Plantar flexor muscles of kangaroo rats (*D. deserti*) shorten at a velocity to produce optimal power during jumping

M. Janneke Schwaner*[1], David C. Lin[2,3,4], and Craig P. McGowan[1,4,5]

[1] Department of Biological Sciences, University of Idaho, Moscow ID, USA

[2] Voiland School of Chemical Engineering and Bioengineering, Washington State University, Pullman WA, USA

[3] Department of Integrative Physiology and Neuroscience, Washington State University, Pullman WA, USA

[4] Washington Center for Muscle Biology, Washington State University, Pullman WA, USA

[5] WWAMI Medical Education Program, Moscow ID, USA

*Corresponding author: M. Janneke Schwaner (mjschwan@uci.edu).

ABSTRACT

During jumping by kangaroo rats, the musculotendon work contributions across all joints are not well understood. Namely, measures of external joint work do not provide information on the contributions from individual muscles or in-series elastic structures. In this study, we examined the functional roles of a major ankle extensor muscle, lateral gastrocnemius (LG), and of a major knee extensor muscle, vastus lateralis (VL), through *in vivo* sonomicrometry and electromyography techniques, during vertical jumping by kangaroo rats. Our data showed that both muscles increased shortening and activity with higher jumps. We found that knee angular velocity and VL muscle shortening velocity were coupled in time. In contrast, the ankle angular velocity and LG muscle shortening velocity were decoupled, and rapid joint extension near the

end of the jump produced high power outputs at the ankle joint. Further, the decoupling of muscle and joint kinematics allowed the LG muscle to prolong the period of shortening velocity near optimal velocity (V_{opt}), which likely enabled the muscle to sustain maximal power generation. These observations were consistent with a LG tendon that is much more compliant than that of the VL.

Introduction

There is a great diversity of musculotendon architectures that are associated with a muscle's motor performance. For example, muscles with relatively long parallel fibers are suggested to be better for high-power outputs. Whereas muscles with short, highly pennate fibers and large cross-sectional areas may be better suited for high force production. These muscles are often attached to relatively long, thin tendons which make the musculotendon units (MTU) well suited for elastic energy storage (Biewener and Roberts, 2000). The importance of energy storage and return from tendons during steady state locomotion has been well studied in terrestrial animals (e.g.; Alexander et al., 1982; Biewener, 1998; Ker et al., 1987) and has been a particular focus for bipedal hopping species and hopping by humans (e.g. Cavagna et al., 1964; Alexander, 1984; Bobbert et al., 1986; Biewener and Blickhan, 1988). However, complex habitats require animals to generate non-steady movements such as maneuvering or moving on inclines, and therefore require mechanical work to be done on the center of mass during a single movement. An extreme example of such a task is predator avoidance, during which some animals use high accelerations to escape.

Kangaroo rats, bipedal hopping rodents (family: Heteromyidae), use vertical jumps to evade predation by snakes and during intra-species encounters (Bartholomew and Cawell, 1951; Webster and Webster, 1980; Freymiller et al., 2019). During submaximal jumps, as performed in a laboratory setting, power outputs approach the limits of what muscles can produce (Schwaner et al., 2018). Yet it remains unclear how these animals generate these high powers. Several studies have assumed that because kangaroo rat tendons are relatively thick, they do not play a substantial role in storing and releasing elastic energy during jumps or steady speed hopping, but rather are adapted to handle the high forces associated with escape jumps (Thompson et al., 1980; Biewener and Blickhan, 1988). For example, in a recent study we calculated that kangaroo rats return up to 3.75% of the required energy for take-off from tendon recoil (Schwaner et al., 2018), a calculation based on the generic value of 1000 MPa for tendon elastic modulus (Ker, 1981). However, Javidi et al. (2019) reported that kangaroo rat tendons are significantly more compliant (330 MPa for the gastrocnemius and 528 MPa for the plantaris muscle) (Javidi et al., 2019). When implementing these new elastic modulus values, our data suggest desert kangaroo rats are able to return ~9.4% of energy needed for a ~0.4m jump from elastic energy stored in the tendons. Although this is still a relatively small contribution to the total energy required for a jump, this re-analysis highlights the importance of tendon compliance and suggests that elastic elements may play a more important and complex role during high-performance tasks by kangaroo rats than previously thought. This also highlights the importance of having species specific tendon data, as studies have shown a substantial range of tendon properties related to animal size and functional task (e.g., LaCroix et al, 2013, Cui et al, 2009).

Tendons can contribute to maximizing or optimizing muscle performance in multiple ways, depending on task demands (Roberts and Azizi, 2011). For example, the elastic capacity of muscle and tendons allows for an increase in whole animal power outputs by storing energy over a longer time and releasing it in a very short amount of time (e.g. Shadwick, 1990; Biewener and Betram, 1991; Roberts, 2002). Tendons also can decouple shortening velocities of the muscle from the shortening velocities of the MTU. The importance of this decoupling is that it may allow the muscle to shorten at its optimal velocity for power production (V_{opt}) (approximately 0.3 times the maximal shortening velocity) (Close, 1972; Rehwaldt et al., 2017; Javidi et al., 2020) for a longer duration. Optimal muscle power output for a longer duration would increase the total work done (the time integral of power) by the muscle, especially within the very limited time that kangaroo rats have to take-off during predator escapes. Note that the work done by the muscle directly adds energy to the mass of the animal and/or is stored in the tendon (to be released later in the form of musculotendon power and work). In both cases, the total work done by the muscle increases the whole-body energy at take-off and results in a greater jump height.

During jumping, the highest power outputs are typically measured at the ankle (e.g. Van Soest et al., 1985; Aerts, 1998; Moore et al., 2017; Schwaner et al., 2018), in part due to the transfer of power generated at the distal joints by bi-articular MTUs, and by dynamic coupling of the limb segments (Gregoire et al., 1984; Jacobs et al., 1996; Zajac et al., 2002). In jumping vertebrates, 25 – 68% of the power measured at the ankle joint originates from more proximal muscles and is transferred over the bi-articular linkage of the ankle extensors (Gregoire et al., 1984; Bobbert et al., 1986; Aerts, 1998; Schwaner et al., 2018). For kangaroo rat jumps, up to

48% of work measured at the ankle originates from more proximal muscles (Schwaner et al., 2018). However, because there are multiple proximal bi-articular linkages and many proximal muscles, it is not clear which proximal muscles are generating this work. During jumps by kangaroo rats, the knee joints do relatively little net external work due to small joint moments during knee extension. Yet, because the origins of the ankle extensors are on the femur, knee extension is the most direct mechanism for energy transfer to the ankle. Therefore, we expect the vastus lateralis, a large knee extensor, to play an important role in jumping. A role that may not be apparent through inverse dynamics analysis alone.

Estimates of MTU length changes based on joint kinematics cannot parse muscle versus tendon length changes, nor reveal muscle strain behavior. To determine the relative contribution of muscle length changes to MTU length changes, we can quantify muscle strain through in vivo measurements such as ultrasound or sonomicrometry and reveal the functional role tendons play in movement tasks (Roberts, 2000; Lichtwark and Wilson, 2006; Lai et al., 2015; Dick et al., 2021). In the present study, we examined the functional role of a major ankle extensor, the lateral gastrocnemius (LG), and a major knee extensor, the vastus lateralis (VL), in kangaroo rats during vertical jumps over a range of jump heights. These muscles have substantially different muscle tendon architecture, with the VL having muscle fascicles that are 3.5 times longer than the tendon (ratio of fascicle to tendon lengths of 18.1: 5.1 mm), compared to the LG which has muscle fascicles that are 0.4 times the length of the tendon (ratio of fascicle to tendon lengths of 16.0 : 40.4) (Rankin et al., 2018). We examined differences (1) through in vivo muscle measures, and (2) by obtaining LG and VL MTU length changes from joint kinematics and moment arms, to compare muscle shortening and MTU

shortening. We hypothesized that LG and VL muscle shortening, and activity patterns will increase as jump height increases, which would suggest that both these muscles contribute to increasing mechanical demands of the task. In addition, we hypothesized that a decoupling between MTU shortening velocity and muscle velocity, consistent with the relatively compliant and longer free LG tendon, enabling more power and work output from the muscle.

