Muscle strain is modulated more with running slope than speed in wild turkey knee and hip extensors

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Summary

We examined the length changes and electromyographic (EMG) activity of two hindlimb muscles in wild turkeys, to determine how these muscles modulate mechanical function with changes in running speed and slope. The muscles studied were the iliotibialis lateralis pars postacetabularis (ILPO), a biarticular knee and hip extensor, and the femorotibialis lateralis (FT), a knee extensor. Muscle length changes were recorded using sonomicrometry, and EMG activity was recorded from indwelling bipolar electrodes as the animals walked and ran at a range of speeds ($1–3.5 \text{ m s}^{-1}$). Treadmill slope was also varied, from a 12° uphill slope to a downhill slope of –12°. To test the hypothesis that the strain pattern in active muscles reflects the demand for mechanical work, we compared strain in the ILPO and FT across the range of slopes. Both muscles underwent active lengthen–shorten cycles during stance. We analyzed the lengthening and shortening part of the strain pattern separately to determine the response of muscle strain to surface slope. In both muscles stance phase shortening strain increased over the range of slopes studied, from 7.8±3.5% (ILPO) and 1.9±2.2% (FT) during downhill running at –12°, to 30.3±3.9% (ILPO) and 8.2±5.6% (FT) during uphill running at 12°. Stance-phase lengthening strain was also modulated with slope, from –15.6±3.2% (ILPO) and –22.1±9.6% (FT) during downhill running at –12°, to –4.2±2.5% (ILPO) and –9.0±5.6% (FT) during uphill running at 12°. The results suggest that for the ILPO and FT a change in net mechanical work output with running slope is likely mediated by a change in both the lengthening, energy absorbing portion of the contraction and the shortening, energy producing part of the contraction. We also found changes in the timing of EMG activity, and the relative portion of the stance period spent lengthening, which were consistent with a shift in muscle function from energy absorption during downhill running, to net energy production during uphill running.

Generally, muscle strain was less affected by speed than by slope. Shortening strains were not significantly correlated with running speed. Only FT lengthening strain changed significantly with speed, ranging from –6.8±4.3% at 1 m s^{-1} to –15.3±4.7% at 3.5 m s^{-1}.

The consistent patterns of strain changes with running slope are evidence that strain pattern is modulated to meet the changes in demand for net mechanical work. The relatively poor relationship between strain and running speed may reflect the fact that changes in running speed during level running are not associated with a change in demand for net mechanical work. Taken together, the speed and slope results suggest that the demand for mechanical work is an important determinant of muscle length patterns in running and walking.

Key words: locomotion, muscle, bird, energetics.

Introduction

Skeletal muscles can act to produce mechanical work or absorb it, depending upon the kind of contraction they undergo. Both of these functions are important in terrestrial locomotion, but the relative demand for muscle energy production *vs* absorption changes with the task: uphill running requires net mechanical energy production to increase the body's potential energy, while downhill running requires net mechanical energy absorption to decrease potential energy. It is reasonable to expect that these changes in the demand for positive or negative work would be associated with a change in the relative amount of lengthening or shortening in individual muscles, because lengthening contractions absorb mechanical energy and shortening contractions produce it.

Measurements of muscle fascicle length changes in several species of animals running on inclined and declined treadmills generally support the prediction that muscle length trajectory is modulated in response to the demand for mechanical work (Roberts et al., 1997; Gillis and Biewener, 2002; Gabaldón et al., 2004; Wickler et al., 2005; Daley and Biewener, 2003). However, studies of muscle function in humans and cats on inclined treadmills suggest that not all muscles modulate strain

or work in response to demand. Ultrasound measurements of human medial gastrocnemius fascicles indicate that there is little change in function in this muscle with running incline (Lichtwark and Wilson, 2006), and EMG data for walking cats suggest that selective recruitment or de-recruitment of certain muscles may provide a mechanism for altering mechanical work with incline (Carlson-Kuhta et al., 1998; Smith et al., 1998). A change in the timing of force production in relation to a given length pattern can also provide a mechanism for altering muscle work output. Some distal muscles in guinea fowl and turkeys, for example, shift the timing of force to occur during a period of muscle shortening in order to produce more work for uphill running (Daley and Biewener, 2003; Gabaldón et al., 2004). Thus, the question of when muscles modulate length trajectory in response to the demand for mechanical work is not fully resolved.

In the present study, we determine the strain (relative length change) and activity of two thigh muscles in wild turkeys across a range of running speeds and slopes. The iliotibialis lateralis pars postacetabularis (ILPO) is a biarticular knee and hip extensor, and the femorotibialis lateralis (FT) is a knee extensor. Distal limb muscles in wild turkeys generally undergo small length changes during force production in level running, and it has been suggested that this is an economical way for muscles to function (Roberts et al., 1997; Gabaldón et al., 2004). However, studies of more proximal muscles in dogs (Carrier et al., 1998), rats (Gillis and Biewener, 2002) and goats (Gillis et al., 2005) have shown large length changes during the period of muscle activity. Thus, our first goal was to determine if more proximal muscles in running turkeys also undergo large length changes.

The second goal of the study was to determine whether the strain trajectory in the ILPO and the FT is modulated in response to the demand for mechanical work. To vary the demand for mechanical work, we ran turkeys on level, inclined (+6°, +12°) and declined (–6°, –12°) slopes. We hypothesized that the strain patterns of the ILPO and FT muscles during level running are influenced by the demand for mechanical work. Specifically, we predicted that increases in slope (uphill running) would be associated with increases in muscle shortening strain, while decreases in slope (downhill running) would be associated with increases in muscle lengthening strain.

The third goal of this study was to determine the effect of running speed on the length trajectory of the ILPO and the FT. From an energetic standpoint, the expected change in muscle strain with speed is not as obvious as it is for a change in slope. Increasing running slope increases the demand for net mechanical work with each step. Changes in running speed may affect the magnitude of cyclic positive and negative work done in each step, but the net work required for level running is negligible at all running speeds. Based on these energetic considerations, we hypothesized that the magnitude of lengthening and shortening strains would be independent of running speed.

