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Patterns of strain and activation in the thigh muscles of goats across gaits during level locomotion

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Summary

Unlike homologous muscles in many vertebrates, which appear to function similarly during a particular mode of locomotion (e.g. red muscle in swimming fish, pectoralis muscle in flying birds, limb extensors in jumping and swimming frogs), a major knee extensor in mammalian quadrupeds, the vastus lateralis, appears to operate differently in different species studied to date. In rats, the vastus undergoes more stretching early in stance than shortening in later stance. In dogs, the reverse is true; more substantial shortening follows small amounts of initial stretching. And in horses, while the vastus strain trajectory is complex, it is characterized mainly by shortening during stance. In this study, we use sonomicrometry and electromyography to study the vastus lateralis and biceps femoris of goats, with three goals in mind: (1) to see how these muscles work in comparison to homologous muscles studied previously in other taxa; (2) to address how speed and gait impact muscle actions and (3) to test whether fascicles in different parts of the same muscle undergo similar length changes. Results indicate that the biceps femoris undergoes substantial shortening through much of stance, with higher strains in walking and trotting [32–33% resting length (L_0)] than galloping (22% L_0). These length changes occur with increasing biceps EMG intensities as animals increase speed from walking to galloping. The vastus undergoes a stretch–shorten cycle during stance. Stretching strains are higher during galloping (15% L_0) than walking and trotting (9% L_0). Shortening strains follow a reverse pattern and are greatest in walking (24% L_0), intermediate in trotting (20% L_0) and lowest during galloping (17% L_0). As a result, the ratio of stretching to shortening increases from below 0.5 in walking and trotting to near 1.0 during galloping. This increasing ratio suggests that the vastus does relatively more positive work than energy absorption at the slower speeds compared with galloping, although an understanding of the timing and magnitude of force production is required to confirm this. Length-change regimes in proximal, middle and distal sites of the vastus are generally comparable, suggesting strain homogeneity through the muscle. When strain rates are compared across taxa, vastus shortening velocities exhibit the scaling pattern predicted by theoretical and empirical work: fascicles shorten relatively faster in smaller animals than larger animals (strain rates near 2 $L\ s^{-1}$ have been reported for trotting dogs and were found here for goats, *versus* 0.6–0.8 $L\ s^{-1}$ reported in horses). Interestingly, biceps shortening strain rates are very similar in both goats and rats during walking (1–1.5 $L\ s^{-1}$) and trotting (1.5–2.5 $L\ s^{-1}$, depending on speed of trot), suggesting that the ratio of *in vivo* shortening velocities (V) to maximum shortening velocities (V_{max}) is smaller in small animals (because of their higher V_{max}).

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

Introduction

While *in vitro* studies of individual fibers, fiber bundles and whole muscles have greatly expanded our knowledge of the physiological properties and contractile mechanisms of skeletal muscle over the past 40 years, our understanding of how muscles actually operate *in vivo* is rather lacking in comparison. Consider the seemingly straightforward question of how much a given muscle changes length during any particular animal movement; for example, a limb muscle during a locomotor stride. Although detailed limb kinematics can provide some insight into the answer, the complex architecture, biarticular nature and/or in-series compliance of many muscles often render joint angular excursions unreliable as surrogates for determining muscle strain trajectories. Hence, unless more direct length-change measurements are made, we are unlikely to know the specific actions of a particular muscle during locomotion, nor can we appreciate more interesting issues that might arise if more complex questions are asked: how do speed or gait influence the way a muscle works; do

fascicles throughout a muscle operate in the same way; do homologous limb muscles in different animals function similarly?

The nature and degree of a muscle's length change during activation, along with the pattern of force it produces, underlie its mechanical function (Josephson, 1999). Hence, to fully understand the workings of skeletal muscles *in vivo* during locomotion, muscle length-change patterns must be characterized, and patterns of muscle force output should be measured. To date, direct *in vivo* measurements of either muscle length change or force output are rare, leaving a major void in our knowledge of the specifics of how muscles are functioning in living animals. This is largely due to complications inherent in doing *in vivo* muscle work. Direct measurements of muscle force are exceedingly difficult and often technically unfeasible to make *in vivo*, and estimating the forces produced by individual limb muscles using force plate and inverse dynamics analyses relies on simplifying assumptions and is complicated by the anatomy of many limb muscles. However, the relatively recent application of sonomicrometry to studying skeletal muscles has revealed that direct muscle strain measurements can be made in animals performing natural activities with much less complication.

In the past decade, sonomicrometry has been used to help elucidate the *in vivo* function of muscles important to various locomotor systems: wing muscles of flying birds (Askew and Marsh, 2001; Biewener et al., 1998a; Tobalske and Dial, 2000), limb muscles of jumping and swimming frogs (Gillis and Biewener, 2000; Olson and Marsh, 1998; Roberts and Marsh, 2003) and the axial muscles of swimming fish (Coughlin, 2000; Coughlin et al., 1996; Katz et al., 1999; Shadwick et al., 1999). In each of these systems, generally similar conclusions have been reached, and muscle fascicles have been shown to undergo active shortening, confirming their importance in providing the mechanical work and power required for propulsion of these different locomotor modes.

By contrast, work on level, terrestrial, limb-based locomotion in mammals has suggested an impressive functional repertoire for the underlying muscles involved. Studies of steady-speed, level locomotion have revealed muscles functioning isometrically (Biewener et al., 1998b, 2004), undergoing substantial shortening (Carrier et al., 1998; Gillis and Biewener, 2001), exhibiting stretch–shorten cycles (Gillis and Biewener, 2001) and fascicles that shorten against a lengthening muscle–tendon unit (Griffiths, 1991; Hoyt et al., 2005). At least some of this functional breadth surely reflects the diverse limb muscles that have been examined (e.g. uni- *vs* bi-articular, pennate *vs* parallel fibered) and the range of joints that they act on (hip *vs* knee *vs* ankle), as well as the range of locomotor styles (e.g. hopping *vs* running) and size disparity (e.g. rat *vs* horse) present among the studies. To better identify patterns and understand how certain parameters, such as body size or gait, may affect the actions of individual limb muscles, the same muscles need to be studied in animals of different size, while controlling for locomotor gait (e.g. walking *vs* trotting *vs* galloping).

In this vein, length-change and activation data from a major knee extensor muscle, the vastus lateralis, have already been reported for trotting mammalian quadrupeds ranging in size from 250 g rats (Gillis and Biewener, 2001) to 25 kg dogs (Carrier et al., 1998) to 400 kg horses (Hoyt et al., 2005). Results suggest that the same muscle in these different animals works quite differently. In rats, the vastus is generally stretched by about 10% of its resting length (L_0) while active in the stance phase during trotting. In dogs, vastus strain patterns during stance are complex but are often characterized by a small degree of stretching (5–10% L_0) followed by more substantial shortening later in stance (up to 20% L_0). In horses, strain patterns in the vastus are also complex but mainly involve shortening (approximately 10% L_0). Clearly, homologous muscles in animals of varying size exhibit distinct strain trajectories even during the same gait.

Gait can also influence the strain regime of limb muscles. Gillis and Biewener (2001) found that in rats, biceps femoris shortening strains increased with speed through walking and trotting; however, once animals transitioned to a gallop, shortening strains were reduced by nearly half. In this same study, the rat vastus lateralis was shown to be stretched more substantially early in stance during galloping than during trotting or walking. Because few studies have examined limb muscle strains directly and systematically across different gaits, it is unclear if these patterns are common to other quadrupeds. If they are, it seems plausible that the transition from a trot to a gallop may dramatically alter muscle actions.

One of the underlying assumptions among studies using sonomicrometry to measure muscle strain is that fractional length changes among fascicles throughout the muscle of interest are largely homogenous. In other words, understanding what happens in a central fascicle provides insight into how the whole muscle behaves. However, there have been few explicit tests of this assumption. Work investigating this issue has shown that strains can differ along the length of an individual fiber (Edman and Reggiani, 1984) or fascicle (Ahn et al., 2003), and in complex muscles such as the bird pectoralis (Soman et al., 2005) and human biceps brachii (Pappas et al., 2002), fascicle strain regimes can vary significantly in different regions of the muscle. Such data suggest that, in some systems, a single measurement of muscle strain from a pair of crystals is inadequate to characterize the strain behavior of an entire muscle. More work needs to be done to assess the extent to which strain homogeneity characterizes major muscles used during animal locomotion.

