were also quantified using the sonomicrometer-measured length and force prior to stimulation. All data were collected at 1000 Hz using a 16-bit A/D converter (National Instruments, TX, USA). Following data collection, the morphological properties of the muscle, including fascicle length, muscle-tendon length, muscle mass and tendon length were measured. The muscle's physiological cross-sectional area was calculated according to Sacks & Roy (1982).

(e) *Data analysis*

All sonomicrometry, EMG and force data were processed and analysed using Igor Pro software (Wavemetrics, OR, USA). Sonomicrometry data required little processing. Occasional level-shifts were removed from the data using a

custom algorithm. EMG data were processed with a band-pass filter with a 100 Hz low cut-off and a 1000 Hz high cut-off.

Data from high-speed video were analysed using Matlab (Mathworks, Inc. MA, USA). The location on the back of the frogs where transducer leads exited was used to approximate the frogs' centre of mass. This location has been previously used as a reliable indicator of the centre of mass (Marsh & John-Alder 1994; Roberts & Marsh 2003). This location was digitized in both camera views. Direct linear transformation of XY coordinates were used to convert data to XYZ positions. The displacements of this digitized point were smoothed with a quintic spline interpolation (s.d. = 0.05) and differentiated to calculate the velocity of the centre of mass during jumps.

Data from isolated muscle experiments were analysed using Igor Pro software. Given the pennate architecture of the plantaris, the aponeurosis of the muscle could not be removed. Therefore, all contractions included some degree of fascicle shortening against the stretch of series elastic structures. As a result, active force and length data were taken, where peak force reached a constant plateau for each contraction. To calculate only the active contribution to force, the passive force at the length corresponding to peak force was subtracted from the total force. This protocol allowed us to account for the fascicle shortening that occurs during the contraction when series elastic elements are present (Macintosh & Macnaughton 2005). Active force–length data were fit with the following function:

$$F_{\text{active}} = e^{-(L^b-1)/s^a} \quad (2.1)$$

where F is force, L is length, and a , b and s represent the roundness, skewness and width of the force–length curve, respectively (Otten 1987). Based on the fit applied to the active force–length data, the peak isometric force (P_0) and the fascicle length at peak force (L_0) were determined for each muscle. The passive force–length data were fit with a standard exponential function (Fung 1967).

$$F_{\text{passive}} = P_0 e^{e^{1+e2(L/L_0)}} \quad (2.2)$$

Each muscle's optimal length (L_0) was used to convert *in vivo* length changes during jumps to strains ($(L - L_0)/L_0$). Total fascicle strain as well as the fascicle strain that occurred prior to centre of mass movement was calculated. The duration of EMG activity for the entire jump as well as the period prior to centre of mass movement was calculated.

In addition to the experimental data collected, a meta-analysis of data from the literature was conducted to compare the passive force–length properties of frog hindlimb muscles to mammalian hindlimb muscles. Studies used for this analysis included a wide range of methodologies from whole muscle *in situ* studies to single fibre preparations. Only data from studies that measured both active and passive force–length properties were included. This allowed normalization relative to peak isometric force (P_0) and optimal length (L_0). Measurements from chemically or mechanically skinned fibres were not included in the analysis because these methods eliminate connective tissue elements surrounding the fibre that have been shown to contribute to passive tension (Prado *et al.* 2005). All passive curves were used to determine the relative length at which the passive force reached 20 per cent of P_0 . This variable was termed L_{20}