Materials and methods
Animals and training
All animal use was approved by the Oregon State University Institutional Animal Care and Use Committee, and conducted in accordance with institutional and federal guidelines. Adult female wild turkeys *Meleagris gallopavo* L. were obtained from a local breeder and housed in an outdoor enclosure (approximately 30 m×7 m). Food and water were provided *ad libitum*. Animals were trained to run on a motor-driven treadmill (Keys Pro 2000 Series, Keys Fitness Products, Dallas, TX, USA) for 4–6 weeks prior to experimental measurements. Treadmill training occurred approximately 4 days a week, for 20 min each day for each animal. Training included a range of speeds, and level, incline (to +12°) and decline (to –12°) running. Data were collected for five animals for the ILPO, and four animals for the FT. The mean (± s.d.) body mass of the animals at the time of the experiments was 3.71±0.23 kg.

Muscles instrumented
Sonomicrometry and EMG transducers were implanted in the iliotibialis lateralis pars postacetabularis (ILPO) and the femorotibialis lateralis (FT; Fig. 1). The ILPO is a triangular-shaped muscle head, with a broad origin on the iliac crest, and an insertion on an aponeurosis that inserts on the patella. The FT is a unipennate muscle. Fibers originate on the femur and insert on the patellar aponeurosis. A comprehensive description
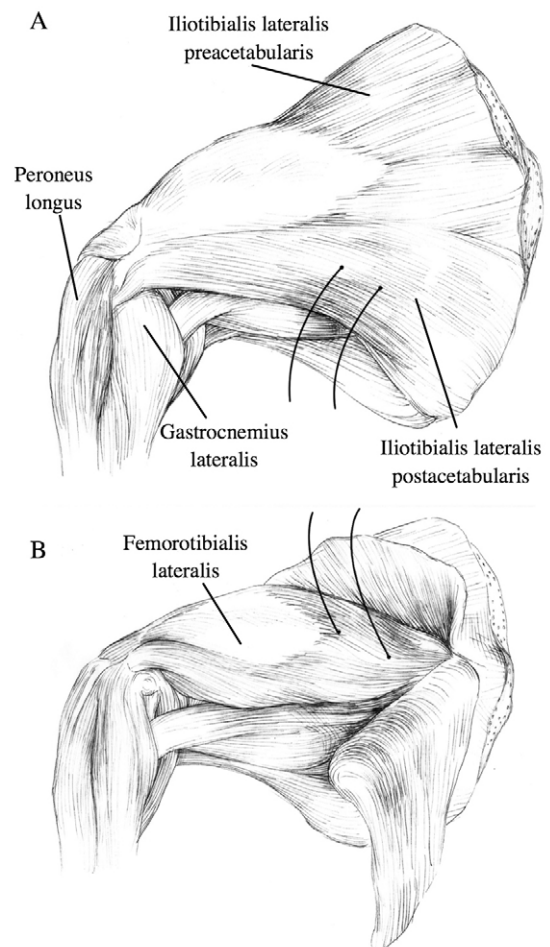

Fig. 1. Lateral views of the iliotibialis lateralis postacetabularis (A) and, with the iliolotibialis reflected (B), the femorotibilialis. The iliotibialis lateralis postacetabularis acts as a knee and hip extensor. The femorotibialis has a broad origin on the femur and is a knee extensor. Approximate location of sonomicrometer crystals are indicated.

of the anatomy of these muscles is given elsewhere (Gatesy, 1999b). Mean (± s.d.) muscle masses for this study were 24.7±5.0 g (ILPO) and 16.8±5.7 g (FT). Mean fiber lengths measured in this study were 61.1±17.9 mm for the ILPO and 22.9±3.2 mm for the FT. Sonomicrometry measurements were taken over a segment of the fascicle. The distance between sonomicrometer crystals, measured postmortem, averaged 18.0±2.4 mm for the ILPO and 12.7±3.1 mm for the FT.

Transducer implantation

Bipolar EMG electrodes and 2 mm sonomicrometry crystals were implanted 1 or 2 days prior to running measurements. Animals were induced and maintained on isoflurane anesthesia (1–2.5%), and a sterile field was maintained for all surgeries. Sonomicrometry crystals (Sonometrics, Inc., London, ON, Canada) were implanted in pairs along the fascicle axis of the ILPO and the FT. Crystals were implanted 12–18 mm apart to measure a segment rather than then entire length of muscle fascicles. Care was taken to implant the crystal pairs in approximately the same location in each bird. Crystals were secured in the muscle with a small drop of Vet-bond^{TM} glue and a single suture of 6-0 silk around the transducer lead. Bipolar electromyograph (EMG) electrodes were inserted near the sonomicrometry crystals. The EMG leads were constructed from 0.076 mm, teflon-coated, stainless-steel wire (Cooner Wire Company, Chatsworth, CA, USA), with 1 mm bared ends. Leads from implanted transducers were routed underneath the skin to a location on the back, where small connectors (Microtech, Inc., Boothwyn, PA, USA) were sutured to the skin. When all measurements were complete, the animals were anesthetized with inhalable isoflurane and euthanized with an injection of Beuthenasia-D. The locations of all sonomicrometry and EMG transducers were confirmed in a *post-mortem* dissection.

