

Functional morphology of the ankle extensor muscle-tendon units in the springhare *Pedetes capensis* shows convergent evolution with macropods for bipedal hopping locomotion

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Running head: Springhare ankle extensors

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Abstract

This study assesses the functional morphology of the ankle extensor muscle-tendon units of the springhare *Pedetes capensis*, an African bipedal hopping rodent, to test for convergent evolution with the Australian bipedal hopping macropods. We dissect and measure the gastrocnemius, soleus, plantaris, and flexor digitorum longus in ten adult springhares and compare it against similar-sized macropods using phylogenetically informed scaling analyses. We show that springhares align reasonably well with macropod predictions, being statistically indistinguishable with respect to the ankle extensors' mean weighted muscle moment arm (1.63 vs 1.65 cm, respectively), total muscle mass (41.1 vs 29.2 g), total muscle physiological cross sectional area (22.9 vs 19.3 cm²), mean peak tendon stress (26.2 vs 35.2 MPa), mean tendon safety factor (4.7 vs 3.6), and total tendon strain energy return capacity (1.81 vs 1.82 J). However, total tendon cross sectional area is significantly larger in springhares than predicted for a similar-sized macropod (0.26 vs 0.17 cm², respectively), primarily due to a greater plantaris tendon thickness (0.084 vs 0.048 cm²), and secondarily because the soleus muscle-tendon unit is present in springhares but is vestigial in macropods. The overall similarities between springhares and macropods indicate that evolution has favored comparable lower hindlimb body plans for bipedal hopping locomotion in the two groups of mammals that last shared a common ancestor ~160 million years ago. The springhare's relatively thick plantaris tendon may facilitate rapid transfer of force from muscle to skeleton, enabling fast and accelerative hopping, that could help to outpace and outmaneuver predators.

Key words: energy, muscle, predation, strain, stress, tendon

Introduction

Bipedal hopping is a specialized form of terrestrial locomotion characterized by the sustained saltatory motion of well-developed elongate hindlimbs that cycle in-phase to propel the animal forward. It has evolved independently in at least five groups of mammals, including springhares (rodents, family Pedetidae), three other rodent lineages (families Dipodidae, Heteromyidae and Muridae), and the marsupial macropods (superfamily Macropodoidea, e.g. Australian rat-kangaroos, wallabies and kangaroos). In the early 1970s, by training two red kangaroos *Macropus rufus* (18 and 28 kg body mass) to undertake sustained hopping on a treadmill, Dawson and Taylor (Dawson & Taylor, 1973) revealed an uncoupling of metabolic rate from travel speed, a pattern later also observed by Baudinette and co-workers (Baudinette et al., 1992) in 13 tammar wallabies *Macropus eugenii* (4.9 kg mean body mass). Both studies defied expectations of a markedly more expensive and linear relationship between the rate of energy use and travel speed, based on earlier measurements of running quadrupeds (Taylor et al., 1970). Subsequent studies on smaller hopping rodents and macropods (0.03 – 3.0 kg body mass) showed that the uncoupling of metabolic rate with travel speed either does not occur (Thompson et al., 1980) or is less pronounced compared with the larger hopping animals (Dawson, 1976; Webster & Dawson, 2003). Indeed, a linear relationship between metabolic rate and travel speed was observed for two hopping springhares (3.0 kg mean body mass), exhibiting a pattern similar to that predicted for a similar-sized running quadrupedal mammal (Figure 1, reproduced from Thompson et al., 1980). It has been noted, although never quantified, that the bipedal hopping pattern of springhares is characterized by hindlimbs that appear more extended when they first make contact with the ground compared to those of larger macropods (Offermans & De Vree, 1987).

Why the energy savings of hopping locomotion become evident, or more pronounced, with increasing body mass remains an open question. During locomotion, muscle-tendon forces must balance the rotational moments generated at limb joints by opposing ground reaction and inertial forces of the swinging limbs, which will increase with travel speed (Alexander, 2003; Biewener & Patek, 2018). In quadrupeds, peak muscle force requirements are attenuated in larger individuals due to changes in limb posture that improve effective mechanical advantage (muscle-to-external moment

arm ratio) of the extensor muscles (Biewener, 1989, 2000, 2005), and decreased peak gravitational body weight-normalized forces experienced during fast running (Biewener, 2000; Alexander, 2003; Dick & Clemente, 2017). In macropods, there is little mechanical assistance by changes in locomotor limb posture afforded to larger individuals, and in fact, the effective mechanical advantage of the ankle extensor muscles may scale independent of body mass, meaning that macropods remain relatively crouched across their body mass range (Bennett & Taylor, 1995; McGowan et al., 2008). Accordingly, the peak force requirements of the ankle extensor muscles increase largely in direct proportion to body mass among the macropods. The increase in peak force requirements is met by a steep (positively allometric) increase in the physiological cross-sectional area (PCSA) of the ankle extensor muscles, but not by a concomitant steep increase in the cross-sectional area (CSA) of the adjoining tendons. Consequently, peak tendon stress is predicted to approach breaking point in the largest macropods for movement at high speeds (Bennett & Taylor, 1995; McGowan et al., 2008; Snelling et al., 2017). Thus, the ankle extensor tendons of smaller macropods, similar to those of small kangaroo rats (Biewener et al., 1981; Biewener & Blickhan, 1988), are relatively thick and likely designed for rapid force transmission to facilitate fast and accelerative hopping; whereas, those of larger macropods are relatively thin and so designed to experience high stresses that facilitate the storage and recovery of strain energy during locomotion. Such body size-related change in functional morphology of the ankle extensor muscle-tendon units of macropods may help to explain the improved hopping efficiency, and decoupling of metabolic rate versus speed, at larger body size (Biewener and Bertram 1991). In larger kangaroos and wallabies, hopping efficiency might also improve with increasing travel speeds up to a point, because faster speeds are achieved primarily by increasing stride length, rather than stride frequency, which should reduce the energetic cost of limb cycling (Webster & Dawson, 2003; Dawson & Webster, 2010; Dawson, 2012).

The ability to engage in fast and accelerative hopping among smaller members of certain rodent and macropod lineages is believed to be an example of convergent evolution driven by predation pressure. For example, despite considerable phylogenetic distance, the large contribution of muscle work over tendon strain energy to the leaping performance of the North African-Middle Eastern jerboas *Jaculus jaculus* (ca. 65 g body mass; Moore et al., 2017) is comparable to that observed

during hopping and leaping in similar-sized North American kangaroo rats *Dipodomys spectabilis* and *D. deserti* (ca. 100 g body mass; Biewener et al., 1981; Biewener & Blickhan, 1988; Schwaner et al., 2018). Presumably, the fitness benefit of outmaneuvering predators outweighed that of energy-efficient locomotion during evolution in both groups of bipedal hopping rodents. It is presently unclear whether convergent evolution might also be evident between bipedal hopping springhares and similar-sized macropods, two groups of mammals that last shared a common ancestor ~160 million years ago (Luo et al., 2011). The springhare represents a unique evolution into a bipedal hopping state, given there are no living hopping or non-hopping close relatives. The ancestral *Megapedetes* occupied grassy woodlands of Namibia and forests of Kenya approximately 20 million years ago before giving rise to the modern species associated more with semi-arid habitats (Senut 2016). Today, springhares occur patchily across southern and eastern Africa (Skinner & Chimimba, 2005). They are herbivorous animals (Peinke & Brown, 2006) that selectively forage above ground at night (Butynski, 1984) and shelter in burrow systems during the day (Butynski & Mattingly, 1979; Peinke & Brown, 2005). Their metabolic rate increases approximately 15 fold from resting to hopping at 2.8 m s⁻¹, a speed reportedly achieved without incurring a significant increase in lactate (Müller et al., 1979; Thompson et al., 1980; Peinke & Brown, 2003). On the one hand, the springhare is of significantly larger body size (ca. 3 kg body mass) than the jerboas and kangaroo rats, and so selection for energy-efficient hopping locomotion may have taken greater priority during its evolution. On the other hand, the springhare is often under intense predation pressure, and so selection may instead have favored fast and accelerative hopping. In support of the latter scenario, behavioral observations report that springhares can “bolt suddenly” to avoid predators (Butynski & Mattingly, 1979) and change “speed and direction of movement and this will reduce the success of predators” (Peinke & Brown, 2005).

