# RESEARCH ARTICLE

# Function of a large biarticular hip and knee extensor during walking and running in guinea fowl (*Numida meleagris*)

Jennifer A. Carr*,†, David J. Ellerby‡ and Richard L. Marsh

Department of Biology, Northeastern University, Boston, MA 02115, USA

*Author for correspondence (carr@fas.harvard.edu)
†Present address: Department of Organismal and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA
‡Present address: Department of Biological Sciences, Wellesley College, 106 Central Street, Wellesley, MA 02481, USA

## SUMMARY

**Physiological and anatomical evidence suggests that in birds the iliotibialis lateralis pars postacetabularis (ILPO) is functionally important for running. Incorporating regional information, we estimated the mean sarcomere strain trajectory and electromyographic (EMG) amplitude of the ILPO during level and incline walking and running. Using these data and data in the literature of muscle energy use, we examined three hypotheses: (1) active lengthening will occur on the ascending limb of the length–tension curve to avoid potential damage caused by stretch on the descending limb; (2) the active strain cycle will shift to favor active shortening when the birds run uphill and shortening will occur on the plateau and shallow ascending limb of the length–tension curve; and (3) measures of EMG intensity will correlate with energy use when the mechanical function of the muscle is similar. Supporting the first hypothesis, we found that the mean sarcomere lengths at the end of active lengthening during level locomotion were smaller than the predicted length at the start of the plateau of the length–tension curve. Supporting the second hypothesis, the magnitude of active lengthening decreased with increasing slope, whereas active shortening increased. In evaluating the relationship between EMG amplitude and energy use (hypothesis 3), we found that although increases in EMG intensity with speed, slope and loading were positively correlated with muscle energy use, the quantitative relationships between these variables differed greatly under different conditions. The relative changes in EMG intensity and energy use by the muscle probably varied because of changes in the mechanical function of the muscle that altered the ratio of muscle energy use to active muscle volume. Considering the overall function of the cycle of active lengthening and shortening of the fascicles of the ILPO, we conclude that the function of active lengthening is unlikely to be energy conservation and may instead be related to promoting stability at the knee. The work required to lengthen the ILPO during stance is provided by co-contracting knee flexors. We suggest that this potentially energetically expensive co-contraction serves to stabilize the knee in early stance by increasing the mechanical impedance of the joint.**

Key words: electromyography, locomotion, muscle physiology.

## INTRODUCTION

Physiological and anatomical evidence suggests that the postacetabular portion of the iliotibialis lateralis muscle (ILPO) is functionally important for rapid running in birds. The ILPO is a biarticular muscle with extensor actions at the hip and the knee (Fig. 1). The changes of energy use with speed during level locomotion in guinea fowl, indicate that the ILPO is most important in running, because energy use by this muscle is very low during walking (Ellerby et al., 2005). Evidence from comparative anatomy also suggests that the evolution (or retention) of a large ILPO is specifically associated with fast running in ground dwelling birds. The ILPO constitutes 13% of the hindlimb muscle mass in guinea fowl, making it the largest single hindlimb muscle, and it is of a similar relative size in flightless ratites (Harris et al., 1994) (http://ag.ansc.purdue.edu/poultry/ratite/ostrich.pdf) (R.L.M., personal observations). The prominence of the ILPO in these groups of able runners, contrasts dramatically with its reduction or absence in other groups of birds that mainly use other forms of locomotion. The ILPO has been reduced or completely lost in many birds that spend a large amount of time swimming, wading or diving, e.g. ducks, geese and gulls, and its reduction or absence is also notable in many

species of birds that are excellent flyers but spend little or no time on the ground, e.g. albatrosses, hawks, swifts and hummingbirds (George and Berger, 1966). The large size of the ILPO in birds adapted for rapid running, its high energy use during this activity and its actions at both the hip and knee suggest that information about the mechanical function of this muscle could be important for understanding stable, economical bipedal running in general.

Previous studies have demonstrated that during running the ILPO in the guinea fowl and turkey actively lengthens and absorbs work during the first part of stance, then actively shortens to produce work during the latter half of stance (Buchanan, 1999; Marsh, 1999; McGowan et al., 2006; Roberts et al., 2007). The function of substantial active lengthening of this large muscle is not clear. Other researchers have suggested that the active lengthening–shortening cycle might enhance economy of force production (McGowan et al., 2006; Roberts et al., 2007). However, active lengthening of muscle fibers beyond their short-range elasticity absorbs work that cannot be recovered, and replacing this negative work could increase the cost of legged locomotion (see Discussion). Lengthening of active muscle can also cause fiber damage (Proske and Morgan, 2001). Given these potential costs one might predict a

counterbalancing benefit for active lengthening, such as enhanced joint stability.

The present study combined data on regional strain and muscle activation from two companion studies of level running (Carr et al., 2011a; Carr et al., 2011b) with data on uphill running collected here, and muscle energy use from previous studies. We used these data to test hypotheses related to alterations in mechanical function induced by speed and slope. The first hypothesis was that active lengthening of the ILPO would occur on the ascending limb of the length–tension curve to avoid potential damage caused by stretch on the descending limb (Proske and Morgan, 2001). The second was that the activity of the muscle would shift to favor active shortening when the birds ran uphill, and shortening would occur on the plateau and shallow ascending limb of the length–tension curve. The third was that changes in active muscle volume, as indicated by electromyographic (EMG) amplitude, would best correlate with changes in muscle energy use when the mechanical function was similar. To test this we examined the activity and mechanical function of the ILPO in relation to muscle energy use as measured by blood flow (Ellerby et al., 2005; Rubenson et al., 2006). We predicted that the relative magnitudes of the changes in volume and energy use would be different when the mechanical function of the muscle changes, e.g. more active shortening compared with active lengthening. Finally, with the information obtained here and in previous studies we reconsider the function of the active lengthening–shortening cycle in the ILPO.