Materials and methods

Animals and Training

Six healthy adult desert kangaroo rats (*D. deserti*) (average body mass: 108.12 ± 9.13 g (S.D.)) were used for these experiments. Animals were wild caught in the Mojave Desert in southeast Nevada (USA) with permission from the Nevada Department of Wildlife. All animals were trained prior to experiments to familiarize them with the experimental environment and the task. During training and experimental sessions, animals were presented obstacles of different heights and were motivated with hand movements to jump over the obstacles. All experimental procedures and husbandry of the animals were approved by and performed in accordance with the University of Idaho Institutional Animal Care and Use Committee (IACUC).

Surgical procedures

To obtain *in vivo* recordings of muscle strain and activity, piezoelectric sonomicrometry (SONO) crystals (1 mm, Sonometrics, London, Canada) and fine-wire bipolar, off-set twist hook electromyography (EMG) electrodes (0.1 mm Teflon coated silver; Cooner Wire, Inc.) were implanted in the VL and LG using aseptic surgical techniques.

Animals were anesthetized in an induction chamber connected to an isoflurane vaporizer (3%). During the surgery animals were maintained at 1 – 2% isoflurane using a nose cone. The left hind leg and the top of the head of the animal were shaved using a small animal clipper and were sanitized with an antiseptic solution and alcohol. Two incisions were made on the hind leg: one parallel to the VL muscle and one parallel to the LG muscle. A third incision was made on the back of the head, right behind the cranium and between the ears. Two EMG electrodes, one common wire, and four SONO crystals, which were connected to a custom-built micro-connector plug, were passed under the skin between the small incision behind the head to the incisions in the hind leg.

A pair of SONO crystals were implanted in the VL and LG muscles within a small incision. We aimed to place crystals as far apart as possible to encompass most of the muscle and to get measurements of muscle shortening and related shortening velocity. Crystals were placed approximately 8 - 12 mm apart, within each muscle. The crystals were aligned for maximum signal-to-noise ratio by observing their output live on an oscilloscope. The incisions in the muscle were closed and the crystals were anchored with 5-0 suture. EMG electrodes were inserted with a 22-gauge needle and secured with 5-0 suture adjacent to the location of the SONO crystals. The common wire was placed between the skin and back musculature. All electrode placements were confirmed post-mortem, and data were excluded from analysis if any wires were found to be loose or in the wrong position. Skin incisions were closed with 3-0 suture and the connector plug was attached to the head with cyanoacrylate adhesive and 3-0 suture. Using permanent marker and non-toxic white paint, markers were placed on the animals' hind leg joint centers of rotation (toe, metatarsophalangeal (MTP), ankle, knee, and

hip joint) and one marker on the ilium. Animals were given a minimum of 24 hours to recover from surgery before experiments were conducted.

Experimental design

Animals jumped, starting at a resting position, from a force plate (AMTI HE6x6, Watertown, MA) inside a plexi-glass enclosure while instrumented with SONO crystals and EMG wires. SONO and EMG data were collected with LabChart (ADIntruments, Colorado Springs, CO). Video data were collected at 200 Hz from a high-speed video camera (Xcitex Inc, Woburn, MA, USA), force plate data were sampled at 600 Hz, and SONO signals were conditioned (Model 120.1, Triton Technologies) and EMG amplified (Grass P511) and then sampled at 2000 Hz. Animals were encouraged with hand gestures to jump over an obstacle (height ranging from 0.12 to 0.55m). The goal was to collect a full range of heights from each animal, which typically included 10-20 jumping trials. Animals landed on a foam pad that was placed on the opposite side of the barrier. Because obstacle height did not equal jump height due to animals overshooting or grabbing the barrier, we calculated the actual jump height for each trial based on the ground reaction force data and ballistic equations (for details, see Schwaner et al., 2018).

Data analysis

Trials were selected for analysis based on completeness of video and force plate data and jump performance (n = 41 trials, 4 to 10 trials per animal). There was a minimum of 4 animals for each of the *in vivo* measures (LG SONO, LG EMG, VL SONO, and VL EMG) (Table 1).

Only jumps that had a sagittal orientation of the animal to the camera and a simultaneous take-off with both legs were used. Total time of a jump trial was determined based on the vertical ground reaction force. Trial start was defined as when the force exceeded the animals' body weight, and trial end as when the vertical force was equal to zero. An initial countermovement occurred during a few trials (< 15%), which was visible in the vertical ground reaction force as a slight decrease below the animal's body weight. When a countermovement did occur, the start of the trial was determined to be the point just before the force decreased below body weight.

Data analysis was performed using a customized analysis script in Matlab (2015a, MathWorks, Natick, MA). Joint markers on metatarsals, ankle, knee, hip and ischium were digitized from the video using ProAnalyst software (Xcitex Inc, Woburn, MA, USA) and low pass filtered (fourth order Butterworth filter) with a cut-off frequency of 30 Hz. Joint angles were calculated based on 2D coordinate data. Ground reaction forces were filtered with a low-pass filter (second order Butterworth filter) with a cut-off frequency of 55 Hz. SONO data were filtered with a second order Butterworth filter with a cut-off frequency of 55 Hz. We used a 50Hz high pass filter on the EMG data to remove motion artefacts and low frequency noise.

Ground reaction force, video, and *in vivo* (EMG and SONO) data were synchronized via a trigger pulse and cut to trial duration. EMG onset and end were determined by an increase and decrease beyond 3 standard deviations of the resting baseline, recorded over a longer period and when the animal was sitting still in the set-up. Because not all animals achieved the same maximal jump height, EMG data were normalized by EMG intensity from a common task of a 0.2-meter-high jump in order to account for magnitude differences due to electrode placement across individual animals. Specifically, individual EMG intensity for each jump, measured as the

integral of the rectified EMG signal for the duration of a burst, was divided by the EMG intensity of this common task jump of 0.2 m. The initial length of a muscle was taken for each trial from a mean SONO length from a period before the jump when the animal was visually sitting still. These values were averaged across trials for each animal to calculate a subject specific initial muscle length (L_{init}) that was used to calculate muscle strain. Muscle strain was calculated by dividing the length change of the SONO crystals by L_{init}. All values and time-series data are reported in text as an average with plus and minus one standard deviation.

To estimate the duration over which a muscle was shortening near $V_{opt,}$, we determined when the muscle strain rate was within the bounds of V_{opt} ±30% of V_{opt}. The percentage of the jump cycle that the respective muscle was operating within the identified range, was obtained for each trial, and averaged over all trials. The value of V_{opt}, 3.0 muscle lengths per second, or a strain rate of 0.3, was taken from our previous study where force-velocity curves were obtained from kangaroo rat LG muscles (Javidi et al., 2020). This study showed the power-velocity curve for the LG has a broad peak, and within the interval of ±30% of V_{opt}, power was greater than 94% of the maximum power value. Force-velocity data were not available for the VL. We assume that the VL, like the LG, are composed of primarily fast twitch muscle fibers (Ross and Meyers, 2021), like other small rodents such as rats (Eng et al., 2008).

MTU length changes were based on joint kinematics and estimates of moment arms measured post-mortem. Initial MTU length was calculated from the individual animals' tibia length and position of ankle and knee joints. Previous studies indicated that changes in moment arms with changing joint angles were relatively small over the range of joint angles in this study, so they were not considered here (Rankin et al., 2018).

Statistical Analysis

To test for significance between the response variables muscle strain and EMG intensity with the predictor variable jump height, we used a mixed-effects model ANOVA. Individual ('Animal') was included as a random effect. Four different mixed effect models were evaluated before choosing final model.

1. 'Y ~ 1 + (1|Animal)'

2. 'Y ~ 1 + JumpHeight + (1|Animal)'

3. 'Y ~ 1 + (1|Animal) + (Jumpheight − 1| Animal)'

4. 'Y ~ 1 + JumpHeight + (1|Animal) + (JumpHeight|Animal)'

The first statistical model ('Y ~ 1 + (1|Animal)'), is the simplest model, corresponding to the null hypothesis that muscle function varies only randomly between individuals and not with jump height. This serves as a reference to rigorously test the alternative hypotheses that muscle function varies with jump height, either as an independent covarying fixed factor

(model 2), as an covarying fixed factor within individuals (model 3) or as a covarying factor both within and across individuals (model 4).

