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# Modulation of proximal muscle function during level *versus* incline hopping in tammar wallabies (*Macropus eugenii*)

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## Summary

**We examined the functional role of two major proximal leg extensor muscles of tammar wallabies during level and inclined hopping (12°, 21.3% grade). Previous *in vivo* studies of hopping wallabies have revealed that, unlike certain avian bipeds, distal hindlimb muscles do not alter their force–length behavior to contribute positive work during incline hopping. This suggests that proximal muscles produce the increased mechanical work associated with moving up an incline. Based on relative size and architectural anatomy, we hypothesized that the biceps femoris (BF), primarily a hip extensor, and the vastus lateralis (VL), the main knee extensor, would exhibit changes in muscle strain and activation patterns consistent with increased work production during incline *versus* level hopping. Our results clearly support this hypothesis. The BF experienced similar activation patterns during level and incline hopping but net fascicle shortening increased (–0.5% for level hopping *versus* –4.2% for incline hopping) during stance when the muscle likely generated force. Unlike the BF, the VL experienced active net lengthening during stance, indicating that it absorbs energy during both level and incline hopping. However, during incline hopping, net lengthening was reduced (8.3% for level hopping *versus* 3.9% for incline hopping), suggesting that the amount of energy absorbed by the VL was reduced. Consequently, the changes in contractile behavior of these two muscles are consistent with a net production of work by the whole limb. A subsidiary aim of our study was to explore possible regional variation within the VL. Although there was slightly higher fascicle strain in the proximal VL compared with the distal VL, regional differences in strain were not significant, suggesting that the overall pattern of *in vivo* strain is fairly uniform throughout the muscle. Estimates of muscle work based on inverse dynamics calculations support the conclusion that both the BF and VL contribute to the additional work required for incline hopping. However, on a muscle mass-specific basis, these two muscles appear to contribute less than their share. This indicates that other hindlimb muscles, or possibly trunk and back muscles, must contribute substantial work during incline hopping.**

Key words: locomotion, hopping, muscle, electromyography, sonomicrometry, vastus lateralis, biceps femoris.

## Introduction

The world in which animals live is not often flat and, as they navigate through their environment, animals encounter a wide range of mechanical demands. These demands include moving up or down hills, which can require considerable changes in potential energy of an animal's center of mass. These mechanical demands and shifting energy requirements must be met by the combined actions of muscles, presumably muscles of the limbs. However, the relative contribution of individual muscles (or muscle groups) to such demands may vary. The range of muscle–tendon architecture that is found in the limbs of animals suggests that individual muscles may be specialized for different tasks. Distal limb muscles are generally composed of short, pinnate fibers, which have been shown to contract under nearly isometric conditions in tammar wallabies (*Macropus eugenii*) (Biewener, 1998) and wild turkeys (*Meleagris gallapavo*) (Gabaldon et al., 2004; Roberts et al., 1997) during steady-speed level locomotion. This enables the muscles to develop high forces economically and to facilitate elastic energy storage and recovery from their long, relatively thin tendons (Biewener and Roberts, 2000). This has also been shown *via* ultrasonography for human ankle extensors during walking (Fukunaga et al., 2001), although the importance of elastic energy recovery is likely to be small in this case. Also, the specialized distal muscles of horses and other ungulates, with extremely short fibers attaching to long tendons,

necessarily perform little net work but are well suited for economical force development to store tendon energy (Dimery et al., 1986) and dissipate loading vibrations (Wilson et al., 2001).

During incline locomotion, distal muscles exhibit a range of functions in different animals (Biewener et al., 2004a; Daley and Biewener, 2003; Gabaldon et al., 2004; Roberts et al., 1997). Whereas the lateral gastrocnemius of guinea fowl (*Numida meleagris*) (Daley and Biewener, 2003) and turkeys (Gabaldon et al., 2004; Roberts et al., 1997) shorten more to increase their net work output on an incline, the ankle extensors (lateral gastrocnemius and plantaris) of tammar wallabies retain their specialized spring-like behavior and continue to generate force nearly isometrically during incline hopping (Biewener et al., 2004a). Consequently, proximal muscles acting at the knee and hip must modulate their function in order to meet the mechanical demands of incline hopping. In contrast to distal muscles, proximal muscles are typically large and composed of relatively longer, more parallel fibers. This allows them to undergo greater length changes, which may make them better suited for generating work (force × length change). In the present study, we examined muscle strain and activation patterns to infer the function of two major, proximal hindlimb extensor muscles during level *versus* incline hopping.

Recent *in vivo* studies of strain and activation patterns of terrestrial animals moving at steady speeds on level ground suggest that limb muscles are capable of a range of mechanical functions. Strain patterns observed during stance phase include isometric activity (Biewener, 1998; Biewener et al., 2004a; Roberts et al., 1997), active shortening (Carrier et al., 1998; Hoyt et al., 2005) and a variety of stretch-shortening cycles resulting in both net shortening and net lengthening (Gillis and Biewener, 2001; Gillis et al., 2005; Hoyt et al., 2005). The vastus lateralis (VL), a principal knee extensor, notably exhibits the greatest variation in strain pattern among terrestrial animals. In rats, the VL undergoes a large initial stretch followed by a small amount of shortening (Gillis and Biewener, 2001). For dogs, goats and, in some cases, horses, the reverse strain pattern is seen, with a relatively small initial stretch followed by greater, and in some cases substantial, shortening (Carrier et al., 1998; Gillis et al., 2005; Hoyt et al., 2005). Given this range of functional diversity observed for the VL, a second aim of our study was to explore how VL strain patterns of a bipedal hopping wallaby compare with VL strain patterns of quadrupeds previously studied.

Further, the majority of studies that have used sonomicrometry to measure muscle fascicle length change assume that a single measurement from the central portion of a muscle is indicative of the fractional length changes across the whole muscle. To date, relatively few studies have tested this assumption. Studies that have examined regional strain variation have shown that strains can differ within complex pinnate muscles (Soman et al., 2005) as well as along a single muscle fascicle (Ahn et al., 2003), whereas other studies have shown fascicle strain to be largely homogenous within a muscle (Gillis et al., 2005). As no clear pattern of regional strain variation has been identified, we sought to determine whether regional strain patterns differed within the VL. The VL was chosen because substantial regional differences in strain could in part explain the wide range of strain patterns observed in this muscle from different species.

Most studies of how muscle function varies during level *versus* incline locomotion have primarily focused on ankle extensors (Biewener et al., 2004a; Daley and Biewener, 2003; Gabaldon et al., 2004; Roberts et al., 1997), largely because it is possible to measure force directly from the muscles' relatively accessible tendons. Although it is not currently possible to measure forces in proximal muscles directly, patterns of muscle strain and activation can provide important insights into how individual muscles and muscle groups accommodate changes in mechanical demand (Gillis et al., 2005). In rats, both the biceps femoris (BF; a hip extensor and knee flexor) and VL exhibit changes in strain patterns in response to an incline grade that are consistent with increased net work output at the hip and knee (Gillis and Biewener, 2002). Whereas the rat BF undergoes increased shortening on an incline, the VL reduces the amount of active lengthening. By contrast, the VL of horses increases active shortening when trotting on an incline compared with on level ground (Wickler et al., 2005). Differences in the VL strain patterns between rats and horses may reflect size-related differences in posture or muscle architecture (Gillis et al., 2005). However, changes in VL strain patterns due to incline for both animals are consistent with a net increase in overall limb work.