Measurements and analysis

Sonomicrometry and EMG signals were recorded as animals ran on a treadmill over a range of speeds and slopes. Muscle fascicle length measurements were recorded at 992 Hz by Sonometrics sonomicrometry hardware and software. EMG signals were recorded with DAM50 differential preamplifiers (World Precision Instruments, Sarasota, FL, USA), with a high-pass filter setting of 10 kHz and a low-pass filter setting of 3 Hz. All signals were recorded to an A/D board (National Instruments PCI-MIO-16-1, Austin, TX, USA) in an Apple Macintosh computer. Sonomicrometry signals were recorded to this system after being exported from the Sonometrics system *via* a D/A converter. The sampling rate was 4000 Hz. In software, sonomicrometer signals were corrected for a 4 ms delay caused by the sonometrics hardware. Sonomicrometer signals were filtered with a 20 Hz low-pass FIR filter. EMG signals were filtered with a 150 Hz high-pass FIR filter.

Waveform analyses were performed in the software application Igor (Wavemetrics, Lake Oswego, OR, USA). The strain patterns were well characterized as lengthen–shorten cycles in both muscles, and we chose to analyze the prominent shortening period and the prominent period of lengthening separately. Small amounts of lengthening and shortening that occurred in some muscles immediately after toe-down or just prior to toe-off were not included in our analysis of muscle strain patterns.

All strain measurements reported are for the stance phase, during which most muscle activity occurred. Stance phase length changes were divided into a lengthening period and a shortening period for separate analysis of lengthening and shortening strains (Fig. 2). Strain was determined as the percentage change in muscle fascicle length relative to resting muscle length:

$$\text{Strain} = 100 \times (L_i - L_f)/L_o, \qquad (1)$$

where L_i is the initial length for the period of measurement, L_f is the final length, and L_o is the muscle resting length. Calculating strain in this way yields negative values for lengthening strains and positive values for shortening strains. A measurement of resting muscle length, L_o, was required to calculate muscle strains and relative shortening velocities. Ideally, L_o should be the muscle length at the midpoint of the plateau of the muscle's length–tension relation. Length–tension measurements were not made for these muscles, so the actual value of L_o was unknown. We chose to use the average length during running, including both stance and swing, as an estimate of L_o. Absolute lengths were generally similar between speeds and slopes; we used the average length for 2 m s^{-1} level running for each bird as the L_o. Our measurements will tend to underestimate actual strains if our calculated L_o is too high, and overestimate them if it is too low. Trends in strain or velocity with speed and slope would be unaffected by the choice of L_o.
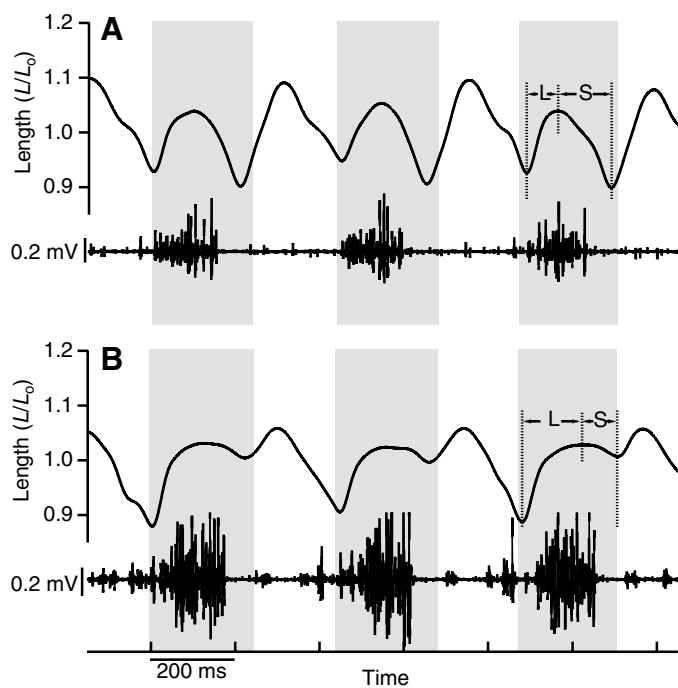
Calculation of EMG timing from a time of EMG onset or

Fig. 2. Representative fascicle length and EMG for the ILPO (A) and the FT (B) for one individual running at 2 m s^{-1} on the level. Shaded areas represent stance. Muscle lengths are presented as a fraction of L_o, calculated as the average muscle length over a stride. Broken lines on the third stride indicate the period of stance lengthening (L) and shortening (S) for which all measurements in this study were taken.

offset are subject to the difficult and often subjective task of identifying what can be a subtle transition between baseline levels and measurable levels of EMG signal. These indicators of EMG timing also cannot account for variation of the timing of EMG intensity within the EMG burst. To determine a measure of EMG timing that avoided these issues, we used the rectified, integrated EMG signal to determine the time necessary to reach half of the total integrated EMG intensity in a burst, or 't50%EMG'. To calculate t50%EMG, we first rectified the filtered EMG signal. The rectified signal was integrated, and we determined the time at which the integrated signal reached one-half its final value. The integration began at 50 ms prior to toe-down, in order to include bursts of activity that preceded stance but were likely associated with stance phase force production, given ordinary electromechanical delay. The integration ended at toe-off. The time to one-half maximum integrated EMG was measured relative to the time of toe-down. This time was then divided by the total stance time to produce a value for t50%EMG, as a percentage of the total stance time.

For each animal and running trial, ten strides were analyzed. Means of these ten strides were then averaged for each bird. All summary statistics are reported as mean ± 1 s.d. To test for the effect of slope or speed on the measured variables, a two-way mixed model analysis of variance (ANOVA) was performed, with individual as the random effect and speed or slope as the fixed effect. Pairwise *post-hoc* analyses were performed with a Tukey honestly significant difference test.

Results

The timing of EMG activity indicates that the ILPO and FT are primarily stance-phase muscles (Fig. 2). The ILPO was active only during stance phase (Fig. 2A). The primary burst of activity in the FT also occurred during stance. Small bursts of EMG activity were observed during swing in the FT, but the relative magnitude of stance and swing phase EMG activity indicates that the FT is predominantly a stance phase muscle in turkeys. All subsequent analyses focus on the stance phase of the ILPO and FT.