## MATERIALS AND METHODS
### Animals and training

Five guinea [*Numida meleagris* (Linnaeus 1758)] fowl averaging 1.54±0.08 kg body mass were used for this experiment. The guinea fowl were housed in individual cages at the Northeastern University Animal Care Facility, with food and water provided *ad libitum*. Before surgery and experimental recordings, the birds were trained to run inside a three-sided box on a motorized treadmill. The birds were trained for 3–5 days per week for a minimum of 5 weeks. Each training session lasted 30 min. After training the birds could, in an individual session, maintain level speeds of $2.5\,\mathrm{m\,s^{-1}}$ for 15 min, $2.78\,\mathrm{m\,s^{-1}}$ for 3 min and $3.0\,\mathrm{m\,s^{-1}}$ for 3 min. Once a bird was sufficiently trained, surgery was performed to insert sonomicrometry and electromyographic (EMG) sensors as described below. After surgery, birds were allowed to recover in individual cages for at least 40 h before any experimental recordings. Animals were killed with Euthasol® (pentobarbital and phenytoin). All *in vivo* procedures were approved by the Northeastern University Animal Care and Use Committee.

### Sonomicrometry and electromyography

More detailed descriptions of sonomicrometry and electromyography surgical techniques, sarcomere measurements and calculations of length and velocity are given in Carr et al. (Carr et al., 2011a).

The anterior fascicles of the ILPO were implanted with two sonomicrometry transducers that spanned the majority of the length of the fascicle (Fig. 1). The posterior fascicle was too long to be spanned by a single set of sonomicrometry transducers and instead was implanted with four transducers that measured length change in the proximal, central and distal segments of the posterior fascicle (Fig. 1). The length changes that occurred in the individual segments of the posterior fascicle were summed. Two fine-wire bipolar EMG
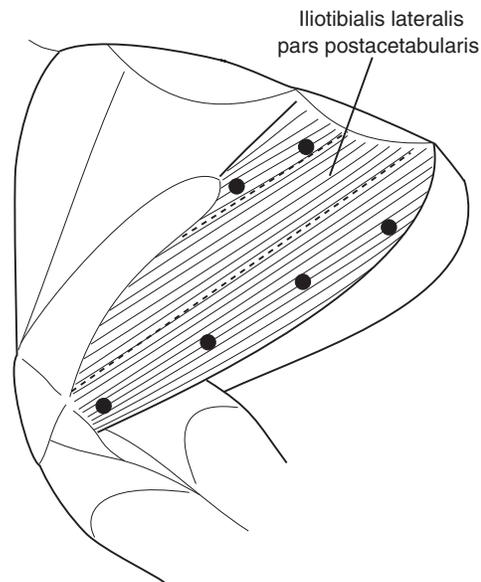


Fig. 1. Position of the iliotibialis lateralis pars postacetabularis (ILPO) in the guinea fowl hindlimb. Black circles indicate the position of the sonomicrometry crystals. EMG electrodes were placed along the fascicles at the approximate mid-point of each pair of sonomicrometer transducers. Dashed lines indicate the divisions used to calculate the weight average strain and velocity. Figure drawn by Dr David Ellerby.

electrodes were implanted at the approximate midpoint of each segment. Sonomicrometry signals were acquired digitally with a sampling frequency of 613 Hz using Sonoview software and a Sonometrics TRX Series 8 interface (Sonometrics Corp., London, ON, Canada). Digital signals were transferred to the application IGOR Pro (Wavemetrics Inc., Portland, OR, USA) and processed using custom-written functions. The EMG signals were amplified by WPI model DAM-50 preamplifiers (World Precision Instruments, Sarasota, FL, USA) with analog high- and low-pass filters set at 10 and 3000 Hz, respectively. The signals were digitized at a frequency of 10 kHz using an ADInstruments Power Lab 16 bit A-D converter (Model 16SP, ADInstruments, Colorado Springs, CO, USA) controlled by a Macintosh computer using the application Chart from ADInstruments. The data were then filtered (finite impulse response band-pass filter, 90–000 Hz) and rectified using the application IGOR Pro (Wavemetrics Inc.). The mean rectified value was calculated both for duration of the burst and the duration of the stride.

*In vivo* segment lengths measured by sonomicrometry ($L_{so}$) were converted to sarcomere lengths using reference sonomicrometer and sarcomere lengths measured in rigor. Following the experimental measurements the animals were killed, allowed to go into rigor, and sonomicrometer segment lengths ($L_{so,ref}$) measured. Portions of the muscle from each segment were then removed, frozen and sectioned to measure mean sarcomere length in rigor ($L_{sc,ref}$). *In vivo* sarcomere lengths ($L_{sc}$) were then calculated using the equation:

$$L_{sc} = (L_{sc,ref} / L_{so,ref}) \times L_{so}. \tag{1}$$

The sarcomere lengths were then converted to fractions of optimal length ($L_0$) by dividing by 2.36, which is the sarcomere length expected in the center of the plateau of the length–tension curve based on filament lengths (Carr et al., 2011a). Fascicle strains are similarly given as $\Delta L_{sc}$ divided by 2.36.

### Calculating average values across the muscle

Small but statistically significant differences in strain and velocity were found between the anterior and posterior fascicles of the ILPO (Carr et al., 2011b). Therefore, we calculated a mass-weighted average of the values from the anterior and posterior regions to arrive at the values presented here. We dissected the muscle and divided it into three regions, as indicated by the dashed lines in Fig. 1. The most posterior region has fascicles running from the pelvis to the knee and was considered to have strains equal to the measured values in the posterior region. The middle region has gradients of decreasing fascicle length and hip moment arm from posterior to anterior and was considered on average to have strains equal to the mean of the anterior and posterior values. The anterior portion was assumed to have strains equal to the measured values in the anterior fascicles. The fractional mass distribution of the three regions was: $0.44 \pm 0.01$, $0.45 \pm 0.01$ and $0.11 \pm 0.01$ (means ± s.e.m., $N=3$) for the posterior, middle and anterior regions, respectively. Therefore, the overall weighted average values were calculated as 0.335 (measured anterior values) + 0.665 (measured posterior values).

