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Dynamics of goat distal hind limb muscle–tendon function in response to locomotor grade

M. Polly McGuigan^{1,2,*}, Edwin Yoo¹, David V. Lee^{1,3} and Andrew A. Biewener¹

¹Concord Field Station, Harvard University, Bedford, MA 01730, USA, ²University of Bath, Bath BA2 7AY, UK and ³University of Nevada, Las Vegas, NV 89154, USA

*Author for correspondence (e-mail: m.p.mcguigan@bath.ac.uk)

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SUMMARY

The functional roles of the lateral gastrocnemius (LG), medial gastrocnemius (MG) and superficial digital flexor (SDF) muscle–tendon units (MTUs) in domestic goats ($N=6$) were studied as a function of locomotor grade, testing the hypothesis that changes in distal limb muscle work would reflect changes in mechanical work requirements while goats walked or trotted on the level, 15 deg. decline and 15 deg. incline. As steep terrain-adapted animals, changes in muscle work output are expected to be particularly important for goats. *In vivo* muscle–tendon forces, fascicle length changes and muscle activation were recorded *via* tendon force buckles, sonomicrometry and electromyography to evaluate the work performance and elastic energy recovery of the three distal MTUs. These recordings confirmed that fascicle strain and force within goat distal hind limb muscles are adjusted in response to changes in mechanical work demand associated with locomotor grade. In general, muscle work was modulated most consistently by changes in fascicle strain, with increased net shortening ($P<0.001$) observed as goats switched from decline to level to incline locomotion. Peak muscle stresses increased as goats increased speed from a walk to a trot within each grade condition ($P<0.05$), and also increased significantly with grade ($P<0.05$ to $P<0.01$). Due to the increase in net fascicle shortening and muscle force, net muscle work per cycle also increased significantly ($P<0.05$ to $P<0.005$) as goats switched from decline to level to incline conditions (LG work: 20 mJ to 56 mJ to 209 mJ; MG work: –7 mJ to 34 mJ to 179 mJ; SDF work: –42 mJ to 14 mJ to 71 mJ, at a 2.5 m s^{-1} trot). Although muscle work was modulated in response to changes in grade, the amount of work produced by these three distal pennate muscles was small (being <3%) in comparison with the change in mechanical energy required of the limb as a whole. Elastic energy recovery in the SDF and gastrocnemius (GA) tendons was substantial across all three grades, with the SDF tendon recovering 2.4 times more energy, on average, than the GA tendon. In parallel with the increase in muscle–tendon force, tendon energy recovery also increased as goats increased speed and changed gait, reaching the highest levels when goats trotted on an incline at 2.5 m s^{-1} (GA: 173 mJ; SDF: 316 mJ). In general, tendon elastic energy exceeded net muscle work across all grade and gait conditions. These results demonstrate, for the first time in a quadruped, similar findings to those observed in ankle extensor muscles in humans, wallabies, turkeys and guinea fowl, suggesting that distal muscle–tendon architecture more generally favors a design for economic force production and tendon elastic energy recovery, with the majority of limb work during incline or decline running performed by larger proximal muscles.

Key words: EMG, fascicle strain, muscle–tendon force, work, tendon elastic energy

INTRODUCTION

Although the daily activities of most animals, including humans, involve locomotion that requires changes in grade, as well as changes in speed and gait, a large majority of past studies have focused on steady level locomotion. During steady level locomotion, limb muscles are required to do little work because the net work of the animal's center of mass associated with potential and kinetic energy fluctuations is zero when averaged over a series of strides. Because of this, the rate and magnitude of muscle force production within the limb, more so than muscle work, determines the energy cost of locomotion across different speeds and gaits, as well as across animals of differing size (Heglund et al., 1982; Kram and Taylor, 1990). Consistent with these findings, ankle extensor (or plantar flexor) muscles of a wide range of animals, including wallabies, kangaroos, wild turkeys, guinea fowl, horses and humans (Ker et al., 1987; Roberts et al., 1997; Biewener, 1998; Biewener et al., 1998; Daley and Biewener, 2003; Fukunaga et al., 2001; Lichtwark et al., 2007), appear to have been selected for economical locomotion. These muscles have a large physiological cross-

sectional area for their volume, due to having short pennate fascicles. They also transmit force *via* long free tendons, which store and return elastic energy as they are stretched and recoil during the gait cycle (Alexander, 1988). This utilization of elastic energy reduces the work that must be performed by muscles, especially during faster locomotion, as the recoil of the tendons helps to propel the animal into the subsequent flight phase.

In various species, including humans (Fukunaga et al., 2001; Lichtwark et al., 2007), these distal muscles have been shown to contract under nearly isometric conditions for the majority of the stance phase when animals move steadily over level ground. Studies on turkeys (Roberts et al., 1997; Galbaldón et al., 2004), guinea fowl (Daley and Biewener, 2003; Higham et al., 2008) and wallabies (Biewener et al., 1998) have used sonomicrometry to demonstrate that the fascicles of distal ankle extensors lengthen slightly or develop force under nearly isometric conditions early in the stance phase, and subsequently contract with little net length change over the period of stance. This strain pattern leads to little net work being performed by the muscles during these conditions, favoring a

reduction in metabolic energy use by the muscles associated with force generation (Taylor, 1994).

But animals are not just required to run at a steady speed on a level surface. What happens when the speed of locomotion is not steady, or the animal is moving up or down hill? Does the architecture of these distal muscles and their tendons constrain them to act as they do on the level, or are they able to modulate the work they perform in order to respond to different locomotor requirements? The evidence to date is equivocal and comes solely from studies on running bipeds for which both muscle force and fascicle strain have been directly recorded (guinea fowl, turkeys and humans, and specialized hopping wallabies). The ankle extensor muscles of running guinea fowl increase their work production on an incline (16 deg.) compared with level locomotion (Daley and Biewener, 2003; Higham et al., 2008), as do those of running turkeys (Roberts et al., 1997; Galbaldón et al., 2004), whose muscles also do work when running on an incline and absorb energy when running on a decline. The modulation of work output by these distal muscles is a result of alterations in both the timing and magnitude of muscle fascicle strain and force production (Daley and Biewener, 2003; Galbaldón et al., 2004). The proximal muscles of rats, wallabies and horses (Gillis and Biewener, 2002; McGowan et al., 2007; Wickler et al., 2005) also increase their net shortening when running up an incline *versus* running on a level. Similarly, the long and lateral heads of the goat triceps exhibit changes in fascicle shortening and lengthening consistent with changes in work output at the shoulder and elbow when goats take off *versus* land from jumps (Carroll et al., 2008). However, differences in work output of proximal muscles are uncertain because the forces produced by these muscles are difficult to determine.

In contrast to these studies, measurements of fascicle length change within the medial gastrocnemius (MG) of humans based on ultrasound (Lichtwark and Wilson, 2006) indicate little change in the muscle's contractile behavior when subjects walked or ran on a level *versus* an inclined (10 deg.) treadmill surface. Under both grade conditions and gaits, the muscles developed force isometrically and only shortened near the end of stance. These findings for the human MG reflect a similar stereotypic isometric behavior of ankle extensors measured previously *via* sonomicrometry when tamarin wallabies hopped on an inclined (16 deg.) *versus* level treadmill (Biewener et al., 2004). Under both conditions the *in vivo* length change trajectories of the lateral gastrocnemius (LG) and plantaris muscles were similar and net strains averaged less than 2%, such that neither muscle contributed significant mechanical work. Whereas similar patterns of isometric behavior and limited work output are observed in the ankle extensors of running humans and hopping wallabies when moving over different grades, this is less the case for the distal muscles of running birds (Roberts et al., 1997; Daley and Biewener, 2003; Galbaldón et al., 2004; Higham et al., 2008), suggesting that muscle–tendon architecture alone may not constrain muscles to contract in a stereotypic manner.

The role of architecture in combination with regional differences between proximal and distal limb muscles for work production, therefore, merits further study (Biewener and Daley, 2007). Because goats are adapted to locomotion across a wide range of substrates, we chose to explore how the functional roles of the distal ankle extensor muscles of domestic goats (*Capra hircus*) vary under level *versus* incline locomotion conditions. As a browsing herbivore, goats travel large distances in search of food, often over rough terrain that requires periods of steep ascent and descent (Prothero and Foss, 2007). Therefore, in addition to economical locomotion, the musculoskeletal system of goats must be capable of responding to

demands for increased work and power production, and energy absorption, to navigate steep inclines and declines. This suggests that goats may be less specialized for economical locomotion than galliform birds and plains-dwelling tamarin wallabies, which are the only animals to date whose distal muscles have thus far been extensively studied.

Although the distal muscle–tendon units (MTUs) of horses and other ungulates appear to be highly specialized for tendon elastic savings and economical locomotion (Biewener, 1998; Biewener, 2003; Ker et al., 1987; Wilson et al., 2001), direct *in vivo* measurements of muscle strain and work behavior have not previously been carried out in the distal muscles of any ungulate species. In doing so, we evaluated the contribution of the goat MG and LG, as well as the superficial digital flexor (SDF), to the total mechanical energy required to move the animal's center of mass. We did so by measuring muscle work and tendon elastic savings directly *via in vivo* recordings of fascicle length (sonomicrometry), myoelectric activation (EMG) and muscle–tendon force (tendon buckle transducers). In combination with kinematic and morphological measurements, we addressed the specific hypothesis that, as mountain terrain-adapted animals, the distal MG, LG, and SDF muscles of goats modulate their function similarly with respect to changes in locomotor grade: from doing little work during level locomotion to net energy production during incline locomotion and energy absorption during decline locomotion. In all cases, however, we anticipated that elastic energy storage and recovery from the muscles' tendons would be substantial. We also hypothesized that changes in muscle work occur mainly *via* changes in fascicle strain, rather than by changes in muscle–tendon force.

MATERIALS AND METHODS

Animals

Six healthy, adult female African pygmy goats (*Capra hircus* L.) ranging in body mass from 16.0 to 27.3 kg (mean \pm s.e.m., 20.1 \pm 2.1 kg) were used in this study. The animals were obtained from a breeding colony at Harvard University's Concord Field Station, housed outdoors (with walk-in shelter) and fed available foliage and additional hay and goat pellets as necessary. The animals were trained to walk and run at a steady speed on a large, motorized treadmill (belt, 2.50 m long and 0.75 m wide) on the level, incline and decline. To achieve incline locomotion one end of the treadmill was elevated and supported by metal struts at an angle of 15 deg. For decline locomotion the animals were turned around and the belt direction reversed. Velocities were chosen that represented a medium paced walk (1.0 or 1.5 m s^{-1}) and trot (2.5 m s^{-1}) for each individual animal, which they could readily maintain on the level, incline and decline. All animal procedures were approved by the Harvard University Institutional Animal Care and Use Committee.