In this study, we explore fascicle strain behavior relative to activation patterns in two major thigh muscles of the goat, the vastus lateralis and biceps femoris, in three major contexts: (1) speed and gait, (2) strain homogeneity and (3) taxonomic variation. We first address the effects of speed and gait on fascicle strain trajectories. Specifically, is length-change behavior in these major hindlimb muscles different between trotting and galloping or walking and trotting? Second, we test the assumption that fascicle strains are homogenous throughout a large uniarticular muscle by recording length-

change patterns from proximal, middle and distal sites within the vastus lateralis. Finally, we explore the question of whether goat thigh muscles exhibit strain patterns comparable to homologous muscles in other species. Of particular interest is whether the vastus exhibits patterns like those observed previously in dogs of similar size (some early stretching followed by more substantive shortening) or if the muscle undergoes length changes more like those observed in rats (i.e. mainly stretching) or horses (i.e. mainly shortening).

Materials and methods

Animals

Seven African pygmy goats (*Capra hircus* L.) ranging in size from 14 to 25 kg (mean, 18 kg) were obtained from the breeding colony at Harvard University's Concord Field Station. All animals were maintained outdoors and fed on available foliage supplemented with hay and goat chow during winter months. For several weeks prior to the experimental recordings, goats were trained to walk and run on a large motorized treadmill (belt, 2.5 m long and 0.75 m wide). Training sessions were meant to familiarize animals with the treadmill and increase their endurance capacity. Training regimens generally alternated between 10–15 min sessions at a moderate trotting speed and 10–15 min sessions in which the belt speed was increased in 0.4–0.5 m s^{-1} increments every 1–3 min to elicit walking, trotting and galloping gaits. Once an animal was able to perform both routines successfully, it was exercised under mock-experimental conditions. This involved running the animal for 1 min at speeds ranging between 1.1 and 4.5 m s^{-1} with a 5-min period of rest between each speed. Once an animal could perform this protocol consistently, it was considered ready for study. All procedures for working with goats were approved by Harvard's IACUC committee.

Surgical procedures

Animals were initially sedated with an intramuscular injection of xylazine (0.05 mg kg^{-1} body mass) or of a ketamine/xylazine mixture (8 mg kg^{-1} body mass/0.05 mg kg^{-1} body mass). Fur covering the experimental (left) hindlimb was shaved and the exposed skin was thoroughly scrubbed and disinfected with a Povidone-iodine solution. Once sedated, animals were intubated and an appropriate level of anesthesia was maintained using vaporized isoflurane. To expose limb muscles for transducer implantation, two incisions were made through the skin and subcutaneous fascia on the lateral surface of the thigh, one over the entire vastus lateralis and the other over the anterior portion of the biceps. An additional small skin incision was made near the ilium and a pathway was cleared between the three incisions for subcutaneous movement of the transducer wires. Prior to surgery, lead wires from electrodes and sonomicrometry crystals were soldered into female connectors (32 pin). All wires were soaked in a bacterial sterilizing solution for an extended period before being pulled subcutaneously from the incision near the ilium down to the incisions exposing the muscles on the thigh.

Pairs of 2.0 mm sonomicrometry crystals (Sonometrics Inc., London, ON, Canada) were implanted 12–18 mm apart along the fascicle axes of each muscle. Small openings were created using small, curved stainless steel scissors, and crystals were embedded into these pockets and aligned until their signals were optimized (as determined by their output on an oscilloscope). The vastus is unipennate (Fig. 1) with fibers running from deep to superficial as they travel from the caudal portion of the muscle proximally to the more cranial portion of the muscle distally, where they attach to the aponeurosis forming the quadriceps tendon. Vastus fibers average 61 mm in length, and this is fairly uniform throughout the muscle, as is pennation angle, which is approximately 20–25°. By contrast, the biceps is parallel fibered, but fibers vary in length considerably in different regions of the muscle. We implanted crystals in the anterior region of the biceps, where fibers average 69 mm in length and act mainly in extension at the hip (more posterior fibers can be over twice as long and also act in knee flexion). Muscle openings were sutured closed using 4-0 silk, and lead wires were sutured to the surface of the muscle to prevent dislocation or misalignment during the experiment. In all animals, a single pair of crystals was implanted into the anterior region of the biceps. Three pairs of crystals were implanted into the vastus. In most individuals these implantations were located in proximal, middle and distal

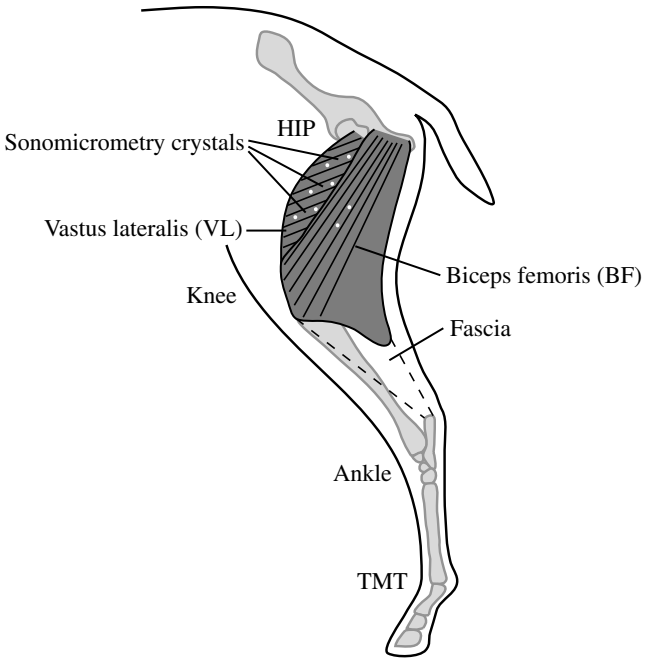

Fig. 1. Schematic illustration showing crystal implantation scheme for the vastus lateralis and biceps femoris. Note the unipennate fiber architecture of the vastus as well as the locations of the three pairs of crystals: proximal, middle and distal. The anterior portion of the biceps, which has parallel fibers, was implanted with a single pair of sonomicrometry crystals.

thirds of the muscle, respectively (Fig. 1). These multiple recordings from the vastus were used to evaluate the extent to which fascicles change length uniformly throughout the muscle during locomotion.

Offset twist hook electrodes (Loeb and Gans, 1986) made of fine, silver wire (California Fine Wire Inc., Grover Beach, CA, USA), with their tips bared of insulation, were implanted into the muscles of interest using a 21-gauge hypodermic needle. In all subjects, two electrodes were implanted into each muscle. In the biceps, electrodes flanked the sonomicrometry crystal pair. In the vastus, electrodes were implanted adjacent to and on either side of the central crystal pair (one electrode was between the proximal and middle pairs of crystals, the other between the middle and distal pairs). Electrode wires were also sutured onto the muscle surface using 6-0 silk. Following all implantations, skin incisions were sutured with 4-0 silk, and the female 32-pin connectors were also anchored to the skin with 4-0 silk.

Table 1. *Individual goats from which muscle activity and strain were recorded and analyzed for the biceps femoris and vastus lateralis*

	Biceps femoris		Vastus lateralis			
				Strain		
Individual	EMG	Strain	EMG	Pro.	Mid.	Dis.
Goat 1	X	X	O	X	X	X
Goat 2	O	X	O	O	X	X
Goat 3	O	O	X	X	X	X
Goat 4	O	O	X	O	X	X
Goat 5	X	X	X	O	X	X
Goat 6	O	X	X	X	X	X
Goat 7	X	X	X	X	X	X

X reflects successful data collection and use in analysis, and O reflects a lack of successful data collection. Pro., Mid. and Dis. represent proximal, middle and distal recording sites from the vastus.

Data collection

Goats were allowed 48–72 h for recovery following surgeries. Before data recording trials began, the female connectors attached to the goats were connected to the recording equipment with lightweight shielded cables and matching male connectors. Electromyographic (EMG) signals were amplified 1000× and filtered (60 Hz notch and 100–3000 Hz bandpass) using Grass P511 amplifiers. Signals from sonomicrometry crystals were processed by a sonomicrometer unit (model 120-1001; Triton Technology Inc., San Diego, CA, USA) and monitored *via* oscilloscope (2235A; Tektronix, Beaverton, OR, USA). Outputs from the Grass amplifiers and Triton sonomicrometer were digitized at 5000 Hz through a 12-bit A/D converter (Digidata 1200B, Axon Instruments Inc., Union City, CA, USA) and recorded onto a computer for later analysis.