No significant regional differences were found in measures of EMG amplitude or timing among the various electrode sites (Carr et al., 2011a; Carr et al., 2011b). Therefore, we calculated the average values for the whole muscles by taking the mean of the values at the different electrode sites.

### Matching blood flow data from the literature to EMG data

Estimates of energy use by the ILPO are based on the rate of blood flow to the muscle during activity (Ellerby et al., 2005; Ellerby and Marsh, 2006; Rubenson et al., 2006). Marsh and Ellerby reviewed the use of muscle blood flow as an indicator of muscle energy use and concluded that for aerobically supported exercise the data from human and animal studies support a linear and proportional relationship between blood flow and energy use (Marsh and Ellerby, 2006). The speeds used in the blood flow studies are in some cases slightly different from those used here to measure the EMG activity, but are not expected to influence the comparisons. For three of the five birds this speed was $2.78\,\mathrm{m\,s^{-1}}$, but for the other birds the speed was $\sim2.5\,\mathrm{m\,s^{-1}}$. For comparison with the running speed of $2.4\,\mathrm{m\,s^{-1}}$ used by Rubenson et al., we used the EMG data collected at $2.5\,\mathrm{m\,s^{-1}}$, and for the fast running speed used by Ellerby et al. we used data collected at $2.78\,\mathrm{m\,s^{-1}}$ (Rubenson et al., 2006; Ellerby et al., 2005).

Average EMG values reported in various publications represent either the integrated value divided by the duration of the burst or the integrated value divided by the entire cycle time. Because the metabolic rate of the muscle is measured over many strides, the average over the stride would seem *a priori* to be the better measure to compare with the estimates of muscle metabolic rate, particularly if the duty factor of the muscle changes. We consider both of these measures in our comparisons.

All blood flow and EMG data were converted to relative values by dividing by the values obtained at a running speed of $1.5\,\mathrm{m\,s^{-1}}$. This running condition was chosen as a reference because it was used in all the relevant studies of muscle blood flow in guinea fowl (Ellerby et al., 2005; Ellerby and Marsh, 2006; Rubenson et al., 2006).

### Statistical analysis

ANOVA was conducted using the general linear model in the application SPSS (version 18.0 for Mac OS, SPSS Inc., Chicago, IL, USA). In addition to the dependent variables, the ANOVA models included: animal identifier as a random factor, and speed and incline as fixed factors. Results of all statistical tests were
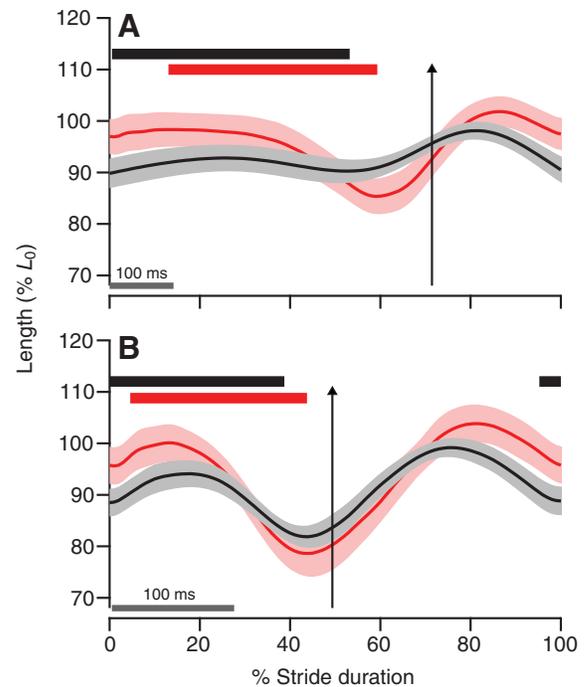


Fig. 2. Mean length of the ILPO as a function of stride duration from foot-down to foot-down when guinea fowl walked or ran on the treadmill with a slope of 0% (black) and 20% (red) at speeds of 0.5 (A) or 2.0 (B) $\mathrm{m\,s^{-1}}$. Horizontal black and red bars indicate the duration of EMG activity at 0.5 and $2.0\,\mathrm{m\,s^{-1}}$, respectively, assuming an electromechanical delay of 25 ms. Black arrows indicate toe-off. Shaded bands indicate ±1 s.e.m.

considered significant if the $P$-values were less than 0.05. Results are reported as means ± 1 standard error of the mean (s.e.m.).

### RESULTS
### Effects of speed and incline on muscle length change and velocity

Under all conditions the fascicles of the ILPO underwent active lengthening–shortening cycles during stance (Fig. 2). During swing, the ILPO showed periods of passive lengthening and shortening (Carr et al., 2011a; Carr et al., 2011b), but these passive length changes will not be considered here.

Active strain during lengthening and shortening was significantly influenced by speed and slope (Table 1; Fig. 3). Both shortening and lengthening strain showed a large increase between speeds of 0.5 and $1.0\,\mathrm{m\,s^{-1}}$, corresponding to a gait change from a walk to a run (Fig. 3). Above $1.0\,\mathrm{m\,s^{-1}}$, shortening strain continued to increase markedly, whereas lengthening strain remained approximately constant. If the $0.5\,\mathrm{m\,s^{-1}}$ data are excluded from the model, the lengthening strain shows no significant increase in speed ($P=0.06$). Lengthening strain decreased significantly with slope, whereas shortening strain increased significantly (Table 1; Fig. 3).

Average active shortening velocity (Fig. 4) increased significantly with both incline and speed (Table 1; Fig. 4). However, lengthening velocity was significantly influenced by speed but not slope.