Surgical procedures

Prior to surgery the lead wires from the force buckles, sonomicrometry crystals and EMG electrodes were soldered to customized female connectors (2 \times 15 pin). All wires were then soaked in a bacterial disinfecting solution (ChlorhexidineTM) for an extended period. Food was removed 12–18 h prior to surgery, and on the morning of surgery the experimental hind limb was shaved and prepped for surgery. Animals were sedated (xylazine: 1 mg kg^{-1} and ketamine: 4 mg kg^{-1} ; i.v. jugular), intubated, and maintained at an appropriate level of anesthesia on a closed system anesthesia machine (Matrix, Orchard Park, NY, USA) at 1.0–1.5% isoflurane. Breathing and heart rate were monitored throughout the procedure and anesthesia adjusted as necessary.

Transducers were implanted under sterile conditions into the gastrocnemius muscle (lateral and medial heads, LG and MG) and the SDF (also referred to as the plantaris in other species; Fig. 1). An approximately 5 cm skin incision was made to expose the LG muscle belly and Achilles tendon. Additional small incisions (1 cm) were made above the ilium and knee in order to pass the transducers subcutaneously to the implantation site. Stainless steel E-type tendon buckle force transducers (Biewener et al., 1998) were attached to the gastrocnemius (GA) and SDF tendons. These tendons run within a common tendon sheath and were separated by blunt dissection. Their identity was confirmed by observing the effect of a gentle pull on the tendon at the ankle and phalangeal joints. The buckles were positioned so as not to interfere with one another during flexion and extension of the ankle joint, and sutured in place by 4-0 silk sewn through the edge of the tendon and in a figure-of-eight loop through the holes on the buckle transducer arms.

The animal was then rotated onto its opposite side to gain access to the MG and SDF muscle bellies. An approximately 3 cm incision was made in the skin over the MG, and two pairs of sonomicrometry crystals and EMG electrodes passed through from the lateral to the medial side of the limb. The MG was then retracted to expose the SDF muscle and a pair of 2 mm sonomicrometry crystals (Sonometrics, London, Ontario, Canada) inserted parallel to the SDF fascicles by piercing the epimyseal fascia of the muscle with small pointed scissors and creating a pocket for each crystal. The goat SDF is multi-pennate, and in its medial region the fascicles course proximo-deep to distal-superficial at an angle averaging 26 deg. (Table 1). Hence, the proximal crystal was positioned at the appropriate depth to account for this pennation. Alignment of the crystals was optimized to maximize their signal-to-noise ratio by monitoring the output of the crystals *via* the recording cable to the sonomicrometry amplifier (Triton 120.2; Triton Technology, San Diego, CA, USA) and oscilloscope (2235A; Tektronix, Beaverton, OR, USA) during implantation. The crystals were then secured in place by closing the pockets and suturing the lead wires to the muscle surface with 4.0 silk. Fine-wire, twisted, offset, hook EMG electrodes made of 0.1 mm enamel insulated silver (California Fine Wire, Grover Beach, CA, USA) with their tips bared of insulation

(~1 mm, with 2–3 mm spacing between electrodes) were then inserted, using a 21 gauge hypodermic needle, adjacent to the sonomicrometry crystals and anchored in place by suturing to the muscle surface with 4.0 silk.

Sonomicrometry crystals (2 mm) and EMG electrodes were then inserted into the MG muscle head, ensuring that the crystals were positioned to follow the proximo-superficial to distal-deep 24 deg. pennation angle course of the MG fascicles. The medial skin incision was then closed with 3.0 vicryl and the animal rotated back onto the original side. A third pair of 2 mm sonomicrometry crystals and EMG electrodes were then implanted into the LG muscle belly following the same procedures. The lateral skin incisions were then closed using 3.0 vicryl and the connectors anchored to the skin using 2.0 vicryl. Finally, an intramuscular injection of the non-steroidal anti-inflammatory drug flunixin meglumine (1 mg kg^{-1}) was administered (and every 12 h thereafter) to reduce any post-surgery soreness, and the connectors were covered with elastic bandage to protect them until data recording. Fascicle strain recordings obtained from each localized muscle region were assumed to represent those of the muscle as a whole. The goats were given 24–48 h to recover after the surgery prior to obtaining experimental recordings. All animals were found to willingly walk and run the day after the surgery, with no apparent sign of lameness.

Data collection

Prior to data collection, the non-surgery limb was shaved and the centers of rotation of the metatarsophalangeal, ankle, knee and hip joints, the ilium, and lateral and medial hoof, of both hind limbs were marked with white, non-toxic paint to facilitate kinematic analysis of the goat. It was necessary to mark both sides of the goat and the medial hoof of the surgery limb to acquire kinematic data for the decline trials as the goat was facing in the opposite direction, and it was only possible to place a camera to one side of the treadmill.

Muscle force, length and activity data were collected *via* a lightweight 10 m shielded cable (NMUF6/30-4046SJ; Cooner Wire, Chatsworth, CA, USA) which connected the female connectors on the back of the goat to the sonomicrometry amplifier, a strain gauge bridge amplifier (Vishay2120, Micromeritics, Raleigh, NC,

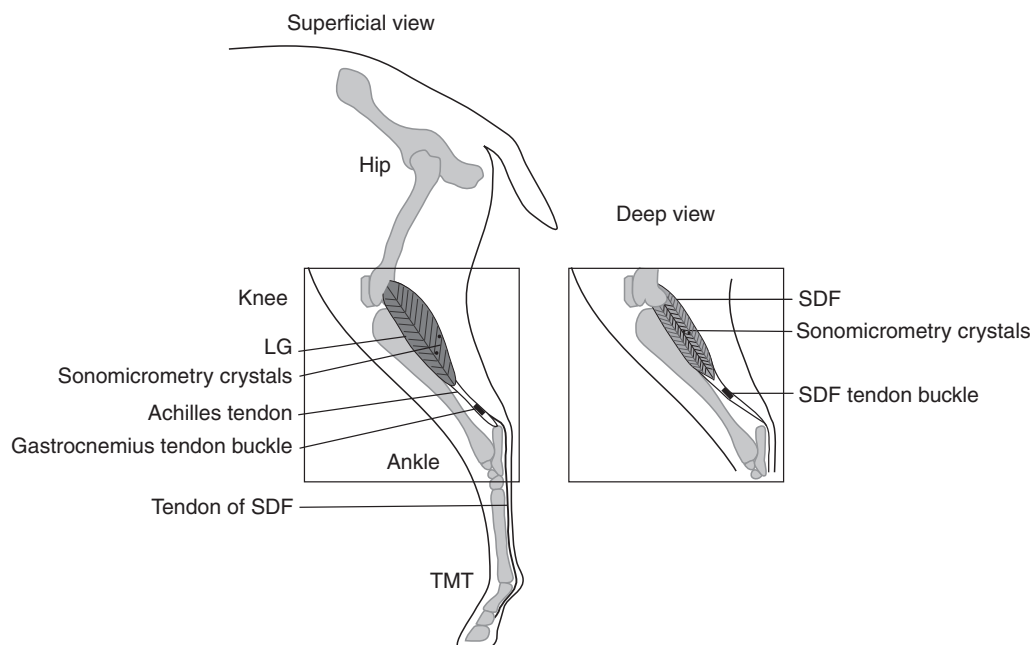


Fig. 1. Lateral view of the goat hind limb showing the anatomical organization of the distal muscle-tendon units [LG, lateral gastrocnemius; MG, medial gastrocnemius (not shown); SDF, superficial digital flexor] studied. TMT, tarso-metatarsal joint. The approximate locations of sonomicrometry crystals and EMG electrodes in the muscle bellies and tendon buckle force transducers are indicated. The inset shows the SDF muscle located deep to the LG muscle (also deep to the MG, which is not shown due to its medial position in the hind limb).

Table 1. Muscle–tendon morphological data

	N	Mass (g)	Fascicle length (mm)	Pennation angle (deg.)	PCSA (mm ²)	Tendon length (mm)	Tendon CSA (mm ²)
LG	5	24.0±1.5	21.6±1.3	25±1	961±49	161.8±5.7	11.8±1.4
MG	5	23.0±3.1	21.2±1.9	24±2	961±159		
SDF	6	21.7±2.7	13.1±0.8	16±2	1386±153	322.9±11.4	10.6±0.7

Values are means ± s.e.m. LG, lateral head of gastrocnemius; MG, medial head of gastrocnemius; SDF, superficial digital flexor; PCSA, physiological cross-sectional area.

USA) and EMG amplifiers (Grass P511, West Warwick, RI, USA). EMG signals were amplified ($\times 1000$) and filtered (60 Hz notch, 100–3000 Hz bandpass). All data were digitized at 5 kHz through a 12-bit A/D converter (Digidata 1200B, Axon Instruments, Union City, CA, USA) and stored on a computer for later analysis. Lateral view high-speed video (PCI-500; Redlake, Morgan Hill, CA, USA) was also taken at 250 Hz. This was synchronized with the muscle data by using a 5 V post-trigger pulse, which stopped the video recording, and was collected *via* the A/D board with the muscle data. A length scale on the treadmill allowed for calibration of the video images.

Data recordings were made while the goats walked and trotted on the treadmill at the speeds determined in training (walk: 1.0 or 1.5 ms⁻¹ and trot: 2.5 ms⁻¹). Two recordings were made at each speed on the level, incline and decline and a sequence of 8–10 strides was saved for analysis for each trial. Data were also collected during quiet standing as a measure of the resting length of the muscle fascicles.

Force buckle calibration

After completing *in vivo* recordings, the animals were killed (sodium pentobarbital, 150 mg kg⁻¹ i.v.) and the tendon force buckles calibrated. The muscles were removed from the bone at their origins and the ankle joint disarticulated, while leaving the distal insertions of the muscles intact. Individual muscle bellies were attached to a force transducer (Kister 9203; Amherst, MA, USA) using 0.0 silk suture and the muscle proximal to the tie frozen with liquid nitrogen to secure the connection. Tension was then applied to the force transducer–muscle–tendon system in a cyclical manner until the forces recorded *in vivo* had been exceeded. Data from the tendon force buckle and the force transducer were collected *via* the A/D system described above. The force transducer was then calibrated using a known weight. Calibration equations were created for each buckle–tendon system using a least squares linear regression fit to the rise and fall of the tendon force buckle output *versus* the calibrated force transducer. Tendon buckle calibrations were linear over the full range of recorded forces, and yielded calibration slopes having $R^2 > 0.998$.

Morphological measurements

After being calibrated the tendon buckles were removed from the tendons and the placements of the sonomicrometry crystals within the muscles were verified. In all cases, crystals were implanted within 0–8 deg. of the fascicle axis, so that errors due to misalignment were less than 1% [$=\cos(8 \text{ deg.})$]. Fascicle length, pennation angle, and wet mass of the LG, MG and SDF muscles were then measured and their physiological cross-sectional area (PCSA) calculated.

Free tendon length of the GA and SDF was measured as the length of the MTU minus mean fascicle length. Average tendon cross-sectional area (CSA) was calculated by measuring the length and mass of a section of each tendon, assuming a density of tendon of 1120 kg m⁻³ (Ker, 1981).