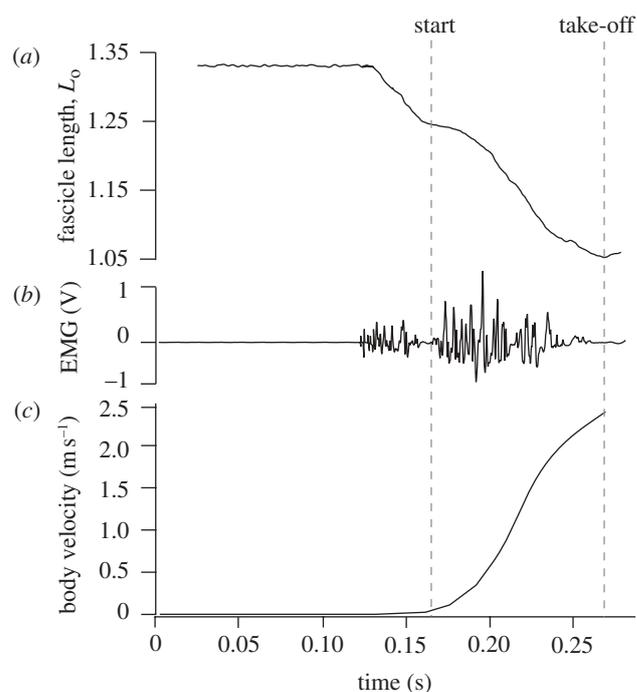


Figure 2. Muscle length, EMG and body velocity for a representative jump. (a) Fascicle lengths measured by sonomicrometry normalized to the muscle's optimal length (L_0). (b) Muscle activity during jumps. The two separate bursts of EMG activity shown occurred in many but not all jumps. (c) Plot of the body velocity during the jump measured from high-speed video. This plot shows that little external movement occurred during the period of initial muscle activation and shortening. The beginning of the jump as measured from external video often coincided with a slowing of fascicle shortening and reduced EMG activity.

and was used to statistically compare frog and mammalian hindlimb muscles with a one-way analysis of variance.

3. RESULTS

The plantaris muscle was active and began shortening in advance of any perceptible movement (figure 2). The plantaris muscle was active for about 42 ms and shortened by about 10 per cent before any jump motion was detectable. The beginning of body movement often corresponded to a short period of reduced muscle activity such that the EMG pattern appeared as two distinct bursts (figure 2b). In addition, initial movements of the body often corresponded to a brief slowing of fascicle shortening (figure 2a). Muscle fascicles continued to shorten throughout the jump, reaching a total shortening strain of 25–30% L_0 before take-off.

The active force–length curve of the plantaris had the commonly described parabolic relationship, consisting of an ascending, plateau and descending portion (figure 3a). Maximum isometric force normalized for cross-sectional area ranged from 25–29 N cm⁻² in the four muscle preparations (table 1). The muscles did not develop passive force until stretched to relatively long lengths (approx. $1.2L_0$), beyond which passive force increased exponentially with increasing length (figure 3a).

The range of fascicle lengths observed during jumping corresponded to the descending limb and plateau of the force–length curve (figure 3b). We found that while

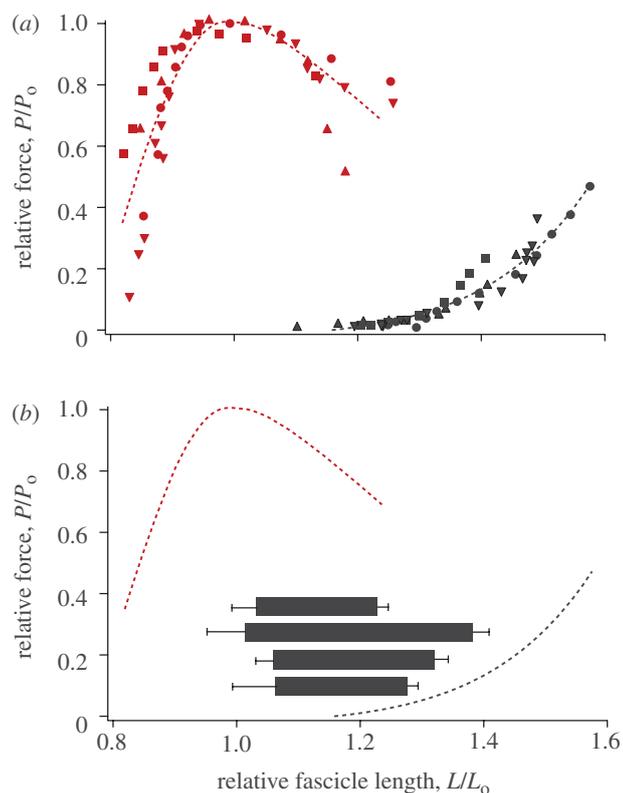


Figure 3. The force–length properties and operating lengths of the plantaris muscle. (a) Normalized force–length curves from plantaris muscles of four individuals. Active properties are shown in red and passive properties are shown in black. Each individual is represented by a different symbol. (b) Muscle operating lengths for the plantaris muscle during jumps. Fascicle length was measured with the same sonomicrometry transducers during both *in vivo* jumps and *in vitro* characterization of the force–length curves, allowing for the accurate determination of operating length during jumping. Each horizontal bar represents the mean (\pm s.d.) operating length for one individual. All muscles started at lengths corresponding to the descending limb of the active force–length curve and shortened onto or near the plateau. Note that the horizontal bars correspond only to values on the x-axis.