### Sarcomere measurements

The mean sarcomere length at the estimated start of active lengthening (25 ms after the EMG onset) got significantly longer with increasing incline, but was not significantly affected by speed (Table 2; Fig. 5). The maximum active length ($L_{max}$) increased significantly as incline was increased, but showed no significant

Table 1. Results of the ANOVA model testing the effects of speed and slope on active strain velocity in the fascicles of the iliotibialis lateralis pars postacetabularis (ILPO)

| | | Active strain | | | | Active velocity | | | |
| | | Shortening | | Lengthening | | Shortening | | Lengthening | |
| Effect | d.f. | F | P | F | P | F | P | F | P |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Bird ID[a] | 3,63 | 42.06 | **<0.001** | 37.59 | **<0.001** | 2.81 | **<0.05** | 0.028 | 0.994 |
| Speed[b] | 6,63 | 44.60 | **<0.001** | 4.697 | **0.001** | 107.1 | **<0.001** | 41.56 | **<0.001** |
| Slope[c] | 3,63 | 94.43 | **<0.001** | 16.80 | **<0.001** | 13.05 | **<0.001** | 2.651 | 0.056 |

Significant *P*-values are shown in bold type.
[a]Identifier for the individual birds entered as a random factor.
[b]Treadmill speed entered as a fixed factor.
[c]Treadmill slope (0, 10, 15 and 20% uphill) entered as a fixed factor.

influence of speed (Table 2; Fig. 5). The minimum active length ($L_{min}$) decreased significantly with both speed and incline (Table 2; Fig. 5).

### EMG activity

The timing and amplitude of the EMG burst was significantly altered by speed and slope (Table 3; Figs 6 and 7). The EMG start time relative to foot-down showed a significant effect of speed. This effect was due to a shift in the start time with speed during uphill running. On the level, the EMG burst started before foot-down and this time was relatively constant across all speeds (mean of $-0.037\pm0.002$ s). When the birds ran uphill the EMG start time shifted significantly later compared with level running, and shifted earlier with increasing speed. For example, when the birds walked uphill at $0.5$ m s$^{-1}$, the mean EMG start time was after foot-down, whereas, when running

uphill at speeds of $1.5$ m s$^{-1}$ or faster the start time was at or before foot-down. The EMG burst duration decreased significantly with speed on all slopes, reflecting the significantly shorter stance time at faster speeds. However, the duration was not significantly affected by slope because start and end times increased by similar amounts. Average EMG amplitude measured as either average value per burst or average value per stride increased significantly with increasing speed and increasing slope (Table 3; Fig. 7).

### DISCUSSION

During level running the ILPO is initially lengthened while active in the first half of stance and then actively shortens during the last half of stance. By inference it first absorbs work and then produces work while active. The lengthening in early stance is related to knee flexion (Carr et al., 2011b), and shortening corresponds to a period
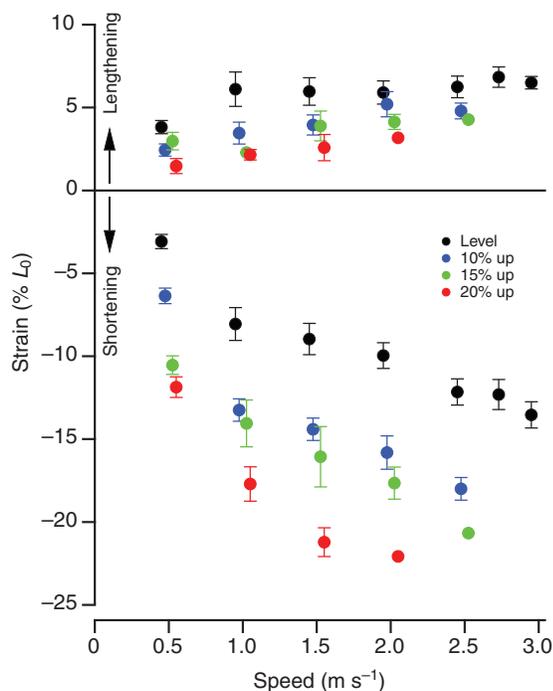


Fig. 3. Strain in the ILPO as a function of speed during active lengthening (positive values) and active shortening (negative values) when guinea fowl ran on the level (black), 10% incline (blue), 15% incline (green) and 20% incline (red) as a function of speed. A significant effect of speed and incline was demonstrated for both active lengthening and active shortening. Values are offset on the speed axis for clarity. Error bars indicate ±1 s.e.m.
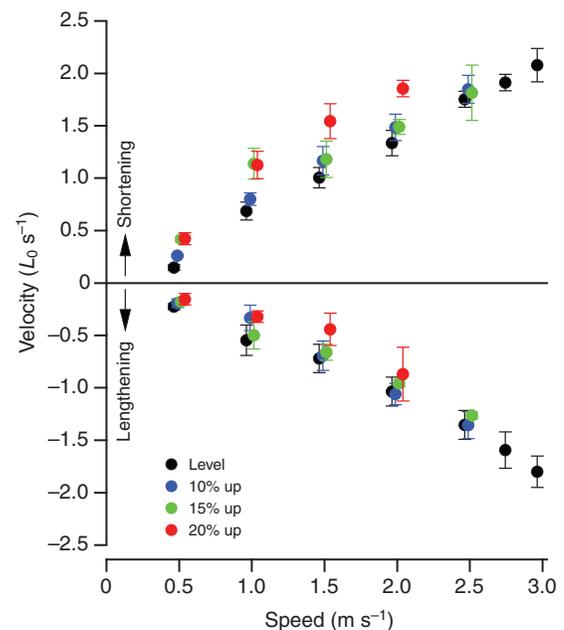


Fig. 4. Velocity during active shortening (positive values: level, black; 10% incline, blue; 15% incline, green; 20% incline, red) and active lengthening (negative values) in the ILPO as a function of speed. A significant effect of speed and incline was demonstrated for active shortening velocity. The velocity during active lengthening was not significantly different across slopes, but there was a significant effect of speed. Error bars indicate ±1 s.e.m.