Results from inverse dynamics analyses further suggest that proximal muscles likely play a major role in providing positive work for activities such as accelerating and running uphill (McGowan et al., 2005; Pandy et al., 1988; Roberts and Scales, 2004; Roberts and Belliveau, 2005). During horizontal accelerations in wallabies and turkeys, muscles acting at both the hip and the ankle provide significant amounts of positive work (McGowan et al., 2005; Roberts and Scales, 2004) whereas the hip generates the large majority of work in humans running uphill (Roberts and Belliveau, 2005). These studies provide important additional insight into which agonist muscle groups are recruited to modulate mechanical work. However, they are limited in their ability to determine individual muscle function because inverse dynamics calculations require simplifying assumptions and often rely on joint kinematics to predict muscle strain. Nevertheless, coupling force estimates from inverse dynamics with direct *in vivo* measurements of muscle strain and activation provide the best available means of interpreting proximal muscle work within the limb of an animal.

In this study, therefore, we used sonomicrometry and electromyography to measure *in vivo* muscle activity and strain patterns of two major thigh muscles, the BF and VL, during level *versus* incline hopping in tammar wallabies. We tested the hypothesis that the BF and VL muscles modulate activation and strain patterns to produce increased positive work during inclined hopping compared with level hopping. Estimates of muscle force timing and magnitude are calculated from inverse

dynamics combined with strain patterns to estimate changes in work output during level *versus* incline hopping.

## Materials and methods

### *Animals*

*In vivo* muscle experiments were conducted at the Harvard University Concord Field Station, USA and the University of Adelaide, Australia. Four healthy adult tammar wallabies (*Macropus eugenii* L.) were obtained from captive breeding colonies in the USA (*N*=2; mean body mass ± s.d., 4.45±0.45 kg) and Australia (*N*=2; body mass 4.30±0.71 kg). Animals in the USA were housed in large indoor/outdoor pens and given food and water *ad libitum.* Animals in Australia were housed in a large system of outdoor pens as members of a larger breeding colony at the Waite Institute Campus of the University of Adelaide. All animals were trained to hop on motorized treadmills in level and inclined positions (12°) at 3.3 and 4.2 m $s^{-1}$. Speeds were selected to match those used in a previous study of distal muscle function of tammar wallabies during level *versus* incline hopping (Biewener et al., 2004a) and are within the range of preferred hopping speeds previously observed (Baudinette et al., 1992; McGowan et al., 2006). The incline selected was the maximum for the treadmill and, although relatively steep, was not beyond what tammar wallabies might encounter in the wild. Training averaged two weeks duration and involved daily bouts lasting approximately 30 min.

Data for joint moment analysis were collected in conjunction with a separate study in which kinematic and ground reaction force data were obtained from additional tammar wallabies as they hopped at preferred speeds (mean ± s.d., 3.84±0.73 m $s^{-1}$) in outdoor runways over level ground (*N*=5; body mass, 6.64±0.52 kg) and up a 14° slope (*N*=4; body mass, 6.26±0.57 kg). The wallabies used for joint moment analysis were, on average, larger than those that hopped on the treadmill; however, muscle forces were normalized to muscle stress, which accounts for differences in body size. The grade of the sloped runway was dictated by the hill available for runway construction. All protocols for this study were approved by the Harvard IACUC and the University of Adelaide Animal Ethics Committee.

### *Muscles*

We chose to examine the vastus lateralis (VL), a uniarticular knee extensor, and the biceps femoris (BF), primarily a hip extensor. In tammar wallabies and other macropods, the BF inserts both above and below the knee joint center of rotation, resulting in a net moment at the knee of approximately zero if the muscle is activated homogeneously (Lodder, 1991). The VL and BF are the two largest muscles in the hindlimb, comprising approximately 33% of total hindlimb muscle mass.

### *Surgical procedures and transducer design*

Prior to *in vivo* recordings, sonomicrometry (SONO) crystals and electromyography (EMG) electrodes were implanted in the VL and BF using sterile surgical techniques. The animals were anesthetized with isoflurane gas delivered *via* a mask. Animals were induced at 4% and maintained throughout the surgery at 1–3%. The surgical field was shaved using a small animal clipper and a razor at the sites of the incisions and was sterilized with an antiseptic solution (Prepodyne, West Argo, Kansas City, MO, USA). Three small incisions were made in the skin: one just above the hip and two on the lateral side of the thigh parallel to the femur. After being sterilized (Nolvasan Solution, Aveco Co., Inc., Fort Dodge, IA, USA or Cetylcide disinfectant, Pennsauken, NJ, USA), the EMG electrodes and SONO crystals, which had previously been soldered into a micro-connector plug (3×GM-6; Microtech, Inc., Boothwyn, PA, USA) and embedded in waterproof epoxy, were passed under the skin through the incision over the hip and fed subcutaneously to the target muscles.

Each pair of 2.0 mm piezoelectric SONO crystals (Sonometrics Inc., London, ON, Canada) were implanted in the muscles by forming two small pockets in the muscle with the tips of a pair of sharp scissors. The pockets were spaced approximately 10–15 mm apart and were arranged parallel to the muscle fascicles. Because the VL has pennate muscle fibers, the depths of the crystals were adjusted to match the pennation angle of the muscle. Two pairs of crystals were

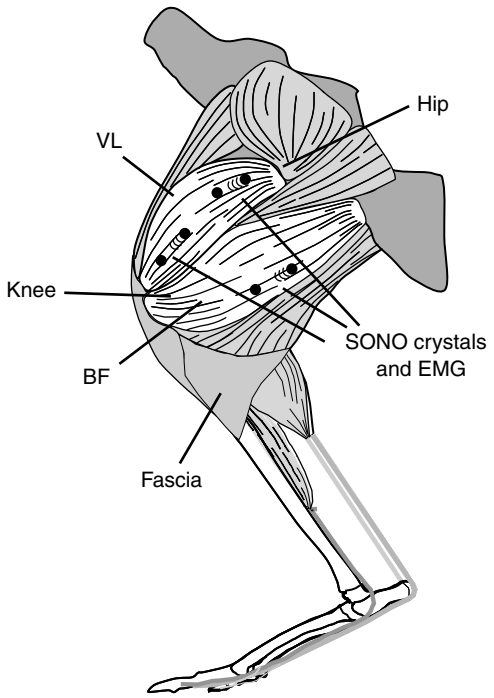


Fig. 1. A schematic drawing of the lateral view of a wallaby hindlimb showing the two muscles examined in this study: biceps femoris (BF) and vastus lateralis (VL). One pair of sonomicrometry crystals (SONO; represented as black circles) was implanted in the BF and two pairs were implanted in the VL, proximally and distally. EMG electrodes (not shown) were implanted in each muscle adjacent to the pairs of SONO crystals.

implanted in the VL, one proximally and one distally, to explore possible regional differences in this muscle (Fig. 1). After insertion, the crystals were aligned to maximize their signal-to-noise ratio by monitoring their output on an oscilloscope. Once a good signal was obtained, the pockets were sutured, and the lead wires were anchored to the surface of the muscle belly using 4.0 silk suture. In the BF, the crystals were implanted in the middle third of the muscle belly, parallel to fascicles that passed closely in line with or slightly below the knee (Fig. 1).