Based on this comparison, model 2 was used for our statistical analysis of VL EMG, VL muscle strain, LG muscle strain, and shortening velocities of VL, LG, and MTU at peak force, as it had the lowest AIC scores (Akaike, 1976). LG EMG response was tested with model 4, as that model had the lowest AIC score. For LG EMG data, the models that had the lowest AIC scores (model 2, AIC = 34.53; model 4, AIC = 33.21) have jump height as a fixed effect and only differ in random effects. Therefore, we concluded that choosing a different model for the LG EMG response data would provide the same comparison of variables with jump height. For the outcomes of the individual mixed-effect model, we reported F-statistics (F), R-squared (R^2), and p-value (p).

We used an ANCOVA to compare strain rates versus angular velocities, between the VL and LG muscle at initial contraction until peak ground reaction force (GRF) to test the hypothesis that muscle strain of VL versus LG, and LG muscle versus distal ankle extensor MTU were significantly different. We also tested the hypothesis if the shortening velocities of the VL versus LG at peak force were significantly different. For all ANCOVA tests, F-statistics (F) and p-values (p) were reported. We visually inspected the distribution for each variable to confirm that it conforms to a normal distribution without pronounced skew and confirmed that the mean adequately represents the center of the distribution. We used a pooled t-test to determine if the VL and the LG muscle operated for different time durations around V_{opt}.

All statistical analyses were performed in the Matlab Statistics and Machine Learning Toolbox. For all statistical analyses used, we considered results significant when $p < 0.05$.

RESULTS

Jumps varied in height, ranging from 0.06 to 0.36 meters. Average initial muscle length (L_{init}) measured by SONO was 10.69 mm (± 0.70 mm) in the LG muscle (range: 9.55-11.97 mm) and 8.65 mm (± 1.14 mm) for the VL muscle (range: 7.19-10.25 mm). Shortest muscle lengths occurred after peak force, and during knee and ankle extension (Figure 1). Normalized VL and LG EMG intensity (Figure 2) increased significantly with increasing jump height ($F = 6.01$, $R^2 = 0.82$, $p = 0.019$; and $F = 12.22$, $R^2 = 0.37$, $p = 0.001$, respectively). The slope of the relative EMG intensity was not found to be significantly different between VL and LG (F-stat = 2.45, $p = 0.12$). Both the VL and LG shortened more with increasing jump height (Figure 3) (VL: $F = 20.64$, $R^2 = 0.79$, $p < 0.001$ and LG: $F = 10.55$, $R^2 = 0.84$, $p = 0.009$); however, the magnitude of VL strain increased significantly more with jump height than the LG strain (F-stat = 6.66, $p = 0.012$). The LG MTU typically showed a brief period of lengthening, on average 2.02% of the rest length (± 1.36%), shown as a positive length change, whereas the VL and VL MTU were more similar in pattern (Figure 4). LG shortening, based on the regression of muscle shortening and jump height, ranged from 1.98 mm at low jumps to 2.92 mm at high jumps, which corresponded to muscle strains of -0.19 and -0.26, respectively. Shortening by the VL muscle ranged from 1.14 mm to 2.46 mm, which corresponded to a muscle strain of -0.12 for a low jump and -0.25 for a high jump.

Both the VL and LG shortened at strain rates ranging near V_{opt} (0.3Vmax ± 30%) during the jump; however, the duration that the LG shortened at this rate was significantly longer than the VL (grey bars in Fig. 5 A&B; t-stat = 10.43, df = 69, sd = 6.29 p < 0.001). The LG began to shorten at approximately V_{opt} (Figure 5B, grey bar graph) at 60.79% (± 11.66%) of the jump and ended at 82.23% (± 7.43%) of the jump. The VL shortening at approximately V_{opt} started at 67 .34% (± 12.13%) of the jump and ended at 73.03% (± 10.79%) of the jump. The highest strain rates for the VL and LG (averages of 7.78 and 8.69 L_{init}/s, respectively) occurred after peak force. At peak force, the strain rates were 4.89 L_{init}/s (± 2.50 L_{init}/s) and 3.28 L_{init}/s (±1.77 L_{init}/s) for the VL and LG, respectively.

The strain rates at peak force were significantly different between the LG and VL (F-stat = 11.65, p = 0.012). Average strain rates for the LG MTU were 3.30 L_{init}/s (± 1.46L_{init}/s) at peak force, with a maximum of 4.46 L_{init}/s (±2.73 L_{init}/s). The LG MTU strain rates at peak force were significantly different from the LG muscle strain rates (F-stat = 7.37, p = 0.08), whereas the LG had a significantly lower maximum shortening velocity, compared to the LG MTU (t-stat = -5.72, df = 80, sd = 3.20, p < 0.001). The relationships between joint angular velocity and strain rate during joint extension (Figure 6) were significantly different between the LG and VL muscle (F-stat = 54.04, p < 0.001), with average correlation coefficients of 0.71 ± 0.15 and 0.93 ± 0.07, respectively.

DISCUSSION

We examined the *in vivo* functional roles of a primary ankle extensor, the LG, and a primary knee extensor, the VL, during jumping by kangaroo rats. During jumping, muscles must shorten to generate mechanical work to raise the animals' center of mass. Our data show that when jump task demand increases, kangaroo rats shorten both their VL and LG muscle over a greater range (Figure 3), suggesting an increase in work contribution by both muscles with higher jumps. In addition to shortening over longer distances, the LG and VL muscle increased EMG intensity with jump height (Figure 2). Further, in agreement with our hypothesis, we found that ankle kinematics and LG muscle kinematics were decoupled in time. Decoupling of muscle and joint kinematics, allows muscles to operate under more optimal conditions while the leg extends at higher, less favorable velocities.

During jumping, MTUs can play multiple different roles, namely, they can transfer energy, store and release energy, and/or produce energy. For example, they can transfer energy over biarticular linkages (Cleland, 1867; Prilutsky and Zatsiorsky, 1994). Transfer of energy between proximal muscles and distal joints during jumping by vertebrates ranges between 25 – 68% of energy measured at ankle joint (Bobbert et al., 1986; Aerts, 1998), and we previously found that for jumping desert kangaroo rats, ~48% of the ankle work is transferred over the distal bi-articular linkage (Schwaner et al., 2018). External work measures indicated that the knee joint was not a substantial contributor to the work of raising the center of mass. Although it is difficult to determine the source of transferred energy measured at the ankle joint, it is expected that large proximal leg extensor muscles contribute to these jumps based

on muscle architecture. Here we show that the VL, a primary knee extensor, does increase shortening and EMG intensity when jump height increases, suggesting increasing mechanical work output. Despite the knee doing limited net external work based on inverse dynamics analysis, here we show that the VL muscle behaves like it is doing net work. This highlights a disconnect between inverse dynamics analysis and muscle mechanics. However, we cannot quantify the VL's contribution to the energy transfer over the bi-articular ankle extensor, due to multiple proximal muscles acting on the knee directly or through bi- and multi-articular linkages.

Additionally, tendons can influence length change patterns of muscles during locomotor tasks (Roberts, 2002), as tendon stiffness determines how tightly muscle kinematics are coupled to joint kinematics (Ker, 1981; Ker et al., 1988). Here we quantified that the VL has a significantly tighter relationship with joint angular velocity (average correlation coefficient 0.93), compared to the LG (average correlation coefficient 0.71). A longer decoupling (21.43 % of jump cycle), in the LG, compared to the VL (5.68% of jump cycle) is consistent with a more compliant tendon for a given change in force over time. We found that at peak ground reaction force, the LG muscle strain rate (3.28 ± 1.77 L_o/s) is near V_{opt}, which is ~3 lengths per second for kangaroo rat plantar flexor muscles (Javidi et al., 2020). Similar decoupling was also observed in the human gastrocnemius and soleus muscle during walking and running, hopping, as well as in jumping tasks (Lichtwark and Wilson, 2006; Lai et al., 2015; Farris et al., 2016; Nikolaidou et al., 2017; Dick et al., 2021). Another mechanism by which fascicle length changes are uncoupled from whole MTU length changes, which has been called variable gearing and includes rotations of the muscle fascicles due to the bulging of the muscle (Wakeling et al., 2011). However, these

could not be assessed using the 2D sonomicrometry methods used in the current study. Although we do not have the mechanical properties of the VL tendon, strain behavior suggests the tendon is relatively stiff. Specifically, the VL muscle shortening patterns mirror knee extension patterns closely and the VL strain rate increases throughout the jump without the plateau seen in the LG, which is consistent with muscles that have similar muscle tendon architecture to the VL in this study (Biewener & Roberts, 2000). As a result, the VL muscle operates near V_{opt} (~3 lengths per second) for a shorter period (5.67 ± 3.16 % jump for VL versus 21.43 ± 7.98 % jump for LG). Here we assume ~10 lengths per second is V_{max}, as reported for a predominantly fast twitch muscle in small rodents (Rehwaldt et al., 2017), which corresponds to V_{opt} of around three lengths per second. Although we do not have exact measures of V_{opt} for the VL, strain rates change over a wide range that likely encompasses V_{opt}. Therefore, a small shift in the assumed value for VL V_{opt} would not impact our conclusions.