Table 2. Results of the ANOVA model testing the effects of speed and slope on *in vivo* sarcomere lengths

| Effect | d.f. | Start lengthening | | Maximum | | Minimum | |
|---|---|---|---|---|---|---|---|
| | | $F$ | $P$ | $F$ | $P$ | $F$ | $P$ |
| Bird ID[a] | 3,63 | 251.3 | **<0.001** | 162.9 | **<0.001** | 49.44 | **<0.001** |
| Speed[b] | 6,63 | 1.259 | 0.289 | 1.803 | 0.113 | 17.11 | **<0.001** |
| Slope[c] | 3,63 | 42.82 | **<0.001** | 19.04 | **<0.001** | 7.698 | **<0.001** |

Significant *P*-values are shown in bold type.
[a]Identifier for the individual birds entered as a random factor.
[b]Treadmill speed entered as a fixed factor.
[c]Treadmill slope (0, 10, 15 and 20% uphill) entered as a fixed factor.

of both knee extension and hip extension (Carr et al., 2011b). The overall strain cycle of the active ILPO found in the present study, taking into consideration regional strain, was qualitatively similar to previous studies of this muscle in guinea fowl and turkeys using a single segment length (Buchanan, 1999; McGowan et al., 2006; Roberts et al., 2007), and quantitative differences can probably be explained by differences in regional strain (Carr et al., 2011a). Buchanan (Buchanan, 1999) and McGowan et al. (McGowan et al., 2006) reported larger strains than those found in the present study, probably because strain was estimated using measurements in the central to distal portions of the posterior fascicles (Carr et al., 2011a). The larger strains found in the ILPO of turkeys (Roberts et al., 2007) may have a similar cause, although a species difference cannot be ruled out. Similar to the ILPO in guinea fowl, the ILPO of turkeys shows increased shortening and decreased lengthening when the birds run uphill. However, unlike the results for guinea fowl, the ILPO of turkeys showed no significant increase in either lengthening or shortening strain with increasing running speed.

Using our data collected on regionally averaged sarcomere strain and EMG activity during level and uphill running along with previously collected data on blood flow, we examined three hypotheses: (1) the ILPO would be lengthened on the ascending limb of the length–tension curve to avoid potential damage caused by stretch on the descending limb; (2) the activity of the muscle would shift to favor active shortening when the birds ran uphill and shortening would occur on the plateau and shallow ascending limb of the length–tension curve; (3) measures of EMG intensity would best match energy use when the mechanical function was similar, but would differ when the mechanical function shifted, e.g. when the birds ran uphill. In addition, we will use the information obtained here and in previous studies to re-consider the function of the active lengthening–shortening cycle in the ILPO.

### Active lengthening occurs on the ascending limb of the length–tension curve

Routine active lengthening of muscle can potentially damage muscle fibers, and damage is hypothesized to be more likely when active lengthening occurs on the unstable descending limb of the length–tension curve (Proske and Morgan, 2001). Muscles subjected to repeated bouts of active stretch have been shown to add sarcomeres in series, presumably to prevent the fibers from lengthening beyond the plateau of the length–tension curve (Lynn and Morgan, 1994; Lynn et al., 1998; Koh and Herzog, 1998; Butterfield et al., 2005). Consistent with the hypothesis that muscles adjust the number of sarcomeres to avoid lengthening on the descending limb, the maximal length of the ILPO was on the ascending limb of the predicted length–tension curve except when the birds ran up the highest slope (20%) when the maximal length was on the plateau (Fig. 5).

### When running uphill, the ILPO increases active shortening and operates at longer lengths

Unlike most major knee extensors in mammals, the biarticular ILPO is also a hip extensor. The ILPO can, therefore, do positive work during both knee and hip extension. However, we predict that the majority of positive work is done at the hip because angular extension is greater at the hip than the knee, and the posterior part of the ILPO, which contains the bulk of the muscle volume, has a larger moment arm at the hip than at the knee (Carr et al., 2011b). Based on what is known about the maximum shortening velocity ($V_{max}$) of muscles in turkeys (Nelson et al., 2004) and considering the size difference between the species, the $V_{max}$ of the ILPO is estimated to be $15L_0 s^{-1}$. During level and uphill running the ILPO actively shortened by 10–22% while shortening at $1$–$2L_0 s^{-1}$, which should allow the active fibers to produce a substantial amount of work.
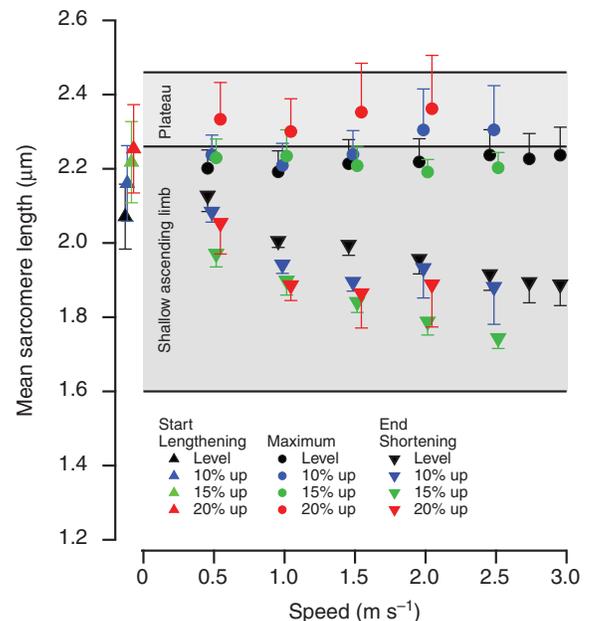


Fig. 5. Estimated mean sarcomere lengths of the ILPO during treadmill exercise at different speeds and slopes. The values at the start of active lengthening varied with slope but not with speed, and thus the mean values across all speeds are plotted as single upward-directed triangles. The mean maximum sarcomere lengths (the values at the end of lengthening and start of shortening) are indicated by closed circles, and values at the end of active shortening by inverted triangles. Treadmill slope is indicated by color: level, black; 10% incline, blue; 15% incline, green; 20% incline, red. Some values are offset on the speed axis for clarity. Error bars indicate ±1 s.e.m. Shaded areas indicate the calculated plateau and ascending limb of guinea fowl ILPO length–tension curve, based on filament lengths.