Two fine-wire bipolar EMG electrodes (offset twist hook, 0.5 mm bared tips with 1 mm spacing) were inserted into each muscle adjacent to the placement of the SONO crystals using a 21-gauge hypodermic needle and secured to the muscle belly with 4.0 suture near the electrode insertion site. The EMG electrodes were constructed from insulated fine silver wire (0.1 mm diameter; California Fine Wire, Inc., Grover Beach, CA, USA). After all electrodes were implanted, the skin incisions were closed with 3.0 sutures and the connector plug was anchored above the hip. Small spots of non-toxic white paint were applied to the skin over the joint centers of rotation and marked with contrasting black ink for obtaining basic joint kinematic data. The animals were administered an analgesic (flunixin meglamine, every 12 h) and antibiotics and were given 24 h to recover.

### *In vivo data and video collection*

*In vivo* muscle data were collected while the animals hopped on a motorized treadmill (belt dimensions: in USA, 2.5 m long×0.75 m wide; in Australia, 2.0 m long×0.6 m wide) at 3.3 and 4.2 m $s^{-1}$ while the treadmill was level and inclined to 12°. During data collection, a lightweight shielded cable ran between the external connector attached above the animal's hip and the recording equipment. Outputs from the EMG electrodes were amplified 1000× and filtered (10 Hz–10 kHz bandpass) using Grass P511 preamplifiers (Grass-Telefactor, West Warwick, RI, USA). Signals from the SONO crystals were connected to a sonomicrometry amplifier (Triton 120.2; Triton Technology Inc., San Diego, CA, USA) and monitored *via* an oscilloscope (Tektronix 2235A; Tektronix Texas, LLC, Richardson, TX, USA). Outputs from the amplifiers were sampled at 5 kHz using a 12-bit A/D converter (in USA, Digidata 1200B system, Axon Instruments, Inc., Union City, CA, USA; in Australia, BioWare™ type 2812A1-3 A/D system, Kistler Instruments Corp., Amherst, MA, USA) and stored on a personal computer. High-speed video (125 Hz; Redlake PCI-500; Morgan Hill, CA, USA) was recorded from the lateral view simultaneously with muscle measurements and was synchronized to the muscle recordings *via* a post-trigger pulse that stopped the video and was recorded by the A/D converter.

### *In vivo data analysis*

Fractional changes in muscle fascicle length were based on changes in length between crystal pairs relative to rest length. Rest length was measured while the animals stood quietly in a burlap sack and while the animals were anesthetized with limbs in approximately a midstance position. Both measurements of rest length were equal. The sonomicrometry signals were corrected for the offset error introduced by the faster speed of sound propagation through the epoxy lens of the crystals relative to the muscle (determined to be 0.82 mm for the Sonometrics 2.0 mm crystals) and for the 5 ms delay introduced by the Triton 120.2 amplifier's filter. Fascicle strain recorded locally within the region of the muscle sampled was assumed to be indicative of the full length of the fascicle, as well as the length of entire muscle. This assumption was tested in the VL by implanting two pairs of crystals. In the BF, crystals were placed in a region believed to be primarily producing hip extension.

*In vivo* data from level and incline grades were analyzed at two hopping speeds: 3.3 and 4.2 m $s^{-1}$ (for a total of four conditions). For each condition, 10 strides were selected for analysis for each animal based on performance (maintaining a steady position on the treadmill) and signal quality. These 10 strides were used to calculate individual means for each variable. Foot-on and foot-off times were determined from the video data. Strain patterns observed in each muscle were separated into periods of shortening and lengthening based on inflections in the strain pattern throughout the stride cycle. Net strain during stance was determined by summing shortening and lengthening strain. Because the goal of this study was to determine how proximal muscles contribute to raising the animals' body during incline hopping, data were only analyzed for the support phase of the hopping cycle.

EMG data were analyzed for the same 10 strides selected for muscle fascicle strain analysis. Several variables were quantified for each signal, including onset time relative to foot-on, duration and mean spike amplitude. Mean spike amplitude, used as a measure of EMG intensity, was normalized for each electrode by dividing by the largest value recorded for that electrode in all conditions (Gillis and Biewener, 2001). Two EMG electrodes were implanted in each muscle to ensure that at least one good signal (high signal-to-noise ratio) was recorded. In cases where both EMG electrodes provided good signals, the values for the two signals were averaged.

Video data corresponding to the *in vivo* analysis were analyzed to determine stride parameters and joint angles at the hip and knee. Stride time, stance time and swing time were determined for all speeds and conditions (at 125 Hz, errors in time are likely to average one frame, or 0.008 s). A subset of five trials from each condition was selected from these data for joint kinematic analysis. Markers at the anterior iliac process, hip, knee, ankle and tarsometatarsal–phalangeal joints were digitized using a customized MATLAB (v. 6.5; The MathWorks, Natick, MA, USA) routine (coded by T. L. Hedrick, University of Washington, USA) and filtered using a quintic spline fit to known RMS (root mean square) data, using a generalized cross-validatory/spline (GCVSPL) program (Woltring, 1986). These coordinates were used to calculate

mean joint angles at the hip and knee, as these joints are directly influenced by the muscles of interest.

### *Joint moments and muscle force patterns*

The data used to calculate net joint moments and muscle strain patterns were collected in association with a separate study, and the values for joint moments during level hopping have been reported elsewhere (McGowan et al., 2005). Individual means were calculated from three trials for each animal during level and incline hopping (level, 15 trials; incline, 12 trials). Individual means were then used to calculate means and standard errors for each condition. Experimental design and analysis techniques for joint moment calculations have also been reported in detail (McGowan et al., 2005) and thus will only be discussed briefly here. The animals hopped over level ground in a 22×0.7 m enclosed outdoor runway in which a force-plate (Kistler type 9286AA; Kistler Instruments Corp., Amherst, NY, USA) with an integrated charge amplifier (crosstalk between channels <1.0%) was embedded in the ground at approximately the midpoint of the runway. For incline hopping, a similar outdoor runway was constructed on a hill with an average slope of 14°. Inverse dynamics analysis was used to calculate the total net moments at the joints. The analysis consisted of combining ground reaction forces (GRF), kinematics and morphometric data to create a linked segment model of the limb, then solving the equations of motion for each segment (Winter, 1990; McGowan et al., 2005). Muscle force patterns for agonist muscle groups were calculated based on a free body analysis of the joint moments, requiring muscle moments and net joints moments to be equal, using the following system of equations (modified from Biewener et al., 2004b):

$$M_a = F_{AE} r_{AE,a} , \quad (1)$$

$$M_k = F_{KE} r_{KE,k} - F_{AE} r_{AE,k} - F_S r_{S,k} , \quad (2)$$

$$M_h = F_{HE} r_{HE,h} - F_{R/S} r_{R/S,h} , \quad (3)$$

where $M_a$, $M_k$ and $M_h$ are the net joint moments at the ankle, knee and hip, respectively, $F_{AE}$, $F_{KE}$ and $F_{HE}$ are the forces exerted by the ankle extensors, knee extensors and hip extensors, $F_S$ and $F_{R/S}$ are the forces exerted by the semitendinosus and rectus femoris/sartorius at the knee and hip, respectively, and $r_{AE,a}$, $r_{KE,k}$, $r_{AE,k}$, $r_{S,k}$, $r_{HE,h}$ and $r_{R/S,h}$ are the moment arms of the respective muscle groups acting at the ankle (a), knee (k) and hip (h). For this study, the ankle extensors are the gastrocnemius and plantaris (the soleus is vestigial in wallabies), the knee extensors are the vastus lateralis, rectus femoris and sartorius and the hip extensors are the biceps femoris (which does not have a net moment at the knee in tammar wallabies), semitendinosis and femorococcygeus.