In addition to decoupling muscle and joint kinematics, elastic structures can play a role in storing and releasing energy by creating an asymmetric loading and unloading time scale. This can enable remarkable power outputs that exceed the physiological limit that muscle alone can produce (e.g. Aerts, 1998; Peplowski and Marsh, 1997). Previously, it was suggested that if an animal had a whole-body power output exceeding the skeletal muscle power output for a given task, the animal must use energy storage in elastic structures to increase their power output over this mechanical limit (e.g. Peplowski and Marsch, 1997; Aerts, 1998). However, elastic energy storage also plays an important role in non-extreme activities. Kangaroo rats can store up to ~10% of work in their tendons for a submaximal laboratory jump (Schwaner et al., 2018), based on their mechanical properties (Javidi et al., 2019). Although energy return values

are substantially greater than earlier estimated based on generic tendon compliance values, energy storage and return by tendon does not seem to be the predominant source of energy powering in kangaroo rat jumps.

While muscle shortening increased with jump height for both the LG and VL, the shortening patterns during jumping were significantly different. LG muscle shortening increased by a little over a third (~36%) from jumps of 0.06 m to jumps of 0.3 m, whereas the VL muscle shortening more than doubled with the same increase in jump height (~208%). The comparatively small change in LG stain with jump height is consistent with maintaining a relatively constant shortening velocity at around $V_{opt.}$ across jump heights, whereas the VL shortening velocity increased throughout the duration of the jump (Figure 6). Additionally, we did not find a significant difference in relative change in EMG intensity over jump heights between the VL and LG. This suggests that the increase in muscle recruitment is similar for both muscles, such that the greater VL shortening is not due greater recruitment. In fact, shortening velocity at peak force increased more with increasing jump height in the VL compared to that of the LG muscle. The shortening velocity of the LG MTU and LG muscle were significantly different. Although we did not directly measure power output in this study, the higher LG MTU velocity, as compared to the LG muscle velocity, indicates the tendon is transferring power to the ankle in addition to power produced by the muscle.

In contrast to wild, snake-evading kangaroo rats, our controlled laboratory conditions allowed animals time to modify body posture prior to take-off, potentially benefiting performance. In the wild, these animals can reach phenomenal jump heights (~0.8m, Freymiller, personal communication), while also seeming to minimize the time it takes to leave the ground in order to avoid a predator's strike. The presence of a compliant tendon, and even elongated hind legs, seem to favor maximizing energy generation over jump quickness, as both of these adaptions likely increase the time needed for a jump. Yet kangaroo rats also have exceptional reaction time and are capable of leaving the ground extremely quickly (Freymiller et al., 2017). The trade-off between jump performance (i.e., height) and quickness (i.e., the time to leave the ground) is an area of ongoing study and will require a more detailed analysis of how these animals perform in natural settings to fully understand. In the laboratory setting, kangaroo rats appeared to sometimes use postural changes that could cause tendon pre-stretch and increase jump performance, but this would require time, which could be detrimental to the kangaroo rat in a ecological predator-prey interaction. This is a potential reason that countermovements are not frequently observed, but our laboratory setting might allow for some postural pre-stretch, exemplified by the MTU lengthening before shortening (Figure 4). A time difference in duration between loading and unloading of elastic elements was not included in the jump interval as we defined it, because jump start was defined based on force pattern. Therefore, we could not account for MTU kinematics prior to the initiation of the jump or calculate estimates of tendon strain relative to the unloaded condition.

We found that kangaroo rats increase jump height with increased recruitment and shortening in muscles acting at both the knee and ankle. In contrast to external net work calculations from our previous studies, this suggests an increase in muscle work at both joints and highlights the limitations of inverse dynamics analysis for understanding individual muscle function. In addition, we show the decoupling of muscle and joint kinematics, in which the strain rate of the LG muscle is decoupled from ankle joint extension and is consistent with a compliant tendon, allows the muscle to operate near V_{opt} for an extended time while the leg extends. Although we do not have VL tendon properties, we hypothesize that the VL tendon is stiffer than the LG tendon, as the VL muscle shortening behavior mirrors knee extension patterns more closely and muscle and joint extension exhibits no explicit decoupling behavior.

ACKNOWLEDGEMENTS

AUTHOR CONTRIBUTION

Conceived and designed the experiments: MJS DCL CPM. Performed the experiments: MJS CPM. Analyzed the data: MJS DCL CPM. Wrote first draft of manuscript: MJS CPM. Edited versions of the manuscript: MJS DCL CPM.

ETHICS STATEMENT

All experimental procedures and husbandry of the animals were approved by and performed in accordance with the University of Idaho Institutional Animal Care and Use Committee (IACUC).

DATA AVAILABILITY

The dataset generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare no competing interests.

REFERENCES

Aerts, P. 1998. Vertical jumping in galago senegalensis: the quest for an obligate mechanical power amplifier. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 353 (1375), 1607 – 1620.

Alexander, R.M., Maloiy, G.M.O., Ker, R.F., Jayes, A.S., Warui, C.N. 1982. The role of tendon elasticity in the locomotion of the camel (Camelus dromedarius). Journal of Zoology London 198, 293 – 313.

Alexander, R.M. 1984. Elastic energy stores in running vertebrates. American Zoologist 24.1, 85 – 94.

Bartholomew, G.A., Caswell, H.H. 1951. Locomotion in kangaroo rats and its adaptive significance. Journal of Mammalogy 32, 155 – 169

Bennett, M.B., Ker, R.F., Dimery, N.J. Alexander, R.McN. 1986. Mechanical properties of various mammalian tendons. Journal of Zoology 209, 537 – 548

Biewener, A.A. 1998. Muscle-tendon stresses and elastic energy storage during locomotion in the horse. Comparative Biochemistry and Physiology 120, 73 – 78.

Biewener, A.A., Bertram, J.E.A. 1991. Efficiency and optimization in the design of skeletal support systems. Chapter in: Efficiency and economy in animal physiology. Editor: Blake, R.W. Cambridge University Press, Cambridge, UK.

Biewener, A.A., Blickhan, R. 1988. Kagnaroo rat locomotion: design for elastic energy storage or acceleration? Journal of Experimental Biology 140, 243 – 255

Biewener, A.A., Roberts, T.J. 2000. Muscle and tendon contributions to force, work, and elastic energy savings: a comparative perspective. Exercise and Sport Science Reviews 28(3), 99 – 107.

Bobbert, M.F., Huijing, P.A., Van Ingen Schenau, G.J. 1986. An estimation of power output and work done by the human triceps surae muscle-tendon complex in jumping. Journal of Biomechanics 19, 899 – 906

Cavagna, G.A., Saibene, F.P., Maragaria, R. 1964. Mechanical work in running. Journal of applied physiology 19.2, 249 – 256.

Cleland, J. 1867. On the actions of muscles passing over more than one joint. Journal of Anatomy and Physiology 1, 85 – 93

Close, R.I. 1972. Dynamic properties of mammalian skeletal muscles. Physiological Reviews 52, 129 – 197

Dick, T.J.M., Clemente, C.J., Punith, L.K., Sawicki, G.W. 2021. Series elasticity facilitates safe plantar flexor muscle-tendon shock absorption during perturbed human hopping. Proceedings of the Royal Society B 288: 20210201.