Data analysis

Stride times were determined from the movements of the hoof of the measurement limb in the high-speed video recordings (4 ms frame⁻¹ resolution). Stance was defined as the first frame in which the limb made contact with the treadmill limb to the last frame in which it was in contact; and swing as the frame after the foot loses contact with the belt until the last frame before it contacts the belt again. All other data were divided into individual strides on the basis of these timings and six strides were analyzed for each condition.

Prior to muscle strain analysis, the following adjustments in the raw sonomicrometry signals were made: (1) a correction for the 5 ms delay introduced by the Triton Sonomicrometer filter, (2) a +2.7% correction in length due to the difference of the speed of sound in water (used by the Triton Sonomicrometer) *versus* skeletal muscle, and (3) a +0.82 mm correction for the faster speed of sound through the epoxy lens of the 2.0 mm SonomicrometryTM crystals (Biewener et al., 1998). After making these corrections, fascicle length measurements were converted to fractional strain by dividing by the distance measured between the crystals during quiet stance [following Gillis et al. (Gillis et al., 2005)]. Fractional strains were converted to total fascicle length changes of the muscle by multiplying by the average length of the muscle fascicles as measured *post mortem*. Again, this method assumes uniform strain along the fascicles. Although this may not always be the case (Ahn et al., 2003; Higham et al., 2008), uniform strain patterns were observed in an earlier study at proximal, mid-belly and distal regions of the unipennate goat vastus lateralis (Gillis et al., 2005). In general, the spacing between crystals (~8–12 mm) provided approximately a 50–60% sampling of fascicle length of these muscles. Lengthening, shortening and net strain (shortening strain+lengthening strain) were calculated when the muscles were active during the stance phase of the stride.

EMG signals were analyzed for the timing of onset and offset, as well as intensity. Timings of EMG onset, offset and duration were expressed both in real time and as a percentage of the stride period. EMG intensity was measured as the mean spike amplitude of the rectified signal over the period of activation and expressed as a percentage of the maximum value recorded for each individual electrode (Gillis et al., 2005).

Muscle length data during individual strides were differentiated to give muscle velocity and multiplied by muscle force to give instantaneous muscle power. The forces generated by the LG and MG, measured as their sum by the GA tendon buckle, were estimated on the basis of the PCSA of each muscle belly assuming equal muscle stress. Muscle power was integrated to give cumulative work over the stride, the final value representing the net work of the muscle per stride. This method assumes that the length change of the whole muscle is equal to that of the individual fascicles and does not account for the pennation angle of the muscle. However, the errors introduced by ignoring fascicle pennation are likely offset by shortening due to angular rotation of fascicles as pennate muscles shorten (Fukunaga et al., 1997;

Lichtwark et al., 2007; Loram et al., 2006) and are likely to be similar for the three muscles investigated here. Consequently, we believe this is unlikely to affect the interpretation of the results. Mass-specific muscle work was calculated using individual muscle masses. The net work required to move the center of mass of the animal during each stride was calculated from the average stride length and the gradient of the slope. The contribution of each muscle to this work was expressed as a percentage of the net work required to move the centre of mass.

Peak stress during each stride was calculated for the GA and SDF tendons based upon average tendon CSA measurements. This was then used to deduce the elastic energy recovered by the tendon using a tendon stiffness of 1 GPa and a hysteresis energy loss of 7% (Ker, 1981; Shadwick, 1990).

Statistical analyses

The data from the six strides in each condition were averaged to produce the mean \pm s.e.m. of each variable for each goat in each condition. A two-way, mixed model ANOVA (Systat, v9) was used to assess the effect of surface grade with respect to gait and speed

on the following variables: net muscle strain, peak muscle stress, muscle work, time of EMG onset, EMG duration, EMG intensity and tendon energy recovery. Pair-wise comparisons were made using a paired *t*-test with a *post hoc* sequential Bonferroni correction. $P < 0.05$ was considered to show a significant difference.

RESULTS

Morphological measurements

Muscle mass, fascicle length and pennation angle for the five LG, five MG and six SDF muscles for which muscle force, length and activation data were recorded are shown in Table 1, along with the length of the free tendon and calculated values of muscle PCSA and tendon CSA. Across the group of goats the PCSA of the two heads of the GA muscle were the same (LG=961 \pm 49 mm² and MG=961 \pm 159 mm²), and smaller than that of the SDF (1385 \pm 153 mm²). This was mainly due to a shorter mean fascicle length of the SDF, rather than its mass, compared with the LG and MG muscles. The length of the free tendon of the SDF (322.9 \pm 11.4 mm) was approximately double that of the GA (161.8 \pm 5.7 mm; Fig. 1).

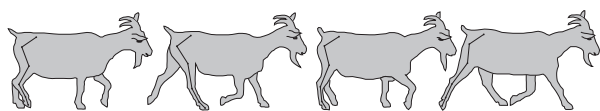
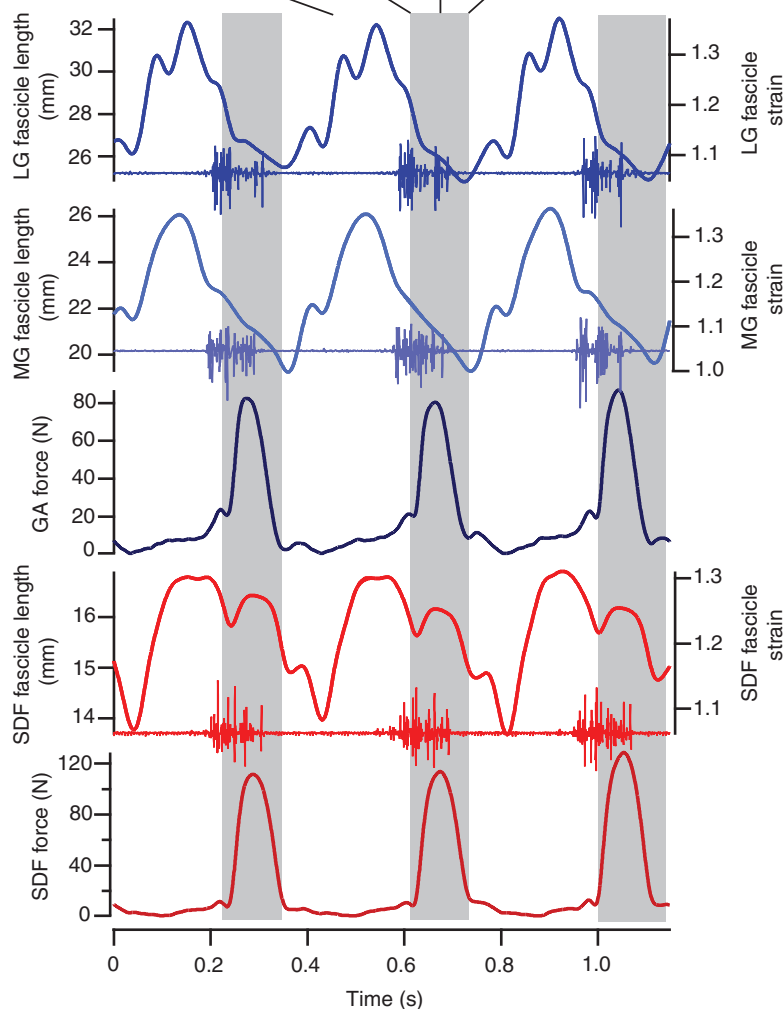


Fig. 2. Representative LG, MG and SDF fascicle strains and EMG, together with gastrocnemius (GA) and SDF muscle–tendon forces, recorded from goat 1 for three sequential strides during level trotting (2.5 m s⁻¹). Gray areas represent stance.



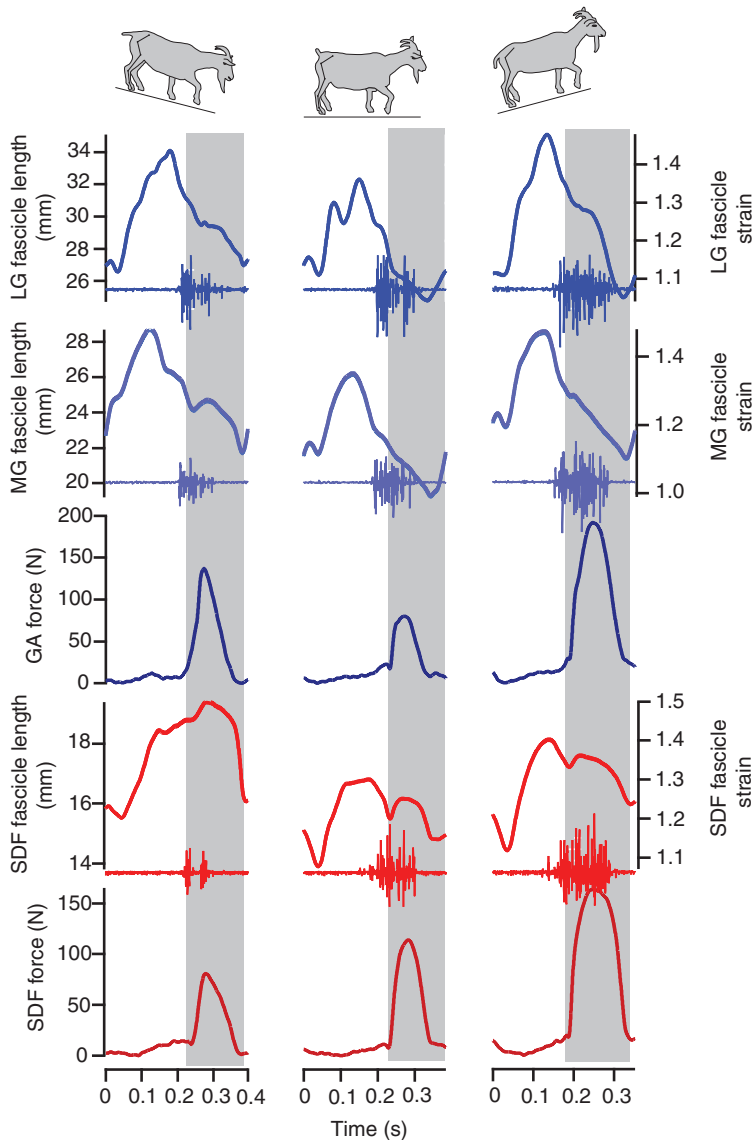


Fig. 3. Representative LG, MG and SDF fascicle strains and EMG, together with GA and SDF muscle–tendon forces, recorded from goat 1 for one stride on a decline (–15 deg.), level and incline (15 deg.) at a trot (2.5 m s^{-1}).

Stride parameters

Stride frequency increased significantly from decline to level and incline locomotion in both walking (decline= $1.712 \pm 0.105 \text{ strides s}^{-1}$; level= $1.817 \pm 0.097 \text{ strides s}^{-1}$; incline= $1.917 \pm 0.102 \text{ strides s}^{-1}$; ANOVA $P < 0.01$) and trotting (decline= $2.538 \pm 0.057 \text{ strides s}^{-1}$; level= $2.795 \pm 0.054 \text{ strides s}^{-1}$; incline= $2.922 \pm 0.077 \text{ strides s}^{-1}$; $P < 0.001$). The proportion of each stride in which the foot was in contact with the ground (duty factor) also increased from decline to level and incline, for both walking (decline= 0.558 ± 0.015 ; level= 0.603 ± 0.014 ; incline= 0.632 ± 0.016 ; $P < 0.0001$) and trotting (decline= 0.393 ± 0.019 ; level= 0.398 ± 0.014 ; incline= 0.485 ± 0.016 ; $P < 0.0001$).