Our calculations assume equal stress in agonist muscles and that no antagonist muscles are active, except for the biarticular muscles, which produce an extensor moment at one joint and a flexor moment at another. Making these assumptions, Eqns 1–3 can be solved simultaneously, yielding estimates of muscle force magnitude and timing for individual muscles.

### *Statistics*

Individual means were calculated from 10 strides for each variable during each condition. A general linear model was used to determine the effects of individual and condition. All values reported in the text are means ± standard error (s.e.m.) unless otherwise noted.

## Results

### *Kinematics*

At each speed, time of contact ($t_c$), swing time and stride time were not significantly different during level *versus* incline conditions (Fig. 2). The resulting duty factors were also not significantly different between grades at 3.3 m s$^{-1}$ (level, 0.44±0.02; incline, 0.46±0.01) or 4.2 m s$^{-1}$ (level, 0.38±0.01; incline, 0.38±0.01). At 4.2 m s$^{-1}$ *versus* 3.3 m s$^{-1}$, $t_c$ and duty factor were significantly smaller during both level and incline hopping ($P$<0.01, $N$=4).

Both hip and knee joint angular excursions changed significantly with grade. The hip joint underwent greater net extension during incline hopping, whereas the knee underwent reduced net flexion (Fig. 3). Increased net extension at the hip during incline hopping is predominately due to greater extension in the latter half of stance, whereas initial hip flexion is small (<5°) in both conditions (Fig. 3B). The knee tended to be more flexed at the time of foot contact (Figs 1, 3C) during inclined hopping, resulting in a significantly reduced change in knee angle during the first half of stance (Fig. 3D). Re-extension of the knee during the latter half of stance tended to be greater during incline hopping, but this difference was not significant. Due to the reduction in initial joint flexion at limb contact with

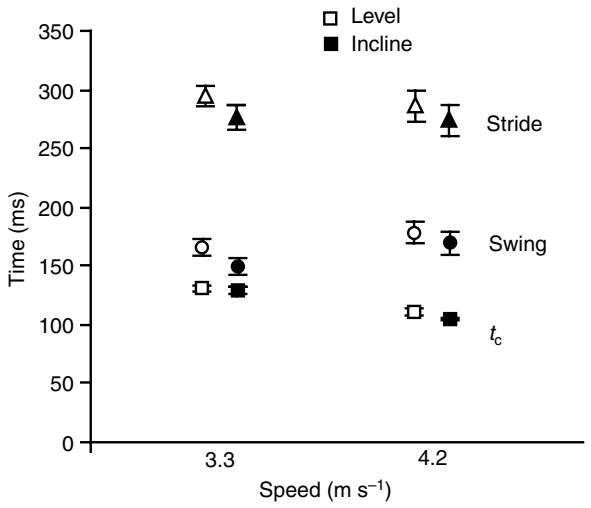


Fig. 2. Stride parameters did not differ significantly between level (open symbols) and incline (filled symbols) hopping at either speed examined. Time of contact ($t_c$; squares) decreased significantly between 3.3 m s$^{-1}$ and 4.2 m s$^{-1}$ on both grades ($P$<0.01), while swing phase duration (circles) and stride time (triangles) did not differ significantly between speeds ($P$>0.05). Symbols are offset on the $x$-axis for clarity. Error bars represent ± 1 s.e.m.

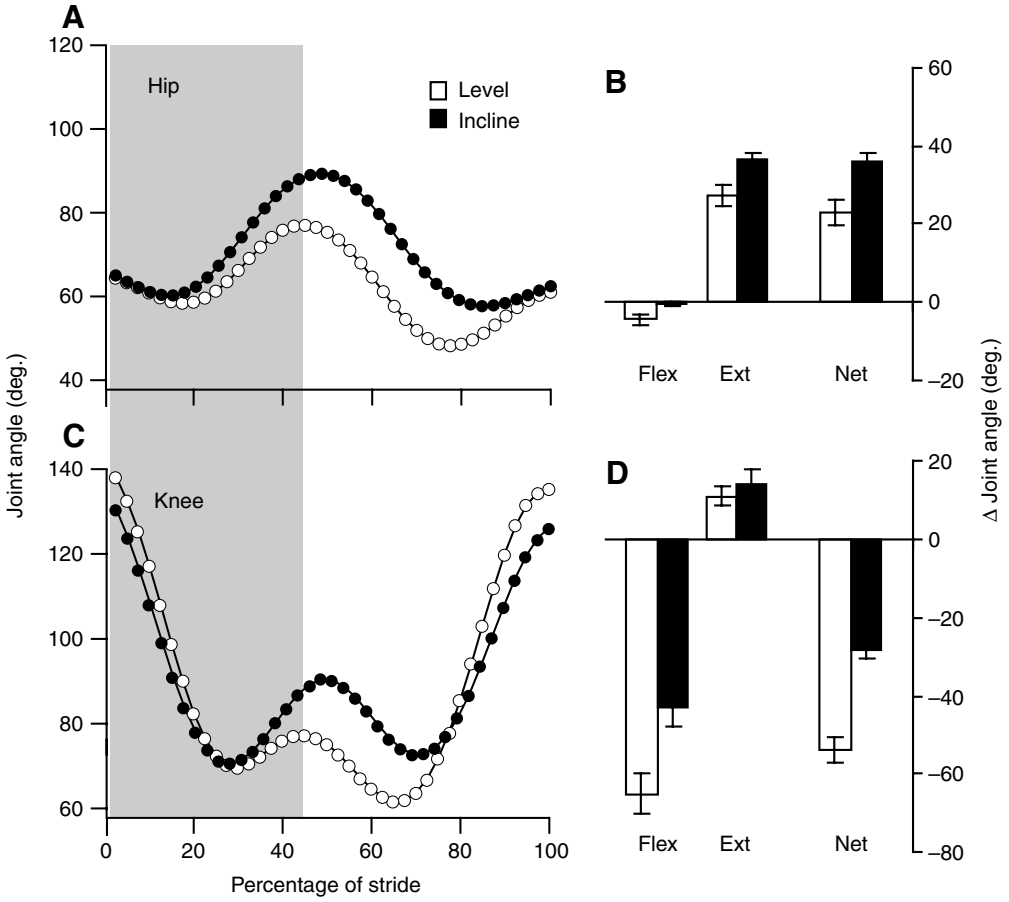


Fig. 3. Representative joint angle patterns normalized to percentage of stride for the (A) hip and (C) knee during level (open) and incline (filled) hopping at 4.2 m $s^{-1}$. The shaded region denotes the stance phase. (B) A significant increase in hip extension ($P$=0.007) produced significantly greater net hip extension ($P$=0.013) during incline hopping. (D) The knee exhibited significantly less net flexion ($P$=0.006) during incline hopping due to a significant decrease in initial joint flexion ($P$=0.021). Initial hip flexion and knee re-extension were not significantly different between level and incline hopping ($P$=0.108 and $P$=0.289, respectively). There were no significant differences in average joint angle changes between speeds, and data for 3.3 and 4.2 m $s^{-1}$ were pooled. Error bars represent ± 1 s.e.m.

the ground, the net angle change at the knee during incline hopping was almost half of that observed during level hopping.