Locomotor trials were performed on the same treadmill used for training and ranged in speed from 1.1 to 4.5 m s^{-1}. Two trials were recorded at each speed, and a sequence of 6–10 strides was saved for analysis from each trial. These sequences were chosen based upon animals holding position on the treadmill belt. A lateral view of each locomotor sequence was recorded as digital video (PCI-500; Redlake, Morgan Hill, CA, USA) at 125 Hz. In a subset of three animals, white markings were used to highlight the anterior border of the ilium, hip joint, knee joint, ankle joint and metatarsal joint. Three strides from each animal during walking, trotting and galloping were used to characterize limb joint kinematics during the different gaits. Electromyography and sonomicrometry data were synchronized with the digital video sequences using a voltage pulse that marked the end of each video recording. Once trials were completed, the positions of EMG electrodes and crystals were confirmed, and all transducers were removed. The first four animals were sacrificed with an overdose of sodium pentobarbital administered intravenously to conduct a post-mortem dissection and confirmation of transducer location. The final three goats were simply reanesthetized as per the initial surgery for this procedure and allowed to recover (in order to maintain the breeding colony's population). Because certain implantations were unreliable or dislodged, simultaneous recordings from all pairs of crystals and EMG electrodes occurred in only one animal; however, in most animals, all but one or two transducers provided successful recordings (Table 1).

Data analysis

Digital video files were used to determine the timing of the stance and swing phases composing each stride. The stance phase was defined as lasting from the frame in which the implanted limb made ground contact to the frame in which it left the ground. The swing phase was defined as lasting from the frame in which the foot of the implanted limb left the ground to the frame in which it again made ground contact. For each locomotor trial, five to six strides were analyzed and the timing of all sonomicrometry and EMG data was determined in the context of these intervals of the stride.

Limb markings were used to digitize two-dimensional Cartesian coordinates characterizing the hip and knee joints in three individuals (Didge Program; Alistair McCullum). Coordinates were translated into angular excursions of these joints in Excel, and average amounts of extension and flexion were calculated for each stride and averaged across individuals.

Muscle fascicle strain was measured as the fractional length change between crystals relative to a resting length defined while animals maintained a stationary standing position. For every animal, several resting lengths were measured during recordings and the average across these measurements was used as the rest length for determining fractional length changes. Because the Triton sonomicrometer underestimates the speed of sound through muscle (it assumes the speed of sound in water), all distances were adjusted by 2.7% prior to the calculation of fractional length change. The sonomicrometer filter also introduces a 5 ms delay, which was corrected for in all timing measurements. The epoxy coating on the crystals introduces an error in the measurement of inter-

crystal distances because sound travels faster in epoxy than muscle. For 2.0 mm crystals, this error averages 0.82 mm and was accounted for in all strain measurements.

Patterns of fascicle strain were complex but consistent and were characterized by regular intervals or phases that could be easily identified. Proximal, middle and distal sites in the vastus lateralis exhibited four such phases during the step cycle, all of which were quantified and analyzed: (1) yield stretching, the amount of fascicle lengthening that took place in early stance; (2) stance shortening, the amount of fascicle shortening that took place following yield stretch; (3) swing stretching, the amount of fascicle lengthening that took place in early swing, and (4) swing shortening, the amount of fascicle shortening following swing stretch. Strain patterns in the biceps femoris were broken down into three phases: (1) swing/stance transition, characterized by small amounts of shortening and stretching that occurred in late swing and early stance; (2) stance shortening, characterized by fascicle shortening following the swing/stance transition, and (3) swing stretching, characterized by fascicle lengthening present during the swing phase. Only the stance shortening strains were quantified and analyzed for the biceps, as length changes during the swing/stance transition tended to be relatively small and those during swing stretching were comparable to those present in stance shortening (but opposite in direction).

EMG signals were analyzed for onset time, offset time, duration and intensity. EMG intensities were calculated by averaging the spike amplitude of each rectified signal. For each muscle, intensities were scaled relative to the maximum intensity observed in that individual.

Statistics

The mean values of vastus and biceps EMG onset, offset, duration and intensity were determined in every locomotor sequence with a successful implantation. Mean levels of fascicle strain, partitioned into phases as described above, were calculated for all working crystal pairs in each locomotor sequence. To test for the effects of gait on EMG activity and fascicle strain behavior, two-way mixed-model analyses of variance (ANOVAs) were performed with gait as the fixed factor and individual as the random factor. To test for strain heterogeneity in the vastus lateralis, three-way mixed model ANOVAs were performed on strain variables with gait and crystal as fixed factors and individual as a random factor. No corrections were performed to account for the performance of multiple tests, but *F*-statistics as well as *P*-values are reported to provide insight into the relative degree of significance found. Scheffe's *post-hoc* tests were used to ascertain details of significant differences when found.

Results

Stride parameters and gaits

Stride durations decreased with increasing speed from approximately 600–700 ms at 1.1 m s^{-1} to below 350 ms at 4.5 m s^{-1}. These decreases reflected decreases in stance phase duration, while swing phase duration remained nearly constant over a broad range of speeds and gaits (Fig. 2). All animals exhibited a walking gait at 1.1 and 1.5 m s^{-1}, a trotting gait from 2.0 to 3.0 m s^{-1} and a galloping gait at speeds of 4.0 m s^{-1} and above. At 3.5 m s^{-1}, animals trotted and/or galloped, depending on the individual.

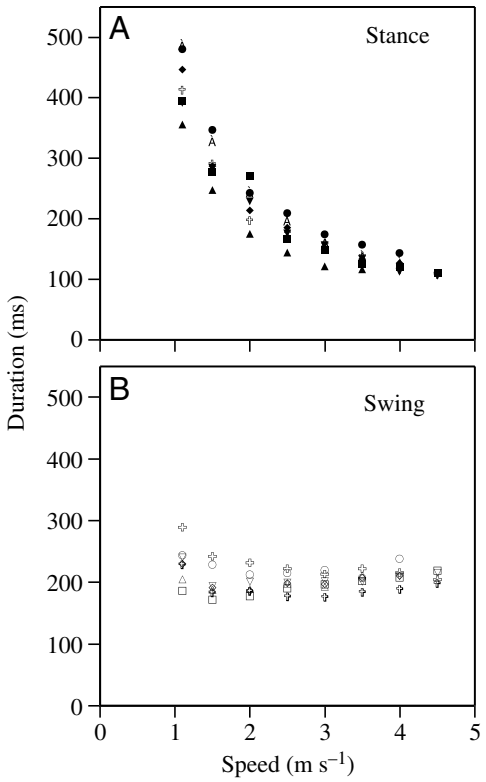

Fig. 2. Plots showing stance duration (A) and swing duration (B) as a function of speed in goats during treadmill locomotion. Note the substantial decrease in stance phase duration with increasing speed in comparison to the relatively constant swing phase duration over the same speed range. Different symbols represent different individuals (*N*=7).