Muscle force and stress

Fig. 2 shows typical traces of muscle fascicle length (and strain), force and EMG activity for the LG, MG and SDF muscles of goat 1 for three strides during trotting (2.5 m s^{-1}) on the level, and Fig. 3 compares similar traces for one stride each on the decline, level and incline. Peak force in the GA and SDF muscles (and tendons) increased significantly with grade during both walking and trotting (GA: walk, $P = 0.0023$; trot, $P < 0.001$; SDF: walk, $P < 0.001$; trot, $P < 0.0001$; ANOVA; Table 2; Fig. 3). Whereas GA muscle–tendon

force did not change from decline to level in either gait, *post hoc* tests revealed that SDF muscle–tendon force increased by 34% ($P < 0.05$) at a walk and 42% ($P < 0.01$) at a trot when goats shifted from decline to level. Both MTUs significantly increased ($P < 0.01$ for all *post hoc* comparisons; Table 2) their force production when goats shifted from level to incline locomotion: the GA increasing by 67% at a walk and 81% at a trot, and the SDF increasing by 52% at a walk and 44% at a trot. Not surprisingly, changes in peak muscle stress and tendon stress showed similar patterns to changes in peak force (Table 2).

The duration of force production by the GA also differed significantly with grade within both gaits (walk: decline= $244 \pm 20 \text{ ms}$, level= $227 \pm 20 \text{ ms}$, incline= $279 \pm 24 \text{ ms}$, $P < 0.01$; and trot: decline= $120 \pm 9 \text{ ms}$, level= $111 \pm 6 \text{ ms}$, incline= $144 \pm 10 \text{ ms}$, $P < 0.01$), being significantly greater in incline than for level locomotion (walk $P < 0.05$; trot $P < 0.01$). Differences in force duration between decline and level gait, however, were not significant. The duration of force production of the SDF muscle also differed significantly with grade in trotting (decline= $123 \pm 18 \text{ ms}$, level= $109 \pm 12 \text{ ms}$, incline= $147 \pm 13 \text{ ms}$, $P < 0.001$), but not in walking (decline= $301 \pm 41 \text{ ms}$, level= $293 \pm 28 \text{ ms}$, incline= $314 \pm 28 \text{ ms}$). The percentage of stance at which peak force (or stress) occurred did not differ with grade in either muscle for either gait (Table 2).

Table 2. Muscle contractile performance across grade and within gait

		Walk (1.0–1.5 m s ⁻¹)			Trot (2.5 m s ⁻¹)		
		Decline	Level	Incline	Decline	Level	Incline
GA	Peak force (N)	63.3±10.1	61.1±8.4	101.8±19.0**	71.0±14.7	77.7±14.9	140.9±18.1**
	Peak stress (kPa)	33.5±1.3	32.6±1.4	53.4±1.4**	37.8±1.7	41.9±1.7	74.9±1.7***
	Time of peak stress (% of stance)	26.9±1.4	28.6±2.9	30.1±2.1	38.9±3.9	39.6±1.7	34.5±2.2
	Peak tendon stress (MPa)	5.4±0.7	5.4±0.7	8.6±1.2***	5.9±0.9	6.9±0.8	12.3±1.5**
	Tendon energy recovery (J kg ⁻¹)	13.2±3.1	13.2±3.7	34.0±8.9**	16.4±3.6	21.1±5.2	67.6±16.7**
LG	Lengthening strain (%)	4.6±2.0	0.5±0.5	0.4±0.3	3.0±1.3	0.5±0.3	0.5±0.5
	Shortening strain (%)	-11.7±2.9	-16.9±4.6	-29.7±4.6**	-12.6±3.0	-16.4±3.0	-27.7±3.9**
	Net strain (%)	-7.0±4.4**	-16.4±4.9	-29.3±4.8**	-9.7±2.6*	-15.9±2.9	-27.1±4.4**
	EMG duration (% of stride)	27.6±4.7	33.3±4.4	52.8±4.6**	25.5±2.2	27.3±1.1	39.7±2.5***
	Relative EMG intensity	0.34±0.04	0.34±0.07	0.48±0.03*	0.47±0.03	0.55±0.08	0.88±0.01***
MG	Net work (J kg ⁻¹)	-0.12±0.96	1.35±0.80	6.48±2.42*	0.63±0.89	2.11±0.95	8.25±2.05**
	Lengthening strain (%)	10.9±2.7**	1.6±0.8	0	9.1±4.4	2.6±1.3	0.2±0.2
	Shortening strain (%)	-9.4±2.8	-12.2±2.3	-23.4±5.2**	-16.0±2.4	-11.3±1.8	-22.1±4.9*
	Net strain (%)	1.5±4.5**	-10.5±2.7	-23.4±5.2**	-6.8±2.5	-8.7±2.8	-21.9±4.9*
	EMG duration (% of stride)	27.0±4.7	32.8±3.0	47.2±2.3**	26.9±3.0	24.9±2.6	40.5±2.5***
SDF	Relative EMG intensity	0.23±0.03	0.29±0.05	0.56±0.04***	0.31±0.04*	0.48±0.06	0.91±0.02***
	Net work (J kg ⁻¹)	-0.47±1.11	1.19±0.32	4.87±1.02*	-0.26±0.65	1.27±0.39	7.46±2.02*
	Peak force (N)	47.3±8.0*	71.4±9.4	108.2±15.7**	58.8±10.3**	102.0±9.3	146.8±15.0**
	Peak stress (kPa)	34.3±1.5*	50.1±1.5	75.0±1.5**	41.9±2.1**	72.0±2.1	102.1±2.1**
	Time of peak stress (% of stance)	24.1±2.9	26. ± 3.2	41.2±7.4**	39.0± 5.4	42.7±2.2	41.6±1.4
SDF	Peak tendon stress (MPa)	4.5±0.7*	6.7±0.7	10.1±1.1**	5.6±0.9**	9.7±0.7	13.9±1.1**
	Tendon energy recovery (J kg ⁻¹)	9.2±2.9	19.8±4.0	45.1±10.3**	14.2±3.7	39.9±5.4	82.4±13.0**
	Lengthening strain (%)	11.6±3.3	4.1±1.4	2.4±1.0	13.1±4.0	4.2±1.6	2.3±1.2
	Shortening strain (%)	-11.0±2.6	-9.4±1.8	-10.3±2.8	-17.2±4.4*	-9.6±2.5	-10.9±2.4
	Net strain (%)	0.6 ± 4	-5.3±1.6	-7.9±3.2	-4.1±2.3	-5.4±2.4	-8.6±3.5
	EMG duration (% of stride)	30.8±2.6***	43.7±2.6	56.8±3.7***	21.5±3.6**	34.4±1.7	46.3±2.8*
	Relative EMG intensity	0.28±0.04	0.36±0.03	0.56±0.04**	0.36±0.06**	0.58±0.03	0.87±0.03**
	Net work (J kg ⁻¹)	-1.91±0.83	0.21±0.43	2.47±1.44	-2.10±0.88	0.49±0.76	3.16±1.95

Values are means ± s.e.m. GA, gastrocnemius; LG, lateral head of gastrocnemius; MG, medial head of gastrocnemius; SDF, superficial digital flexor.

Asterisks represent significant differences from the level condition in that gait in *post hoc* comparisons (* $P<0.05$; ** $P<0.01$; *** $P<0.001$).

Muscle fascicle length change

The LG underwent net shortening when the muscle was active during the stance phase on all grades in both walking and trotting (Table 2; Figs 2 and 3). Net fascicle shortening strain increased significantly with grade for both walking (decline=-7.0±4.4%, level=-16.4±4.9%, incline=-29.3±4.8%, $P<0.0001$) and trotting (decline=-9.7±2.6%, level=-15.9±2.9%, incline=-27.1±4.4%, $P<0.001$; Fig. 4). The difference in net strain with grade was due mainly to a significant increase in shortening strain in both gaits (walk and trot, $P<0.001$; Table 2), although a small, but insignificant, decrease in lengthening strain was also observed.

The MG similarly underwent net shortening when the muscle was active during the stance phase on all grades, apart from decline walking (Table 2; Figs 3 and 4). In a manner similar to the LG, the degree of net shortening strain in the MG increased with grade in both walking (decline=1.5±4.5%, level=-10.5±2.7%, incline=-23.4±5.2%, $P<0.01$) and trotting (decline=-6.8±2.5%, level=-8.7±2.8%, incline=-21.9±4.9%, $P<0.01$; Fig. 5). At a walk, this was due to a significant decrease in lengthening strain ($P<0.003$), as well as a significant increase in shortening strain ($P<0.02$), but at a trot it resulted solely from an increase in shortening strain ($P<0.05$; Table 2). Only on a decline was a distinct stretch-shortening cycle observed in the MG fascicles.

In contrast to the LG and MG, the SDF muscle underwent a stretch-shortening cycle on all three grades (Fig. 3), resulting in significant net fascicle shortening on all grades apart from walking on a decline (Table 2). Net shortening strain of the SDF fascicles,

however, did not change significantly with grade for either walking (decline=0.6±2.4%, level=-5.3±1.6%, incline=-7.9±3.2%) or trotting (decline=-4.1±2.3%, level=-5.4±2.4%, incline=-8.9±3.5%; Fig. 4). There was, however, a significant decrease in lengthening SDF strain with grade in walking ($P<0.02$) and trotting ($P<0.05$) and a significant decrease in shortening SDF strain with grade in trotting ($P<0.05$) that resulted from a decrease between decline and level locomotion (Table 2; Fig. 4).

Muscle EMG

Consistent with increased limb support times, EMG duration and intensity generally increased with grade in all three muscles within both gaits (Figs 3, 5 and 6). The duration of LG muscle activity during walking averaged 172±34 ms on the decline, 196±130 ms on the level and 286±29 ms on the incline, and during trotting averaged 98±7 ms on the decline, 98±3 ms on the level and 134±9 ms on the incline. LG EMG duration, expressed as a percentage of stride duration, increased significantly with grade within both gaits (walk, $P=0.0018$; and trot, $P=0.0001$) due mainly to the large increase between level and incline locomotion (Fig. 5; Table 2).