Table 3. Results of the ANOVA model testing the effects of speed and slope on electromyographic (EMG) timing and amplitude

| Effect | d.f. | Start | | Stop | | Duration | | Average per burst | | Average per stride | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | F | P | F | P | F | P | F | P | F | P |
| Bird ID[a] | 4,88 | 19.32 | **<0.001** | 10.85 | **<0.001** | 17.09 | **<0.001** | 16.79 | **<0.001** | 48.59 | **<0.001** |
| Speed[b] | 6,88 | 6.688 | **<0.001** | 310.4 | **<0.001** | 64.48 | **<0.001** | 53.454 | **<0.001** | 42.10 | **<0.001** |
| Slope[c] | 3,88 | 8.324 | **<0.001** | 14.25 | **<0.001** | 0.285 | 0.836 | 12.73 | **<0.001** | 10.054 | **<0.001** |

Significant *P*-values are shown in bold type.
[a]Identifier for the individual birds entered as a random factor.
[b]Treadmill speed entered as a fixed factor.
[c]Treadmill slope (0, 10, 15 and 20% uphill) entered as a fixed factor.

Roberts et al. found that the turkey ILPO shifted function with changing slope (Roberts et al., 2007). They noted increased lengthening and decreased shortening during downhill running and decreased lengthening and increased shortening when running uphill. They hypothesized that work absorption by the muscle (negative work) predominated when the birds ran downhill and positive work output predominated during uphill running. We did not examine running on a downhill slope, but did demonstrate a decrease in active lengthening and an increase in active shortening when the birds ran uphill (Fig. 3). The shift from negative to positive strain will tend to favor work output when running uphill. However, the net work output of the ILPO will also be influenced by other features of the strain trajectory. A second factor tending to increase work is a shift to longer sarcomere lengths (Fig. 5), placing a portion of the shortening on the plateau of the length–tension curve. However, the increase in positive strain during

uphill running is accompanied by an increase in shortening velocity (Fig. 4), which would be predicted to decrease force and thus work. Of course, the net work will also be influenced by altered recruitment, and average EMG per burst and average EMG amplitude increased as incline increased (Fig. 7). On balance it seems probable that positive work per stride increases above the value in level running when the birds run uphill. This conclusion is consistent with the substantial increase in energy use by the ILPO during uphill running (Rubenson et al., 2006).

### The relationship of energy use to EMG activity changes with mechanical function

EMG amplitude has been used for many years (Inman et al., 1952; Bigland and Lippold, 1954) as the most common *in vivo* measure of the relative volume of muscle recruited. Thus, intense interest
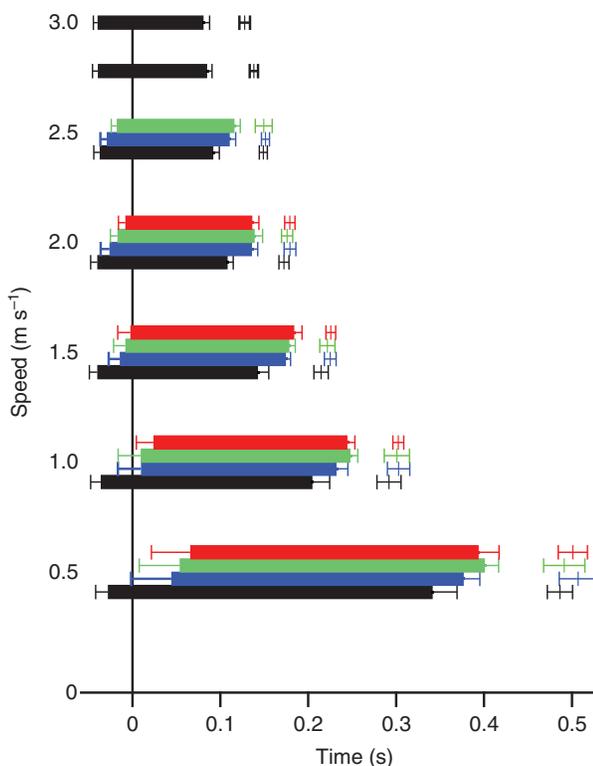


Fig. 6. The period of electromyographic (EMG) activity in the ILPO at different speeds and slopes. Zero time indicates foot-down. The mean start and stop times for the EMG burst are connected with a thick bar, with thin error bars at the ends indicating ±1 s.e.m. Values are grouped by speed on the vertical axis with the slope indicated by color (0%, black; 10%, blue; 15%, green; 20%, red). The thin bars on the right indicate the mean stance times ± 1 s.e.m.
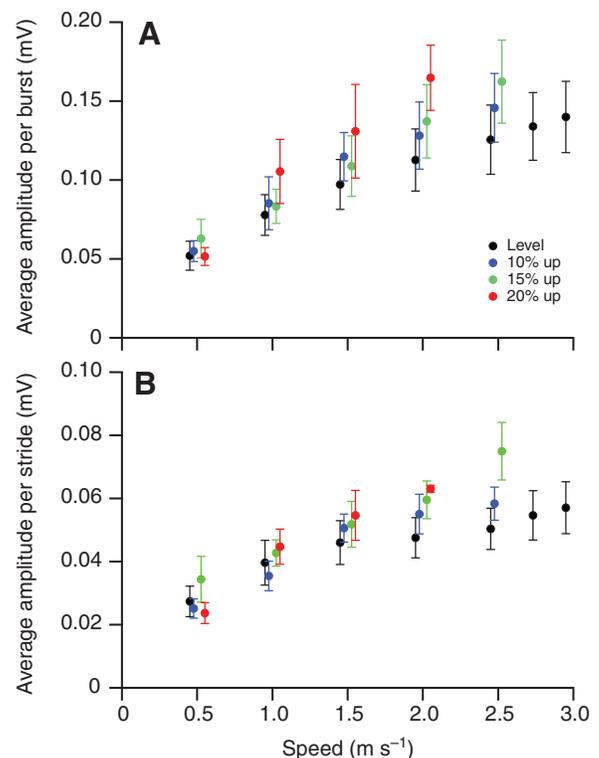


Fig. 7. Mean values for average EMG per burst (A) and average EMG values per stride (B) in the ILPO on the level (black), 10% incline (blue), 15% incline (green) and 20% incline (red) as a function of speed. Values are offset on the speed axis for clarity. Error bars indicate ±1 s.e.m.