### *In vivo muscle strain*

Among all four wallabies, muscle strain patterns were similar in the BF and VL during steady-speed level hopping and when they hopped on a 12° incline (Fig. 4). During both level and incline hopping, the BF and VL underwent an initial stretch, as the hip and knee flexed, followed by shortening during joint extension. In some trials, an initial muscle shortening was recorded at the beginning of stance, however it was usually small and occurred briefly when force in the muscle was presumably low. Consequently, it was not included in the analysis of strain pattern. Because no significant differences in strain were observed in either muscle between the two speeds measured (3.3 and 4.2 m $s^{-1}$), fascicle strain data were pooled for the two speeds to compare level *versus* incline hopping.

### *Biceps femoris*

Biceps femoris net muscle fascicle shortening increased significantly between level and incline hopping, predominately due to an increase in shortening strain (reported as a negative value by convention) (Fig. 5A). During level hopping, the BF was stretched and shortened a similar amount (6.5±1.7 and –6.9±2.2% strain, respectively) yielding little or no net strain (–0.5±1.0%). During incline hopping, fascicle stretch was slightly, but not significantly, reduced (5.0±1.3%; $P$=0.121) whereas fascicle shortening during the second half of stance increased significantly (–9.2±2.2%; $P$<0.001, $N$=4) relative to level hopping. As a result, net shortening strain in the BF during incline hopping was –4.2±1.5%. The timing of the reversal in strain tended to occur earlier in stance during incline trials (36.9±5.2% of stance) than during level trials (41.6±4.4%); however, the difference in timing was not significant ($P$=0.163).

### *Vastus lateralis*

Muscle fascicle strain in the VL was measured in two sites (proximal and distal) to explore possible regional differences in this muscle. In two of the four wallabies, both sites yielded reliable data. However, in the remaining two animals, data from only one site could be analyzed for each animal. Thus, a sample size of three was analyzed for each site. For the two animals for which recordings from both sites were reliable, significant differences in strain magnitudes were found. Therefore, proximal and distal sites were analyzed separately.

During both level and incline hopping, the VL underwent net lengthening strain (Fig. 5B), which tended to be reduced during incline hopping, especially at the distal site. However, due to the variability among animals and smaller sample size ($n$=3), no significant differences were observed between level and incline

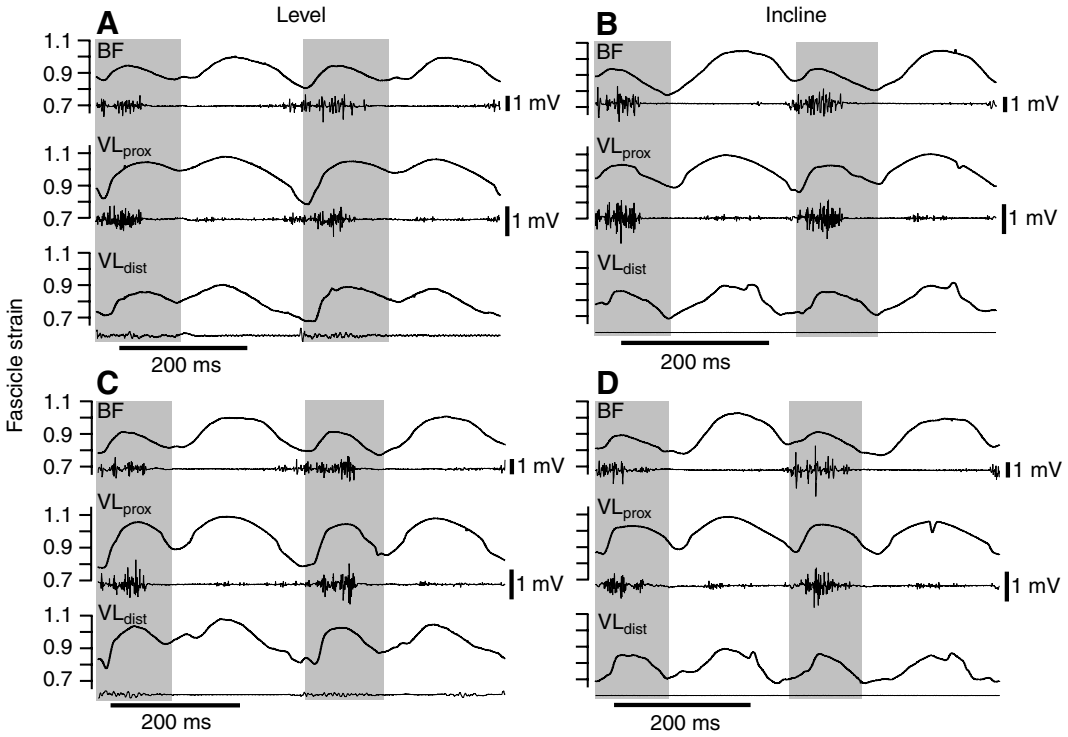


Fig. 4. Representative muscle fascicle strain and activation patterns from wallaby #3 during level and incline hopping at (A,B) 3.3 and (C,D) 4.2 m s$^{-1}$. Shaded regions indicate stance phase. Muscle fascicle strain was recorded from one sight in the biceps femoris (BF) and two sights in the vastus lateralis, proximally (VL$_{prox}$) and distally (VL$_{dist}$). In general, both muscles exhibited decreased initial stretch followed by increased shortening during incline as compared with level hopping. There were no significant differences in fascicle strain due to speed. Note: the second EMG electrode in the VL (bottom trace) did not provide reliable data in these trials.

hopping at either site ($P$>0.05). Proximal (P) and distal (D) sites exhibited similar strain patterns and, although the proximal site on average experienced greater strain magnitudes, the differences were not significant ($P$>0.05). During level hopping, the VL experienced a large initial stretch (P, 18.0±5.4% strain; D, 14.7±4.1%) followed by moderate fascicle shortening (P, –9.5±2.8%; D, –7.4±2.4%), which resulted in a net stretch of 8.7±3.0% at the proximal site and 7.9±2.4% at the distal site. Slightly lower magnitudes of stretch were observed during inclined hopping at both sites (P, 14.8±3.7%; D, 12.3±3.7%) whereas fascicle shortening only appeared to show an increase at the distal site (P, –10.2±1.4%; D, –9.7±3.5%). The resulting net strain was 4.9±3.7% at the proximal site and 2.8±3.1% at the distal site. The reversal in VL fascicle strain occurred at the same time in proximal and distal sites (level P, 54.5±4.6% of stance; D, 53.8±5.6%) and, similar to the BF, tended to occur earlier during incline hopping (incline P, 48.4±8.0%; D, 48.4±6.7%).