Farris, D. J., Lichtwark, G. A., Brown, N. A. T. Cresswell, A. G. 2016. The role of human ankle plantar flexor muscle–tendon interaction and architecture in maximal vertical jumping examined in vivo. Journal of Experimental Biology 219, 528–534

Freymiller et al., 2017. Recent interactions with snakes enhance escape performance of desert kangaroo rats (Rodentia: Heteromyidae) during simulated attacks. Biological Journal of the Linnean Society 122 (3), 651 – 660

Freymiller, G.A., Whitford, M.D., Higham, T.E., Clark, R.W. 2019. Escape dynamics of free-ranging desert kangaroo rats (Rodentia: Heteromyidae) evading rattle snake strikes. Biological Journal of the Linnean Society 127 (1), 164 – 172

Gregoire, L., Veeger, H.E., Huijling, P.A., Van Ingen Schenau, G.J. 1984. Role of mono- and biarticular muscles in explosive movements. International Journal of Sports Medicine 5, 301 – 305

Jacobs, R., Bobbert, M.F., Van Ingen Schenau, G.J. 1996. Mechanical output from individual muscles during explosive leg extensions: the role of biarticular muscles. Journal of Biomechanics 29, 513 – 523

Javidi, M., McGowan, C.P., Schiele, N.R., Lin, D.C. 2019. Tendons from kangaroo rats are exceptionally strong and tough. Scientific reports 9 (1), 1 – 9

Javidi, M., McGowan, C.P., Lin, D.C. 2020. Estimation of the force-velocity properties of individual muscles from measurement of the combined plantarflexor properties. Journal of Experimental Biology, 10.1242/jeb.219980

Ker, R.F. 1981. Dynamic tensile properties for the plantaris tendon of sheep (Ovis aries). Journal for Experimental Biology 93, 283 – 302

Ker, R.F., Alexander, R.McN., Bennett, M.F. 1988. Why are mammalian tendons so thick? Journal of Zoology London, 216, 309 – 324

Ker, R.F., Bennet, M.B., Bibby, S.R., Kester, R.C., Alexander, R.M. 1987. The spring in the arch of the human foot. Nature 325, 147 – 149.

Lai, A., Lichtwark, G.A., Schache, A.G., Lin, Y.C., Brown, N.A.T., Pandy, M.G. (2015). In vivo behavior of the human soleus muscle with increasing walking and running speeds. Journal of Applied Physiology 118: 1266 – 1275.

Lichtwark, G.A., Wilson, A.M. (2006). Interactions between the human gastrocnemius muscle and the Achilles tendon during incline, level and decline locomotion. Journal of Experimental Biology 209, 4379 – 4388.

Lutz, G.J., Rome, L.C. 1994. Built for jumping: the design of the frog muscular system. Science 263 (5145), 370 – 372.

Moore, T.Y., Rivera, A.M., Biewener, A.A. 2017. Vertical leaping mechanics of the Lesser Egyptian Jerboa reveal specialization for maneuverability rather than elastic energy storage. Frontiers of Zoology 14, 32

Nikolaidou, M. E., Marzilger, R., Bohm, S., Mersmann, F., Arampatzis, A. 2017. Operating length and velocity of human M. vastus lateralis fascicles during vertical jumping. R Soc Open Sci 4, 170185

Peplowski, M.M., Marsh, R.L. 1997. Work and power output in the hindlimb muscles of Cuban tree frogs Osteopilus septentrionalis during jumping. Journal of Experimental Biology 200, 2861 – 2870

Prilutsky, B.I. Zatsiorsky, V.M. 1994. Tendon action of tow-joint muscles: transfer of mechanical energy between joint during jumping, landing, and running. Journal of Biomechanics 17, 24 – 34

Rankin, J.W., Doney, K.M., McGowan, C.P. (2018). Functional capacity of kangaroo rat hindlimbs: adaptations for locomotor performance. *Journal of the Royal Society Interface* 15, 20180303.

Rehwaldt, J.D. Rodgers, B.D. Lin, D.C. 2017. Skeletal muscle contractile properties in a novel murine model for limb girdle muscular dystrophy 2i. Journal of Applied Physiology 123: 1698 – 1707

Roberts, T.J. 2002. The integrated function of muscles and tendons during locomotion. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 133(4), 1087 – 1099

Schwaner, M.J. Lin, D.C. McGowan, C.P. 2018. Jumping mechanics of desert kangaroo rats. The Journal of Experimental Biology 221 (22).

Roberts, T.J., Azizi, E. 2011. Flexible mechanisms: the diverse roles of biological springs in vertebrate movement. Journal of experimental biology 214, 353 – 361.

Ross, C.D., Meyers, R.A. 2021. Immunohistochemistry of kangaroo rat hindlimb muscles. The Anatomical Record, 1-13.

Shadwick, R.E. 1990. Elastic energy storage in tendons: mechanical differences related to function and age. Journal of Applied Physiology 68, 1033 – 1040

Thompson, S.D., MacMillen, R.E., Burke, E.M., Taylor, C.R. 1980. "The energetic cost of bipedal hopping in small mammals." *Nature* 287, 223-224

Van Soest, A.J., Roebroeck, M.E., Bobbert, M.F., Huijling, P.A., Van Ingen Schenau, G.J. 1985. A comparison of one-legged and two-legged countermovement jumps. Medical Sciences Sport and Exercise 17, 635 – 639

Wakeling, J. M., Blake, O. M., Wong, I., Rana, M., and Lee, S. S. M. 2011. Movement mechanics as a determinate of muscle structure, recruitment, and coordination. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 366, 1554–1564. doi: 10.1098/rstb.2010.0294

Webster, D.B., Webster, M. 1980. Morphological adaptations of the ear in the rodent family Heteromyidae. American Zoologist 20, 247 − 254

Zajac, F.E., Neptune, R.R., Kautz, S.A. 2002. Biomechanics and muscle coordination of human walking. Part I: Introduction to concepts, power transfer, dynamics and simulations. Gait Posture 16, 215 − 232