Joint kinematics

Joint angular excursions were cyclical, and consistently identifiable periods of flexion and extension could be identified during all strides, regardless of gait. The hip joint generally extended throughout the stance phase and flexed throughout the swing phase (Fig. 3). Hip extension averaged just over 40° in walking and trotting but was reduced to approximately 35° during galloping. The angular excursion pattern of the knee consisted of both flexion and extension during stance and swing (Fig. 3). Initial knee flexion during stance averaged 14° during walking, 22° during trotting and 31° during galloping. Re-extension of the knee later in stance averaged 13–19° regardless of gait. Thus, stance-related knee flexion was generally less than or equal to knee extension during walking, but flexion was often greater than re-extension during trotting and galloping. During swing, as the foot is lifted off the ground, the knee flexes approximately 30–40°, regardless of

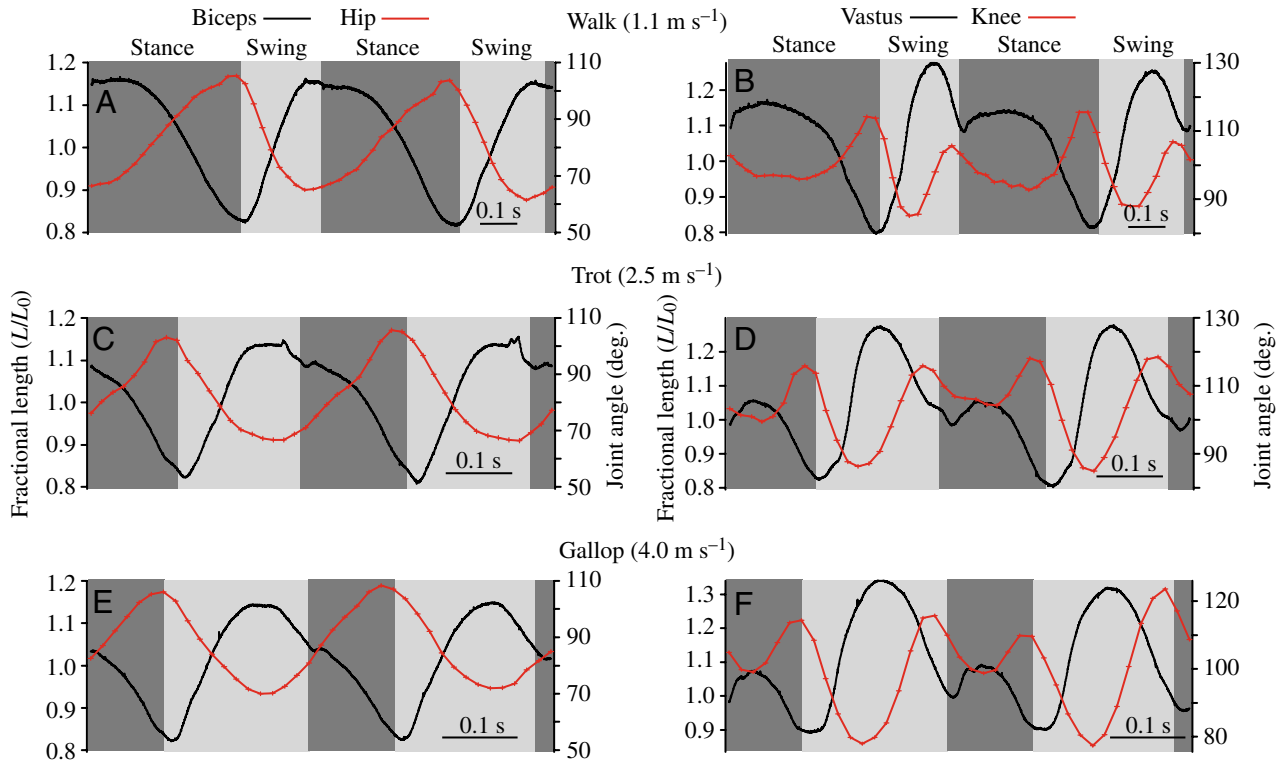

Fig. 3. Patterns of hip (A,C,E) and knee (B,D,F) joint angle excursions from one individual at all gaits, in red, superimposed onto fascicle strain patterns of the biceps and vastus, respectively, in black. The right-hand *y*-axis corresponds to the joint angles, the left-hand *y*-axis to the strain. Stance and swing phases are shaded dark and light gray, respectively. Note how biceps fascicles generally shorten during hip extension and lengthen during hip flexion. Vastus fasicles are generally stretched during knee flexion and shorten during knee extension.

gait, but as the limb is swung forward, it re-extends an average of 26° during walking, 36° during trotting and 51° during galloping. Hence, knee flexion during early swing remains consistent at all speeds, but re-extension in late swing becomes more exaggerated at faster gaits.

Muscle activity and strain

Electromyographic and/or muscle strain data were collected from seven individuals; see Table 1 for more specific sample sizes of different muscle recordings. Both the biceps and vastus exhibited consistent patterns of activation and length change with each stride (Fig. 4). Certain features of these patterns changed consistently with gait, and such changes are elaborated on below.

Biceps

EMG activity in the biceps occurred in discrete bursts during each stride (Fig. 4A). Burst onset began prior to foot-down and activity generally ceased in the middle third of stance (Figs 4A, 5). Onset timing was significantly different between gaits ($P<0.01$, $F=37.1$) and began at the earliest during galloping and at the latest (closer to foot-down) during

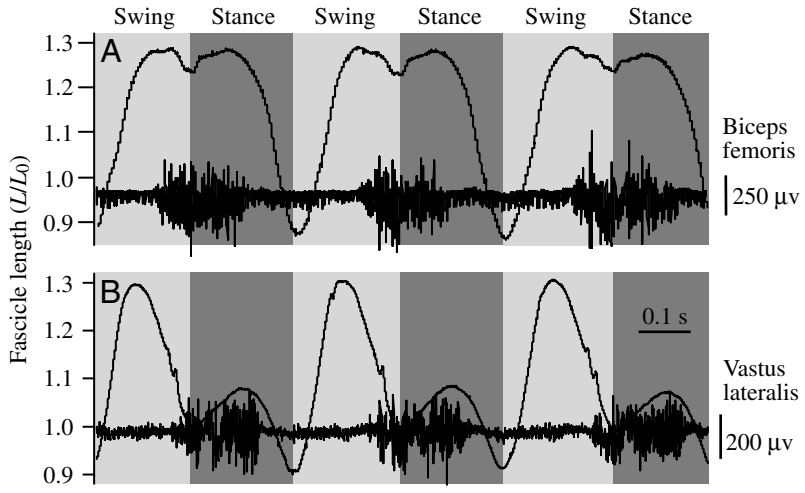

Fig. 4. Patterns of activation and strain in the biceps femoris (A) and vastus lateralis (B) during three strides of slow trotting. Data are from the same individual trotting at 2.0 m s^{-1}. Dark gray shading represents the stance phase; light gray shading represents the swing phase. The biceps remains fairly isometric during the interval in which electromyographic activity is present in late swing and early stance and then shortens rapidly and substantially later in stance. The vastus is generally stretched during much of its activation period early in stance before shortening late in stance.

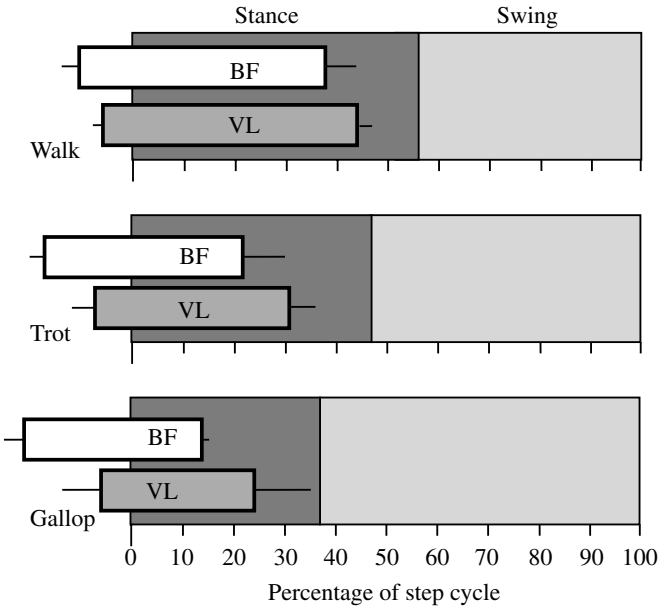

Fig. 5. Schematic diagram showing the relative timing of electromyographic activity in the biceps femoris (BF) and vastus lateralis (VL) during walking, trotting and galloping. Stance and swing phases are shaded dark and light gray, respectively. Note how the stance phase takes up a relatively small fraction of the stride during galloping. Both muscles are consistently activated prior to foot-down at the start of stance, and both become inactive during stance. The biceps is consistently activated and deactivated earlier than the vastus.