For both muscles, the timing of length change and EMG activity suggests that the period of active force production in these muscles is well characterized as a lengthen–shorten contraction. A brief period of shortening immediately following toe-down, and a brief period of lengthening immediately before toe-off, were also present in the ILPO (Fig. 2A) and occasionally in the FT. These length changes were small, and given the observed timing of EMG activity, likely occurred during periods when the muscles were not generating force. Therefore, we quantified only the strains observed during the prominent stance lengthen–shorten cycle for all analyses.

With changes in running slope, the relative magnitudes of lengthening and shortening strains were altered for the ILPO (Fig. 3) and the FT (Fig. 4). We measured shortening and lengthening strain separately to test the hypothesis that shortening would increase with the demand for mechanical work as running slope increased, and that lengthening strain would decrease. This hypothesis was supported for both muscles (Fig. 5). Across the range of running slopes, there was a clear role for modulation of both lengthening and shortening strains in response to changes in the demand for mechanical
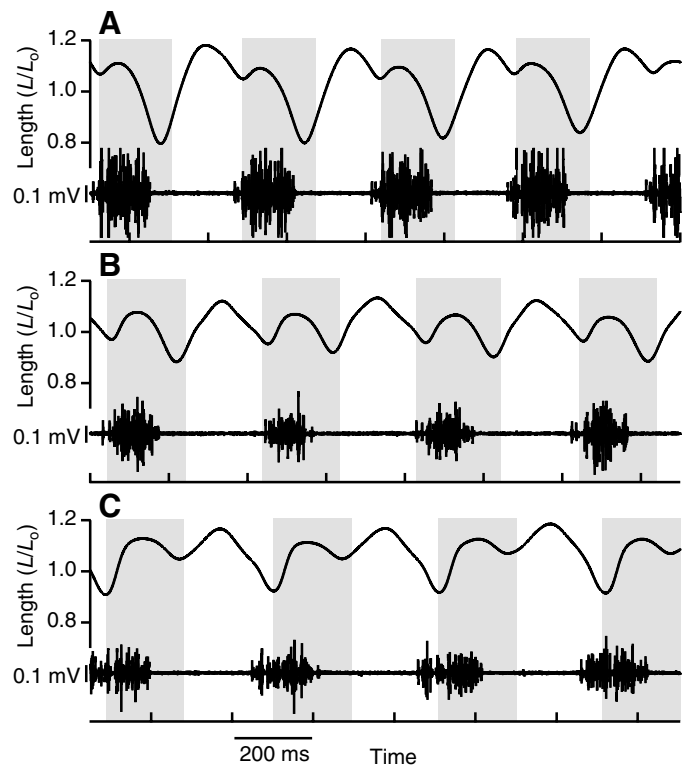

Fig. 3. Representative fascicle lengths and EMG for the ILPO during 12° uphill (A), level (B) and 12° downhill (C) running at 2 m s^{-1}. Shaded areas indicate stance phase. The trend towards increased shortening and decreased lengthening as slope increases is apparent in these sample strides. Data shown are for a different individual than depicted in Fig. 2.

work (Fig. 5). Shortening strains increased significantly across the range of slopes studied for both the ILPO ($P<0.0001$) and the FT ($P<0.05$). The relationship between lengthening strain and running slope was significant for both the ILPO ($P<0.0001$) and the FT ($P<0.004$), with downhill running eliciting more lengthening than uphill running. At the extremes of the range of slopes used here, shortening and lengthening strains were quite large. The FT showed the largest lengthening strains, at −22.1±9.6% (mean ± s.d.) during downhill running on a −12° slope at 2 m s^{-1}. The ILPO showed the largest shortening strains, with 30.3±3.9% (mean ± s.d.) during uphill running on a 12° slope at 2 m s^{-1}.

The trend in average muscle shortening and lengthening velocities with slope paralleled the trend in strains (Fig. 6). Stance phase time did not vary with running slope, thus changes in muscle fascicle velocity paralleled changes in muscle strain. Muscles shortened faster and lengthened more slowly as incline increased in both the ILPO ($P<0.0004$ for lengthening; $P<0.0001$ for shortening) and the FT ($P<0.004$ for lengthening; $P<0.04$ for shortening).

The increase in muscle shortening strain and decrease in lengthening strain observed with increasing incline are consistent with the idea that the length trajectory of these muscles is modulated in response to the demand for mechanical work. We also wanted to determine whether changes in the
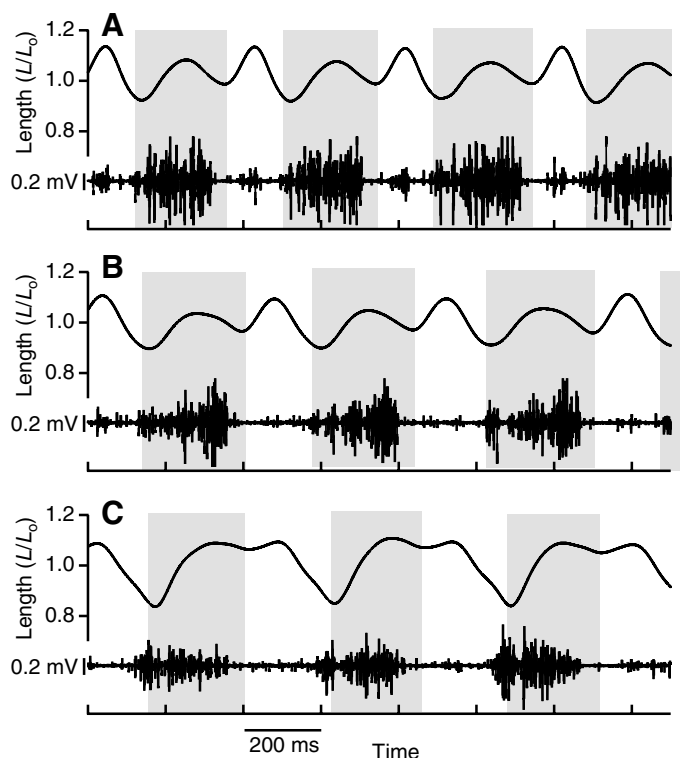
Fig. 4. Representative fascicle lengths and EMG for the FT during 12° uphill (A), level (B) and 12° downhill (C) running at 2 m s^{-1}. Shaded areas indicate stance phase. Data shown are for a different individual than depicted in Fig. 2.
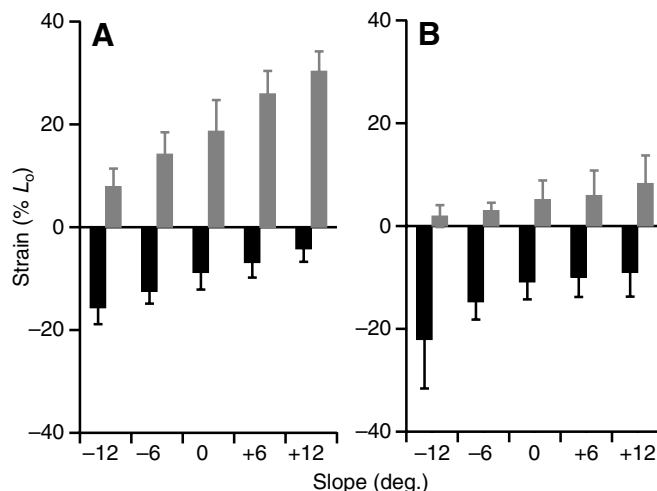