We therefore assessed the functional morphology of the four ankle extensor muscle-tendon units that balance rotational moments at the ankle joint during bipedal hopping in the springhare, to test for convergent evolution with similar-sized bipedal hopping macropods. This unique mode of locomotion that is shared between the springhare (a placental) and macropods (marsupials) offers an opportunity to assess the degree to which evolution has selected for similar morphological solutions to

corresponding functional tasks in two phylogenetically unrelated groups of mammals. We measured springhare moment arms, muscle volumes, muscle PCSAs and tendon CSAs, and calculated peak tendon stresses, tendon safety factors, and tendon strain energy return capacities in the gastrocnemius, soleus, plantaris, and flexor digitorum longus muscle-tendon units. We extend the analysis by comparing the mean value of selected traits in the springhare against a series phylogenetically informed scaling analyses using published data of 16 species of bipedal hopping macropods. We show that for most traits, springhares align well with macropod predictions, except they have a plantaris tendon CSA significantly greater than predicted in an equivalent-sized macropod, and they have a soleus muscle-tendon unit that is vestigial in macropods. The springhare's ability to engage in fast and accelerative hopping is considered with particular reference to the plantaris tendon thickness, life history and body size.

Materials and methods

Animal collection

The carcasses of ten adult springhares *Pedetes capensis* (Forster 1778), comprising four males and six females, and weighing 2.73 ± 0.24 kg body mass (mean \pm SD), were collected opportunistically during management culls in the Kalahari, Northern Cape, South Africa, under the approval of the University of the Witwatersrand Animal Research Ethics Committee (2018/01/02/O). All carcasses were placed into plastic bags, tied shut, frozen within 4 h of death, and kept frozen for 4 to 8 weeks. On the day of dissection, each carcass was thawed at room temperature, weighed to 0.05 kg on a calibrated strain gauge scale (PCE-CS 300; PCE Instruments, Southampton, UK), and the hindlimbs were then removed and wrapped in damp cloth to prevent water loss.

Moment arms

The lower hindlimbs contain four ankle extensor muscle-tendon units (MTUs) that balance rotational moments at the ankle joint. The muscle bellies are located in the posterior compartment, caudal to the partly-fused tibia and fibula, and comprise the medial and lateral heads of the gastrocnemius (GAS), the soleus (SOL), plantaris (PL) and flexor digitorum longus (FDL). The GAS and SOL tendons both insert directly on the calcaneus (ankle extensors), whereas the PL tendon passes behind the calcaneus and inserts on the intermediate phalanges of the toes (ankle extensor and digital flexor), and the FDL tendon passes around the ankle and inserts on the distal phalanges of the toes (digital flexor).

The GAS, SOL and PL muscle moment arms ($r_{\text{gas,sol,pl}}$; cm), and the FDL muscle moment arm (r_{fdl} ; cm), were estimated as the perpendicular distance between the tendon's line of action and the center of rotation of the ankle joint. The GAS, SOL and PL muscle moment arms were treated as one because their respective tendons follow very similar paths toward the calcaneus and thus their moment arms at the ankle are virtually identical. The external (ground reaction force) moment arm (R_{ext} ; cm) was estimated as the perpendicular distance between the estimated ground reaction force and the center of rotation of the ankle joint (Biewener, 1989; Bennett & Taylor, 1995; McGowan et al., 2005; McGowan et al., 2008; Snelling et al., 2017). The ground reaction force was assumed to act

in a vertical direction halfway along the proximal phalanx of the longest toe, digit III (Bennett & Taylor, 1995; Bennett, 2000; Snelling et al., 2017). We standardized our measurements by holding the ankle joint at approximately 90 degrees (McGowan et al., 2008; Snelling et al., 2017), which we assume corresponds to the *in vivo* mid-stance hopping position when peak ground forces acting in a vertical direction are expected. Our measurements of muscle and external moment arms are estimates only informed by published traces of a single springhare hopping on a treadmill recorded at 100 fps by high-speed cinematography (Offermans and De Vree 1987). We used a set of 15-cm digital vernier calipers to measure the muscle moment arms to 0.01 cm, and we used a 30-cm ruler to measure the external moment arm to 0.1 cm.

To evaluate the combined action of the four muscles at the ankle joint, a mean weighted muscle moment arm (\bar{r} ; cm) was calculated as $\bar{r} = (r_1 A_{m,1} + r_2 A_{m,2} + r_n A_{m,n}) / (\sum A_m)$, where $r_n A_{m,n}$ is the product of the muscle moment arm and the PCSA (see calculation below) for each individual muscle, and $\sum A_m$ is the total summed PCSA of all four muscles (Biewener, 1989; McGowan et al., 2008), implying equal stresses across the four muscle agonists. The effective mechanical advantage of the GAS, SOL and PL ($ema_{gas,sol,pl}$), and of the FDL (ema_{fdl}), was calculated as the quotient of the relevant muscle moment arm ($r_{gas,sol,pl}$ or r_{fdl}) and the estimated external moment arm (R_{ext} ; cm). A mean weighted effective mechanical advantage ($e\bar{m}a$) was then calculated as the quotient of the mean weighted muscle moment arm (\bar{r}) and the external moment arm (R_{ext}).

Muscle-tendon unit morphology

Each MTU was dissected free from the bone, and its total length (l_{mtu} ; cm) measured to 0.01 cm with the calipers, and its total mass (m_{mtu} ; g) measured to 0.01 g on a calibrated digital balance (BJ-1000C; Precisa, Dietikon, Switzerland). The muscle belly was isolated from each MTU, and its length (l_m ; cm) and mass (m_m ; g) recorded to the same precisions. Muscle volume (V_m ; cm³) was calculated as $V_m = m_m / \rho_m$, where ρ_m is the mass-density of mammalian muscle [= 1.06 g cm⁻³ (Méndez & Keys, 1960)]. An incision was then made along the length of the muscle, so that it could be parted along its central axis, photographed with a scale, and 3 – 5 measurements made of fascicle

length (l_f ; cm), and 3 – 5 of pennation angle (θ), using computer graphics software (CorelDRAW 15; Corel Corporation, Ottawa, ON, Canada) (Figure 2). The PCSA of each muscle (A_m ; cm²) could then be obtained given, $A_m = V_m \cos\theta / l_f$, where l_f and θ are the average values from each muscle (Sacks & Roy, 1982). The PCSA calculation adjusts for the askew alignment, and thus direction of force, of the fascicles in a pennate muscle.

The extra-muscular fraction of the tendon of each MTU was laid straight and its length ($l_{t,ext}$; cm) measured to 0.01 cm, and its mass ($m_{t,ext}$; g) recorded to 0.01 g, using the same calipers and balance. The small part of the PL and FDL tendons that pass behind the calcaneus and ankle, respectively, have low elastic moduli relative to other parts of the tendon (Ker et al., 1986), and thus were removed prior to measurement (McGowan et al., 2008; Snelling et al., 2017). The intra-muscular fraction of the tendon could not be measured directly, but its length ($l_{t,int}$; cm) was determined given $l_{t,int} = l_{mtu} - l_{t,ext} - l_f \cos\theta$. Thus, total tendon length ($l_{t,int+ext}$) was obtained from the sum of $l_{t,int}$ and $l_{t,ext}$. The CSA of each tendon (A_t ; cm²) was calculated as $A_t = m_{t,ext} / (\rho_t l_{t,ext})$, where ρ_t is the mass-density of tendon [= 1.12 g cm⁻³ (Ker, 1981)]. The total volume of each tendon (V_t ; cm³) was then calculated given $V_t = A_t l_{t,int+ext}$.