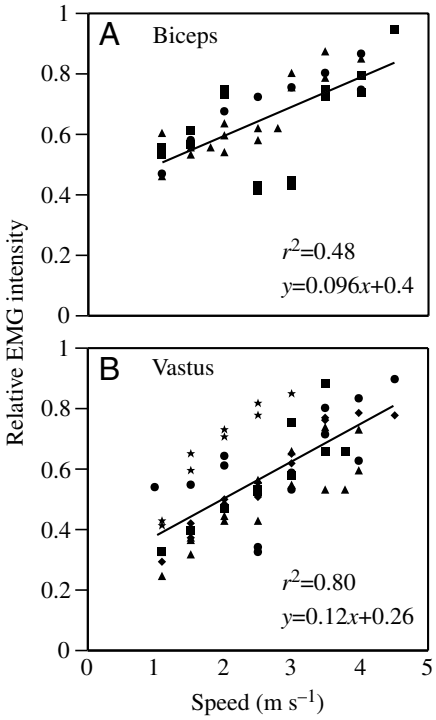

Fig. 6. Plots of relative EMG intensity in the biceps (A) and vastus (B) over a range of speeds. Intensity increases with speed in both muscles and is highest during galloping and lowest during walking. Different symbols represent different individuals (N=3 for biceps, N=5 for vastus).

walking (Fig. 5). Similarly, offset timing also varied significantly between gaits (P<0.01, F=28.2) and occurred at the earliest (relatively soonest after foot-down) during galloping and at the latest during walking (Fig. 5). EMG burst durations decreased with speed in a manner similar to stance phase durations, such that the ratio of EMG duration to stance duration was similar at all gaits (P>0.25, F=1.31) and averaged 0.87. EMG signal intensity increased with speed in the biceps (Fig. 6) and was significantly greater during galloping and trotting than during walking (P<0.05, F=7.8).

Biceps fascicle strain patterns were consistent across individuals and could be characterized by three distinct phases, regardless of gait (Fig. 7B). The first phase, which extended from late swing into early stance and coincided with the initial period of hip extension, consisted of a brief bout of fascicle shortening followed by a brief period of fascicle lengthening (Fig. 7A,B). The relative amounts of shortening *versus* lengthening varied among individuals and gaits (Figs 4A, 7A), but the excursions were typically smaller than the more substantial length changes observed in the other phases (Fig. 7A). The second phase consisted exclusively of fascicle shortening that coincided with hip extension through the rest of stance. This phase generally began during the first third of stance (Fig. 7A,B). Phase three consisted exclusively of lengthening as the hip flexed over much of the swing phase (Fig. 7A,B). The time at which phase 2 began was affected significantly by gait (P<0.01, F=16.0) and was earliest during walking and latest during galloping, where shortening did not begin, on average, until 34% of the way through stance. Shortening strains during phase 2 were also affected significantly by gait (P<0.05, F=4.6) and were greater during walking (mean, –0.32) and trotting (mean, –0.31) than during galloping (mean, –0.22) (Fig. 8A). Biceps shortening velocities increased nearly linearly with speed from walking to trotting before leveling off during galloping (Fig. 8D). As a result, velocities were significantly lower during walking (mean, –1.12 L s^{-1}) than during trotting and galloping (means, –2.10 and –2.06 L s^{-1}, respectively) (P<0.001, F=61.8).

Vastus

As in the biceps, a single burst of vastus EMG activity was present during each stride (Fig. 4B). Activity began late in the swing phase, just prior to foot-down (Fig. 5), and this onset timing was not affected by gait (P>0.25, F=0.67). EMG activity generally ended in the last third of the stance phase, regardless of gait (Fig. 5), and EMG duration, as a fraction of stance duration, remained nearly constant between 0.85 and 0.90 across gait and speed. EMG burst intensity in the vastus differed significantly between gaits (P<0.01, F=28.4), increasing as animals moved from slow walking speeds to fast galloping speeds (Fig. 6B). In comparing activity patterns between the two thigh muscles, vastus activity began after

biceps activity in all three gaits, and the relative difference in onset timing was greatest during galloping (Fig. 5). Vastus bursts always ended after biceps bursts as well, but the difference in relative offset timing remained consistent in all gaits (Fig. 5).

Vastus strain patterns were consistent across all animals and gaits and could be characterized by four phases (Fig. 7D). Phase 1 occurred in the first half of stance as the knee was generally flexing and consisted of stretching of vastus fascicles (Fig. 7C,D). The amount of stretching in phase 1 was affected significantly by gait (P<0.05, F=6.3) and was greater during galloping (mean, 0.14) than during walking (mean, 0.09) or trotting (mean, 0.08) (Fig. 8B). Strain rates during this stretching interval were also affected by gait (P<0.001, F=37.2) and were lowest during walking (mean, 0.84 L s^{-1}), intermediate during trotting (mean, 1.25 L s^{-1}) and highest during galloping (mean, 2.40 L s^{-1}) (Fig. 8E). Phase two occurred in the latter half of stance and consisted of rapid shortening of vastus fascicles, during knee extension (Fig. 7C,D). The amount of shortening in phase 2 differed significantly between gaits (P<0.01, F=7.3) and was greatest during walking (mean, –0.23), intermediate during trotting (mean, –0.185) and smallest during galloping (mean, –0.16) (Fig. 8B). Similarly, strain rates in this phase also differed significantly between gaits (P<0.001, F=33.7) but were highest in galloping (mean, –2.44 L s^{-1}), intermediate in trotting (mean, –1.95 L s^{-1}) and lowest during walking (mean, –1.16 L s^{-1}) (Fig. 8F). Phase 3 consisted of rapid stretching of vastus fascicles as the knee flexed in the first half of swing (Fig. 7C,D). Stretching strains were much larger than those in phase 1, during stance, but were not different between gaits (P>0.25, F=1.0), averaging 0.31–0.35 at all speeds (Fig. 8C). Phase 4 consisted of rapid and substantial shortening in the second half of swing as the knee re-extended prior to foot-down (Fig. 7C,D). Phase 4 shortening differed significantly between gaits (P<0.01, F=17.5) and was greatest in galloping (–0.30), intermediate in trotting (–0.25) and least during walking (–0.18) (Fig. 8C).

Recordings from proximal, middle and distal sites in the vastus lateralis were successful in four animals and revealed similar overall strain trajectories in fascicles from these