Fig. 5. Strain during shortening (grey) and lengthening (black) for the ILPO (A) and the FT (B). With increases in slope, shortening strain increased and lengthening strain decreased. There was a significant effect of slope on shortening and lengthening strain for both muscles.

timing of strain, or muscle activity, might occur across incline in a manner that was consistent with a modulation of muscle work. It has been demonstrated that for a given strain pattern, changes in the relative timing of activation can alter muscle work output by altering the phase relationship between force and strain (Daley and Biewener, 2003; Gabaldón et al., 2004). We tested whether timing of muscle activity was modulated with running slope by measuring the time taken during stance for half of the total integrated, rectified EMG activity to be developed (t50%EMG). Both the ILPO and FT showed the same pattern (Fig. 7). The time taken to develop half the EMG activity was significantly affected by slope. A *post-hoc* pairwise analysis indicated that for both muscles, only the −12° condition was significantly different from values for level running. Thus, the shift in EMG timing relative to values for level running appears to occur for downhill running but not uphill. EMG activity occurred earlier in the contact phase for decline running than for level or uphill running. Presumably, this shift in EMG timing indicates a mechanism for increasing negative work output for decline running by increasing the relative proportion of force production that occurs during the lengthening, rather than the shortening phase of the lengthen–shorten cycle in these muscles.

Increases in running slope were also associated with an increase in rectified integrated EMG activity for the FT and ILPO. To account for variation in signal between different electrode placements, we determined integrated rectified EMG for all birds relative to the value for 2 m s^{-1}, level running. For

the FT, there was an increase in activity from level to incline running, with values at +12° of 1.52±0.13 the value for level running. ILPO activity increased from 0.71±0.29 for −12° running to 1.58±0.46 for +12°.

Changes in the relative portion of the lengthen–shorten cycle spent in lengthening *vs* shortening might also affect the work output of muscles for different slopes. The duration of the lengthening phase and the shortening phase changed significantly with slope in the ILPO (P<0.001 for lengthening; P<0.0001 for shortening). As a result of the changes in timing of lengthening and shortening, the relative fraction of the time spent lengthening was significantly greater during downhill running compared with uphill running (Fig. 8). For example, during −12° running, lengthening occupied 56% (±4.3%) of the lengthen–shorten cycle time, while for uphill running at +12°,
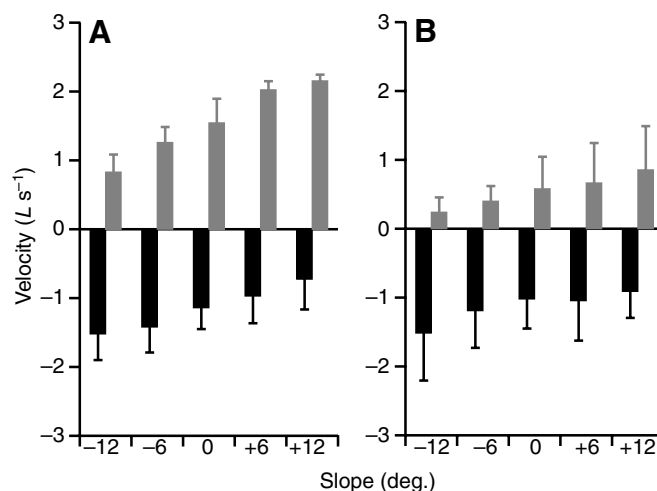

Fig. 6. Average fascicle velocity during shortening (grey) and lengthening (black) for the ILPO (A) and the FT (B). With increases in slope, shortening velocity increased and lengthening velocity decreased.
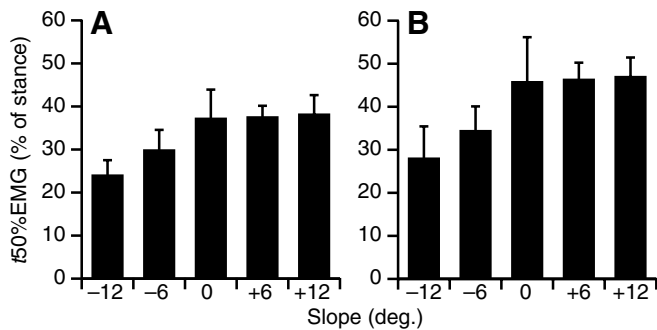
Fig. 7. Timing of EMG activity, measured as the fraction of the stance phase taken to develop one-half of the rectified integrated EMG signal (*t*50%EMG) for the ILPO (A) and the FT (B).

the portion of the cycle spent lengthening was only 23% (±13%). The relative proportion of time spent shortening and lengthening did not vary significantly with slope for the FTL. The relative time spent lengthening *vs* shortening also did not vary significantly across speed for either muscle.