Tendon stress loading, safety factor and strain energy return capacity

The muscle and tendon are connected in series, meaning that total muscle PCSA force is distributed across the adjoining tendon CSA, thus allowing peak tendon stress ($\sigma_{t,max}$; MPa) to be estimated from $\sigma_{t,max} = \sigma_{m,max} (A_m / A_t)$ (Ker et al., 1988; Pollock & Shadwick, 1994a), where $\sigma_{m,max}$ is maximum isometric stress of mammalian muscle [= 0.3 MPa (Biewener, 2003)]. We assume that A_m corresponds approximately to the optimal sarcomere length for producing maximum force. The tendon safety factor (S_t) was calculated as $S_t = \sigma_{t,fail} / \sigma_{t,max}$, where $\sigma_{t,fail}$ is the stress under which tendons are typically expected to rupture [= 100 MPa (Bennett et al., 1986)]. Lastly, the strain energy return capacity of the tendon (U_t ; J) was estimated according to the equation, $U_t = V_t \left(\frac{\sigma_{t,max}^2}{2E} \right) 0.93$ (Biewener & Baudinette, 1995; McGowan et al., 2008; Snelling et al., 2017), where E is the elastic modulus of tendon calculated for tammar wallaby [= 1000 MPa (Biewener & Baudinette, 1995)] and

0.93 accounts for tendon's 7% energy loss due to mechanical hysteresis (Bennett et al., 1986; Biewener, 2008). Although E can vary with species, location and pathology (LaCroix et al., 2013), our assumed value of 1000 MPa is functionally applicable to the present investigation having been derived for the ankle extensor tendons in the tammar wallaby (4.8 kg mean body mass) operating over their normal stress range during hopping locomotion.

Phylogenetically informed comparisons with bipedal hopping macropods

To provide an interspecific backdrop on which to assess the springhare, we used published morphological and phylogenetic information, to undertake scaling analyses of selected traits as a function of body mass, across 16 species of bipedal hopping macropods (species inventory in Table S1 of Supplementary Material; data taken from McGowan et al., 2008; Snelling et al., 2017). The comparison of the springhare against the macropods is validated, in part, by consistent methodology among the studies. Macropod trait and body mass data were \log_{10} -transformed and analysed using a phylogenetic generalized least squares (PGLS) regression model (Grafen, 1989; Martins & Hansen, 1997; Garland & Ives, 2000) in the caper v1.0.1 package (Orme, 2018) within R v3.6.1 (R Core Team, 2013). The phylogenetic tree used for the analysis was constructed from a published supertree of 5020 extant mammals (Fritz et al., 2009). A measure of phylogenetic correlation, λ (Pagel, 1999), was estimated by fitting PGLS regression models with different values of λ and finding the value that maximizes the log likelihood.

Statistical analyses

All mean values are reported with 95% confidence intervals (CI) unless stated otherwise. Measurements were taken from both the left and right lower hindlimbs and averaged for each individual (except for two specimens where measurements could be obtained only from a single intact leg). Mean values thus represent an average corresponding to a single leg only. Statistical differences among mean values ($P < 0.05$) were tested with either a paired t-test, or a repeated-measures ANOVA followed by Tukey's post hoc test, as appropriate, within the R v3.6.1 software environment (R Core Team, 2013). We also wished to test if springhares are significantly different from a typical bipedal

hopping macropod of the same body mass. To this end, we superimposed the mean value of selected traits of springhares onto the PGLS regression models derived for the macropods. A statistical difference was identified when the springhare's mean value fell outside the macropods' 95% prediction belt, containing the area in which 95% of all data points are expected to fall (as recommended in Cooper & Withers, 2006). This analysis was performed with R v3.6.1 and with commercial statistical software (Prism 8; GraphPad Software, La Jolla, CA, USA).

Results

Moment arms

The springhares were all adults of similar body mass (2.40 – 3.25 kg body mass) and so we present absolute mean values, with statistical comparisons across the MTUs, in Tables 1 – 3. The ankle extensors' estimated mean weighted muscle moment arm (\bar{r}), which characterizes the combined action of the GAS, SOL, PL and FDL at the ankle joint, was 1.63 ± 0.09 cm, whereas the estimated mean external (ground reaction force) moment arm (R_{ext}) was 7.91 ± 0.23 cm (Table 1). Thus, the mean weighted effective mechanical advantage of these four MTUs ($e\bar{m}a$) was 0.21 ± 0.01 . The effective mechanical advantage of the GAS, SOL and PL ($ema_{\text{gas,sol,pl}} = 0.23 \pm 0.01$) being approximately three-fold greater than that of the FDL ($ema_{\text{fdl}} = 0.08 \pm 0.01$).

Muscle-tendon unit morphology

Mean muscle mass was greatest in the GAS (18.9 ± 1.3 g) and PL (12.4 ± 0.6 g), but less than one-half in the FDL (6.0 ± 0.4 g) and SOL (3.8 ± 0.3 g). Mean fascicle length varied from 1.3 to 1.9 cm and mean pennation angle from 24.4 to 31.9 degrees among the different muscles (Table 2). Mean muscle PCSA was greatest in the GAS (9.4 ± 1.2 cm²) and PL (8.0 ± 0.9 cm²), and again less than one-half in the FDL (3.8 ± 0.4 cm²) and SOL (1.8 ± 0.3 cm²). Mean tendon CSA was greatest in the PL (0.084 ± 0.008 cm²), statistically indistinguishable in the GAS and FDL (pooled mean of 0.070 ± 0.004 cm²), and smallest in the SOL (0.032 ± 0.007 cm²). The ratio of muscle PCSA-to-tendon CSA was greatest in the GAS (141 ± 16), followed by the PL (95 ± 7), and then by the SOL and FDL with statistically indistinguishable ratios (pooled mean of 57 ± 8).

Tendon stress loading, safety factor and strain energy return capacity

Mean estimated peak tendon stress was greatest in the GAS (42.3 ± 4.9 MPa), followed by the PL (28.6 ± 2.2 MPa), and then by the SOL and FDL with statistically indistinguishable stresses (pooled mean of 17.0 ± 2.5 MPa) (Table 3). Thus the estimated tendon safety factor was lowest in the GAS and PL (pooled mean of 3.0 ± 0.3) and greatest in the SOL and FDL (pooled mean of 6.4 ± 0.8). The

estimated tendon strain energy return capacity was greatest in the GAS and PL (pooled mean of 0.77 ± 0.12 J), followed by the FDL (0.22 ± 0.04 J), and then by the SOL (0.06 ± 0.02 J). Accordingly the GAS and PL contribute approximately 42.5% each, the FDL only 12.0%, and the SOL only 3.0%, to the total energy return capacity of the ankle extensors.

Phylogenetically informed comparisons with bipedal hopping macropods

Scaling analyses of selected traits as a function of body mass across 16 species of bipedal hopping macropod indicate a consistent absence of phylogenetic signal among the residuals, with estimated maximum log likelihood of $\lambda = 0$, rendering the PGLS equivalent to an OLS regression model. For simplicity, we either summed or averaged trait values across the multiple MTUs, before regressing the overall value against body mass, and presenting the data in Figures 3 – 5 (regressions of individual MTUs in Supplementary Material).

In the macropods, mean weighted muscle moment arm (\bar{r}) scales against body mass with an exponent 0.43 ± 0.03 (hyperallometry), and the springhare's value of 1.63 ± 0.09 cm is almost exactly as predicted (1.65 cm) for a macropod of the same body mass (Figure 3). Total muscle mass ($\sum m_m$) in the macropods scales with an exponent 1.18 ± 0.08 (hyperallometry), and the springhare's value of 41.1 ± 2.3 g is approximately 40% higher than predicted (29.2 g), but nonetheless falls within the 95% prediction bands (Figure 4A). Total muscle PCSA ($\sum A_m$) in the macropods scales with an exponent 0.87 ± 0.09 (hyperallometry), and the springhare's value of 22.9 ± 2.3 cm² is approximately 20% higher than predicted (19.3 cm²), and once again falls within the 95% prediction bands (Figure 4B). Total tendon CSA ($\sum A_t$) in the macropods scales with an exponent 0.64 ± 0.06 (isometry), and the springhare's value of 0.26 ± 0.02 cm² is approximately 50% greater than predicted (0.17 cm²), representing a significant difference by falling outside the 95% prediction bands (Figure 4C). Closer examination shows that the springhare's large total tendon CSA results primarily from having a disproportionately thick PL tendon relative to its body mass, with a mean \pm SD of 0.084 ± 0.012 cm² (Figure 4C inset).