frogs were at rest in the crouched pre-jump position, fascicles were on average 30 per cent longer than the muscle's optimum length ($1.3L_0$). During the take-off phase of the jump, fascicles shortened by an average of 27 per cent, resulting in final lengths corresponding to the plateau of the force–length curve (figure 3b). On average, fascicle length at take-off did not differ significantly from muscle optimal length ($p < 0.001$).

A comparison of fixed-end contractions *in vitro* demonstrates the influence of the initial and final length on maximum force output in a contraction (figure 4). In contraction (1), the muscle begins on the plateau but shortens down to a final length corresponding to the ascending limb of the force–length curve (figure 4). In contraction (2), the muscle begins on the descending limb but shortens down to a final length within the plateau region (figure 4). As a result of differences in the final length, peak force developed in the second contraction is much higher than the force of the first contraction. This example shows that peak force production may be

Table 1. Plantaris muscle properties. L_0 , length at max isometric force; P_0 , max isometric force; L_{20} , length at which passive force reaches 20 per cent.

individual no.	mass (g)	L_0 (mm)	P_0 (N cm^{-2})	L_{20} (L_0)
1	2.31	11.32	26.59	1.46
2	2.29	10.26	25.91	1.39
3	2.41	9.35	24.96	1.45
4	2.56	10.95	28.76	1.43

more influenced by muscle lengths at the end of a contraction than by the muscle's initial length.

The relatively long fascicle lengths in the frog plantaris before the jump would, in many muscles, be associated with high passive tension. However, we found that the passive tension at these lengths (approx. $1.3L_0$) corresponded to less than 10 per cent of P_0 (figure 3). This result prompted a comparison of the passive properties of various hindlimb muscle in order to understand whether this pattern was unique to our study. Our meta-analysis confirmed that the passive compliance found in our study was broadly observed in other studies of frog hindlimb muscles (figure 5). A comparison of the passive force–length properties of frog and mammalian hindlimb muscles showed that frog muscles do not develop passive tension until much longer lengths (figure 5). To provide a statistical comparison of the passive properties of frog and mammalian hindlimb muscles, we quantified the length (L_{20}) at which each muscle develops passive tensions corresponding to 20 per cent of P_0 (table 1). We found that frog muscles have a significantly higher L_{20} than mammalian muscles ($p < 0.0001$; figure 5b). Based on this analysis, we conclude that increased passive compliance is an important functional feature of frog hindlimb muscles.

4. DISCUSSION

(a) Operating lengths of the frog plantaris

The plantaris muscle shortens significantly during a jump, with fascicles shortening by about 30 per cent of their initial length during the take-off phase. This relatively large magnitude of shortening is consistent with some previous studies (Olson & Marsh 1998; Roberts & Marsh 2003). However, other studies have shown strains to be lower in the plantaris of *Bufo marinus* (Gillis & Biewener 2000) and the semimembranosus of *Rana pipiens* (Lutz & Rome 1994, 1996). These differences may, in part, be due to variation in the jump distance in different experiments, as shorter jumps will require less mechanical work, which may be produced with less muscle shortening. Nonetheless, the large strains observed in the plantaris suggest that excursions are not likely to be restricted to the plateau of the force–length curve.