Figures
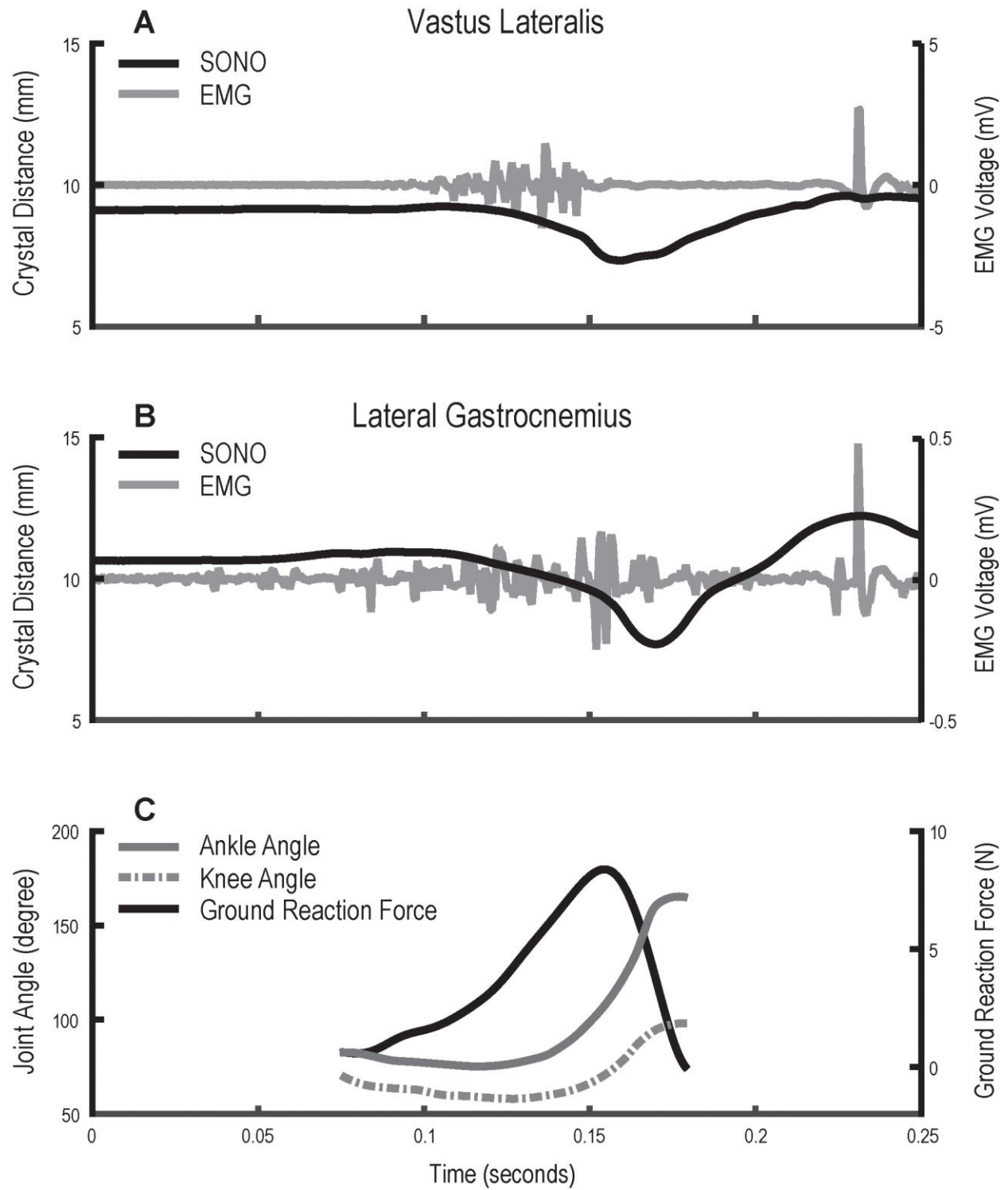
Figure 1. Representative data for a jump of 0.28m. Electromyography (EMG, right vertical axis) and sonomicrometry (SONO, left vertical axis) data from the Vastus Lateralis (VL, graph A) and Lateral Gastrocnemius (LG, graph B), as well as angles (graph C, left axis) for the ankle (grey solid line) and knee (dashed grey line) and ground reaction force (solid black line, right vertical axis). EMG and SONO data in graph A and B are raw data (not filtered).
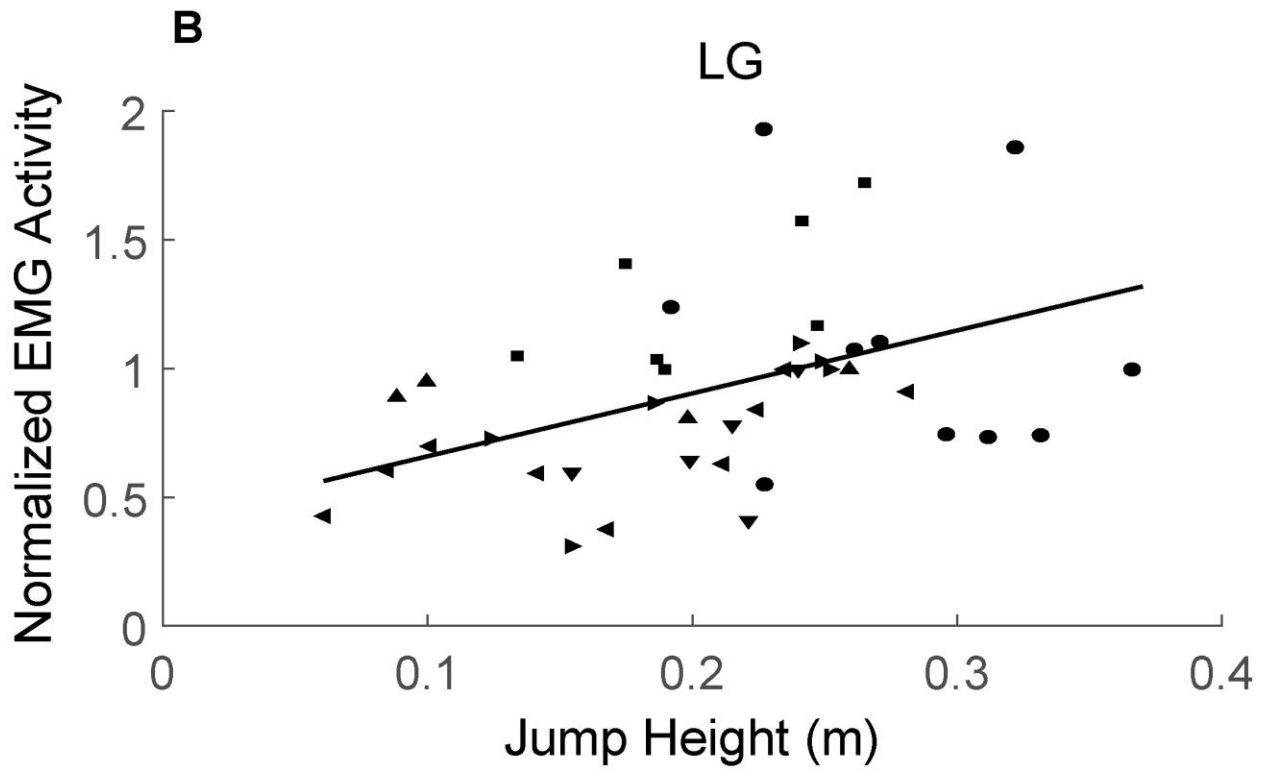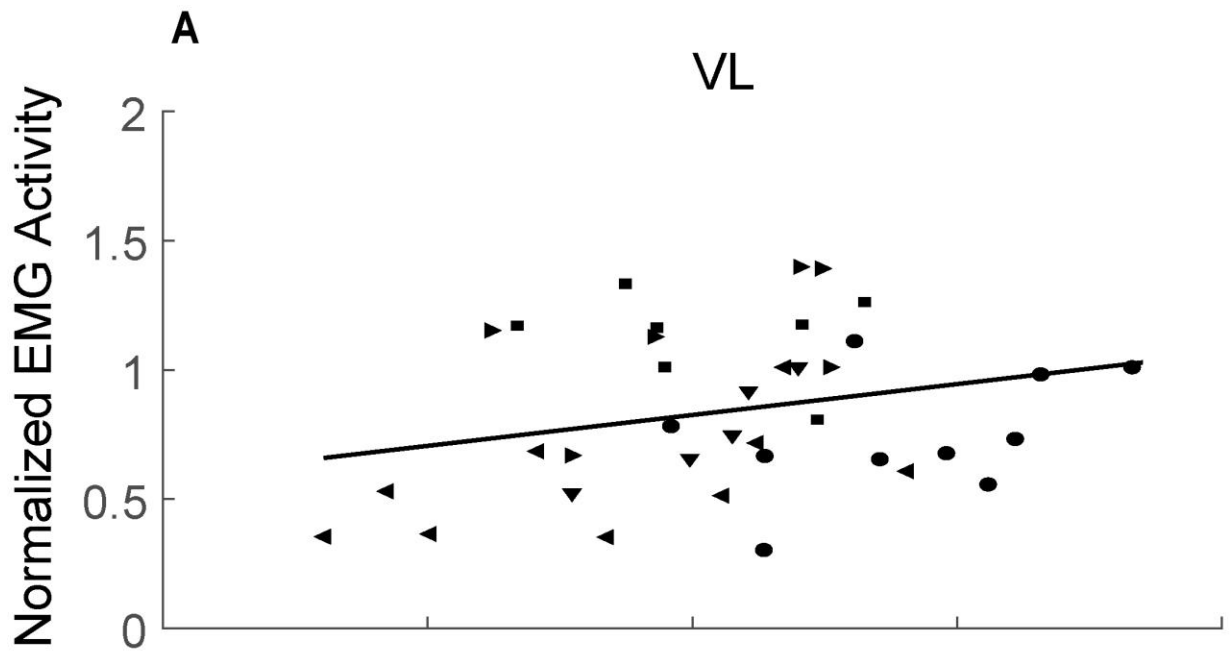
Figure 2. Relative EMG intensity for VL (A) and LG (B). Relative EMG intensity in the VL and LG increased significantly with increasing jump height (VL: F = 6.01, R^2 = 0.81, p = 0.019, LG: F = 12.22, R^2 = 0.37, p = 0.001). Relative EMG intensity relationship with jump height was not significantly different between muscles (F-stat 2.45, p = 0.12). Each individual is represented by a different marker. Solid lines indicate regression lines. EMG was normalized by EMG intensity from a 0.2 m high jump for each animal.