We also ran scaling analyses on the estimated tendon stress loading, safety factor and strain energy return capacity in the bipedal hopping macropods. Mean peak tendon stress ($\bar{\sigma}_{t,\max}$) increases with body mass in the macropods scaling with an exponent 0.21 ± 0.08 , and the springhare's value of 26.2 ± 2.5 MPa is approximately 30% lower than predicted (35.2 MPa) for a macropod of the same body mass, falling within the 95% prediction bands (Figure 5A). Mean tendon safety factor (\bar{S}_t) decreases with body mass in the macropods, scaling with an exponent -0.22 ± 0.11 , and the springhare's value of 4.7 ± 0.5 is approximately 30% higher than predicted (3.6), once again within the 95% prediction bands (Figure 5B). These differences in tendon stress and safety factor reflect the springhare's greater total tendon CSA. Lastly, total tendon strain energy return capacity ($\sum U_t$) increases with body mass in the macropods, scaling with a very steep exponent of 1.56 ± 0.19 , and the springhare's value of 1.81 ± 0.34 J is almost exactly as expected (1.82 J) for a macropod of the same body mass (Figure 5C).

Discussion

This study is the first to assess the functional morphology of the ankle extensor MTUs in the bipedal hopping springhare. The reason for studying the ankle extensor MTUs is because of their important role in locomotion, particularly during bipedal hopping, with variation in their position, arrangement and architecture apparently facilitating profound variation in function, from the accelerative (but less energy efficient) movement typical of smaller hoppers, to the energy-efficient (but less accelerative) movement characteristic of larger hoppers. To place our results into context, we superimposed the mean value of selected traits in the springhare onto a series of PGLS regression models using published data derived from 16 species of bipedal hopping macropods weighing 0.79 to 32.7 kg body mass. By taking a comparative approach, we make a number of findings that inform on the biomechanics and physiology of hopping locomotion in the springhare (a placental) compared to that of macropods (marsupials). We find the mean weighted muscle moment arm, total muscle mass, and total muscle PCSA of the springhare are not significantly different to that predicted for an equivalent-sized macropod. However, total tendon CSA in the springhare is significantly greater than predicted, driven primarily by the springhare's disproportionately thick PL tendon, and secondarily by the springhare's soleus muscle-tendon unit, which is vestigial in macropods. As such, the springhare's mean peak tendon stress and mean tendon safety factor tend to be somewhat lower and higher than predicted, respectively, for an equivalent-sized macropod, but the total tendon strain energy return capacity is almost exactly as predicted. We discuss these findings and their implications for fast and accelerative hopping in the springhare.

The ankle extensors' mean weighted muscle moment arm scales with body mass with a hyperallometric exponent of 0.43 among the macropods, which may partly assist with opposing larger gravitational forces and joint moments experienced by larger individuals (McGowan et al., 2008). The springhare's mean weighted muscle moment arm of 1.63 cm is almost exactly as predicted for a macropod of the same body mass (1.65 cm) and falls well within the macropods' 95% prediction bands (Figure 3). Indeed the springhare's individual muscle moment arms are all quite comparable to those of a macropod of the same body mass (Figure S1 in Supplementary Material). Furthermore, the springhare's GAS, SOL and PL muscle moment arm of 1.84 cm, the springhare's external (ground

reaction force) moment arm of 7.91 cm, and the springhare's GAS, SOL and PL effective mechanical advantage of 0.23, each agree within ~10% of that predicted in a similar-sized macropod (1.70 cm, 7.09 cm, and 0.24, respectively) (Bennett & Taylor, 1995). The similarity of the various moment arms is significant because it indicates that, despite the independent evolution of hopping bipedalism in springhares and macropods, the two groups have converged towards a very similar mechanical posture and body plan, particularly with respect to the lower hindlimbs and their position at mid-stance, to achieve the same mode of locomotion.

The ankle extensors' total muscle mass scales with body mass with a hyperallometric exponent of 1.18 among the macropods, primarily to accommodate the increasing total muscle PCSA, which also scales with a hyperallometric exponent of 0.87. The hyperallometry of total muscle PCSA presumably translates to hyperallometry of peak muscle force, which may act in partnership with the hyperallometry of the mean weighted muscle moment arm, to oppose the larger gravitational forces experienced by larger individuals (McGowan et al., 2008). The springhare's total muscle mass of 41.1 g is 40% larger than predicted for a macropod of the same body mass (Figure 4A) due to the contribution of a SOL muscle, as well as the GAS and PL muscle masses being somewhat larger than predicted, but nonetheless falling within the macropods' 95% prediction bands (Figure S2 in Supplementary Material). The springhare's total muscle PCSA of 22.9 cm² is only 20% greater than predicted for a macropod of the same body mass (Figure 4B), which occurs despite the contribution of the SOL, because the other muscles have PCSAs quite similar to that predicted for a macropod of similar body size (Figure S3 in Supplementary Material). The reason that the springhare's total muscle mass is 40% larger than predicted, whereas its total muscle PCSA is only 20% greater than predicted, reflects modest differences in the springhare's fascicle length and pennation angle compared to macropod predictions. Our measure of total muscle PCSA is informative because it largely determines the muscle's capacity to generate force. Hence, the similarity of total muscle PCSA indicates that springhares can produce peak muscle forces that are comparable to those predicted by a macropod of similar body mass. The way these forces are transmitted from the muscle to the skeleton, however, depends in large part on the morphology of their adjoining tendons.

The ankle extensors' total tendon CSA scales with body mass with a near-isometric exponent of 0.64 among the macropods. Because the specific mechanical properties of tendons are fairly uniform among mammals (Bennett et al., 1986; Pollock & Shadwick, 1994b), adaptations of tendon CSA (i.e. tendon thickness) and variation in tendon length largely determine the amount of strain energy stored and released by elastic recoil under a given muscle load during locomotion. Due to their geometric scaling, the ankle extensor tendons of larger macropods are relatively thin compared to the PCSA, and hence the peak forces generated by their adjoining muscles. Muscle forces therefore are concentrated over a relatively smaller tendon area, increasing stress and augmenting the tendon's capacity to store and release strain energy. Indeed, this likely helps to explain the uncoupling of metabolic energy from travel speed in large wallabies and kangaroos (Biewener & Baudinette, 1995). The ankle extensor tendons of smaller macropods by comparison are relatively thick compared to muscle PCSA, resulting in lower stress and reduced capacity for strain energy recovery. As has been shown for yellow-footed rock wallabies (McGowan et al., 2005), relatively thicker tendons are less compliant, and by stretching less under a given force, facilitate the rapid and accelerative movements of rat kangaroos and other small hoppers, similar to North American kangaroo rats (Biewener et al., 1981).