Despite the large strains observed in the plantaris during a jump, our hypothesis that the muscle would shorten to lengths well below the plateau of the force–length curve was not supported. Rather than starting at the plateau and shortening to the ascending limb of the curve, the plantaris started at long lengths, on the descending portion of the curve, and shortened to final

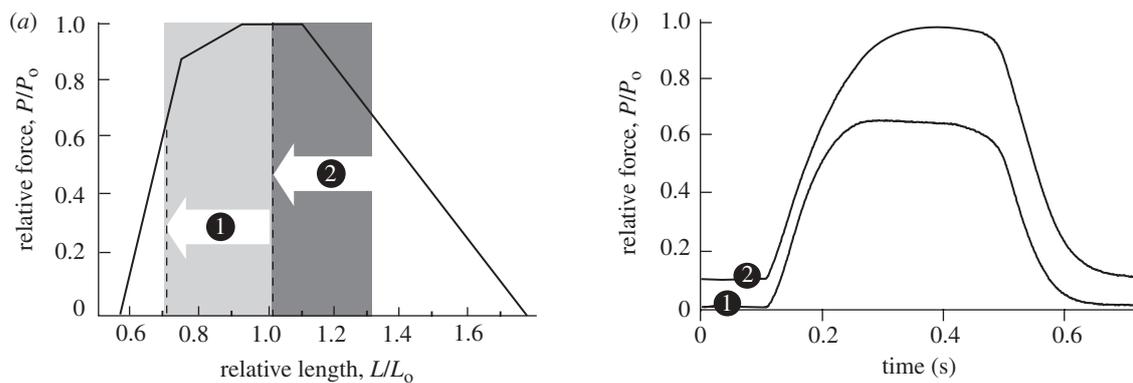


Figure 4. The effect of operating length on the peak force production in a muscle. Using an *in vitro* protocol we show the same muscle undergoing two contractions (1 and 2) where the fascicles shorten by about 30 per cent. (a) Operating lengths for two ‘fixed-end’ contractions in the same muscle shortening against elastic structures *in vitro*. In both contractions the muscle shortens by about 30 per cent, but contraction (1) starts on the plateau, while contraction (2) starts on the descending limb. The classic Gordon Huxley & Julian (1966) force–length curve illustrates the hypothetical effect of length on force for maximally activated, isometric muscle. (b) Developed force versus time for two sample contractions consisting of length changes depicted in panel (a). Higher peak force is developed when the muscle starts at a longer length. This example shows that peak force production may be influenced more by a muscle’s length at the end of a contraction than by its initial length.

lengths on the plateau. Operating on either the ascending or descending limb of the curve comes at a cost of reduced average force. A consideration of the dynamics of force production in a single contraction, however, suggests that the pattern observed in the bullfrog plantaris may be advantageous for generating higher peak forces.

The consequences for force production for two different starting lengths are illustrated in figure 4. The figure depicts two contractions, where a muscle starts at an initial length corresponding to either (i) the plateau, or (ii) the descending limb and then actively shortens by 30 per cent of L_0 (figure 4). In both contractions, the muscle–tendon length is kept constant and fascicle shortening is done against the stretch of elastic structures. Although in both cases the muscle operates over lengths where force production on average would be submaximal, the peak force developed is much higher in the second contraction, when the muscle begins on the descending limb (figure 4b). The reason for this difference is that when the muscle begins on the plateau, it shortens beyond the optimal lengths for force production during the early period of force development, when the muscle is not yet fully active. In contrast, when the contraction begins on the descending limb, the muscle operates at the optimal lengths for force production when the force is fully developed. As a result, the peak force ultimately developed for a contraction that began on the descending limb is nearly twice as high when compared with the contraction that began on the plateau (figure 4). Thus, under the contractile conditions approximating those expected during a jump, peak forces are more likely to be determined by fascicle lengths near the end of force production as opposed to lengths during the initial period of force development. Developing high peak forces may be particularly important for muscles like the plantaris that operate with an in-series tendon, as energy storage and recovery in elastic elements will be proportional to peak force.

One challenge in relating the *in vivo* behaviour of muscles to their *in vitro* properties is that recruitment patterns of a muscle are difficult to recreate *in vitro*. As

a result, an important caveat to our results is that the force–length curve of the muscle is characterized under maximal stimulation. One advantage to frog jumping as a model system is that an anti-predator behaviour like a frog jump likely involves maximal recruitment (Lutz & Rome 1994). However, even under conditions of submaximal recruitment, a muscle’s force–length curve should accurately describe the effect of length on force in the subset of fibres that are active. One other concern in translating force–length behaviour measured *in vitro* to *in vivo* function is a potential effect of stimulation frequency. Several studies have shown that a muscle’s force–length curve shifts toward longer lengths at lower stimulation frequencies (Rack & Westbury 1969; Roszek *et al.* 1994; Zuurbier *et al.* 1998; Brown *et al.* 1999). Brown and colleagues (1999) found that a threefold drop in stimulation frequency (from 120 to 40 pps) resulted in a shift of approximately 10 per cent L_0 . Such a shift, if it exists *in vivo*, would affect the values of operating range that we measured for the plantaris. However, even with a 10 per cent shift in the value of L_0 , the plantaris would operate largely on the descending limb.