### *Muscle activity*

Based on EMG patterns, activation of the BF and VL remained largely unchanged between level and incline hopping

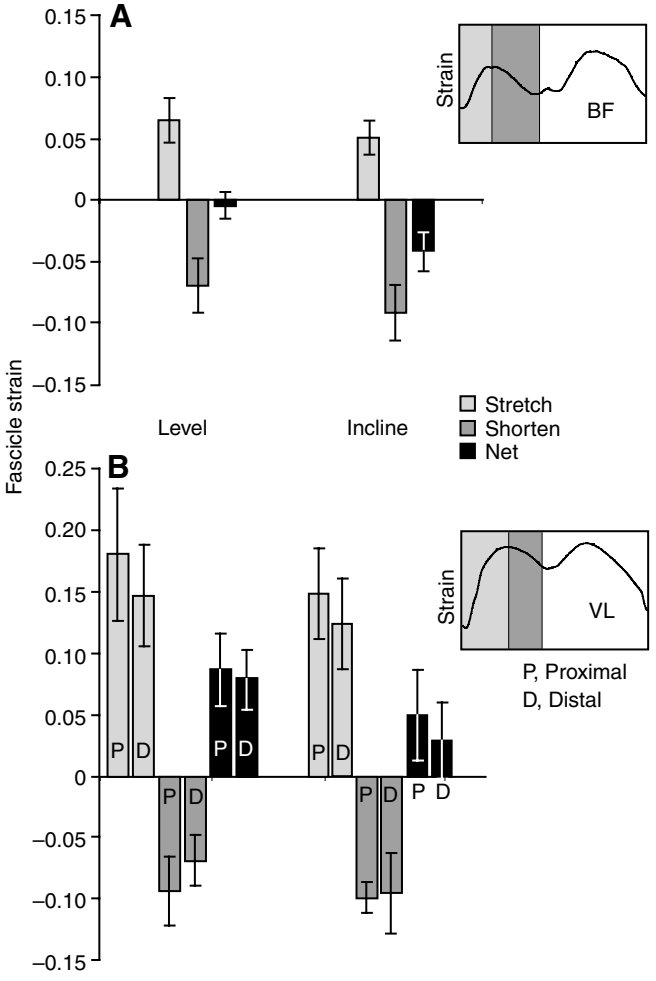


Fig. 5. Histograms showing mean stretch, shortening and net strain between level and incline hopping in the (A) biceps femoris (BF) and (B) vastus lateralis (VL) (averaged for 3.3 and 4.2 m s$^{-1}$). Insets indicate regions for which strains were measured during stance. The BF exhibited significantly greater shortening during inclined hopping ($P$<0.001), which produced significantly greater net shortening during stance ($P$<0.001). Due to high levels of variation, there were no significant differences in strain between level and incline hopping or between sites in the VL. Error bars represent ± 1 s.e.m.

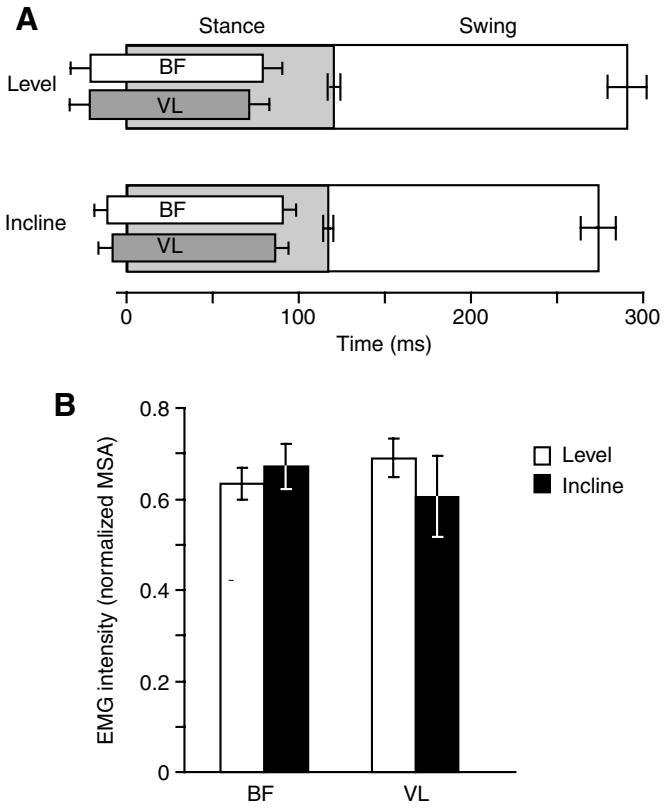


Fig. 6. (A) A schematic showing the timing of electromyogram (EMG) activity in the biceps femoris (BF) (white) and vastus lateralis (VL) (dark grey) relative to the stride cycle. EMG onset occurred at the same time in both muscles and tended to occur later in the cycle during incline hopping, which led to the muscle tending to be active for a greater portion of stance phase. (B) EMG intensity, measured as normalized mean spike amplitude (MSA), did not differ significantly between level and incline hopping in either muscle. Error bars represent ± 1 s.e.m.

conditions. However, the onset time of muscle activity tended to occur closer to the time of foot contact (Fig. 6A) during incline hopping (BF; level, –21.3±3.5 ms *versus* incline, –11.7±8.1 ms; VL; level, –21.5±5.7 ms *versus* incline, –6.5±7.2 ms). By contrast, EMG duration did not change (BF; level, 99.2±10.2 ms *versus* incline, 102.7±6.7 ms; VL; level, 92.6±11.2 ms *versus* incline, 95.3±6.9 ms). As a result, both muscles were active for a greater fraction of stance during incline hopping (BF; level, 0.71±0.08 ms *versus* incline, 0.82±0.07 ms; VL; level, 0.57±0.14 ms *versus* incline, 0.75±0.10 ms). However, none of the differences were statistically significant ($P$>0.05). EMG intensity, measured as mean spike amplitude, was also not significantly different between level and incline hopping for either muscle (BF, $P$=0.105; VL, $P$=0.090). However, mean EMG intensity in the VL tended to decrease during incline hopping (Fig. 6B). Because no significant differences in EMG parameters existed between speeds, the data were pooled for comparison of level and incline hopping.

### *Joint moments and muscle stress*

Net joint moments showed a significant change in pattern between level hopping and hopping on a 14° incline (Fig. 7). Hip extensor moments increased in magnitude during incline hopping (level, 14.1±1.7 N m; incline, 22.4±1.7 N m), and the peak moment occurred slightly later in stance (Fig. 7A). At the knee, the net moment was negative (requiring flexor muscle activity) for the initial 60% of stance during incline hopping whereas during level hopping the knee moment was negative for only the initial 25% of stance before rising (Fig. 7C). Peak knee extensor moments were over fourfold greater during level hopping trials than during incline hopping trials (level, 5.5±1.1 N m; incline, 1.3±1.0 N m). Despite significant differences in peak hip joint moments, estimates of BF stress were not significantly different between level incline hopping (Fig. 6B) (level, 137.6±14.7 kPa; incline, 169.9±21.7 kPa). Due to the biarticular muscles crossing the knee, estimates of VL stress were relatively large, but similar in both level and incline hopping (Fig. 7D; level, 191.3±18.8 kPa; incline, 190.0±37.5 kPa). During both level and incline hopping, the VL experienced greater peak stress than the BF.