The 3-kg springhare is within the intermediate body mass range of bipedal hoppers where it is hypothesized that a transition may occur from relatively thick tendons that assist with acceleration, to relatively thin tendons that facilitate energy savings (Baudinette, 1994). We found that the springhare's total tendon CSA of 0.26 cm² is approximately 50% greater than predicted for a macropod of the same body mass and falls outside the macropods' 95% prediction bands (Figure 4C). This reflects the contribution of a SOL tendon, as well as the PL tendon being disproportionately thick compared to that of a similar-sized macropod (Figure 4C inset). In fact, the springhare PL tendon CSA of 0.084 cm² is approximately 80% greater than predicted for a macropod of the same body mass (Figure S4 in Supplementary Material). In theory, the thick PL tendon should transmit forces more rapidly from the muscle to the tendon's skeletal attachment points, favoring speed and acceleration performance. The ability to engage in rapid locomotion may assist the springhare to outpace and outmaneuver predators (e.g. birds of prey, canine and feline predators), particularly at night, when it is foraging above ground. For instance, springhares in the southern Kalahari are

frequently targeted by cheetahs. However, a successful hunt rate of only ~20% was the lowest from a total of 15 recorded prey species and 989 hunting interactions, and has been attributed to the springhare's ricochetal hopping enabling sudden and sharp turns, as well as the proximity of burrows for escape (Mills & Mills, 2017). The disproportionately thick PL tendon of springhares may also be an adaptation to assist with burrow excavation in these semi-fossorial animals. Although the long-clawed forelimbs are used for digging, the hindlimbs are also used for kicking clear the excavated soil (Butynski & Mattingly, 1979). Conceivably, this task might impose reasonably high forces transmitted by the hindlimbs, which would be facilitated by relatively stiff tendons.

The mismatch in the scaling of the ankle extensors' total muscle PCSA (exponent of 0.87) and total tendon CSA (exponent of 0.64) among macropods indicates that peak tendon stresses are expected to increase with body mass, scaling with an exponent of 0.21, such that mean tendon safety factors decrease with body mass, scaling with an exponent of -0.22. It is inevitable, therefore, that larger macropods are at greater risk of tendon rupture due to reduced safety margins, especially as forces increase during fast hopping locomotion (Biewener & Bertram, 1991). Indeed, the increased likelihood of ankle extensor tendon rupture has been argued to impose an upper body mass limit of approximately 140 – 160 kg among hopping macropods (McGowan et al. 2008, Snelling et al. 2017). The tradeoff benefit for operating tendons closer to failure during locomotion is that the high stresses provide increased strain energy storage and recovery, reducing the metabolic cost of locomotion. Thus, the ankle extensors' total strain energy return capacity increases with body mass among macropods with a steep exponent of 1.56, affording energy savings of approximately 50% (versus muscle work) during hopping locomotion in larger wallabies and kangaroos (Biewener et al., 1998).

Our findings indicate that the springhare's mean peak tendon stress estimate of 26.2 MPa and mean tendon safety factor of 4.7 are approximately 30% lower and 30% higher, respectively, than predicted for a macropod of the same body mass, yet within the macropods' 95% prediction bands (Figure 5A,B). Once again, it is primarily the springhare's disproportionately thick PL tendon, with estimates of relatively low peak stress and high safety factor that influence these relationships (Figures S5 and S6 in Supplementary Material). The springhare's total tendon strain energy return capacity of 1.81 J is almost exactly as expected for a macropod of equivalent body mass (Figure 5C),

and indeed the springhare's individual tendon strain energy return capacities are all very similar to predictions (Figure S7 in Supplementary Material). Apparently, even the springhare PL tendon retains a reasonable capacity to store and return elastic strain energy, because although its relatively large CSA reduces stress, tendon CSA and length increase tendon volume, providing more material to store strain energy. Thus, the springhare's total tendon strain energy return capacity is as predicted for a macropod of similar body mass, but is nonetheless modest when compared to that of larger macropods. Consistent with prior metabolic measurements of springhares showing no evidence of energy-speed uncoupling as observed in larger wallabies and kangaroos (Thompson et al., 1980), our results here further indicate that energy savings during hopping locomotion is likely not a strong selective pressure in this species. This view is further reinforced by the observation that springhares normally do not travel far while foraging (Peinke & Brown, 2005), whereas larger macropods can cover vast distances, economically, across the Australian Outback in search of sparsely distributed food and water sources (Dawson, 2012).

In summary, our analysis of the functional morphology of the ankle extensor MTUs in the springhare reveals generally remarkable agreement with that of similar-sized macropods, despite the two groups independently evolving bipedal hopping locomotion. We observed close agreement with regard to the ankle extensors' muscle moment arms, estimated ground reaction force external moment arm, and effective mechanical advantage at the ankle joint, indicating that the springhare shares similar mid-stance hopping mechanics of an equivalent-sized macropod. Likewise, the springhare's GAS and FDL muscle tendon units have very similar muscle PCSAs, tendon CSAs, peak tendon stresses, safety factors, and strain energy return capacities compared with an equivalent-sized macropod. However, we found that although the springhare's PL muscle PCSA is reasonably similar to macropod predictions, its PL tendon CSA greatly exceeds predictions, indicating estimated peak tendon stress and tendon safety factor in the springhare somewhat lower and higher, respectively, than predicted for a macropod of similar body size. We posit that the relatively overbuilt PL tendon may have evolved to assist in translating forces more rapidly from the muscle to the skeletal attachment points, facilitating fast and accelerative hopping, likely to be advantageous in predator-dense habitats.

Abbreviations

CSA, (tendon) cross-sectional area; FDL, flexor digitorum longus; GAS, gastrocnemius; MTU, muscle-tendon unit; PCSA, (muscle) physiological cross-sectional area; PGLS, phylogenetic generalized least squares; PL, plantaris; SOL, soleus

Acknowledgments

The authors acknowledge the humane method of the management culls and we thank the marksman for allowing access to carcasses. We thank Craig White from Monash University for his assistance with the phylogenetically informed analyses, Channen Long from the University of Pretoria for her advice on R, Amy Cheu of basiliskos.com for the springhare illustration in Figure 1, and Roger Seymour at Adelaide University for his comments on an earlier draft of the manuscript. This research was supported by a South African Claude Leon Foundation Postdoctoral Fellowship to EPS and a University of the Witwatersrand FRC Individual Grant to EPS and AF.

Author contributions

All authors contributed significantly to the study.

Conflict of interest

No conflicts of interest, financial or otherwise, are declared by the authors.

Data availability

Data available at ResearchGate (www.researchgate.net/profile/Edward_Snelling).

References

- Alexander RM** (2003) *Principles of Animal Locomotion*, Princeton University Press, Princeton, NJ, USA.
- Baudinette RV** (1994) Locomotion in macropodoid marsupials: Gaits, energetics and heat balance. *Aust J Zool* **42**, 103-123.
- Baudinette RV, Snyder GK, Frappell PB** (1992) Energetic cost of locomotion in the tammar wallaby. *Am J Physiol Regul Integr Comp Physiol* **262**, R771-778.
- Bennett MB** (2000) Unifying principles in terrestrial locomotion: Do hopping Australian marsupials fit in? *Physiol Biochem Zool* **73**, 726-735.
- Bennett MB, Taylor GC** (1995) Scaling of elastic strain energy in kangaroos and the benefits of being big. *Nature* **378**, 56-59.
- Bennett MB, Ker RF, Dimery NJ, et al.** (1986) Mechanical properties of various mammalian tendons. *J Zool (Lond)* **209**, 537-548.
- Biewener AA** (1989) Scaling body support in mammals: Limb posture and muscle mechanics. *Science* **245**, 45-48.
- Biewener AA** (2000) Scaling of terrestrial support: Differing solutions to mechanical constraints of size. In *Scaling in Biology* (eds Brown JH, West GB), pp. 51-66. Oxford, UK: Oxford University Press.
- Biewener AA** (2003) *Animal Locomotion*, Oxford University Press, New York, NY, USA.
- Biewener AA** (2005) Biomechanical consequences of scaling. *J Exp Biol* **208**, 1665-1676.
- Biewener AA** (2008) Tendons and ligaments: Structure, mechanical behavior and biological function. In *Collagen: Structure and Mechanics* (ed Fratzl P), pp. 269-284. New York, NY, USA: Springer.
- Biewener AA, Blickhan R** (1988) Kangaroo rat locomotion: Design for elastic energy storage or acceleration? *J Exp Biol* **140**, 243-255.
- Biewener AA, Bertram JEA** (1991) Efficiency and optimization in the design of skeletal support systems. In *Efficiency and Economy in Animal Physiology* (ed Blake RW), pp. 65-82. Cambridge, UK: Cambridge University Press.
- Biewener AA, Baudinette RV** (1995) *In vivo* muscle force and elastic energy storage during steady-speed hopping of tammar wallabies (*Macropus eugenii*). *J Exp Biol* **198**, 1829-1841.
- Biewener AA, Patek SN** (2018) *Animal Locomotion, second edition*, Oxford University Press, Oxford, UK.
- Biewener AA, Alexander RM, Heglund NC** (1981) Elastic energy storage in the hopping of kangaroo rats (*Dipodomys spectabilis*). *J Zool (Lond)* **195**, 369-383.
- Biewener AA, Konieczynski DD, Baudinette RV** (1998) *In vivo* muscle force-length behavior during steady-speed hopping in tammar wallabies. *J Exp Biol* **201**, 1681-1694.
- Butynski TM** (1984) Nocturnal ecology of the springhare, *Pedetes capensis*, in Botswana. *Afr J Ecol* **22**, 7-22.