The MG displayed a similar activation pattern to the LG (Figs 2, 3 and 5). The duration of MG activity during walking averaged 154±24 ms on the decline, 182±19 ms on the level and 250±18 ms on the incline, and during trotting averaged 103±10 ms on the decline, 88±8 ms on the level and 139±10 ms on the incline. As a percentage of stride duration, MG EMG duration also increased significantly during walking ($P<0.001$) and trotting ($P<0.0001$),

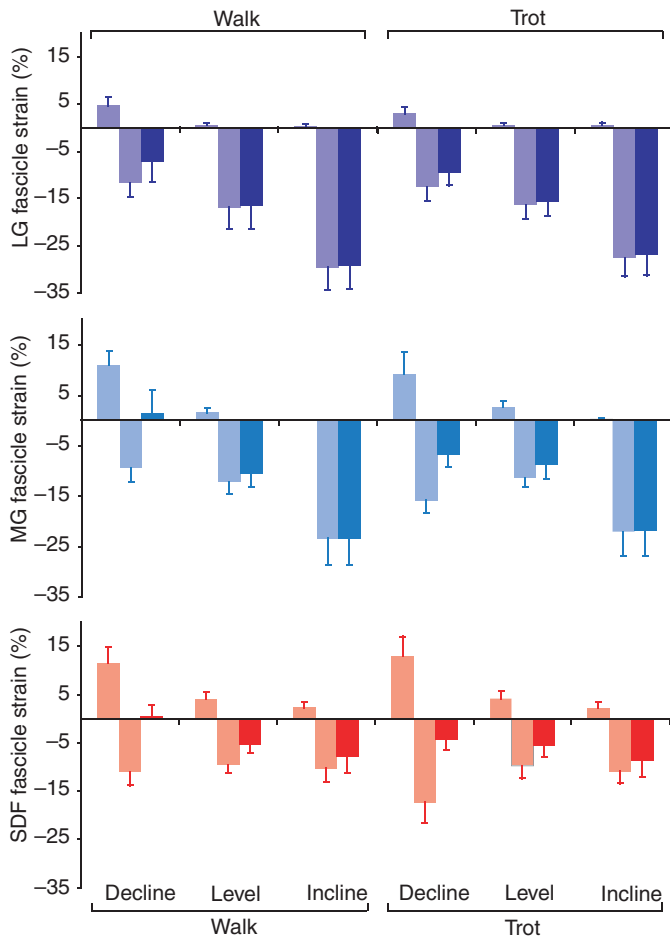


Fig. 4. Muscle fascicle strains (lengthening, shortening and net %) recorded via sonomicrometry for the LG, MG and SDF muscles for decline, level and incline conditions averaged across both walking (1.0 and 1.5 m s^{-1}) and trotting (2.5 m s^{-1}) conditions (values shown are means \pm s.e.m.). Positive strains represent fascicle lengthening and negative strains fascicle shortening. Net strain is shown as the darker bars.

again due mainly to a large increase between level and incline locomotion (Fig. 5; Table 2).

Similar to the LG and MG, EMG duration of the SDF also increased with grade in both gaits (walk: decline= 185 ± 22 ms, level= 242 ± 15 ms, incline= 300 ± 24 ms; and trot: decline= 82 ± 13 ms, level= 123 ± 5 ms, incline= 159 ± 11 ms), as well as when expressed as a percentage of stride duration (walking and trotting: $P<0.0001$). However, in the SDF this was due to increases from decline to level, as well as from level to incline (Fig. 5; Table 2).

Relative EMG intensity, expressed as a percentage of the maximum mean spike amplitude, was maximal in all three muscles when animals trotted on an incline. As with EMG duration, relative EMG intensity also increased significantly with grade for both walking and trotting gaits in all three muscles (LG walk $P<0.01$; LG trot $P<0.001$; MG walk $P<0.0001$; MG trot $P<0.0001$; SDF walk $P<0.001$; SDF trot $P<0.0001$; Fig. 6). *Post hoc* tests revealed that this was generally due to a significant increase in EMG intensity between level and incline locomotion (Table 2). When examined across all three grades and both gaits, EMG intensity was significantly correlated with differences in peak muscle stress in all three muscles (Fig. 7; LG: Pearson $r=0.6069$,

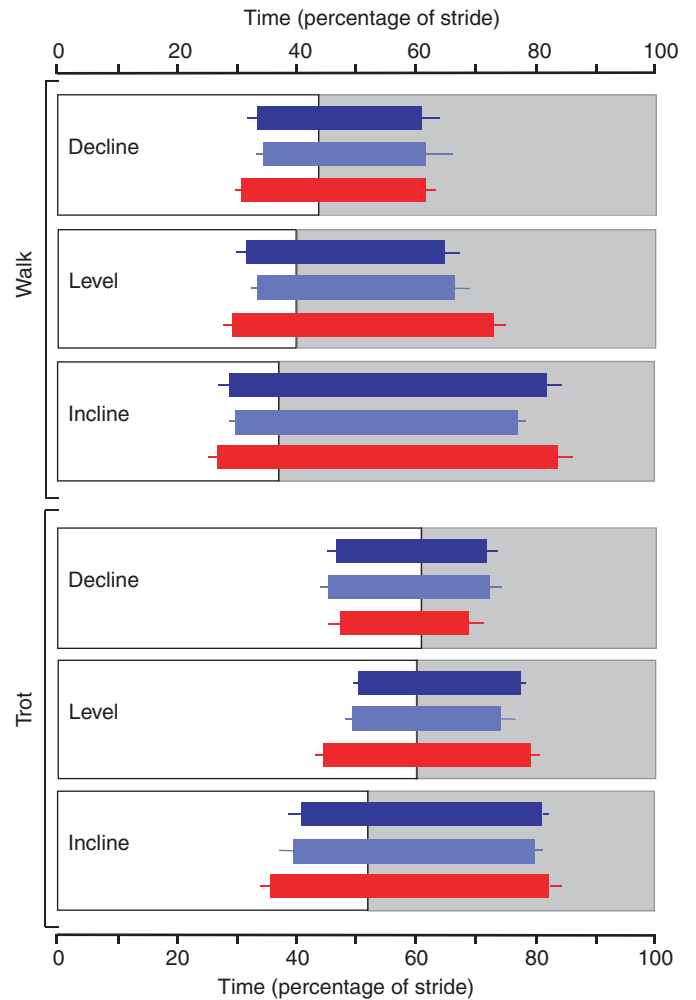


Fig. 5. Timing and duration of EMG activity recorded in the LG (dark blue), MG (light blue) and SDF (red) normalized as a percentage of stride duration relative to the onset of stance for (A) walk (averaged for 1.0 and 1.5 m s^{-1}) and (B) trot (2.5 m s^{-1}) across the three grade conditions. Stance is depicted by gray shading, and swing by white. Values shown are means \pm s.e.m.

$P<0.0001$; MG: Pearson $r=0.6816$, $P<0.0001$; SDF: Pearson $r=0.5585$, $P<0.0001$), but was more variable in significance when considered within a grade.

Muscle work

Fig. 8 shows typical work loops obtained from our *in vivo* force and fascicle strain recordings for the LG, MG and SDF during one stride when goats trotted at 2.5 m s^{-1} on the decline, level and incline. Mean values of muscle mass-specific work produced by each muscle across the group of goats are given in Table 2 and shown Fig. 9. At a walk, the MG and SDF absorbed energy on the decline (-29 ± 9 mJ and -41 ± 19 mJ, respectively), while the LG performed little net work (-1 ± 23 mJ). When walking on the level, the LG and MG produced energy (37 ± 23 mJ and 21 ± 30 mJ, respectively), while the SDF performed little net work (4 ± 10 mJ). On the incline, all three muscles produced energy, but the increase in energy production was greater in the LG (171 ± 72 mJ) and MG (123 ± 40 mJ) than in the SDF (54 ± 35 mJ).

At a trot, the MG and SDF absorbed energy on the decline (MG= -7 ± 14 mJ; SDF= -42 ± 17 mJ) but produced energy on the

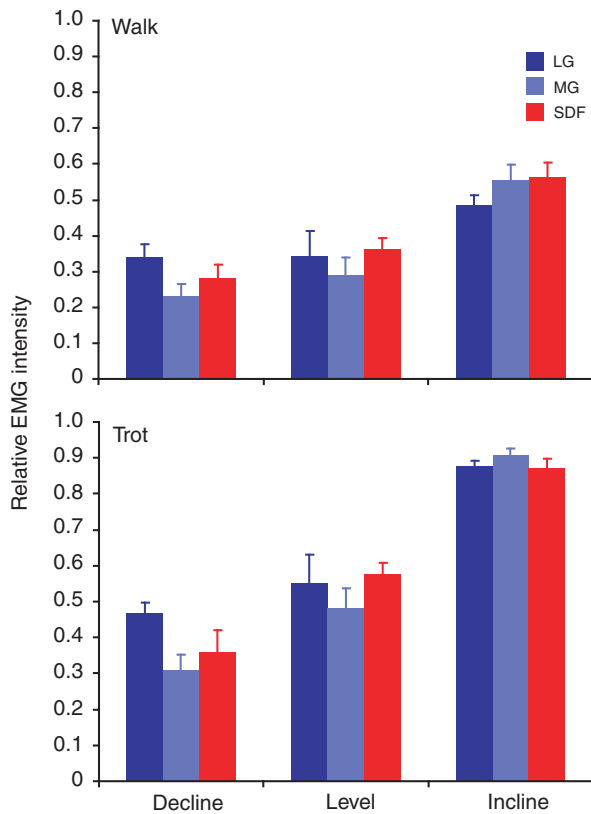


Fig. 6. Relative EMG intensity (mean spike amplitude normalized to the maximum value obtained during incline trotting) of the LG, MG and SDF for walking and trotting across the three grade conditions. Values shown are means + s.e.m.

level (MG=33.9±14.9 mJ; SDF=14.0±16.6 mJ) and incline (MG=178.7±53.7 mJ; SDF=71.1±47.6 mJ). However, the LG produced energy on all three grades (decline=20.0±24.4; level=56.0±28.1; incline=208.9±60.4). The increase in net work with grade was again greater for the LG and MG than for the SDF. When muscle work was normalized for muscle mass, a significant increase in mass-specific muscle work from level to incline was still observed for the LG ($P<0.02$) and MG ($P<0.01$), but not for the SDF (walk: $P=0.062$, trot: $P=0.053$; Table 2). No significant change in mass-specific work in either gait was observed in all three distal limb muscles for decline *versus* level locomotion (Table 2).