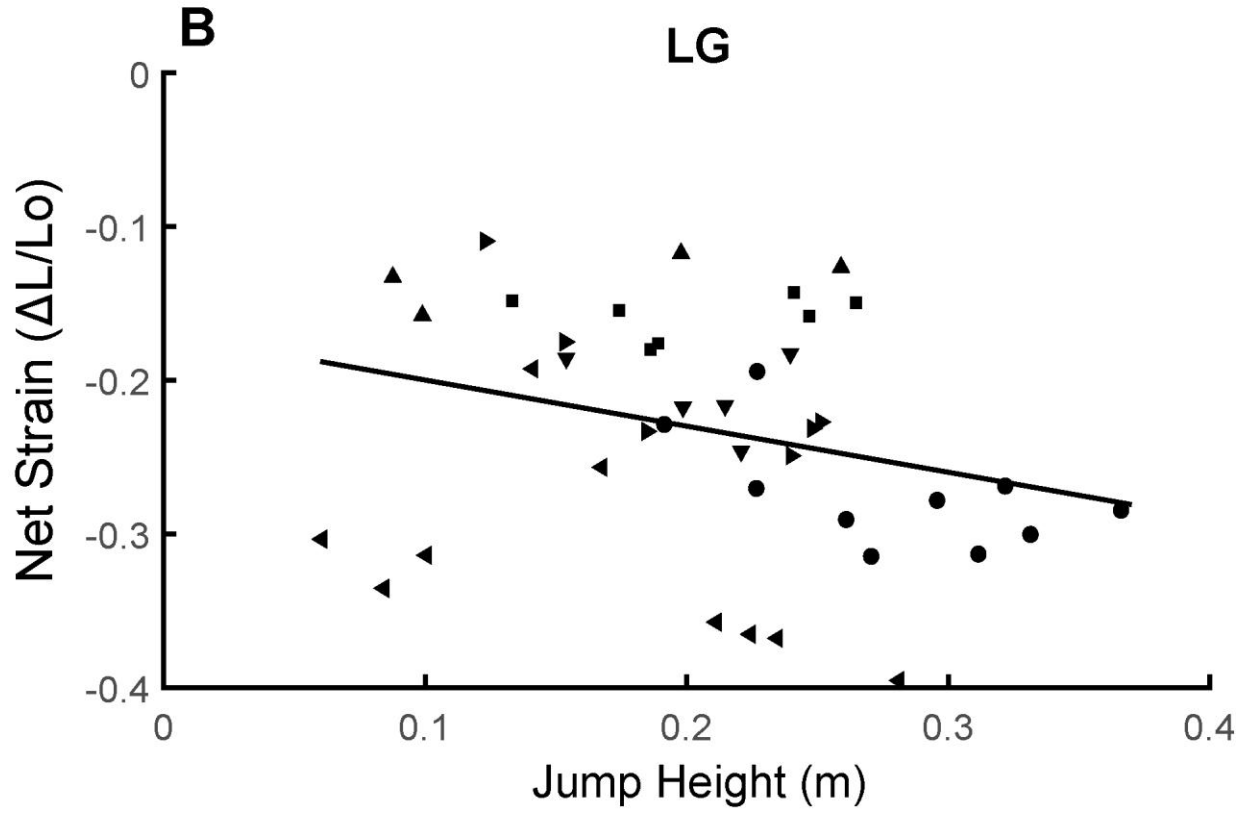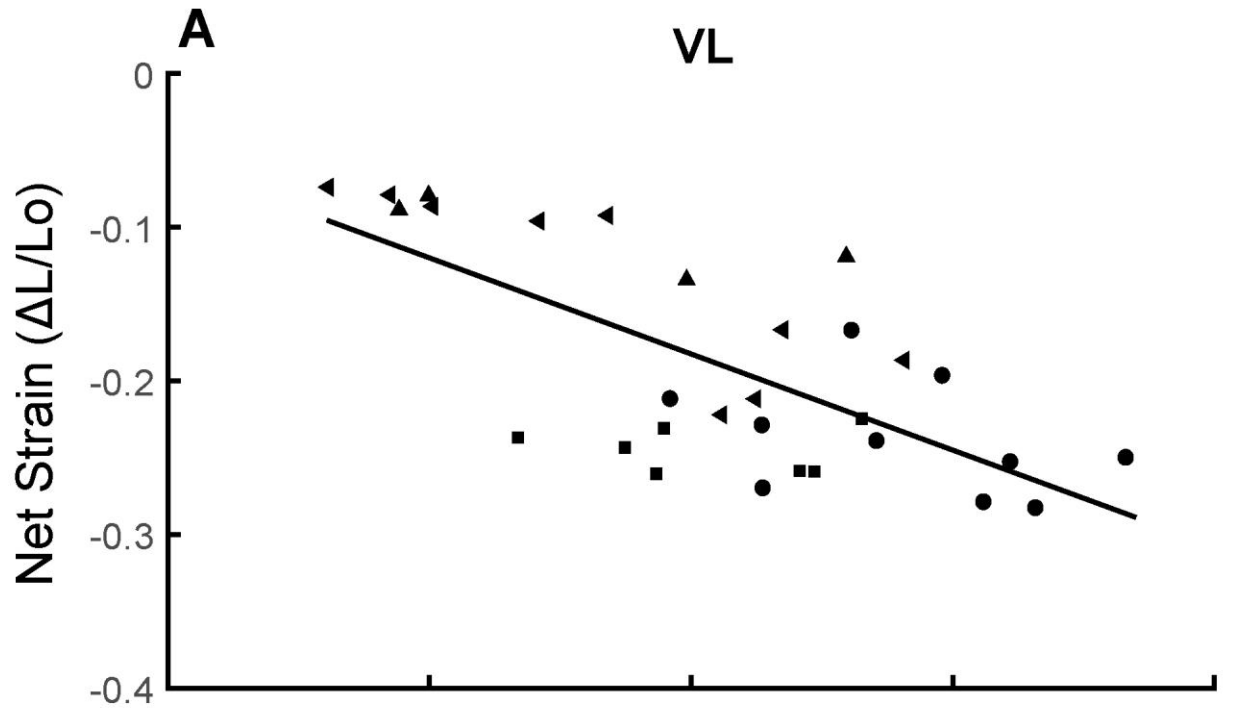
Figure 3. Net muscle strains from the VL and LG muscle across jump heights. Strain in both the VL (A) and the LG (B) decreased significantly (increased shortening) with jump height (VL: F = 20.63 , R^2 = 0.80, $p < 0.001$ and LG: F = 7.56, R^2 = 0.83, p = 0.009, respectively). Net strain increase over jump heights was significantly different when comparing between muscles (F = 6.66, p = 0.012). Different markers represent different individuals. Solid lines indicate the regression lines. Muscle length change was normalized to muscle strain by average initial muscle length per individual.
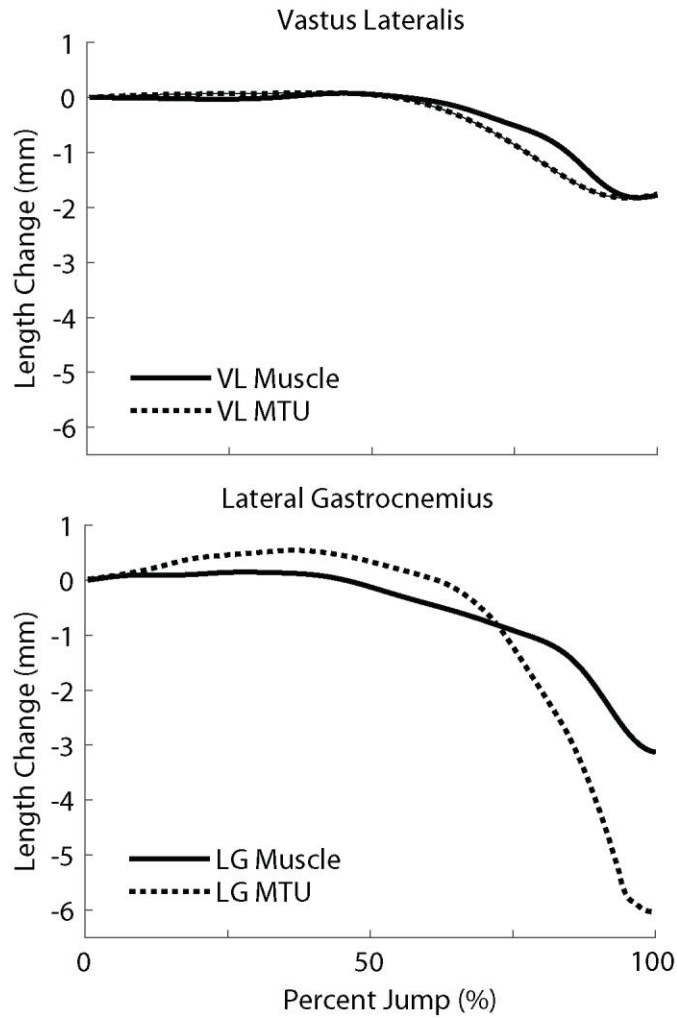
Figure 4. Length change (mm) of the VL and LG muscle tendon unit (MTU, dashed line) and the VL and LG muscle (solid line), measured by SONO, for a representative jump trial of 0.25 meters.