Generally, shortening and lengthening strains in the ILPO and FT were much less affected by speed than by slope (Fig. 9). The strain measured during shortening was not significantly correlated with speed for either muscle. The only significant change in strain across slope occurred for lengthening in the FT ($P<0.0001$). FT lengthening strain ranged from –6.8±4.3% at 1 m s^{-1} to –15.3±4.7% at 3.5 m s^{-1}. Integrated rectified EMG was statistically unchanged across running speed for both muscles.

Because the duration of stance decreases with increasing speed, the effect of speed on average muscle velocity during lengthening and shortening was significant even when there was little or no change in muscle strain (Fig. 10). The FT showed relatively high variance in average shortening velocity, primarily due to variation in the duration of the lengthening and
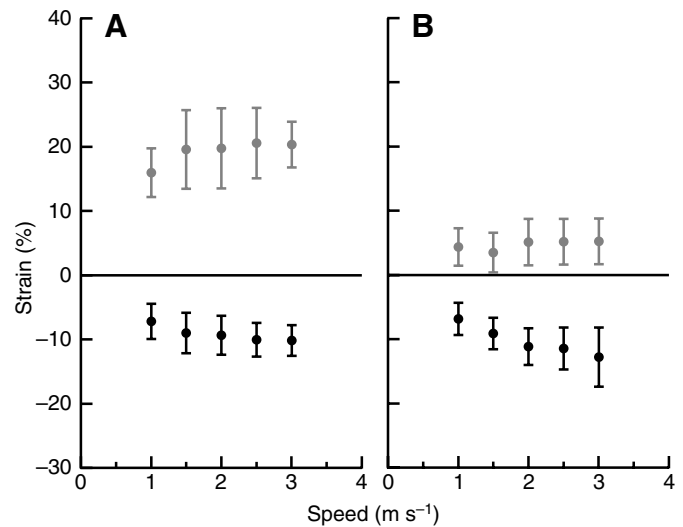

Fig. 9. Shortening (grey) and lengthening (black) strains for the ILPO (A) and the FT (B). There was no significant relationship between shortening strain and running speed. Lengthening strain for the FT was the only variable significantly affected by running speed.

shortening phase of the muscle between individuals (see, for example Figs 2 and 4). Both muscles operated at higher average shortening and lengthening velocities as speed increased ($P<0.0001$ for shortening and lengthening velocities, both muscles).

Discussion

Both of the muscles studied underwent an active lengthen–shorten cycle during stance phase. The ILPO and FT insert on a substantial aponeurotic sheet, thus length changes of muscle fascicles may not be well predicted by joint movements. Nevertheless, there is at least a qualitative agreement between joint movements and muscle fascicle
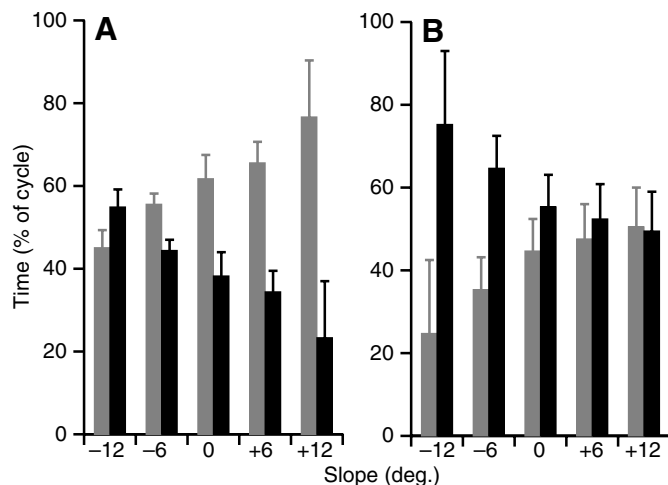

Fig. 8. Relative time spent shortening (grey) and lengthening (black), expressed as a proportion of the total time of the lengthen–shorten cycle of the ILPO (A) and the FT (B). Relatively more time was spent shortening as the demand for mechanical work increased with increasing slope.
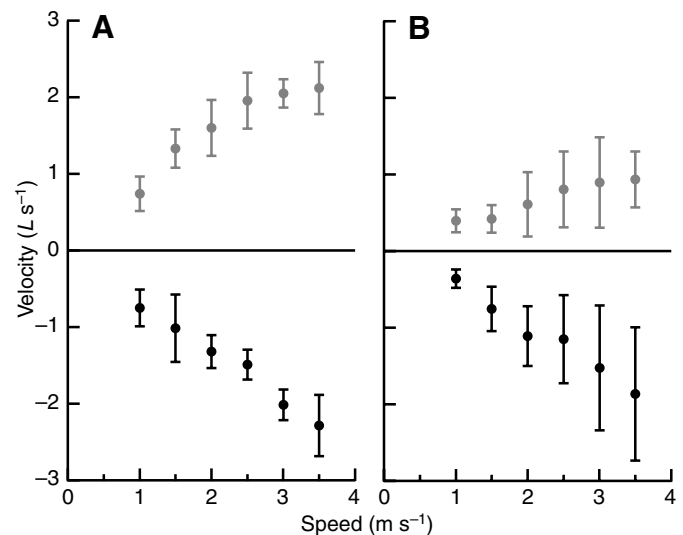

Fig. 10. Velocities of shortening (grey) and lengthening (black) were significantly correlated with running speed for both the ILPO (A) and the FT (B).

length changes for the ILPO and FT during running. The knee flexes during most of the stance phase, followed by a brief and small extension near the end of stance (Roberts and Scales, 2004). This is consistent with the generally extended period of lengthening or isometry in the FT and the briefer period of shortening at the end of stance (Fig. 2, Fig. 4). The ILPO acts as an extensor at both the hip and the knee. During much of stance (approximately the middle 50%), simultaneous hip extension and knee flexion would tend to counteract one another in terms of muscle length changes. Joint angle measurements during running would suggest that lengthening of the ILPO in the first half of stance is due primarily to knee flexion, while shortening of the ILPO in the second half of stance is associated primarily with hip extension.