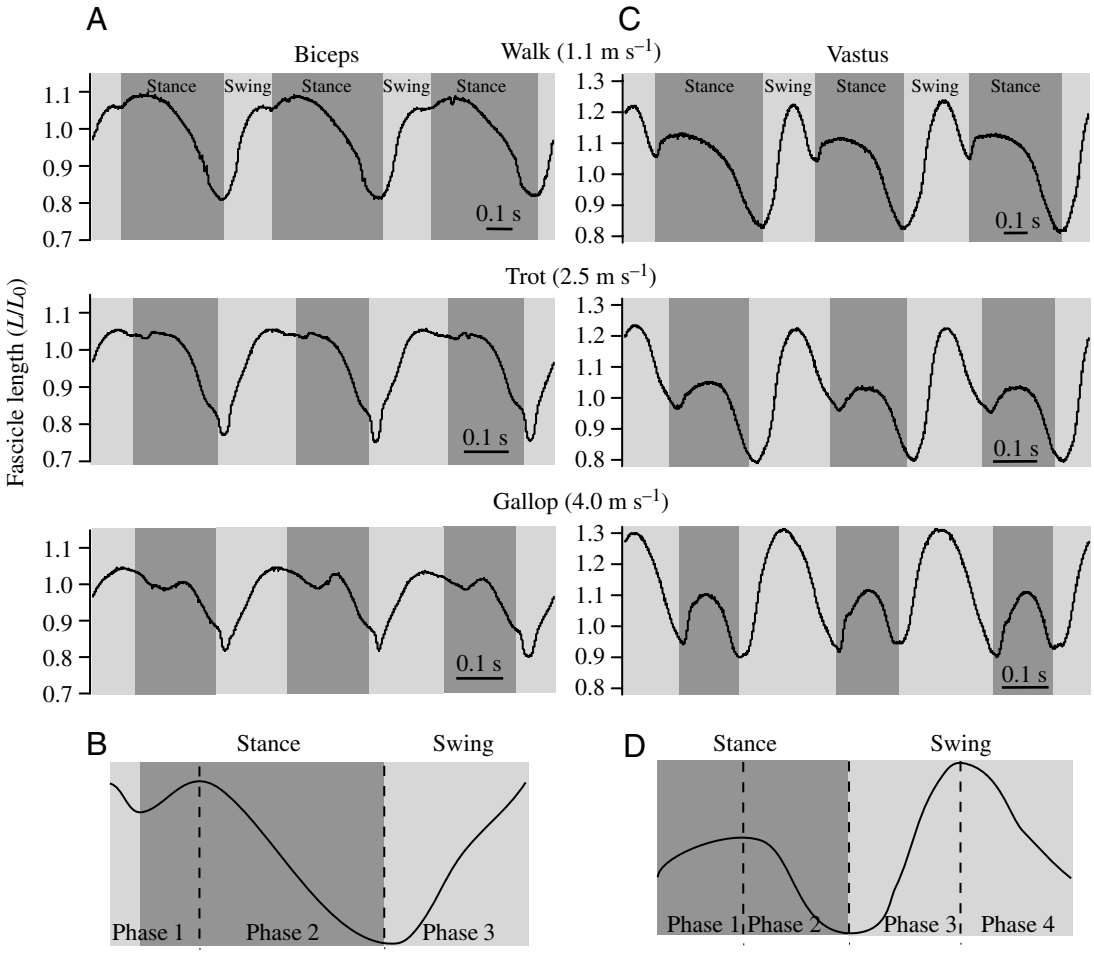

Fig. 7. Patterns of strain in the biceps (A) and vastus (C) during walking, trotting and galloping from three strides to show characteristic length-change trajectories during different gaits. Idealized strain trajectories are shown for the biceps (B) and vastus (D). In the biceps, strain trajectories can be divided into three discrete phases; in the vastus, four discrete phases are defined (described in more detail in the text). Dark gray shading represents the stance phase; light gray shading represents the swing phase.

portions of the muscle (Fig. 9). In some animals, fairly substantial differences in the magnitude of fascicle strain between sites were observed (Fig. 9B). However, this was not a consistent pattern across the four individuals, and statistical analyses revealed no significant effect of site implantation on strain magnitude during phases 1–4. The timing of strain events was also generally comparable across sites, although there was a significant difference among sites with respect to the onset timing of phase 1 (the start of stretching during stance), such that proximal implantations began stretching significantly, but only slightly (0.02 stride cycles), after more distal sites.

Discussion

Our aim in this study was to characterize the patterns of activation and strain in two goat thigh muscles, the biceps femoris and vastus lateralis, with three major goals in mind: (1) to explore how these patterns differ across gaits; (2) to test whether fascicle strain is homogeneous within the vastus lateralis and (3) to compare strain patterns with those found in rats, dogs and horses to determine if homologous muscles operate differently in these different species.

Speed and gait

Animals exercise over a wide variety of speeds in nature and often exhibit specific gaits in association with particular speed ranges. In many quadrupeds, walking is used for slower speeds, trotting for intermediate speeds and galloping for fast speeds. Although animals can use either hindlimb as the leading or trailing limb during a gallop, in this study all animals used the implanted limb as the trailing limb at some point during each experiment, whereas only three of seven animals used it as the leading limb. While this may reflect an effect of the implantation (i.e. animals more often chose to use the implanted limb as the trailing limb), we doubt that such an effect significantly altered the functional roles of the muscles under study, as both strain and activation patterns in the trailing limb were similar between goats that interchanged the implanted limb and those that did not. In addition, no visible signs of movement impairment were obvious during recordings. Because of the larger sample size, we chose to focus only on trailing limb sequences. Thus, whenever galloping is referred to, it is the trailing limb that is being discussed.

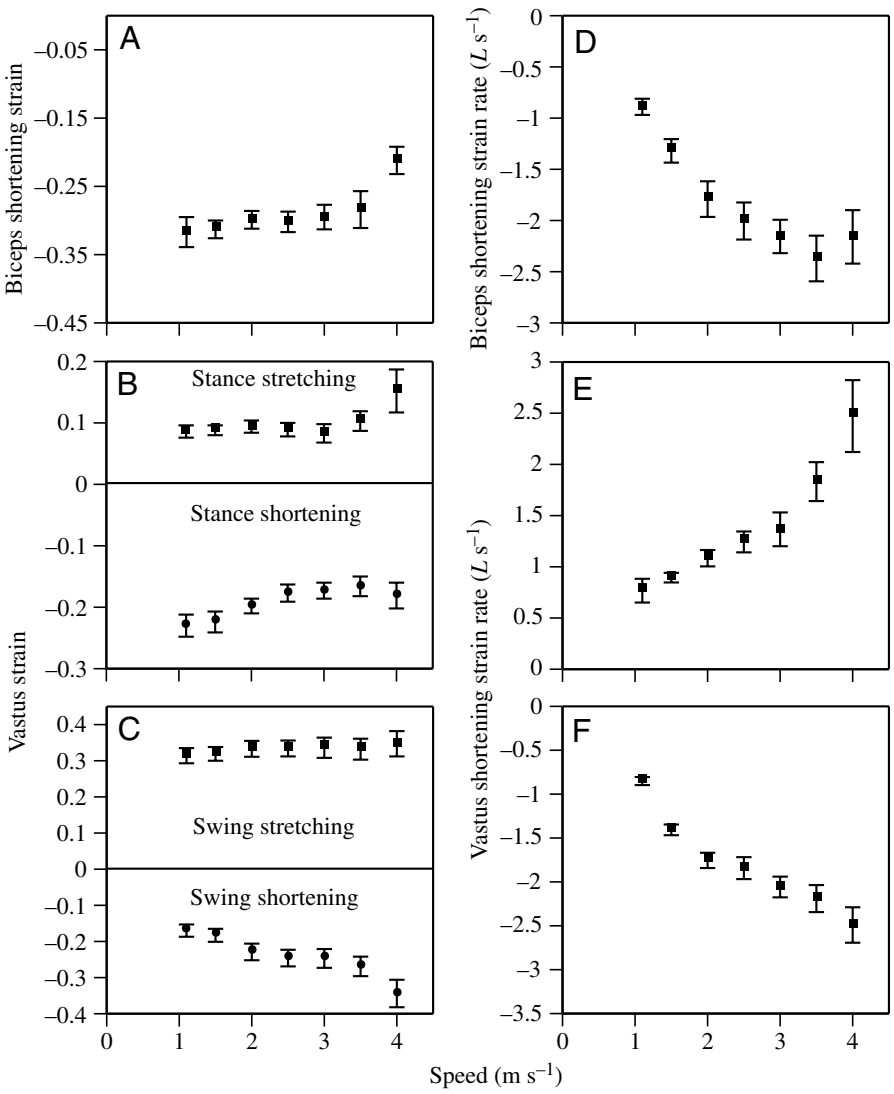

Fig. 8. Plots of fascicle strain (A–C) and strain rate (D–F) in the biceps and vastus as a function of speed. For the biceps (A,D), N=5 for all speeds except 4.0 m s^{-1} where N=4. All animals exhibited walking at 1.1 and 1.5 m s^{-1}, trotting at 2.0, 2.5 and 3.0 m s^{-1} and galloping at 4.0 m s^{-1}; at 3.5 m s^{-1}, two animals used trotting, one galloping, and two both trotting and galloping. For the vastus (B,C,E,F), N=7 for all speeds except 4.0, where N=6. All animals exhibited walking at 1.1 and 1.5 m s^{-1}, trotting at 2.0, 2.5 and 3.0 m s^{-1} and galloping at 4.0 m s^{-1}; at 3.5 m s^{-1}, four animals used trotting, two galloping, and two both trotting and galloping. All vastus data are from middle implantation locations.

EMG activity

As is generally the case for limb extensor muscles, the onset of activation in both the vastus and biceps of the goat consistently preceded ground contact (Fig. 5). In the vastus, this ‘phase advance’ averaged 20–30 ms, or 5–8% of the stride cycle, at all speeds and gaits. In the biceps, the phase advance was greater than in the vastus and increased at higher speeds, averaging 45 ms during walking and over 70 ms during galloping. Because of the reduced stride durations at

faster gaits, this increase in the absolute phase advance translated into a substantial difference when times were scaled relative to the stride duration (biceps femoris phase advance was 9% of the stride cycle during walking, 21% during galloping). EMG activity generally ended in the last third of stance in the vastus, although cessation was earlier, on average, during trotting and galloping than during walking (Fig. 5). A similar, yet more exaggerated, pattern was true of the biceps, where activity ended relatively late in stance during walking but ceased earlier in stance during trotting and especially galloping (Fig. 5). In rats, the only other animal we know of for which vastus and biceps activity has been reported at different gaits, a general shift to relatively earlier activity timing is also apparent at faster gaits (Gillis and Biewener, 2001). The overall burst duration of both muscles is a nearly constant fraction of the stance phase duration (~0.85), regardless of speed or gait. Similar results, albeit different fractions, have been found in thigh muscles of rats (~0.7) and horses (~0.6) (Gillis and Biewener, 2001; Hoyt et al., 2005).