(b) Dangers of the descending limb

Few muscles have been shown to operate primarily on the descending limb of the force–length curve during locomotion. It is generally accepted that the descending limb of the force–length curve is avoided because of the increased susceptibility of muscle fibres to damage when actively stretched at long lengths (Lieber & Friden 1993; Proske & Morgan 2001). Many locomotor activities (i.e. downhill running, braking, jump landing) require active muscle lengthening in order to absorb energy and decelerate the body (Lindstedt *et al.* 2001). These eccentric contractions can disrupt the cytoskeletal components of myofibrils and result in decreased capacity for force generation (Lieber *et al.* 1996; Proske & Morgan 2001). The likelihood of eccentric muscle damage has been shown to increase with muscle length such that muscles operating on the descending limb of the

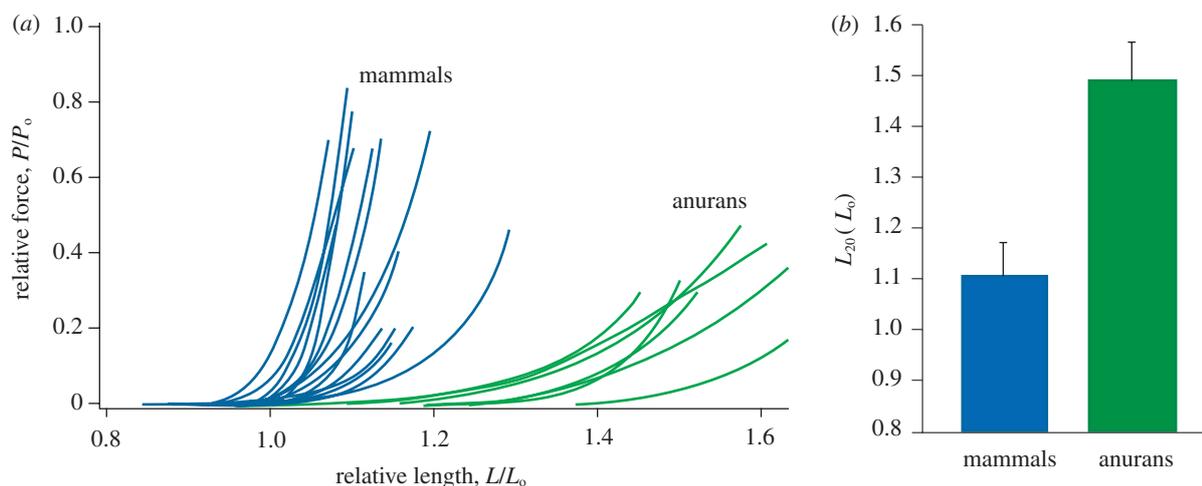


Figure 5. Meta-analysis of passive muscle properties. (a) Passive force–length curves are shown for 15 mammalian and 7 anuran hindlimb muscles. The data show that frog muscles can be stretched to longer muscle lengths relative to their optimal length (L_0) for a given force. (b) A comparison of the length (L_{20}) at which muscles reach passive forces that correspond to 20 per cent of maximum active isometric force. Anuran muscles can be stretched to significantly ($p < 0.0001$) longer lengths for the same level of force. Data included in this analysis were limited to published studies of hindlimb muscles that measured both active and passive force–length properties and could therefore be normalized to L_0 . Mammalian data are from: Gareis *et al.* (1992), Woittiez *et al.* (1983), Hawkins & Bey (1997), Davis *et al.* (2003), Witzmann *et al.* (1982), Rack & Westbury (1969) and Askew & Marsh (1998). Anuran data are from: Bagni *et al.* (1988), Julian & Moss (1980), this study, Talbot & Morgan (1998), Edman (1979), Altringham & Bottinelli (1985) and Ter Keurs *et al.* (1978). See electronic supplementary material, figure for more detail.

force–length curve suffer greater disruption at the level of the sarcomere (Morgan 1990; Gosselin & Burton 2002). Therefore, in muscles that may be required to absorb energy, avoiding the descending limb of the force–length curve may be considered a protective mechanism against the potential of eccentric muscle damage.