The ILPO and FT both modulate length trajectory in response to the demand for mechanical work. The muscles underwent more net shortening as incline increased, and more net lengthening with increasing decline. Although muscle force and work cannot be determined from these measurements, the timing of EMG activity suggests that the muscles were active during the stance lengthen–shorten cycle. Thus, increases in muscle shortening were likely associated with an increase in the net positive work developed by the muscles, and increases in muscle lengthening were associated with an increase in the energy absorbed by the muscles. These results add to the growing body of evidence that individual muscles modulate their mechanical output in response to the demand for mechanical work. Strain is altered in response to surface slope in the vastus lateralis and biceps femoris of rats (Gillis and Biewener, 2002; Butterfield et al., 2005), the vastus and triceps in horses (Wickler et al., 2005), the lateral gastrocnemius and digital flexor IV of guinea fowl (Daley and Biewener, 2003) and the lateral gastrocnemius and peroneus of wild turkeys (Roberts et al., 1997; Gabaldón et al., 2004). However, not all muscles modulate strain in response to the demand for mechanical work. Ultrasound measurements indicate that strain and muscle work are constant across slope in the medial gastrocnemius of running humans (Lichtwark and Wilson, 2007). Ankle extensor muscles of a hopping animal, the tammar wallaby also do not modulate strain with incline (Biewener et al., 2004), though the medial gastrocnemius does modulate strain between steady-speed hopping and rapid take-off jumps in another species of wallaby, the Tasmanian pademelon (Griffiths, 1989).

In addition to changes in muscle fascicle strain, several other mechanisms can alter the mechanical power output of the limb musculature in response to demand. Selective recruitment or de-recruitment of individual muscles or muscle groups (Carlson-Kuhta et al., 1998; Smith et al., 1998; Roberts and Belliveau, 2005), changes in muscle force output (Daley and Biewener, 2003) and changes in the timing of force output relative to length changes (Daley and Biewener, 2003; Gabaldón et al., 2004) have all been demonstrated. In the present study, there was generally an increase in recruitment, as measured by rectified, integrated EMG, from downhill to uphill slopes. Given that both muscles underwent lengthen–shorten cycles, it is impossible to determine whether this increase in activity was associated with an increase in positive work, negative work, or both. The timing of stimulation, relative to stance time, was also altered in the ILPO and FT from level to decline running. The EMG signal occurred earlier in the stance period during decline running compared with level or uphill. This change in timing of muscle activity would tend to increase force output during the lengthening portion of the lengthen–shorten cycle of these muscles, thus increasing mechanical energy absorption. Timing of EMG activity and time spent in the shortening or lengthening phase of the lengthen–shorten cycle were not altered from level to incline running. The ability to extend the period of muscle activity to late in stance may be limited by the need to allow sufficient time for the muscle to relax before the beginning of swing phase.

Changes in running speed had much smaller effects on muscle length trajectory compared with changes in running slope. We hypothesized that there would be no significant change in muscle fascicle strain with running speed, based on the assumption that the net work required to move the body in each step is unchanged with running speed. This hypothesis was not supported, as significant changes in strain with speed occurred for the lengthening phase in the FT. However, running slope generally had a much stronger influence on strain than did running speed. For example, shortening strain increased by more than fourfold in the FT and more than threefold in the ILPO from $-12°$ to $+12°$, while there was no significant difference between shortening strains measured at a $1~\mathrm{m~s^{-1}}$ walk and a $3.5~\mathrm{m~s^{-1}}$ run. Lengthening strain was also not significantly correlated with speed for the ILPO. The idea that strain is more strongly influenced by slope than by speed is supported by all of the measurements in the present study with the exception of the lengthening strains measured in the FT. FT lengthening strains changed nearly as much across the range of speeds measured here as they did across the range of running slopes. The increase in lengthening strain with running speed in the turkey FT is consistent with the speed-related kinematic patterns for another galliform, guinea fowl (Gatesy, 1999a), which showed an increase in knee flexion with running speed.

One feature of muscle function that was constant across all running slopes and speeds was the general pattern of length change during muscle activity, that of a cyclical pattern of lengthening followed by shortening. Lengthen–shorten cycles in active muscle fascicles have been observed previously in the ILPO of running guinea fowl (McGowan et al., 2006), as well as in the knee extensors of dogs (Carrier et al., 1998), rats (Gillis and Biewener, 2002) and goats (Gillis et al., 2005). The lengthen–shorten pattern observed here in knee and hip extensors involve larger strains than observed for turkey ankle extensors. For example, a net strain during force production of only 6.6% was measured for turkey lateral gastrocnemius during level running at $4~\mathrm{m~s^{-1}}$ (Roberts et al., 1997). This value also represents the approximate shortening strain for the LG, as lengthening strains were negligible. The shortening strains during fast ($3.5~\mathrm{m~s^{-1}}$) level running in the present study for the FT were lower than those of the LG, at only 4.7±1.8%, but the muscle underwent significant lengthening ($-15.3±5.8\%$). In contrast, the ILPO shortened by 19.6±4.9% for the fastest level running speeds measured here ($3.5~\mathrm{m~s^{-1}}$), and it lengthened by $-9.6±2.1\%$.