A consistent result among past studies of hindlimb extensor muscle activity over a range of speeds and/or gaits is that EMG intensity increases with speed. The same result was found in this study for both the biceps and vastus. EMG intensity (quantified as mean spike amplitude) appeared to increase approximately linearly with speed, so that values were generally highest during galloping, intermediate during trotting and lowest during walking (Fig. 6).

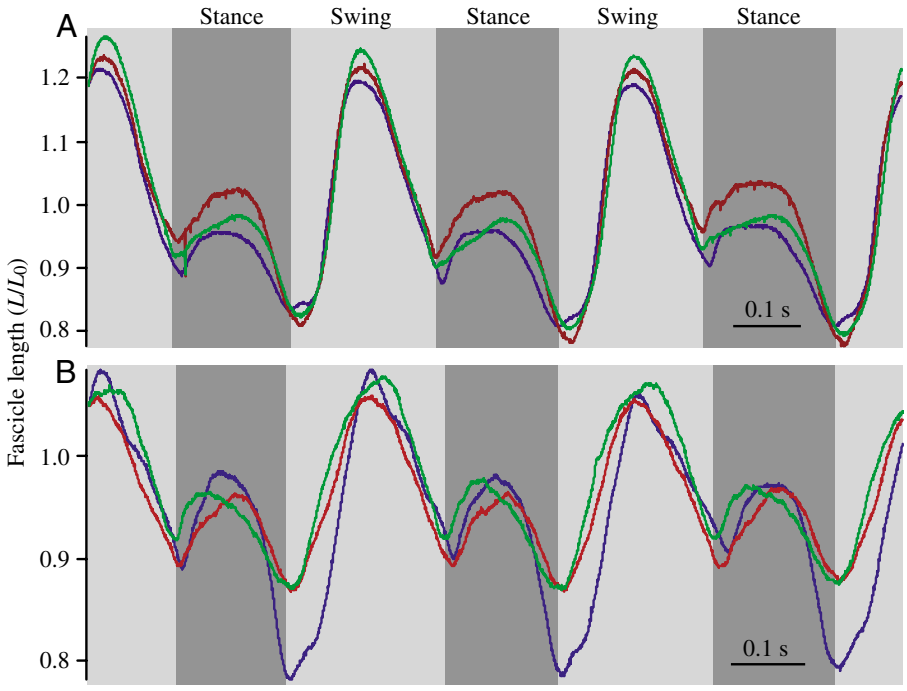

Fig. 9. Patterns of vastus strain in proximal (blue), middle (red) and distal (green) sites from two individuals showing subtle (A) and more substantive (B) variation. Note the overall similarity in strain trajectory in timing, even in B, where strain magnitude was much greater in the proximal location.

Joint kinematics and fascicle strain

As limb joints flex and extend over the course of a stride, the underlying muscles acting about the joints are often assumed to change length in a predictable way based on joint angular excursions. In this study, fascicle length changes of the vastus and biceps do generally reflect the pattern of joint angle changes observed at the hip and knee (Fig. 3). For example, during hip and knee flexion, the biceps and vastus, respectively, are stretched, and when the muscles shorten, the hip and knee extend. However, the precise timing, degree and even direction of length changes may not always be predictable based on limb kinematics. Tendons/aponeuroses can stretch and shorten in response to muscle contractions and ground reaction forces in ways that make predicting the strain patterns of the muscle fascicles in series with them quite complicated. We elaborate on this below when discussing comparative aspects of vastus function under 'Taxonomic comparisons'.

The actual length-change patterns muscle fascicles undergo when active largely determine their functional role. In the anterior regions of the biceps femoris, where fascicles act largely to extend the hip, shortening is expected to be a major component of the fascicles' strain trajectory as the hip extends throughout the entire stance phase. Not surprisingly, in goats, large degrees of shortening (25–35% of resting length) were characteristic of the biceps during stance. But the onset of this shortening was not coincident with foot-down. Instead, small amounts of stretching and/or shortening (generally 5% resting length or less) were present immediately after foot-down, preceding the more substantial shortening later in stance. This more isometric phase increasingly occupied a larger portion of stance as speed increased, ranging from approximately one-fifth of stance during walking to one-third of stance during galloping. Interestingly, muscle activity in the biceps ceased about two-fifths of the way through stance during galloping, indicating that during this gait EMG activity ended shortly after the onset of biceps shortening. The timing of muscle force development is unknown in these muscles, but it is unlikely that force production ceases when EMG activity comes to an end. Rather, in various studies from diverse taxa, it has been shown that muscle force production generally peaks near the end of EMG activity (Biewener et al., 1998a,b; Daley and Biewener, 2003; Roberts et al., 1997; Walmsley et al., 1978) and continues well after EMG activity ceases (up to one-third of the stance phase in wallabies and guinea fowl). Consequently, this period of more isometric activity before

shortening may reflect high force generation prior to the production of work during stance-related shortening.

Total biceps shortening strain following the more isometric interval early in stance remained nearly constant in walking and trotting and averaged 0.30–0.35. As stance phase durations decreased with increasing speeds, shortening strain rates in the biceps more than doubled between slow walking and fast trotting. These increases in strain rate with speed were concomitant with increases in EMG intensity and have been observed in hindlimb extensor muscles in a variety of mammalian quadrupeds (Gillis and Biewener, 2001; Hoyt et al., 2005; Prilutsky et al., 1996). Few studies have explicitly examined the effects of gait on fascicle strain, but previous work on the rat biceps suggested a reduction in fascicle shortening upon transition to the gallop (Gillis and Biewener, 2001). Goats exhibited a slight reduction in hip extension excursions during galloping (~5°), and a reduction in biceps shortening was also observed at the trot–gallop transition, adding evidence to the idea that a shift to galloping may reduce fascicle strain in hip extensors in a wider variety of mammals.

In line with the flexion–extension cycle of the knee, vastus fascicles exhibit a stretch–shorten cycle during the stance phase of all gaits. The onset of stretching closely coincided with foot-down at the start of the stance phase, and fascicles stretched for approximately one-third to half of stance, depending on gait. EMG activity was present during all of this stretching, suggesting energy dissipation during this interval, as well as high levels of force production. These roles seem particularly prominent during galloping, where fascicles stretched nearly twice as much and as fast as during other gaits, and for slightly over half of the stance interval, on average. Such results are comparable to those found in rats, where the rate and magnitude of vastus stretching over the first half of stance were also much higher during galloping than other gaits (Gillis and Biewener, 2001).

Shortening of vastus fascicles followed this initial stretch, and the amount of shortening was reduced at faster gaits (Fig. 8B). During walking and trotting, shortening strains were 2–3 times greater than the initial stretch, and shortening velocities were approximately 1.5 times greater than stretching velocities (Fig. 8B,E,F). Although we again do not know the time course of force production in the vastus, these strain patterns suggest that positive work is more substantive than negative work during stance in these gaits (i.e. much more and faster fascicle shortening than stretching). During galloping, the mechanical role of the vastus is less clear, as shortening and lengthening strains and strain rates were similar. It would seem that both positive and negative work are important in all gaits, as the knee yields to dissipate energy before re-extending to accelerate the body forward, but their relative amounts obviously depend heavily on the time course of the muscle's force production, which we cannot address in this study.

Strain homogeneity in the vastus

Few studies have directly measured fascicle strain in different regions of the same muscle *in vivo*. It is generally assumed that fractional length changes are comparable enough throughout the muscle that a single, typically centralized, recording provides insight into how the entire muscle operates. Strain heterogeneity within a muscle could exist in a variety of forms. For example, strain regimes might differ along the length of a fiber or fascicle. Ahn et al. (2004) found that shortening strains in segments along the same fascicle of a toad's semimembranosus differed substantially. Distal regions shortened much less than proximal and middle regions and were even stretched initially while other parts of the muscle shortened. This corresponds with work on individual fibers *in vitro* in which shortening of the more central sections occurs while those nearer the fiber's ends remain isometric or are stretched (Edman and Reggiani, 1984). Strain regimes might also differ among fascicles in different parts of the muscle. Soman et al. (2005) showed this to be true in the pigeon pectoralis, where fascicles in the posterior part of the sternobrachial region shortened much less than fascicles in the anterior and middle regions of this part of the muscle.