over the years has focused on whether EMG amplitude correlates with muscle force, mechanical power output and metabolic rate (Bigland-Ritchie and Woods, 1976; De Luca, 1997; Kooistra et al., 2006; Disselhorst-Klug et al., 2009). Studies with isolated muscle (Woledge et al., 1985; DeHaan et al., 1989; Beltman et al., 2004; Trinh and Syme, 2007) and *in vivo* studies (Bigland and Lippold, 1954; Bigland-Ritchie and Woods, 1976; Aura and Komi, 1986; Takarada et al., 1997) predict that the relationship between active fiber volume, as estimated by EMG intensity, and all these variables will vary with the mechanical state of the muscle, including fiber length and shortening velocity. However, *in vivo* data for individual muscles are limited, particularly with regard to metabolic rate.

Estimates of energy use based on blood flow for all of the individual muscles in the guinea fowl hindlimb (Marsh et al., 2004; Ellerby et al., 2005; Ellerby and Marsh, 2006; Rubenson et al., 2006) allows us to compare variation in EMG intensity of the ILPO (McGowan et al., 2006) (this study) with the variation in the metabolic rate of this muscle under conditions of varying speed, slope and trunk loading. Because of the tight coupling of increases in blood flow and increases in oxygen consumption in vertebrate skeletal muscle, blood flow can be used as a quantitative measure of metabolic rate (Marsh and Ellerby, 2006). As a measure of EMG intensity, we have used averaged filtered and rectified EMG signals and for metabolic rate we have used the increases in blood flow above the blood flow at rest. To compare EMG intensity with metabolic rate we have recalculated both the EMG values and the increases in muscle blood flow relative to the values obtained in the birds running on the level at $1.5\,\mathrm{m\,s^{-1}}$ (see Materials and methods).

Our data, collected during level running, indicate that although measures of EMG intensity are correlated with energy use by the ILPO the quantitative changes in EMG intensity and metabolic rate sometimes differ widely, probably because of differences in mechanical function, and perhaps other factors (Fig. 8). The metabolic rate of the ILPO estimated from blood flow increases dramatically when the birds switch from walking to running and increases further with increasing speed during level running. When the birds walked at $0.5\,\mathrm{m\,s^{-1}}$, the metabolic rate of the ILPO was only 4% of the value found during running at $1.5\,\mathrm{m\,s^{-1}}$. When the birds increased speed from 1.5 to over $2.4\,\mathrm{m\,s^{-1}}$, the metabolic rate increased by 75%. The average EMG intensity per stride also changed substantially with running speed, but the changes were much smaller than the changes in metabolic rate. At $0.5\,\mathrm{m\,s^{-1}}$ the average EMG was ~60% of the value at $1.5\,\mathrm{m\,s^{-1}}$, and when running speed was increased to $2.78\,\mathrm{m\,s^{-1}}$ the average EMG per stride increased by only 20% over the value at $1.5\,\mathrm{m\,s^{-1}}$. The change in the relationship of EMG intensity to muscle metabolic rate with running speed corresponds to major changes in mechanical function with speed and gait. During walking at $0.5\,\mathrm{m\,s^{-1}}$ the ILPO lengthens and shortens very slowly while active, only changing length by a few percent (Fig. 3) (also McGowan et al., 2006). Both active lengthening and active shortening velocity and strain increase substantially when the birds switch to a running gait and increase further with increasing running speed (Figs 3 and 4) (McGowan et al., 2006). Isolated muscle and *in vivo* studies predict that for the same level of activation, metabolic rate will be substantially higher during active shortening than during active lengthening or isometric contractions (Aura and Komi, 1986; Beltman et al., 2004; Bigland-Ritchie and Woods, 1976; Woledge et al., 1985). Nevertheless, the magnitude of the differences between recruitment measured by EMG and muscle metabolic rate are very large, particularly when comparing the EMG intensity found during walking and with that
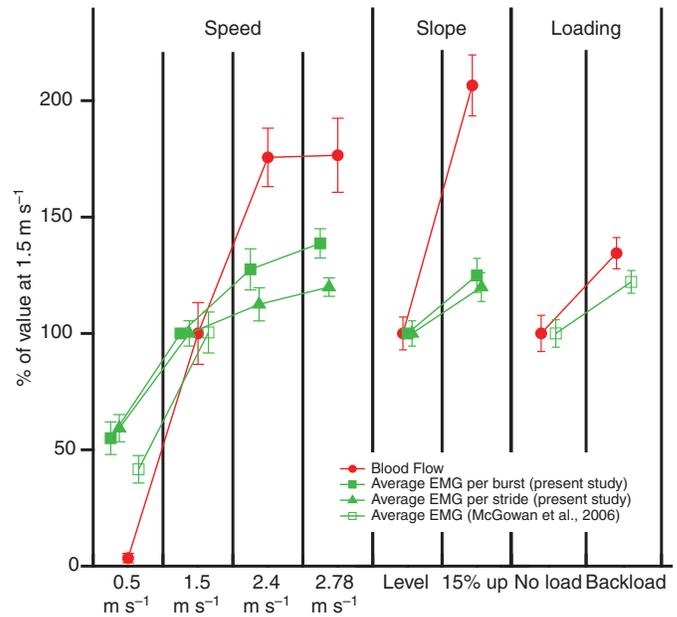


Fig. 8. Relative changes in blood flow (red) and average EMG amplitude (green) in the ILPO under various exercise conditions. Values are expressed relative to the values measured during level, unloaded running at $1.5\,\mathrm{m\,s^{-1}}$. Error bars indicate ±1 s.e.m.

obtained during running at $1.5\,\mathrm{m\,s^{-1}}$. Other factors can alter the relationship of EMG intensity to the recruited volume, including the fiber type and motor unit synchrony (Milner-Brown and Stein, 1975; De Luca, 1997; Farina et al., 2004).