The implications of the lengthen–shorten pattern of muscle length change for the energetics of running are not entirely

clear. It has been proposed that isometric force production may allow muscles to operate economically because muscles produce high forces at low shortening velocities (Roberts et al., 1997; Taylor, 1994). Lengthen–shorten cycles might also be economical, because while the force is reduced in shortening muscle relative to isometric, the force produced in actively lengthening muscle exceeds that of isometric. The question of muscle energy consumption during lengthen–shorten cycles has been addressed primarily in the context of measurements of muscle efficiency (e.g. van Ingen Schenau et al., 1997; Curtin, 1997; Barclay, 1997). Analyses of *in vivo* efficiency during cyclical movements involving stretch–shorten cycles are complicated by the inability to separate out contributions of series elasticity and muscle contractile elements (in the present study 'lengthen–shorten' is used to distinguish the observed behavior of muscle fascicles from a 'stretch–shorten' cycle which might imply contributions from series elastic elements). Isolated muscle studies suggest that efficiency of cyclic lengthen–shorten cycles may exceed that of isovelocity contractions (Barclay, 1997; Barclay, 1994). However, studies comparing the force economy of isometric contractions to the cost of force production in lengthen–shorten contractions are lacking.

The ILPO in running guinea fowl shows significant increases in EMG activity and strain with the addition of external weights to the body (McGowan et al., 2004). Studies of muscle blood flow also indicate that the ILPO and FT show a disproportionate increase in energy consumption with body loading in guinea fowl (Ellerby and Marsh, 2006). These investigators suggested that these muscles may be selectively recruited in response to a load because their anatomy allows an increase in joint moments to support body weight without increasing antagonistic forces that can be associated with most biarticular muscles. It is also possible that the lengthen–shorten cycles of these muscles allow them to increase activity and force output without increasing net work output. Loading, like incline running, increases the metabolic demand but the mechanical challenge is fundamentally different. Loading increases the demand for force and cyclical positive/negative work, but does not change the demand for net mechanical work. A muscle such as the ILPO might be particularly well-suited to respond to this demand, because an increased force output in this muscle might lead to an approximately equal increase in positive and negative work in each cycle, so that the net work remains unchanged. If a muscle that underwent only shortening during force production were to increase its force output, it would also increase its net work output.

In conclusion, the iliotibialis lateralis pars postacetabularis and the femorotibialis lateralis both modulate muscle strain in response to the demand for mechanical work: fascicle lengthening increases and shortening decreases with increased demand for energy absorption (e.g. downhill running), while lengthening decreases and shortening increases with increased demand for positive work (e.g. uphill running). The ability to vary both lengthening and shortening strain, as well as to vary the timing of activation in relation to the length cycle, may allow these muscles flexibility in their mechanical function.

References

Barclay, C. J. (1994). Efficiency of fast- and slow-twitch muscles of the mouse performing cyclic contractions. *J. Exp. Biol.* **193**, 65-78.

Barclay, C. J. (1997). Initial mechanical efficiency in cyclic contractions of mouse skeletal muscle. *J. Appl. Biomech.* **13**, 418-422.

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

Butterfield, T. A., Leonard, T. R. and Herzog, W. (2005). Differential serial sarcomere number adaptations in knee extensor muscles of rats is contraction type dependent. *J. Appl. Physiol.* **99**, 1352-1358.

Carlson-Kuhta, P., Trank, T. V. and Smith, J. L. (1998). Forms of forward quadrupedal locomotion. II. A comparison of posture, hindlimb kinematics, and motor patterns for upslope and level walking. *J. Neurophysiol.* **79**, 1687-1701.

Carrier, D. R., Gregersen, C. S. and Silverton, N. A. (1998). Dynamic gearing in running dogs. *J. Exp. Biol.* **201**, 3185-3195.

Curtin, N. A. (1997). First catch the fish: efficiency during stretch-shortening cycles that mimic natural locomotion. *J. Appl. Biomech.* **13**, 429-432.

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.

Ellerby, D. J. and Marsh, R. L. (2006). The energetic costs of trunk and distal-limb loading during walking and running in guinea fowl *Numida meleagris*. II. Muscle energy use as indicated by blood flow. *J. Exp. Biol.* **209**, 2064-2075.

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.

Gatesy, S. M. (1999a). Guineafowl hind limb function. I: Cineradiographic analysis and speed effects. *J. Morphol.* **240**, 115-125.

Gatesy, S. M. (1999b). Guineafowl hind limb function. II: Electromyographic analysis and motor pattern evolution. *J. Morphol.* **240**, 127-142.

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.

Griffiths, R. I. (1989). The mechanics of the medial gastrocnemius muscle in the freely hopping wallaby (*Thylogale billardierrii*). *J. Exp. Biol.* **147**, 439-456.

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.

McGowan, C. P., Duarte, H. A., Main, J. B. and Biewener, A. A. (2006). The effects of load carrying on metabolic cost and hindlimb muscle dynamics in guinea fowl (*Numida meleagris*). *J. Appl. Physiol.* **101**, 1060-1069.

Roberts, T. J. and Belliveau, R. A. (2005). Sources of mechanical power for uphill running in humans. *J. Exp. Biol.* **208**, 1963-1970.

Roberts, T. J. and Scales, J. A. (2004). Adjusting muscle function to demand: joint work during acceleration in wild turkeys. *J. Exp. Biol.* **207**, 4165-4174.

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.

Smith, J. L., Carlson-Kuhta, P. and Trank, T. V. (1998). Forms of forward quadrupedal locomotion. III. A comparison of posture, hindlimb kinematics, and motor patterns for downslope and level walking. *J. Neurophysiol.* **79**, 1702-1716.

Taylor, C. R. (1994). Relating mechanics and energetics during exercise. In *Advances in Veterinary Science and Comparative Medicine*. Vol. 38A (ed. J. H. Jones), pp. 181-215. San Diego: Academic Press.

van Ingen Schenau, G. J., Bobbert, M. F. and de Haan, A. (1997). Does elastic energy enhance work and efficiency in the stretch-shortening cycle. *J. Appl. Biomech.* **13**, 389-415.

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