- Butynski TM, Mattingly R** (1979) Burrow structure and fossorial ecology of the springhare *Pedetes capensis* in Botswana. *Afr J Ecol* **17**, 205-215.
- Cooper CE, Withers PC** (2006) Numbats and aardwolves—how low is low? A re-affirmation of the need for statistical rigour in evaluating regression predictions. *J Comp Physiol B* **176**, 623-629.
- Dawson TJ** (1976) Energetic cost of locomotion in Australian hopping mice. *Nature* **259**, 305-307.
- Dawson TJ** (2012) *Kangaroos, second edition*, CSIRO Publishing, Melbourne, Victoria, Australia.
- Dawson TJ, Taylor CR** (1973) Energetic cost of locomotion in kangaroos. *Nature* **246**, 313-314.
- Dawson TJ, Webster KN** (2010) Energetic characteristics of macropodoid locomotion. In *Macropods: The Biology of Kangaroos, Wallabies, and Rat-kangaroos* (eds Coulson G, Eldridge M), pp. 99-108. Melbourne, Victoria, Australia: CSIRO Publishing.
- Dick TJM, Clemente CJ** (2017) Where have all the giants gone? How animals deal with the problem of size. *PLoS Biol* **15**, e2000473.
- Fritz SA, Bininda-Emonds ORP, Purvis A** (2009) Geographical variation in predictors of mammalian extinction risk: big is bad, but only in the tropics. *Ecol Lett* **12**, 538-549.
- Garland T, Ives AR** (2000) Using the past to predict the present: Confidence intervals for regression equations in phylogenetic comparative methods. *Am Nat* **155**, 346-364.
- Grafen A** (1989) The phylogenetic regression. *Philos Trans R Soc Lond B Biol Sci* **326**, 119-157.
- Ker RF** (1981) Dynamic tensile properties of the plantaris tendon of sheep (*Ovis aries*). *J Exp Biol* **93**, 283-302.
- Ker RF, Dimery NJ, Alexander RM** (1986) The role of tendon elasticity in hopping in a wallaby (*Macropus rufogriseus*). *J Zool (Lond)* **208**, 417-428.
- Ker RF, Alexander RM, Bennett MB** (1988) Why are mammalian tendons so thick? *J Zool (Lond)* **216**, 309-324.
- LaCroix AS, Duenwald-Kuehl SE, Lakes RS, et al.** (2013) Relationship between tendon stiffness and failure: a metaanalysis. *J Appl Physiol* **115**, 43-51.
- Luo ZX, Yuan CX, Meng QJ, et al.** (2011) A Jurassic eutherian mammal and divergence of marsupials and placentals. *Nature* **476**, 442-445.
- Martins EP, Hansen TF** (1997) Phylogenies and the comparative method: A general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am Nat* **149**, 646-667.
- McGowan CP, Skinner J, Biewener AA** (2008) Hind limb scaling of kangaroos and wallabies (superfamily Macropodoidea): Implications for hopping performance, safety factor and elastic savings. *J Anat* **212**, 153-163.
- McGowan CP, Baudinette RV, Usherwood JR, et al.** (2005) The mechanics of jumping versus steady hopping in yellow-footed rock wallabies. *J Exp Biol* **208**, 2741-2751.
- Méndez J, Keys A** (1960) Density and composition of mammalian muscle. *Metabolism* **9**, 184-188.

- Mills G, Mills M** (2017) *Kalahari Cheetahs: Adaptations to an Arid Region*, Oxford University Press, Oxford, UK.
- Moore TY, Rivera AM, Biewener AA** (2017) Vertical leaping mechanics of the Lesser Egyptian Jerboa reveal specialization for maneuverability rather than elastic energy storage. *Front Zool* **14**, 32.
- Müller EF, Kamau JMZ, Maloiy GMO** (1979) O₂-uptake, thermoregulation and heart rate in the springhare (*Pedetes capensis*). *J Comp Physiol* **133**, 187-191.
- Offermans M, De Vree F** (1987) Locomotion patterns in the springhare *Pedetes capensis* (Rodentia: Pedetidae). *Revue Zool Afr* **101**, 271-274.
- Orme D** (2018) The caper package: comparative analysis of phylogenetics and evolution in R, version 1.0.1. <https://CRANR-projectorg/package=caper>.
- Pagel M** (1999) Inferring the historical patterns of biological evolution. *Nature* **401**, 877-884.
- Peinke DM, Brown CR** (2003) Metabolism and thermoregulation in the springhare (*Pedetes capensis*). *J Comp Physiol B* **173**, 347-353.
- Peinke DM, Brown CR** (2005) Burrow utilization by springhares (*Pedetes capensis*) in the Eastern Cape, South Africa. *Afr Zool* **40**, 37-44.
- Peinke DM, Brown CR** (2006) Habitat use by the southern springhare (*Pedetes capensis*) in the Eastern Cape Province, South Africa. *S Afr J Wildl Res* **36**, 103-111.
- Pollock CM, Shadwick RE** (1994a) Allometry of muscle, tendon, and elastic energy storage capacity in mammals. *Am J Physiol Regul Integr Comp Physiol* **266**, R1022-1031.
- Pollock CM, Shadwick RE** (1994b) Relationship between body mass and biomechanical properties of limb tendons in adult mammals. *Am J Physiol Regul Integr Comp Physiol* **266**, R1016-R1021.
- R Core Team** (2013) R: A language and environment for statistical computing, version 3.6.1. <http://wwwR-projectorg/>.
- Sacks RD, Roy RR** (1982) Architecture of the hind limb muscles of cats: Functional significance. *J Morphol* **173**, 185-195.
- Schwaner MJ, Lin DC, McGowan CP** (2018) Jumping mechanics of desert kangaroo rats. *J Exp Biol* **221**, jeb186700.
- Senut B** (2016) Morphology and environment in some fossil Hominoids and Pedetids (Mammalia). *Journal of Anatomy* **228**, 700-715.
- Skinner JD, Chimimba CT** (2005) *The Mammals of the Southern African Subregion, third edition*, Cambridge University Press, Cambridge, UK.
- Snelling EP, Biewener AA, Hu QH, et al.** (2017) Scaling of the ankle extensor muscle-tendon units and the biomechanical implications for bipedal hopping locomotion in the post-pouch kangaroo *Macropus fuliginosus*. *J Anat* **231**, 921-930.
- Taylor CR, Schmidt-Nielsen K, Raab JL** (1970) Scaling of energetic cost of running to body size in mammals. *Am J Physiol* **219**, 1104-1107.

Thompson SD, MacMillen RE, Burke EM, et al. (1980) The energetic cost of bipedal hopping in small mammals. *Nature* **287**, 223-224.

Veiga GN, Snelling EP (2020) Dataset of 'Functional morphology of the ankle extensor muscle-tendon units in the springhare *Pedetes capensis* shows convergent evolution with macropods for bipedal hopping locomotion', www.researchgate.net/profile/Edward_Snelling

Webster KN, Dawson TJ (2003) Locomotion energetics and gait characteristics of a rat-kangaroo, *Bettongia penicillata*, have some kangaroo-like features. *J Comp Physiol B* **173**, 549-557.