Tendon energy recovery

Peak GA and SDF tendon stress increased significantly with grade within both gaits (walk: GA, $P=0.0004$; SDF, $P<0.0001$; and trot: GA, $P<0.001$; SDF, $P<0.0001$; Table 2; Fig. 10). This reflected the increase in muscle recruitment (EMG intensity) and force that was observed with increased grade. Assuming an elastic modulus of goat tendon of 1 GPa (applicable for the range of peak tendon stresses observed), the amount of elastic strain energy recovered by the GA and SDF tendons during each stride was determined based on their estimated free tendon volume (Table 1). On average, the SDF tendon was responsible for recovering 2.4±0.9 times more energy than the GA tendon across all grade and gait conditions. Mass-specific tendon energy recovery is shown in Table 2 and Fig. 10 for the purpose of comparison with mass-specific muscle work values. Overall, there was a significant increase in energy recovery by each tendon with grade in both gaits (GA: walk,

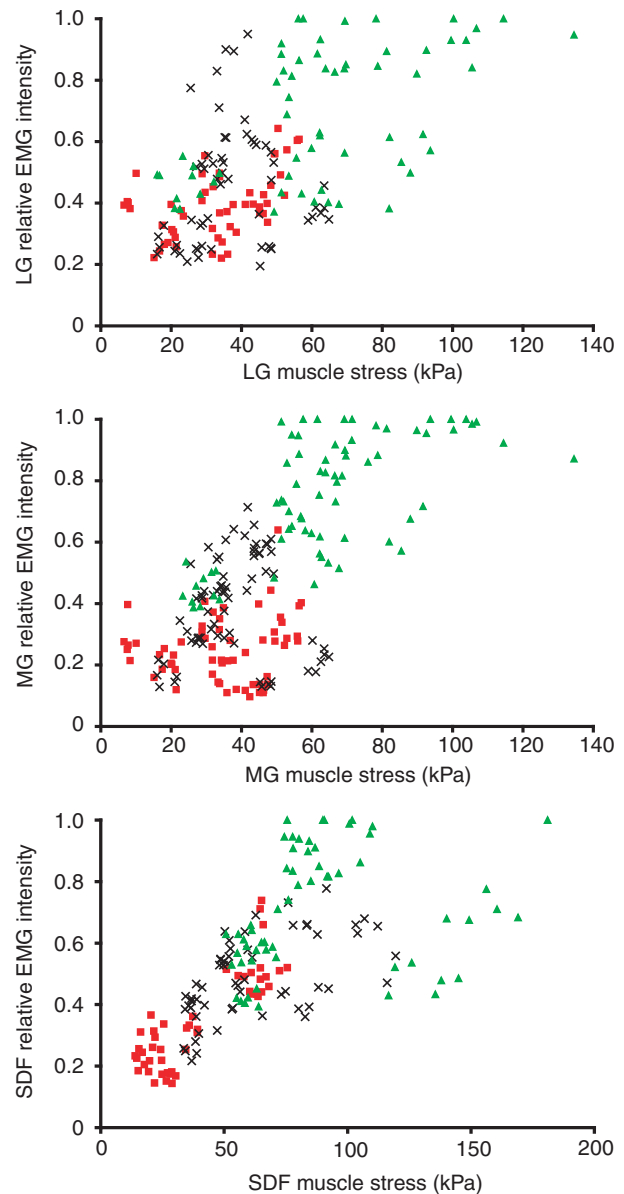


Fig. 7. Relative EMG intensity (mean spike amplitude normalized to the maximum value obtained during incline trotting) of the LG, MG and SDF plotted against muscle stress (kPa) for the two gaits and three grade conditions. Decline strides are represented by red squares, level strides by black crosses and incline strides by green triangles. Muscle stress in the MG and LG was derived from their shared tendon force and was therefore assumed to be the same for the two muscle heads (see Materials and methods). A significant correlation between muscle stress and EMG activation intensity was observed in all muscles when compared across the gaits and grades (Table 2).

$P<0.005$ and trot, $P<0.01$; SDF: walk, $P<0.001$ and trot, $P<0.001$; Fig. 10; Table 2). At a walk, the GA tendon recovered 27.8±7.0 mJ on the decline, 26.5±6.3 mJ on the level and 72.8±21.7 mJ on the incline, and at a trot recovered 35.0±9.1 mJ on the decline, 41.1±6.6 mJ on the level and 137.3±30.2 mJ on the incline. In comparison, at a walk the SDF tendon recovered 36.0±12.2 mJ on the decline, 76.5±17.9 mJ on the level and 177.0±45.9 mJ on the incline and at a trot it recovered 54.7±15.6 mJ on the decline, 152.1±24.4 mJ on the level and 315.9±54.4 mJ on the incline. Across both gaits and the three grade conditions, GA and SDF

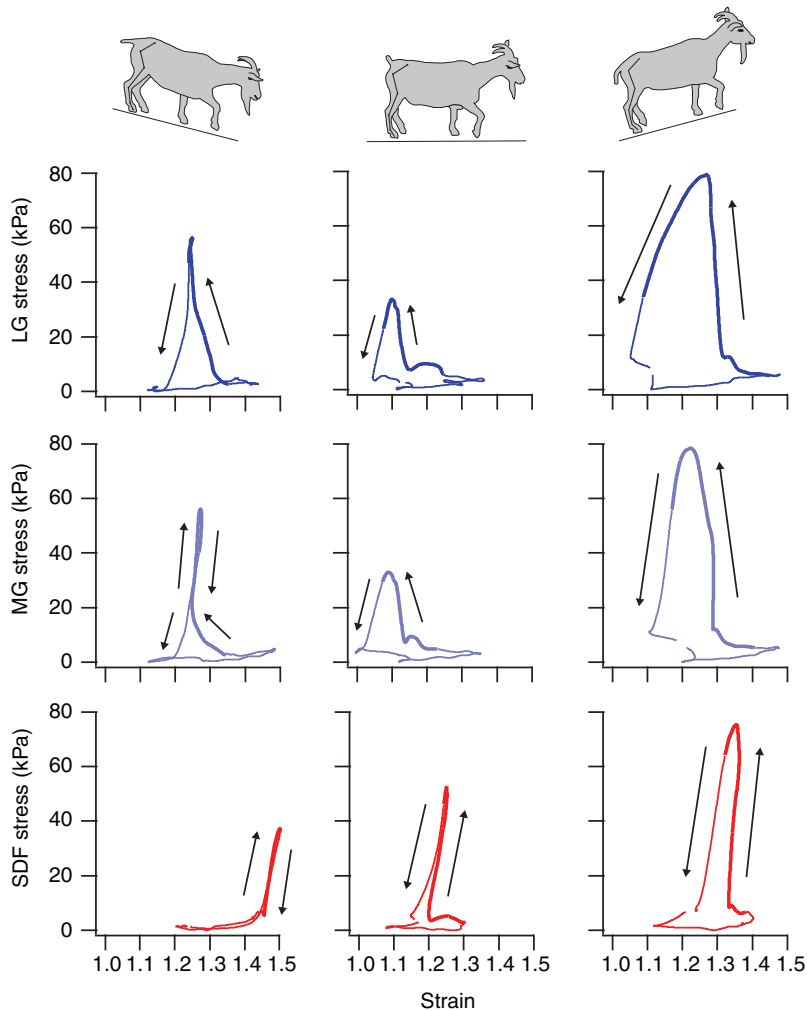


Fig. 8. Representative muscle work loops (shown as *in vivo* muscle stress plotted *versus* fascicle strain) of the LG, MG and SDF for a single stride cycle during decline, level and incline trotting (2.5 m s^{-1}). Thicker portions of the line in each work loop denote the period of muscle activation. Arrows denote the direction of changes in muscle stress *versus* fascicle strain (strain=1.0 denotes the resting length of the fascicles recorded during quiet stance). Net work per cycle is denoted by the area within the work loop (here normalized as muscle stress and strain), with counter-clockwise loops denoting net positive work. In all three muscles, muscle stress (force) and net shortening strain increase as animals move from decline to level, to incline conditions.

tendon energy recovery substantially exceeded muscle fascicle work (Table 2; Figs 9 and 10), averaging more than 14-fold in the GA and 38-fold in the SDF on a muscle mass-specific basis. Thus, despite the modulation of muscle fascicle work, tendon energy recovery remained a key function of the distal tendons during locomotion on the different grades.

DISCUSSION

We sought to analyze how the contractile behavior and work performance of three distal hind limb muscles of domestic goats respond to changes in locomotor grade. Given that goats naturally inhabit mountainous terrain and regularly move over steeply graded surfaces, we hypothesized that the LG, MG and SDF, which act as synergists to extend the ankle joint, would similarly adjust their work output to absorb energy when goats moved on a decline and produce increasing energy when goats shifted from level to incline locomotion. Our results confirmed this hypothesis (Figs 8 and 9). For each muscle, similar changes in work occurred for walking and trotting gaits, although the shift in muscle work was generally greater at a trot. Nevertheless, changes in muscle work were much less than the amount of energy stored and recovered elastically in the muscles' tendons (Fig. 10), indicating the importance of distal tendon energy savings in goats while moving over different grades. Even so, goats have less effective tendon energy savings than more highly specialized tammar wallabies (Biewener et al., 1998; Biewener et al., 2004), red kangaroos (Dawson and Taylor, 1973; Alexander

and Vernon, 1975), and likely larger ungulates that have been indirectly assessed (Alexander, 1988; Biewener, 1998).

Muscle–tendon architecture in relation to tendon elastic savings and muscle work

As in other runners, the distal MTUs of goats studied here are well designed for economically producing force (short, pennate fascicles) and recovering elastic energy from their long tendons (Ker et al., 1987; Roberts et al., 1997; Biewener et al., 1998; Biewener and Roberts, 2000). The fact that tendon energy savings were less than those observed in more specialized wallabies, kangaroos and larger ungulates suggests goats may reflect a compromise for locomotion in mountainous terrain. For example, compared with tammar wallabies that hop, goat distal tendon energy savings were lower in both absolute and body mass-adjusted terms. Whereas the GA and plantaris tendons of wallabies recover 890 mJ during level hopping and 900 mJ during incline hopping (Biewener et al., 2004), the equivalent tendons of goats recovered 183 mJ during level trotting and 453 mJ during incline trotting: 50% or less than that achieved by tammar wallabies in absolute terms. The threefold smaller size of tammar wallabies makes this difference even greater. This comparison, however, ignores the likelihood that energy savings are also achieved in goat forelimb tendons. The lower energy savings in goat tendons reflect their lower operating stresses [GA: 5.4–12.3 MPa and SDF: 4.5–13.9 MPa; Table 2; compared with wallaby tendons, GA: 24.2–26.3 MPa and plantaris: 23.9–26.0 MPa;

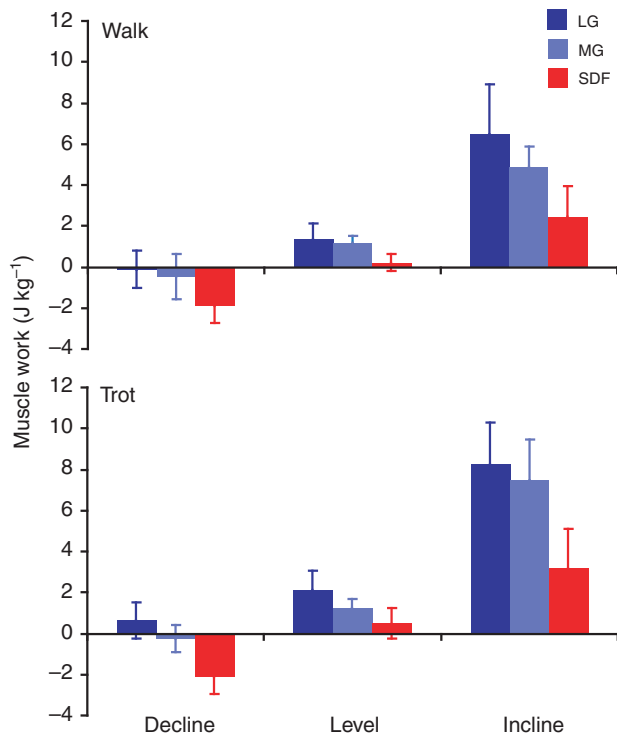


Fig. 9. Mass-specific muscle work (J kg^{-1}) performed by the LG, MG and SDF during walking (averaged for 1.0 and 1.5 m s^{-1}) and trotting (2.5 m s^{-1}) across the three grade conditions. Net work output increased in all three muscles as a function of grade, and was greater within the LG and MG than in the SDF (Table 2). Values shown are means \pm s.e.m.