### *Muscle work estimates*

Estimates of muscle work during stance, based on muscle stress calculations and mean strain patterns, indicated that both muscles likely contribute to generating the work required to lift the animal's center of mass during incline hopping (Fig. 8). Collectively, the BF and VL shift from absorbing ~17 J kg$^{-1}$ muscle stride$^{-1}$ during level hopping to generating ~20 J kg$^{-1}$ muscle stride$^{-1}$ on an incline, a net difference of ~37 J kg$^{-1}$ stride$^{-1}$. During level hopping, the BF undergoes a relatively symmetric strain pattern under relatively low stress, resulting in the absorption of a small amount of work (estimated to be 3.6 J kg$^{-1}$ muscle). By contrast, during incline hopping the muscle undergoes increased net shortening and experiences higher stress and thus produces substantially more positive work (estimated to be 12.6 J kg$^{-1}$ muscle). The VL switches from absorbing a relatively large amount of energy at the knee (estimated to be 13.6 J kg$^{-1}$) during level hopping to producing positive work during incline hopping (estimated to be 7.3 J kg$^{-1}$).

## Discussion

The results of our study clearly support our hypothesis that the BF, primarily a hip extensor in tammar wallabies, and the VL, a large knee extensor, exhibit changes in muscle strain and activation patterns consistent with increased work production during incline *versus* level hopping. The BF experienced similar phase and activation patterns during level and incline hopping, but fascicle shortening increased significantly, resulting in net shortening during stance, when the muscle is presumably generating force. Changes in VL fascicle strain during level *versus* incline hopping also support our hypothesis, although in a slightly less intuitive way. Unlike the BF, the VL experienced active net lengthening during stance, presumably absorbing energy during both level and incline hopping. During incline

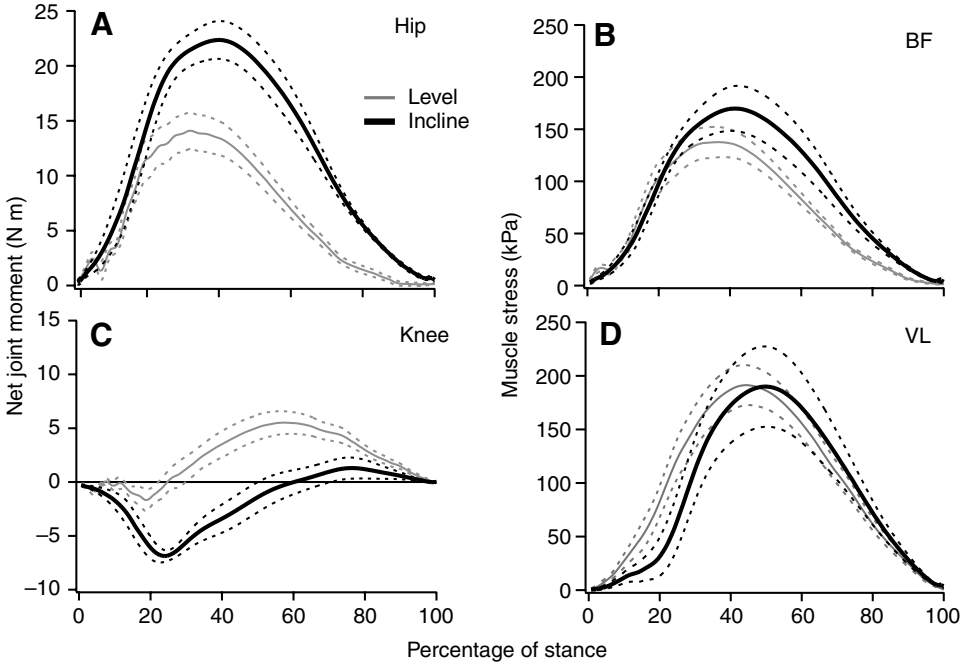


Fig. 7. Mean net joint moments (A,C) and muscle stresses (B,D) from a wallaby (body mass, 6.76 kg) hopping on a level runway and a 14° incline (three trials each). Hip joint moment (A) and resulting biceps femoris (BF) muscle stress (B) increased significantly between level and incline hopping. Joint moment at the knee (C) decreased significantly during incline hopping; however, due to the action of biarticular muscles, vastus lateralis (VL) stress (D) remained similar between level and incline trials. Broken lines represent ± 1 s.e.m.

hopping, however, net lengthening was reduced, suggesting less energy absorption by the VL. Consequently, this would favor a net increase in overall hindlimb work and contribute to raising the animals' center of mass. Although muscle strain and activation patterns are suggestive of changes in mechanical output by the BF and VL, actual work values cannot be determined without direct force measurements. A second aim of our study was to explore possible regional variations of fascicle strain within the VL. Although slightly higher fascicle strains tended to occur in the proximal VL, the observed differences were generally small and not significant. Consequently, we interpret the overall pattern of strain as being homogeneous within the muscle, consistent with what has been reported previously for the goat VL (Gillis et al., 2005).

To indirectly assess muscle work, we employed inverse dynamics to estimate individual muscle forces through time. Work loops generated from this analysis further support our hypothesis for the functional roles of the BF and VL. Consistent with our interpretations of fascicle strain patterns, the BF was estimated to generate relatively little net work during level hopping (–3.6 J kg$^{-1}$) but to produce a substantial amount of net work when hopping on an incline (12.6 J kg$^{-1}$). Our analysis and estimate of VL work showed that the VL absorbs energy (negative work) during level hopping but, interestingly, despite undergoing net lengthening, produced positive work (7.3 J kg$^{-1}$) during incline hopping. Positive work is produced because of the phase relationship between muscle strain and force development, which is an important determinant of a muscle's mechanical output (Daley and Biewener, 2003; Gabaldon et al., 2004; Josephson, 1999).

An important question is how these estimates of work compare with the total work required by the animal to raise its center of mass. The net positive power required to raise the

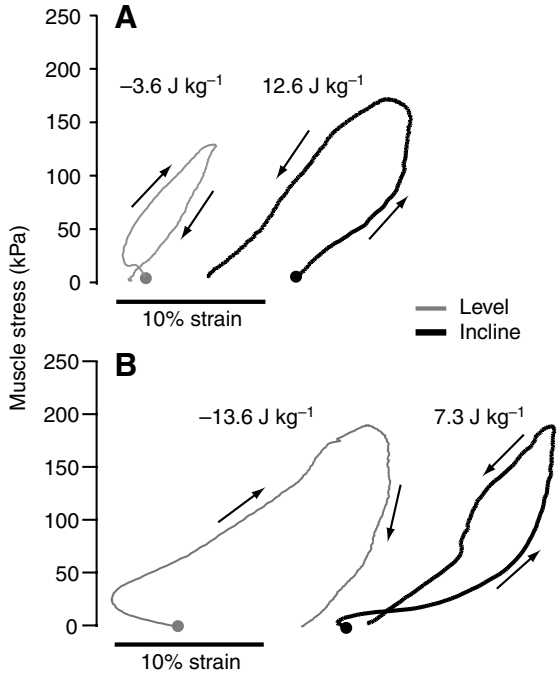


Fig. 8. Work loops predicted for the (A) biceps femoris (BF) and (B) vastus lateralis (VL) during level and incline hopping calculated from muscle stress (Fig. 7) and mean fascicle strain. The BF likely does little work during level hopping but produces a substantial amount of positive work during incline hopping (counter-clockwise work loop). The VL likely shifts from absorbing a large amount of energy (negative work) during level hopping to producing a small amount of positive work on an incline. Estimated work values are in J kg$^{-1}$ muscle. Circles on the ends of the work loops indicate the beginning of stance. The scale bar along the *x*-axis represents 10% fascicle strain in this direction.