Figures

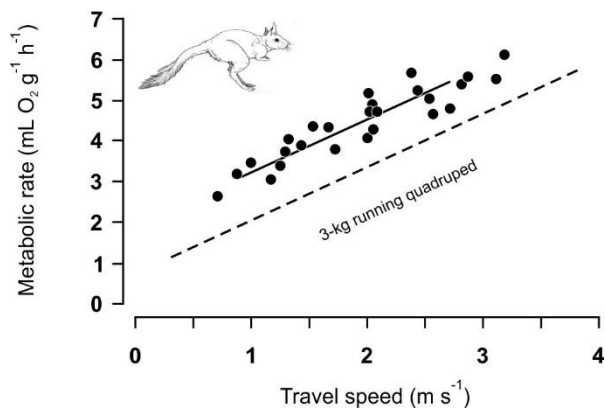


Figure 1. Metabolic rate increases linearly with travel speed during bipedal hopping in the springhare *Pedetes surdaster* (solid line), a pattern similar to that predicted for a running quadrupedal mammal (dashed line) of equivalent body mass (reproduced from Thompson et al. 1980). Originally published as *P. capensis* prior to taxonomic revision that now separates Africa’s southern species *P. capensis* from its eastern species *P. surdaster*. Note arithmetic data plotted on arithmetic axes.



Figure 2. Fascicle length (l_f) and pennation angle (θ) were measured from bisected muscle bellies of the ankle extensor muscle-tendon units (SOL shown here) in ten adult springhares *Pedetes capensis*.

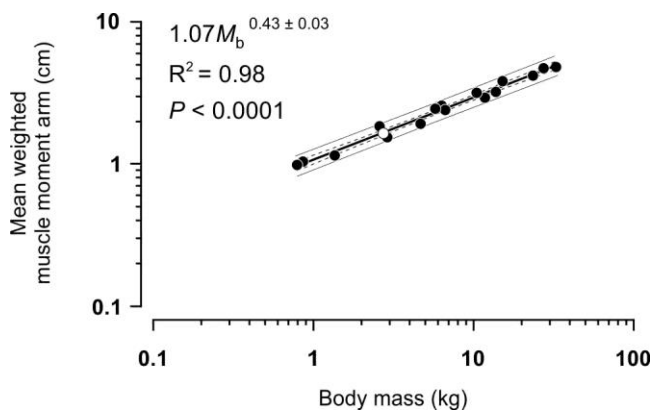


Figure 3. Mean weighted muscle moment arm at the ankle joint (\bar{r}) in the springhare (empty circle), superimposed onto a scaling analysis of \bar{r} against body mass (M_b) in 16 species of bipedal hopping

macropods (filled circles) plotted on logarithmic axes. Solid inner line is the regression mean, dashed outer lines represent the 95% confidence band, and solid outer lines represent the 95% prediction band. Note that \bar{r} in the springhare includes the small contribution of the SOL, which is vestigial in the macropods. The small contribution of the FDL to \bar{r} is unavailable in one macropod species (*Macropus eugenii*). The springhare (2.73 kg body mass) is straddled to the immediate left by the Pearson Island rock-wallaby *Petrogale lateralis pearsoni* (2.59 kg) and to the immediate right by the quokka *Setonix brachyurus* (2.89 kg). Macropod data were taken from the literature (species inventory in Table S1 of Supplementary Material; McGowan et al., 2008; Snelling et al., 2017).

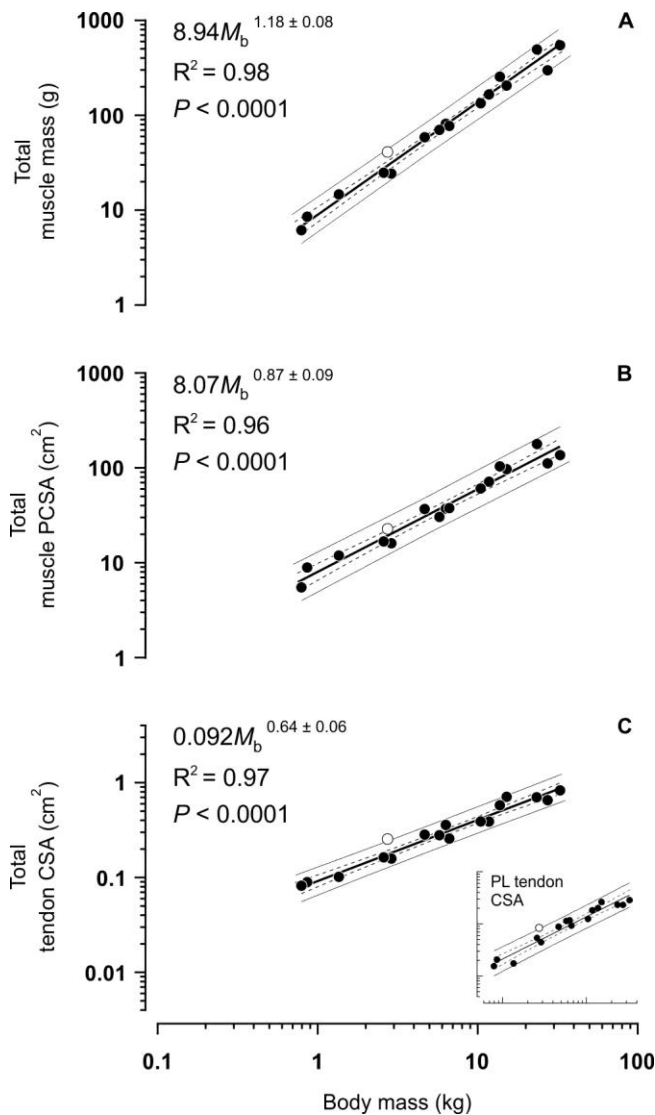


Figure 4. Total muscle mass (A; $\sum m_m$), total muscle PCSA (B; $\sum A_m$), and total tendon CSA (C; $\sum A_t$, with PL tendon CSA inset) of the ankle extensor muscle-tendon units in the springhare (empty

circles), superimposed onto scaling analyses of $\sum m_m$, $\sum A_m$, and $\sum A_t$ against body mass (M_b) in 16 species of bipedal hopping macropods (filled circles) plotted on logarithmic axes. Solid inner line is the regression mean, dashed outer lines represent the 95% confidence band, and solid outer lines represent the 95% prediction band. Note that $\sum m_m$, $\sum A_m$, and $\sum A_t$ in the springhare includes the small contribution of the SOL, which is vestigial in the macropods. PCSA is muscle physiological cross-sectional area and CSA is tendon cross-sectional area.