Table 2 (Biewener et al., 2004)]. The lower stresses of goat tendons likely indicate the need for having thicker, stiffer tendons to negotiate mountainous terrain. Estimates of tendon energy recovery based on direct measurements of muscle–tendon forces in other ungulates have not yet been made, but indirect estimates of tendon energy recovery in horse tendons based on external joint moment analysis during level trotting (Biewener, 1998) also indicate

considerably more energy savings ($110\text{--}215 \text{ mJ kg}^{-1}$ body mass) than achieved in goats ($9.1\text{--}22.6 \text{ mJ kg}^{-1}$ body mass) and comparable to the levels achieved by wallaby tendons during hopping (136 mJ kg^{-1} body mass).

Despite exhibiting an apparent compromise for reduced energy recovery to maintain sufficient stiffness, when normalized on a muscle mass-specific basis tendon elastic savings substantially exceeded net muscle work for the three goat distal MTUs (Table 2). Thus, although muscle work significantly changed with locomotor grade, the changes were small in comparison with elastic energy recovered from each muscle's tendon, suggesting a functional specialization and constraint due to muscle–tendon architecture. A modeling approach recently used to investigate joint compliance and actuation during downhill, level and uphill running of goats supports such a constraint on the function of ankle extensor MTUs (Lee et al., 2008). Consistent with the presence of real physical tendon springs, modeled ankle joint stiffness was constant across level and grade running conditions. Net ankle work decreased during downhill and increased during uphill running compared with being nearly zero on the level. Yet specific net work (the ratio of net to total work) was constant across level and grade running. Together, these findings support the conclusion that ankle function is constrained by the tendon elasticity and short fibers of the ankle extensors.

Evaluating how the work output of the LG, MG and SDF compares with other hind limb muscles of goats is difficult to assess at this time, given limited data for proximal muscle function. Gillis and colleagues (Gillis et al., 2005) found that the goat biceps femoris and vastus lateralis both exhibit net shortening strains (-15 to -32%) during level locomotion across different gaits, indicating that these muscles perform net positive work. However, the difficulty of assessing muscle force in proximal muscles makes evaluating their work output a major challenge. When goats moved on decline and incline grades, net work of their distal muscles was extremely small compared with overall potential energy changes of their center of mass. For example, when trotting at 2.5 m s^{-1} , the goats' average potential energy change was $-50.3 \text{ J stride}^{-1}$ when they moved down a 15 deg. decline and $43.7 \text{ J stride}^{-1}$ when they moved up a 15 deg. incline (reflecting differences in stride length and frequency in relation to center of mass height change under these two grade

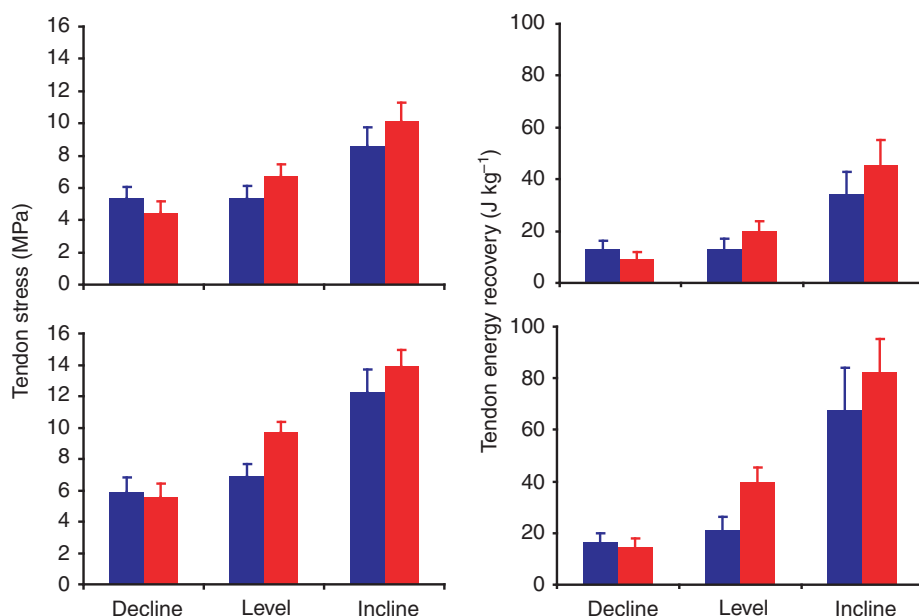


Fig. 10. Peak tendon stress (MPa) and elastic energy recovery for the GA (dark blue) and SDF (red) tendons for (A) walking (averaged for 1.0 and 1.5 m s^{-1}) and (B) trotting (2.5 m s^{-1}) across the three grade conditions. Mass-specific tendon energy recovery (J kg^{-1} , normalized to muscle mass) is shown for comparison with mass-specific muscle work (Fig. 9). In both tendons, stress and energy recovery increase from a walk to a trot and as animals switch from level to incline conditions, but not when they switch to a decline. Tendon energy recovery is substantial and significantly greater than muscle work across all grade and gait conditions (Table 2). Values shown are means \pm s.e.m.

conditions). Assuming that the hind limbs contribute 40% to net changes in mechanical work during decline locomotion and 60% during incline locomotion (Biewener, 2003), this amounts to an estimated total work for a single hind limb at a trot of $-10.1 \text{ J stride}^{-1}$ on a decline and $13.1 \text{ J stride}^{-1}$ on an incline. In comparison, the combined work of the LG and MG was 0.02 J on a decline and 0.33 J on an incline, while the SDF's work output varied from only -0.05 J to 0.01 J . Consequently, the total work performed by these three distal muscles averaged less than 3% of the estimate for hind limb work as a whole, clearly indicating that proximal limb and trunk muscles must contribute most of the change in work output required to move down and up steeper grades. While this analysis ignores other components of work (mainly kinetic energy changes of the limbs and antagonistic muscle work), these are unlikely to change as substantially as center of mass potential energy changes associated with differing locomotor grades, upon which these estimates are based.

Muscle fascicle strain, work and recruitment in relation to locomotor grade

The generally uniform contractile behavior observed across grade and gaits in the distal muscle fascicles of goats has also been observed in distal leg muscles of tammar wallabies hopping on level and incline grades (Biewener et al., 2004) and in human MG fascicles measured *via* ultrasound when subjects walked and ran on decline, level and incline surfaces (Lichtwark and Wilson, 2006). Across these conditions, the human MG was observed to develop force isometrically and shorten only late in stance as the Achilles tendon was recoiling. Although the MG likely performs net positive work, Lichtwark and Wilson (Lichtwark and Wilson, 2006) determined that elastic energy recovery from the tendon was likely much greater than the estimated positive work of the muscle's fascicles.

To the extent that the distal muscles of the goat modulated their work output in response to changes in locomotor grade, we hypothesized that most of the change in work would be linked to changes in fascicle strain, rather than muscle force. However, we found that both fascicle strain (Fig. 4) and muscle force (Table 2) contributed to the observed changes in muscle work. When goats trotted or walked on a decline, muscle–tendon forces and net fascicle shortening generally decreased, although no change in GA force was observed during decline locomotion. When goats trotted or walked on an incline, distal muscle–tendon forces and net shortening strains consistently increased. In general, changes in muscle–tendon force and net fascicle strain were greatest when animals shifted to incline locomotion *versus* when they shifted from level to decline locomotion.

Similar changes in muscle–tendon force and strain have been observed in the distal muscles of other animals when moving on different grades. In running turkeys, Gabaldón and colleagues (Gabaldón et al., 2004) found that changes in LG and peroneus longus muscle work were closely matched to changes in mechanical energy requirements across different grade conditions (-12 deg. to $+12 \text{ deg.}$), but that the majority of work modulation was achieved *via* changes in net fascicle strain, with little change in muscle–tendon force. In studies of the LG, MG and digital flexor-IV of guinea fowl (Daley and Biewener, 2003; Higham et al., 2008), varying contributions of force and net fascicle strain have been observed to change muscle work when animals run up an incline *versus* on a level. As noted above, however, the highly specialized distal MTUs of hopping wallabies (Biewener et al., 2004) show little change in force or net fascicle strain when hopping on an incline *versus* the level. In addition,

adjustments in the relative timing of muscle force and fascicle strain have been shown to also contribute to changes in work output (Daley and Biewener, 2003; Gabaldón et al., 2004). Clearly, muscle activation and recruitment (Fig. 7) are adjusted to vary muscle force relative to fascicle strain and work output, irrespective of muscle–tendon architecture. Further studies will be needed to probe how the modulation of muscle work is achieved both within and among different muscles across differing mechanical tasks.

Similar to horses (Wickler et al., 2005; Parson et al., 2008), goats exhibited an increased stride frequency when moving on an incline compared with a level (and decreased during decline locomotion). However, whereas goats also increased their hind limb duty factor (from decline to level to incline), no change in limb contact time was observed for the hind limb of horses (Wickler et al., 2005) when trotting on an incline *versus* level. In humans (Minetti et al., 1994) and rats (Gillis and Biewener, 2002), stride frequency also increases when shifting from decline to level to incline running conditions, resulting in a greater duty factor for both decline and incline compared with level conditions. Consistent with several muscles in other animals [rat (Gillis and Biewener, 2002), turkey (Gabaldón et al., 2004), horse (Wickler et al., 2005)], EMG intensity increased significantly with grade in the three distal goat muscles, reflecting increased activation to produce greater muscle force and work. Interestingly, in distal guinea fowl muscles (Daley and Biewener, 2003), no significant change in EMG intensity was recorded for incline *versus* level locomotion, despite significant increases in muscle force and work output on an incline.

Evidence for a proximo-distal gradient of muscle function

Differences in muscle architecture in relation to fascicle strain patterns suggest the possibility of a proximo-distal gradient of work performance in the limbs of many terrestrial animals (Biewener and Daley, 2007). Although large proximal muscles likely modulate the majority of net limb work, it is clear that distal muscles also can play a role. Our results for the distal MTUs of the goat generally support this. The distal ankle extensors of running turkeys and guinea fowl similarly adjust their work output in response to changes in grade (Roberts et al., 1997; Daley and Biewener, 2003; Gabaldón et al., 2004; Higham et al., 2008). Nevertheless, the work output of the distal muscles of these running birds is still less than that predicted by their mass (Daley and Biewener, 2003), indicating that proximal muscles of these running birds play the major role in adjusting limb work output to changes in mechanical demand.