center of mass of a 4.3 kg wallaby (mean mass for this study) up a 12° incline at 4.2 m $s^{-1}$ is 37 W. [=$Mgu$ sin(α)= 4.3×9.81×4.2×sin(12°), where $M$ is body mass, $g$ is gravitational acceleration and $u$ is speed]. At 4.2 m $s^{-1}$, wallabies have a stride frequency of 3.52 $s^{-1}$, which means 10.5 J of work is required per stride (5.25 J $limb^{-1}$). For a 4.3 kg wallaby, BF mass (0.049 kg) and VL mass (0.035 kg) together comprise 33% of total hindlimb muscle mass. Based on the work values estimated from our inverse dynamics analysis above, the difference in net work between level and incline hopping is 0.79 J for BF and 0.73 J for VL, totaling a net change of 1.53 J $leg^{-1}$ $stride^{-1}$. This suggests that the BF and VL contribute ~29% of the work required to hop up an incline, which is close to what would be expected for their mass but less than would be predicted given that their distal muscles do not contribute positive work during incline hopping (Biewener et al., 2004a). Consequently, this implies that other proximal hindlimb muscles contribute more than their share of work (on a mass percentage basis) and/or that other musculature is recruited to help power incline hopping. In a related inverse dynamics study of tammar wallaby level accelerations (McGowan et al., 2005), we found the sum of the work done by the limbs was less than the work done on the animal's center of mass. From these results we concluded that back and trunk extension, powered by the trunk musculature, likely provide additional positive work. It seems likely that trunk musculature also generates work during inclined hopping; however, more research is required to test this.

It is important to point out that our calculations of work are based on an inverse dynamics analysis of animals hopping over ground and strain measurements from different group of animals hopping on a treadmill. While we view these results as a reasonable representation of level and incline hopping for tammar wallabies, it is possible that individual variation and/or behavioral differences when hopping over ground *versus* on a treadmill may influence our predictions for work. Further, inverse dynamics relies on several assumptions that may contribute to error when predicting muscle force patterns, particularly force magnitude. Despite these caveats, we feel that our calculations make reasonable predictions of muscle work, which corroborate our conclusions for muscle function in the BF and VL based on activation and strain data alone.

### *Effects of grade on muscle activity patterns*

The activity patterns of the tammar wallaby BF and VL remained consistent during level *versus* incline hopping, despite changes observed for the contractile performance and estimates of force for the BF. Mean spike amplitude (MSA), a measure of EMG intensity, did not change significantly in either muscle. However, during incline trials, BF MSA tended to be greater and VL MSA tended to be lower than during level hopping. Onset of activity of the BF and VL tended to occur later in the cycle during incline hopping, and both muscles were active for a greater fraction of stance. However, there was substantial variation in these data, and differences were not statistically significant.

Previous studies (Gillis and Biewener, 2002; Pierotti et al., 1989; Roberts et al., 1997) have shown that limb muscle EMG intensity generally increases when animals move up an incline relative to level locomotion. However, in a study of guinea fowl running in which EMG and direct force measurements were made simultaneously, Daley and Biewener found that, although force increased when animals ran on an incline, there was no change in lateral gastrocnemius MSA due to grade (Daley and Biewener, 2003). Daley and Biewener did report a difference in the timing of EMG activity of the lateral gastrocnemius, in which the onset of muscle activation occurred later in the cycle and for a greater percentage of the stance period (Daley and Biewener, 2003). This difference in timing was associated with force production over a longer period of muscle shortening, increasing net muscle work during incline running. The duration of EMG activity as a percentage of stance has also been shown to increase during incline *versus* level running in the BF and VL of rats (Gillis and Biewener, 2002). Unlike the EMG patterns in the present study, the increase in EMG burst duration of rats was mainly due to a change in EMG deactivation time. Based on currently available data, therefore, it is clear that a range of neuro-motor responses for locomotion on an incline is possible. It is important to note that EMG intensity alone does not predict well the mechanical output of a muscle, due to a number of complex interacting factors such as nonlinear force–length and force–velocity properties and the potential to recruit different muscle fiber types (Josephson, 1999). It is possible that an increased sample size or a frequency analysis of the EMG signals (Wakeling, 2004) may yet reveal differences in muscle recruitment patterns between level and incline hopping in the BF and VL of tammar wallabies and other species, but such an analysis has yet to be carried out.

### *Comparative analysis of the VL*

The VL appears to exhibit a greater diversity of contractile strain patterns across species than any other muscle for which data are currently available. One aim of this study was to determine how tammar wallabies, as bipedal hoppers, compare to quadrupeds that have been measured. In general, across those species studied to date, the VL undergoes an initial stretch followed by a shortening phase. In rats, the magnitude of stretch exceeds shortening (Gillis and Biewener, 2001; Gillis and Biewener, 2002) whereas in dogs (Carrier et al., 1998) and goats (Gillis et al., 2005) the reverse is true. The VL of horses exhibits a pattern similar to dogs and goats, but the strain trajectory of horses is more complex (Hoyt et al., 2005; Wickler et al., 2005). As noted by Gillis et al. (Gillis et al., 2005), it is tempting to suggest that these differences may be related to limb posture and body size; however, additional studies are needed to explore this hypothesis. Results from our study show that the VL of tammar wallabies functions similar to that of rats (Gillis and Biewener, 2001; Gillis and Biewener, 2002). The VL undergoes net lengthening and likely absorbs energy during both level and incline locomotion. Given the difference in size between tammar wallabies and rats, it seems likely that the similarity in VL strain patterns reflects functional similarities

due to limb posture. Unlike quadrupeds, larger species of kangaroos and wallabies do not adopt a more upright limb posture (Bennett and Taylor, 1995), and knee joint angle changes between trotting rats and hopping wallabies are very similar. Therefore, both species experience relatively large external joint moments at the knee, which require high force production by the knee extensor muscles.

From an energetics perspective, it would appear detrimental to have a muscle, such as the wallaby VL, that absorbs energy during steady-speed locomotion. However, active stretching also enables a muscle to produce higher forces. Based on our calculations, the VL experiences muscle stresses of nearly 200 kPa during both level and incline hopping, even though the external moment at the knee is quite low during incline hopping. These high muscle stresses occur because the knees of wallabies (and other species) are spanned by several bi-articular muscles, which also act to flex the joint. During incline hopping, the forces acting in the hamstrings are increased due to an increased hip extensor moment. The ankle extensors also cross the knee and produce high forces during hopping. These forces are transmitted across the knee and must be balanced by the VL and other knee extensors. Thus, although the mechanical role of the VL is to absorb energy, active stretching may be required to produce the forces necessary to stabilize the knee and resist the bi-articular forces transmitted across the knee during hopping. As joint moments are likely to be relatively higher in small, more crouched quadrupeds, it is possible that high force production could be the primary function of the VL in animals with more crouched postures.

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