In this study, strain patterns were measured and compared from fascicles in parallel with one another from the proximal, middle and distal third of the vastus lateralis in four goats. Unlike the pigeon pectoralis, which has a complex bipennate architecture, the vastus of the goat is unipennate (Fig. 1). On average, stretching and shortening strains during stance were lowest in the proximal region of the muscle (Fig. 10), and this pattern was evident in three of four individuals. However, the pattern was reversed in the other animal, where the highest strains were observed proximally. Thus, while the degree of muscle length change can be different in different regions of the muscle, these differences were not consistent across the four animals we studied. These differences were also relatively small and were not statistically significant. As a result, we conclude that fascicle strains do not differ systematically from proximal to distal regions within the goat vastus.

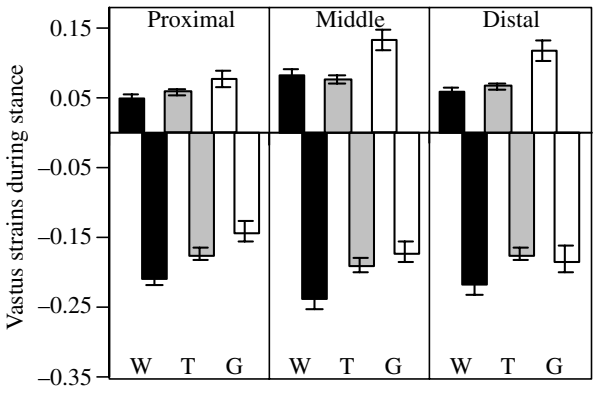

Fig. 10. Stretching (positive) and shortening (negative) strains in the vastus during stance from proximal, middle and distal fascicles during walking (W; black), trotting (T; gray) and galloping (G; white). Although strains tend to be highest in the middle region, this pattern was not present in all individuals, and differences between sites were not significant. Data shown are means from four individuals. Error bars represent standard errors.

Taxonomic comparisons

Direct *in vivo* measurements of fascicle strain have been recorded in the anterior biceps femoris in two mammalian quadrupeds: rats (Gillis and Biewener, 2001) and goats (present study). Despite gross differences in size, limb posture, life history and phylogenetic affinities, these species share a number of features with respect to the actions of the biceps femoris during treadmill-based locomotion. Biceps activation occurs prior to the onset of stance in both species, and muscle activity ceases late in stance during walking but relatively earlier in stance during trotting and galloping. With respect to strain patterns, biceps shortening is present over the majority of stance, with strains of approximately 25–35% in both species at speeds below galloping. As noted above, the transition to galloping decreases biceps shortening strains significantly. Thus, the anterior region of the biceps femoris seems to play similar roles in these different species, exhibiting substantial levels of shortening as the hip extends during stance.

Strain data from the vastus lateralis have been recorded in a broader range of animals: rats (Gillis and Biewener, 2001), dogs (Carrier et al., 1998), horses (Hoyt et al., 2005) and goats (present study), affording a larger comparative framework for understanding how this muscle operates during locomotion. In all species, the muscle is typically activated very near foot-down and remains active over approximately the first two-thirds of stance. Unlike in the biceps, however, strain patterns differ markedly among species. It is tempting to posit that these differences may be related in some way to body size. Limb posture and muscle pennation angles generally differ in animals of different size (Alexander et al., 1981; Biewener, 1989) and personal observations (G.B.G.) suggest that in muscles like the vastus, the relative amount of collagenous connective tissue in the form of aponeuroses and fascial sheaths is greater in large than small animals. If parameters such as limb posture, pennation angle and series elasticity of limb muscles change with body size, it might not be surprising to find certain muscles undergoing different length-change patterns in large and small animals. However, to begin to understand whether and how body size affects locomotor muscle function, more closely related species that differ in size and posture will need to be compared, and detailed comparative analyses of muscle architecture will need to be performed in relation to functional studies.

In the smallest animals, rat vastus fascicles typically undergo much more stretching early in stance (10–15%) than shortening later in stance (0–10%). This pattern corresponds well with knee joint kinematics, which shows much more flexion than extension during stance. In goats, as in dogs, the knee exhibits ample flexion followed by varying degrees of extension during stance (in dogs, knee extension is comparable to or greater than knee flexion depending on gait, whereas in goats, knee extension can be less than, greater than or equivalent to knee flexion, depending on gait). Regardless, in both dogs and goats, initial bouts of vastus stretching generally involve lower strains (5–15%) than the shortening bouts that follow later in stance (10–25%). Finally, in horses, vastus fascicles exhibit a rather complex strain trajectory but essentially shorten throughout much of stance (10–15%), with some of this shortening occurring as the knee flexes. Such shortening against what must be a lengthening muscle–tendon unit has also been documented in an ankle extensor of cats (Griffiths, 1991) and is also likely to be present on occasion in the goat vastus. For example, during walking, knee extension does not begin until midway through stance, whereas vastus shortening begins about a quarter of the way through stance. Thus, in the second quarter of stance, goat vastus fascicles begin shortening despite continued knee flexion and, therefore, a still-lengthening muscle–tendon unit. Moreover, despite several instances of comparable levels of knee flexion and extension during stance at slower speeds, vastus shortening always exceeded vastus stretching. Series elasticity, in the form of compliant tendons or aponeuroses at the distal end of these muscles, likely accounts for such findings (Griffiths, 1991; Hoyt et al., 2005). In any case, it is clear that knee joint kinematics are not necessarily reliable for inferring the time course or magnitude of fascicle strains in muscles acting at the knee, even for those that are uniarticular such as the vastus.

Shortening strain rates among species are also interesting to compare. Various theoretical and empirical studies have predicted or shown that the maximum shortening velocity (V_{max}) of muscle fibers decreases with increasing body size (Hill, 1950; Lindstedt et al., 1985; McMahon, 1975; Rome et al., 1990). Fiber type impacts the scaling exponent – slow oxidative fibers show a greater scaling effect than fast glycolytic fibers (Rome et al., 1990) – but maximum shortening velocity is highest in small animals and progressively decreases with increasing size regardless of fiber type. If muscles in animals of different size operate over a similar range of V/V_{max}, as is often assumed to be the case, then shortening velocity *in vivo* should scale similarly and should be higher at physiologically equivalent speeds in smaller animals than larger animals. Results from shortening in the vastus support this general relationship. Vastus shortening velocities during trotting are much lower, on average, in 400 kg horses (–0.6–0.8 $L\ s^{-1}$) than in 15–25 kg dogs (mean, –2.0 $L\ s^{-1}$) and goats (mean, –1.95 $L\ s^{-1}$). Rats could not be included in the comparison as their vastus fascicles exhibit little or no shortening during stance in trotting. By contrast, shortening strain rates in the biceps seem quite comparable in animals that differ in size by two orders of magnitude. Fascicles in the anterior region of the biceps shorten at rates ranging between –0.8 to –1.5 $L\ s^{-1}$ during walking and –1.5 to –2.5 $L\ s^{-1}$ during trotting in both rats and goats. If V_{max} scales negatively with size in this muscle, but V does not, then V/V_{max} differs at equivalent speeds and is lower in smaller animals. As more fascicle length-change data are collected *in vivo*, empirical scaling relationships for V in different muscles will be interesting to compare with one another and with those already derived empirically for V_{max}.

In summary, biceps fascicles of the goat shorten

substantially following an initial, more isometric period at the start of stance. Shortening strains are similar in walking and trotting but decrease significantly after the transition to galloping. Strain rates increase with speed from walking to trotting and level off in galloping. Despite a predicted decrease in muscle shortening velocities between small and large animals, anterior biceps fascicles in rats and goats shorten at similar rates during the same gait. Vastus fascicles are initially stretched in stance before shortening, and shortening strains are generally much greater than lengthening strains. A similar pattern has been reported in the vastus of similarly sized dogs, but rather different strain trajectories are present in significantly larger and smaller mammalian quadrupeds. Different regions of the vastus exhibit qualitatively similar strain regimes and, on average, stretch and shorten to the same degree and over a similar time course. Finally, vastus shortening velocities are lower in horses than in dogs and goats, as predicted by empirical and theoretical studies of the scaling of muscle function.

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