A **VL**

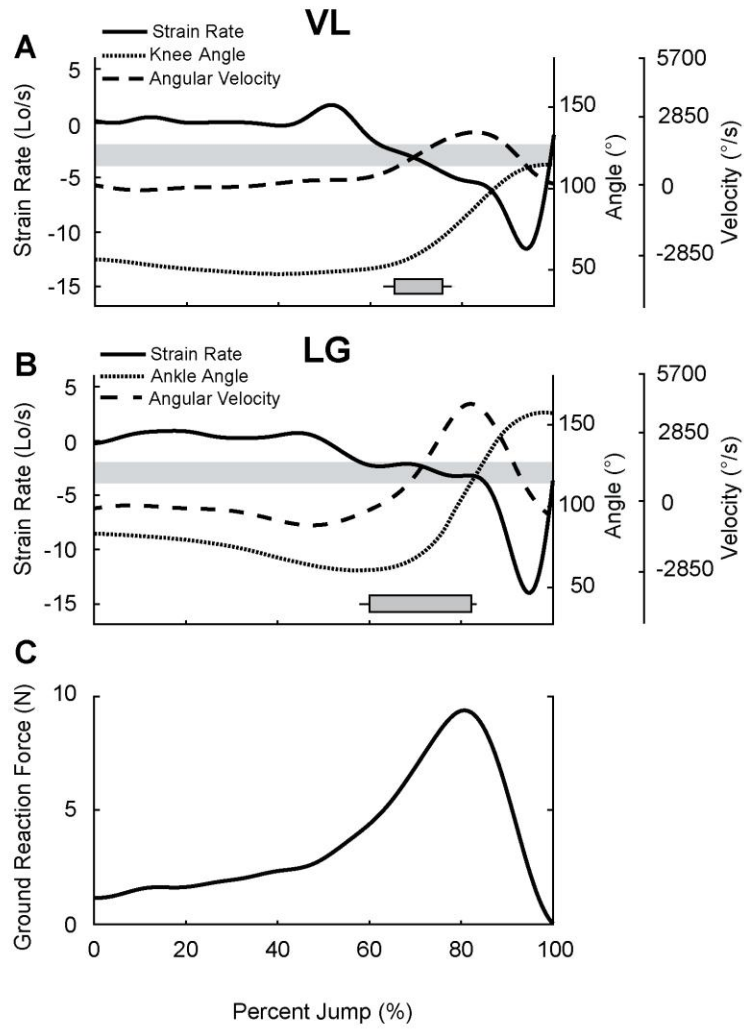B **LG**

C

Percent Jump (%)

Figure 5. VL (A) and LG (B) muscle strain rates (solid lines, left axis), knee (A) and ankle (B) joint angles (small dashed lines) and joint angular velocities (large dashed lines) (right axes), and a ground reaction force (C) for a representative jump of 0.26 meters. Variables are plotted against percentage jump. The shaded bands indicate V_{opt} (± 30%). Bar graphs indicate average time that a muscle spends in this shaded area for all trials, error bars represent s.e.m. The LG operates near V_{opt} for a significantly longer percentage of the jump compared to the VL muscle (t-stat = 10.43, df = 69, sd = 6.28, p < 0.001).
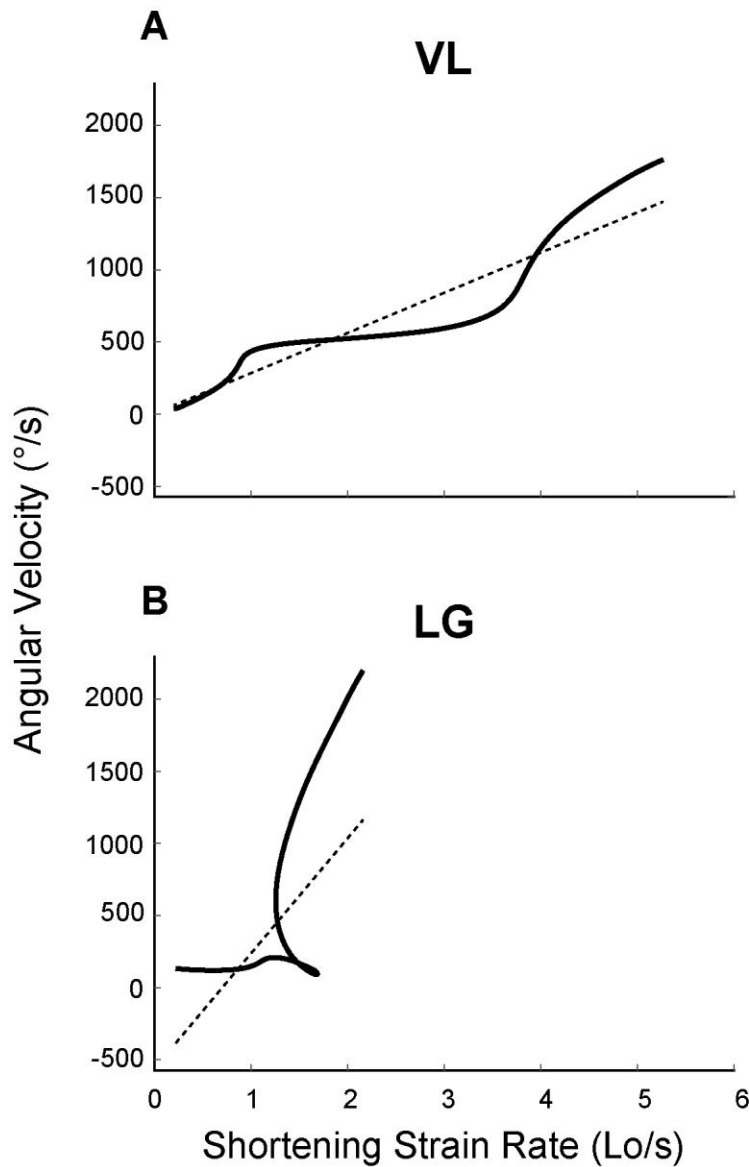
Figure 6. Joint angular velocity vs muscle shortening strain rate for the VL (A) and LG (B) from the time of initial muscle shortening until the time of peak force for a representative jump of 0.2 meters. Dashed lines represent the regression lines. Knee angular velocity is more tightly correlated with VL shortening rate (R = 0.94) compared to ankle angular velocity and LG shortening rate (R = 0.57). On average, the Pearson coefficient was significantly different for the knee and VL (R = 0.93 ± 0.073, n = 30) compared to the ankle and LG (R = 0.71 ± 0.15, n = 41) (F-stat = 54.04, p < 0.00).

TABLE 1. KANGAROO RATS (D. DESERTI) FROM WHICH MUSCLE STRAIN (SONO) AND EMG INTENSITY FOR THE VASTUS LATERALIS (VL) AND LATERAL GASTROCNEMIUS (LG) MUSCLE WERE RECORDED DURING JUMPING TASKS.

Animal	VL SONO	VL EMG	LG SONO	LG EMG
1	X		X	X
2	X	X	X	X
3		X	X	
4		X	X	X
5	X	X	X	X
6	X	X	X	X