A difficulty for testing hypotheses of proximo-distal regionalization of limb muscle function and work is the inability to record forces from proximal muscles directly. One approach is to infer proximal muscle function indirectly from joint work patterns. However, because of biarticular muscle force and energy across proximal and distal joints, as well as muscle redundancy and antagonist activity (Herzog and Leonard, 1991; Prilutsky and Zatsiorsky, 1994; Winter, 1990), inferences of muscle function made by relating *in vivo* muscle strain and activation patterns to joint work patterns must be cautiously drawn. To date, analysis of proximal muscle function in rats (Gillis and Biewener, 2002), horses (Wickler et al., 2005) and wallabies (McGowan et al., 2007) during level *versus* incline locomotion indicates activation patterns linked with increased fascicle shortening strains (or reduced lengthening, as in the wallaby vastus lateralis) consistent with a shift to increased muscle work output. Using inverse dynamics of knee and hip joint moments to estimate wallaby biceps femoris and vastus lateralis forces (assuming equal stress among muscle synergists and no velocity-dependent effects on muscle force), McGowan and colleagues (McGowan et al., 2007) estimated

a shift of 0.79 J in the biceps femoris and 0.73 J in the vastus lateralis work output, which accounted for approximately 29% of the mechanical energy required to hop on an incline, comparable to these muscles representing 33% of total hind limb muscle mass.

By incorporating a Hill-type muscle-modeling approach to estimate muscle force due to velocity and length effects (determined from direct measures of fascicle strain), as well as PCSA, the roles of the biarticular long head of the goat triceps *versus* its monoarticular lateral head have also been recently assessed in relation to jumping tasks (Carroll et al., 2008). Estimating time-varying forces of the two heads of the triceps from their *in vivo* fascicle strain and activation patterns relative to elbow joint moments, Carroll and colleagues (Carroll et al., 2008) observed that the monoarticular lateral triceps modulates its work output more closely with changes in elbow joint work when compared between jump take-offs (net shortening and energy production) and landings (lengthening and energy absorption). In comparison, the biarticular long head of the goat triceps generally showed reduced net fascicle strains associated with its role in providing force and energy transmission between the elbow and shoulder. Consequently, adopting such an approach may also be of value for pursuing analysis of proximal muscle function in relation to patterns of the distal muscles observed here.

In summary, it seems clear that distal MTUs in goats, like those of other terrestrial animals adapted for economical locomotion, are designed to promote tendon elastic energy savings with varying contributions of muscle work associated with changing mechanical demands due to changes in locomotor grade. Net fascicle strain and muscle force both varied to modulate muscle work, with shifts in fascicle strain being most consistent and substantial across the three distal muscles studied here, in comparison to muscle-tendon force. Although distal muscle work varied with changes in locomotor grade, the magnitude of work output was small in comparison with the mechanical energy requirements estimated for the limb as a whole. Consequently, goat distal limb MTUs appear well suited to generating forces economically across different grades, but their energy recovery may be limited by the requirements of moving over mountainous terrain. Despite the challenge of characterizing and quantifying work patterns of proximal limb muscles, it seems clear that larger proximal muscles contribute disproportionately to required changes in total limb work. Future analysis of proximal muscles will help to explore the extent to which a proximo-distal gradient in limb muscle function generally exists within the limbs of terrestrial animals.

LIST OF ABBREVIATIONS

CSA	cross-sectional area
GA	gastrocnemius
LG	lateral gastrocnemius
MG	medial gastrocnemius
MTU	muscle-tendon unit
PCSA	physiological cross-sectional area
SDF	superficial digital flexor

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REFERENCES

Ahn, A. N., Monti, R. J., Biewener, A. A. (2003). *In vivo* and *in vitro* heterogeneity of segment length changes in the semimembranosus muscle of the toad. *J. Physiol.* **549**, 877-888.

Alexander, R. M. (1988). *Elastic Mechanisms in Animal Movement*. Cambridge: Cambridge University Press.

Alexander, R. M. and Vernon, A. (1975). The mechanics of hopping by kangaroos (Macropodidae). *J. Zool. (Lond.)* **177**, 265-303.

Biewener, A. A. (1998). Muscle-tendon stresses and elastic energy storage during locomotion in the horse. *Comp. Biochem. Physiol. B* **120**, 73-87.

Biewener, A. A. (2003). *Animal Locomotion*. Oxford: Oxford University Press.

Biewener, A. A. and Daley, M. A. (2007). Unsteady locomotion: integrating muscle function with whole body dynamics and neuromuscular control. *J. Exp. Biol.* **210**, 2949-2960.

Biewener, A. A. and Roberts, T. J. (2000). Muscle and tendon contributions to force, work and elastic energy saving: a comparative perspective. *Exerc. Sport Sci. Rev.* **28**, 99-107.

Biewener, A. A., Konieczynski, D. D. and Baudinette, R. V. (1998). *In vivo* muscle force-length behavior during steady-speed hopping in tamar wallabies. *J. Exp. Biol.* **201**, 1681-1694.

Biewener, A. A., McGowan, C., Card, G. M. and Baudinette, R. V. (2004). Dynamics of leg muscle function in tamar wallabies (*M. eugenii*) during level *versus* incline hopping. *J. Exp. Biol.* **207**, 211-223.

Carroll, A., Lee, D. V. and Biewener, A. A. (2008). Differential muscle function between muscle synergists: long and lateral heads of the triceps in jumping and landing goats (*Capra hircus*). *J. Appl. Physiol.* **105**, 1262-1273.

Daley, M. A. and Biewener, A. A. (2003). Muscle force-length dynamics during level *versus* incline locomotion: a comparison of *in vivo* performance of two guinea fowl ankle extensors. *J. Exp. Biol.* **206**, 2941-2958.

Dawson, T. J. and Taylor, C. R. (1973). Energy cost of locomotion by kangaroos. *Nature* **246**, 313-314.

Fukunaga, T., Ichinose, Y., Ito, M., Kawakami, Y. and Fukashiro, S. (1997). Determination of fascicle length and pennation in a contracting human muscle *in vivo*. *J. Appl. Physiol.* **82**, 354-358.

Fukunaga, T., Kubo, K., Kawakami, Y., Fukashiro, S., Kanehisa, H. and Maganaris, C. N. (2001). *In vivo* behavior of human muscle tendon during walking. *Proc. Biol. Sci.* **268**, 229-233.

Gabaldón, A. M., Nelson, F. E. and Roberts, T. J. (2004). Mechanical function of two ankle extensors in wild turkeys: shifts from energy production to energy absorption during incline *versus* decline running. *J. Exp. Biol.* **207**, 2277-2288.

Gillis, G. B. and Biewener, A. A. (2002). Effects of surface grade on proximal hindlimb muscle strain and activation during rat locomotion. *J. Appl. Physiol.* **93**, 1731-1743.

Gillis, G. B., Flynn, J. P., McGuigan, P. and Biewener, A. A. (2005). Patterns of strain and activation in the thigh muscles of goats across gaits during level locomotion. *J. Exp. Biol.* **208**, 4599-4611.

Heglund, N. C., Fedak, M. A., Taylore, C. R. and Cavagna, G. A. (1982). Energetics and mechanics of terrestrial locomotion. IV. Total mechanical energy changes as a function of speed and body size in birds and mammals. *J. Exp. Biol.* **97**, 57-66.

Herzog, W. and Leonard, T. R. (1991). Validation of optimization models that estimate the forces exerted by synergistic muscles. *J. Biomech.* **24**, 31-39.

Higham, T. E., Biewener, A. A. and Wakeling, J. M. (2008). Functional diversification within and between muscle synergists during locomotion. *Biol. Lett.* **4**, 41-44.

Ker, R. F. (1981). Dynamic tensile properties of the plantaris tendon of sheep (*Ovis aries*). *J. Exp. Biol.* **93**, 283-302.

Ker, R. F., Bennett, M. B., Bibby, S. R., Kester, R. C. and Alexander, R. M. (1987). The spring in the arch of the human foot. *Nature* **325**, 147-149.

Kram, R. and Taylor, C. R. (1990). Energetics of running: a new perspective. *Nature* **346**, 265-267.

Lee, D. V., McGuigan, M. P., Yoo, E. H. and Biewener, A. A. (2008). Compliance, actuation and work characteristics of the goat foreleg and hindleg during level, uphill and downhill running. *J. Appl. Physiol.* **104**, 130-141.

Lichtwark, G. A. and Wilson, A. M. (2006). Interactions between the human gastrocnemius muscle and the Achilles tendon during incline, level and decline locomotion. *J. Exp. Biol.* **209**, 4379-4388.

Lichtwark, G. A., Bougoulas, K. and Wilson, A. M. (2007). Muscle fascicle and series elastic element length changes along the length of the human gastrocnemius during walking and running. *J. Biomech.* **40**, 157-164.

Loram, I. D., Maganaris, C. N. and Larkie, M. (2006). Use of ultrasound to make noninvasive *in vivo* measurement of continuous changes in human muscle contractile length. *J. Appl. Physiol.* **100**, 1311-1323.

McGowan, C. P., Baudinette, R. V. and Biewener, A. A. (2007). Modulation of proximal muscle function during level *versus* incline hopping in tamar wallabies (*Macropus eugenii*). *J. Exp. Biol.* **210**, 1255-1265.

Minetti, A. E., Ardigo, L. P. and Saibene, F. (1994). Mechanical determinants of the minimum energy cost of gradient running in humans. *J. Exp. Biol.* **195**, 211-225.

Parson, K. J., Pfau, T. and Wilson, A. M. (2008). High-speed gallop locomotion in the Thoroughbred racehorse. I. The effect of incline on stride parameters. *J. Exp. Biol.* **211**, 935-944.

Priulitsky, B. I. and Zatsiorsky, V. M. (1994). Tendon action of two-joint muscles: transfer of mechanical energy between joints during jumping, landing and running. *J. Biomech.* **27**, 25-34.

Prothero, D. R. and Foss, S. E. (2007). *The Evolution of Artiodactyls*. Maryland, USA: JHU Press.

Roberts, T. J., Marsh, R. L., Weyand, P. G. and Taylor, C. R. (1997). Muscular force in running turkeys: the economy of minimizing work. *Science* **275**, 1113-1115.

Shadwick, R. E. (1990). Elastic energy storage in tendons: mechanical differences related to function and age. *J. Appl. Physiol.* **68**, 1033-1040.

Taylor, C. R. (1994). Relating mechanics and energetics during exercise. *Adv. Vet. Sci. Comp. Med.* **38A**, 181-215.

Wickler, S. J., Hoyt, D. F., Biewener, A. A., Cogger, E. A. and DeLapaz, K. L. (2005). *In vivo* muscle function vs speed: II. Muscle function trotting up an incline. *J. Exp. Biol.* **208**, 1191-1200.

Wilson, A. M., McGuigan, M. P., Su, A. and van den Bogert, A. J. (2001). Horses damp the spring in their step. *Nature* **414**, 895-899.

Winter, D. A. (1990). *Biomechanics and Motor Control of Human Movement*. New York: Wiley.