The lack of quantitative correspondence between muscle recruitment measured by EMG intensity and muscle metabolic rate is also evident during uphill running. When the birds ran up a 15% grade at $1.5\,\mathrm{m\,s^{-1}}$, the average rectified EMG increased by ~20% over the value for level running at the same speed, whereas the metabolic rate of the ILPO doubled between level and uphill running on a 15% grade (Fig. 8). For uphill running, two mechanical changes probably contribute to this difference in relative values. First, the ILPO shortens farther and faster during uphill compared with level running. Second, less time is spent in active lengthening, and, therefore, more of the EMG burst serves to activate the muscle during the more costly shortening phase of activity.

Data from guinea fowl carrying trunk loads are also consistent with the hypothesis that the changes in EMG intensity and metabolic rate in the ILPO are quantitatively more similar if similar mechanical conditions are maintained when metabolic effort changes. McGowan et al. measured average EMG intensity in the guinea fowl ILPO during unloaded level running and when birds carried a trunk load of 22% of their body mass (McGowan et al., 2006). This protocol matched that used by Ellerby et al. who measured blood flow under the same conditions. In this case muscle metabolic rate, as indicated by blood flow, increased by 35% in loaded running, whereas average EMG intensity per burst increased by 22% (Ellerby and Marsh, 2006). Given the measurement errors reported in both studies the 13% difference between the relative increase in EMG intensity and the relative increase in metabolic rate is not statistically significant. Correspondingly, the mechanical conditions for the active ILPO, as indicated by the shortening velocity and strain, are similar in loaded and unloaded running (McGowan et al., 2006).

### Function of the active lengthening–shortening cycle in the ILPO

The active lengthening during knee flexion in early stance may be functionally similar to that seen in the single joint vastus lateralis of quadrupedal mammals and the femerotibialis of birds (Gillis and Biewener, 2001; Gillis et al., 2005; Hoyt et al., 2005; Roberts et al., 2007). Active lengthening of fascicles in the knee extensors of humans may also occur during stance knee flexion (Cutts, 1989), but *in vivo* data on this point are lacking. All these knee extensors co-contract with knee flexors during early stance (Ford et al., 2008; Gatesy, 1999; Gillis and Biewener, 2001; Gillis et al., 2005; Mann and Hagy, 1980; Osternig et al., 1995).

Minimizing energy use has usually been considered an important selective force in the evolution of walking and running. In this regard, substantial lengthening of the fascicles in large muscles during level legged locomotion, as occurs in the ILPO and other knee extensors during running, presents a puzzle. McGowan et al. and Roberts et al. suggested that active lengthening of the ILPO in running birds could function to improve the economy of force production and possibly the efficiency of subsequent work production (McGowan et al., 2006; Roberts et al., 2007). However, if the muscle fibers in the ILPO absorb work during early stance knee flexion this mechanical energy will have to be subsequently restored by active muscle shortening leading to an increase in energy cost. When a muscle–tendon unit is stretched, lengthening can occur in the tendon and/or the active muscle fibers. Tendon stretch and elastic recoil can save energy during legged locomotion by decreasing the positive work done by the muscle fibers during a stride (Roberts et al., 1997). However, stretch of the muscle fibers beyond their short-range stiffness results in mechanical energy loss. Small stretches during activation may increase net work output during subsequent shortening (Josephson, 1993), but during longer stretches mechanical energy is lost because muscle force is enhanced during stretch and decreases during shortening (Chapman et al., 1985; Ettema et al., 1990; Ettema, 1996; Takarada et al., 1997).

If the volume of actively stretched muscle is large, supplying the mechanical work to lengthen the active fibers could have a substantial metabolic cost and decrease the economy of locomotion. Although the metabolic rate of the active muscle during stretch is expected to be relatively low (Bigland-Ritchie and Woods, 1976), costly positive muscle work will have to be done to restore the mechanical energy balance during the stride. Positive muscular work will be required either to directly supply the mechanical work during the stretch, or to replace the mechanical work supplied from another source. For example, if work was supplied in early stance from the kinetic energy of the body, this energy would have to be replaced by positive work later in the stride. In the case of the ILPO, fiber lengthening is caused by active knee flexion. During this part of the stride, inverse dynamics calculations in quail (Clark and Alexander, 1975), turkeys (Roberts, 2001) and guinea fowl (R.L.M., unpublished data) indicate that net positive work is required to produce knee flexion. The work to stretch active knee extensors will add to this externally calculated work and thus must be provided directly by the knee flexors, increasing their energy use. The hypothesis that co-contraction increases metabolic cost is supported by evidence from humans (Frost et al., 2002; Mian et al., 2006).

If active lengthening of the knee extensors in early stance costs metabolic energy, it seems likely that this muscle activity has balancing beneficial functions. We hypothesize that the system of co-contracting muscles at the knee, including the ILPO, functions to improve stability by increasing the mechanical impedance (stiffness and damping) of the joint. The ILPO early in stance is actively lengthened by the co-contracting knee flexors, including the flexor cruris lateralis and the posterior iliofibularis (Gatesy, 1999; Hoogendyk, 2005). Impedance control has been identified as a major neural control strategy (Burdet et al., 2001; Full and Kotitschek, 1999; Hogan, 1985) that allows stable kinematic trajectories in the face of externally (Wagner and Blickhan, 2003) or internally (Selen et al., 2005) generated variability in joint moment. Numerous human studies suggest that co-contraction increases in situations that increase loading variability or uncertainty, and thus require higher joint impedance for stable movement (Hewett et al., 2005; Lamontagne et al., 2000).

## LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| ILPO | iliotibialis lateralis pars postacetabularis |
| $L_0$ | optimal length (length at the middle of the plateau region of the length–tension curve) |
| $L_{max}$ | maximum active length |
| $L_{min}$ | maximum active length |
| $L_{sc}$ | *in vivo* sarcomere length |
| $L_{sc,ref}$ | reference sarcomere length |
| $L_{so}$ | *in vivo* segment lengths measured by sonomicrometry |
| $L_{so,ref}$ | sonomicrometer length |

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