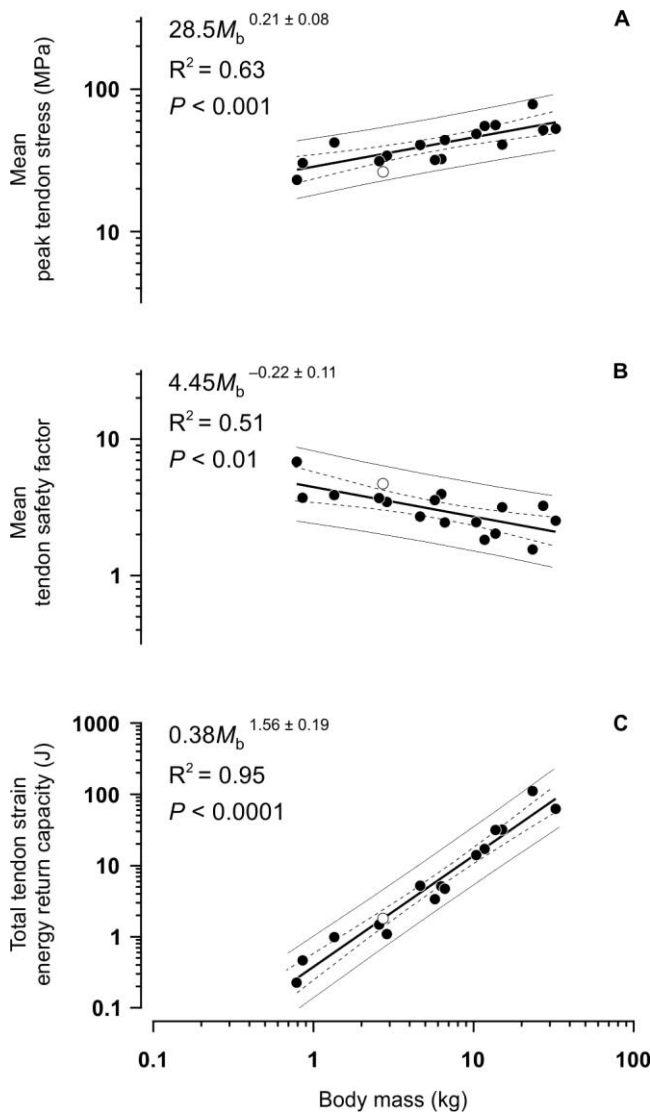


Figure 5. Estimated mean peak tendon stress (A; $\bar{\sigma}_{t,max}$), mean tendon safety factor (B; \bar{S}_t), and total tendon strain energy return capacity (C; $\sum U_t$) of the ankle extensor muscle-tendon units in the springhare (empty circles), superimposed onto scaling analyses of $\bar{\sigma}_{t,max}$ and \bar{S}_t against body mass (M_b) in 16 species of bipedal hopping macropods, and $\sum U_t$ against M_b in 15 species of macropods

(filled circles), plotted on logarithmic axes. Solid inner line is the regression mean, dashed outer lines represent the 95% confidence band, and solid outer lines represent the 95% prediction band. Note that $\bar{\sigma}_{t,\max}$, \bar{S}_t and $\sum U_t$ in the springhare includes the contribution of the SOL, which is vestigial in the macropods. The small contribution of the FDL to $\sum U_t$ is unavailable in two macropod species (*Macropus eugenii* and *Petrogale xanthopus*) and $\sum U_t$ is entirely unavailable in another (*M. rufus*).

Tables

Table 1. Mean \pm 95% CI values of the moment arms and effective mechanical advantages of the ankle extensor muscle-tendon units in the springhare *Pedetes capensis* ($N = 10$ individuals).

	GAS, SOL & PL	FDL	t-test
muscle moment arm, r (cm)	1.84 ± 0.10	0.61 ± 0.07	$t_{1,9} = 25.8, P < 0.0001$
effective mechanical advantage, ema (r/R_{ext})	0.23 ± 0.01	0.08 ± 0.01	$t_{1,9} = 25.3, P < 0.0001$

The mean weighted muscle moment arm is $\bar{r} = 1.63 \pm 0.09$ cm, mean external (ground reaction force) moment arm is $R_{ext} = 7.91 \pm 0.23$ cm, and mean weighted effective mechanical advantage is $e\bar{m}a = 0.21 \pm 0.01$. Values correspond to a single leg only. GAS, SOL and PL were treated as one because their moment arms at the ankle are virtually identical. GAS is gastrocnemius, SOL is soleus, PL is plantaris and FDL is flexor digitorum longus.

Table 2. Mean \pm 95% CI values of the morphology of the ankle extensor muscle-tendon units in the springhare ($N = 10$).

	GAS	SOL	PL	FDL	ANOVA
muscle-tendon unit					
mass, m_{mtu} (g)	19.4 \pm 1.3 ^a	4.0 \pm 0.3 ^b	14.1 \pm 0.7 ^c	7.5 \pm 0.5 ^d	$F_{3,27} = 580, P < 0.0001$
length, l_{mtu} (cm)	14.0 \pm 0.3 ^a	13.2 \pm 0.4 ^b	24.3 \pm 0.5 ^c	24.0 \pm 0.3 ^c	$F_{3,27} = 1432, P < 0.0001$
muscle belly					
mass, m_m (g)	18.9 \pm 1.3 ^a	3.8 \pm 0.3 ^b	12.4 \pm 0.6 ^c	6.0 \pm 0.4 ^d	$F_{3,27} = 592, P < 0.0001$
volume, V_m (cm ³)	17.8 \pm 1.2 ^a	3.6 \pm 0.3 ^b	11.7 \pm 0.5 ^c	5.6 \pm 0.3 ^d	$F_{3,27} = 592, P < 0.0001$
muscle length, l_m (cm)	7.1 \pm 0.3 ^a	9.4 \pm 0.4 ^b	8.5 \pm 0.3 ^c	8.8 \pm 0.3 ^c	$F_{3,27} = 62.6, P < 0.0001$
fascicle length, l_f (cm)	1.6 \pm 0.2 ^a	1.9 \pm 0.2 ^b	1.3 \pm 0.2 ^c	1.3 \pm 0.2 ^c	$F_{3,27} = 23.7, P < 0.0001$
pennation angle, θ (deg)	31.9 \pm 2.7 ^a	24.4 \pm 2.2 ^b	28.8 \pm 2.7 ^a	31.5 \pm 3.0 ^a	$F_{3,27} = 11.5, P < 0.0001$
PCSA, A_m (cm ²)	9.4 \pm 1.2 ^a	1.8 \pm 0.3 ^b	8.0 \pm 0.9 ^c	3.8 \pm 0.4 ^d	$F_{3,27} = 134, P < 0.0001$
tendon					
volume, V_t (cm ³)	0.85 \pm 0.08 ^a	0.36 \pm 0.07 ^b	2.09 \pm 0.18 ^c	1.90 \pm 0.11 ^d	$F_{3,27} = 368, P < 0.0001$
length, $l_{t,int+ext}$ (cm)	12.6 \pm 0.4 ^a	11.4 \pm 0.4 ^b	23.1 \pm 0.5 ^c	22.9 \pm 0.4 ^c	$F_{3,27} = 1871, P < 0.0001$
CSA, A_t (cm ²)	0.067 \pm 0.005 ^a	0.032 \pm 0.007 ^b	0.084 \pm 0.008 ^c	0.073 \pm 0.005 ^a	$F_{3,27} = 91.0, P < 0.0001$
PCSA-to-CSA ratio, A_m/A_t	141 \pm 16 ^a	62 \pm 16 ^b	95 \pm 7 ^c	52 \pm 4 ^b	$F_{3,27} = 57.6, P < 0.0001$

Values correspond to a single leg only. ^{a,b,c,d}Tukey's post hoc test for significant differences among means ($P < 0.05$). PCSA is muscle physiological cross-sectional area and CSA is tendon cross-sectional area. See Table 1 for muscle abbreviations.

Table 3. Mean \pm 95% CI estimates of the peak tendon stress, tendon safety factor and tendon strain energy return capacity of the ankle extensor muscle-tendon units in the springhare ($N = 10$).

	GAS	SOL	PL	FDL	ANOVA
peak tendon stress, $\sigma_{t,max}$ (MPa)	42.3 \pm 4.9 ^a	18.5 \pm 4.8 ^b	28.6 \pm 2.2 ^c	15.5 \pm 1.3 ^b	$F_{3,27} = 57.6, P < 0.0001$
tendon safety factor, S_t	2.4 \pm 0.3 ^a	6.3 \pm 1.6 ^b	3.5 \pm 0.3 ^a	6.6 \pm 0.5 ^b	$F_{3,27} = 24.1, P < 0.0001$
strain energy return capacity, U_t (J) *	0.73 \pm 0.20 ^a	0.06 \pm 0.02 ^b	0.81 \pm 0.14 ^a	0.22 \pm 0.04 ^c	$F_{3,27} = 156, P < 0.0001$

Values correspond to a single leg only. ^{a,b,c,d}Tukey's post hoc test for significant differences among means ($P < 0.05$). *Data \log_{10} -transformed to attain normality prior to ANOVA. See Table 1 for muscle abbreviations.