Unlike most terrestrial vertebrates, the hindlimb muscles of frogs are rarely loaded eccentrically. During swimming, hindlimb muscles shorten and produce positive work to generate the necessary hydrodynamic forces (Richards & Biewener 2007). Similarly, during the take-off phase of jumping and hopping, hindlimb muscles shorten and produce positive work in order to accelerate the mass of the frog. Although the landing phase of a jump or a hop requires the absorption of energy, most frogs use their forelimbs and bodies rather than their hindlimbs for this function (Nauwelaerts & Aerts 2006). In fact, no EMG activity is observed in the hindlimb muscles of anurans during the landing phase of a jump or a hop (Olson & Marsh 1998; Ahn *et al.* 2003). It is possible that the lack of eccentric loading allows frog hindlimb muscles to safely operate on the descending limb of the force–length curve with little threat of eccentric muscle damage.

(c) *Passive muscle forces and anuran muscle function*

In many muscles, the feasibility of operating on the descending limb of the force–length curve may also be limited by high passive tensions at long muscle lengths. Stretching muscles to initial lengths that correspond to the descending limb can require high forces, which must ultimately come from either antagonistic muscles, or gravitational or inertial forces on the body. Since passive forces in muscles increase exponentially with length, getting to such long lengths may simply require too much force in many muscles.

A comparison of anuran and mammalian passive muscle forces reveal a striking difference in passive muscle properties that explains why frog muscles are capable of operating at such long lengths (figure 3). Compared with mammalian muscles, frog hindlimb muscles can be stretched to significantly longer lengths before developing high passive tensions. Literature values for passive force–length relationships indicate that, if frog muscles had the same passive properties as those of mammals, the passive forces developed at the pre-jump resting length of $1.3L_0$ would be extremely high. Most literature values for passive muscle stiffness indicate that mammalian muscles would develop forces as much as or exceeding the muscle's peak active force at $1.3L_0$ (figure 5). In fact, such high passive tensions may limit the ability to assume the highly crouched pre-jump limb posture common in most frogs.

It has previously been suggested that increased passive muscle stiffness limits a limb's range of motion (Brown *et al.* 1996). Therefore, the observed compliance of frog hindlimb muscles may allow for the large joint excursions associated with a jump and may imply a broader relationship between changes in passive muscle properties and diversity in limb posture.

Variation in a muscle's passive stiffness can arise from changes in either intra-sarcomeric proteins or extra-sarcomeric connective tissues. Studies have shown that the spring-like sarcomeric protein titin is responsible for some of the passive elastic properties of muscle (Wang *et al.* 1991). Titin isoforms vary in size and an increase in size has been shown to be inversely proportional to passive stiffness (Granzier & Labeit 2006). A muscle's passive elastic properties can also be attributed to extra-sarcomeric collagenous structures surrounding myofibers (Williams & Goldspink 1984; Purslow 1989). An increase in the collagen content of the perimysium and endomyosium can result in a significant increase in a muscle's

passive stiffness (Williams & Goldspink 1984). Although it is well established that both titin and extra-sarcomeric connective tissues contribute to passive tension, the relative contribution of each component can vary substantially between different muscles (Prado *et al.* 2005). It remains unclear whether the differences in the passive properties of frog and mammalian hindlimb muscles observed in this study arise from differences in myofibrillar proteins like titin or ultrastructural differences in extra-sarcomeric connective tissues. Future comparative studies may shed light on the passive structures responsible for changes in stiffness and how such changes may ultimately accompany shifts in locomotor function.

5. CONCLUSIONS

During a jump the plantaris muscle begins on the descending limb of the force-length curve and shortens onto the plateau. The observed operating lengths suggest little decrement in force production despite significant fascicle shortening. Our findings suggest that the passive compliance of frog hindlimb muscles is a critical and unique feature of the system, which facilitates the use of the descending limb of the force-length curve and improves force production during jumping.

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