Mechanical function of two ankle extensors in wild turkeys: shifts from energy production to energy absorption during incline *versus* decline running

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Summary

We investigated the mechanical function of two ankle extensor muscles, the lateral gastrocnemius (LG) and peroneus longus (PL), in wild turkeys *Meleagris gallopavo* during steady speed running. We hypothesized that mechanical work output of the LG and PL during running parallels the demand for mechanical work on the body. The turkeys ran on level, inclined ($+6°$, $+12°$) and declined ($-6°$, $-12°$) treadmills to change the demand for mechanical work. Simultaneous measurements of muscle length (from sonomicrometry) and muscle force (from tendon strain gauges) were used to calculate mechanical work output.

During level running at a speed of $2 \ m \ s^{-1}$, the LG and PL were both active in stance but produced peak force at different times, at approximately 21% of stance duration for the LG and 70% for the PL. The LG and PL also had different length patterns in stance during level running. The LG underwent little shortening during force production, resulting in negligible net positive work ($2.0±0.8 \ J \ kg^{-1}$). By contrast, the PL produced force across a stretch–shorten cycle in stance and did significant net positive work ($4.7±1.6 \ J \ kg^{-1}$). Work outputs for both the LG and PL were directly proportional to running slope. When we increased the demand for net positive work by running the turkeys on an incline, the LG and PL increased stance net positive work output in direct proportion to slope ($P<0.05$). Stance net positive work output increased to $7.0±1.3 \ J \ kg^{-1}$ for the LG and $8.1±2.9 \ J \ kg^{-1}$ for the PL on the steepest incline. Increases in stance net positive work for the LG and PL were associated with increases in net shortening strain and average shortening velocity, but average force in stance remained constant. The LG and PL muscles were also effective energy absorbers during decline running, when there is demand for net negative work on the body. During decline running at $2 \ m \ s^{-1}$ on the steepest slope, the LG absorbed $4.6±2.2 \ J \ kg^{-1}$ of net work in stance and the PL absorbed $2.4±0.9 \ J \ kg^{-1}$ of net work. Shifts in muscle mechanical function from energy production during incline running to energy absorption during decline running were observed over a range of running speeds from $1–3 \ m \ s^{-1}$ for both the LG and PL.

Two fundamentally different mechanisms for changing work output were apparent in the mechanical behavior of the LG and PL. The LG simply altered its length pattern; it actively shortened during incline running to produce mechanical energy and actively lengthened during decline running to absorb mechanical energy. The PL changed mechanical function by altering its length pattern and by shifting the timing of force production across its stretch–shorten cycle. During incline running, the PL produced force during late stance shortening for positive work, but during decline running, the timing of force production shifted into early stance, to align with lengthening for negative work. In addition, during decline running, the PL greatly reduced or eliminated late stance shortening, thus reducing the potential for positive work.

Our results show that the changing demands for whole body work during steady speed running are met, at least in part, by an ability of single muscles to shift mechanical function from net energy production to net energy absorption.

Introduction

The muscles that power animal movement must perform a wide range of mechanical functions. Many muscles perform mechanical work. For example, the scallop adductor muscle performs hydrodynamic work as it forcefully shortens to rapidly close the shell and propel the animal through water (Marsh et al., 1992). Flight also requires work to overcome fluid forces, and measurements of pigeon pectoralis indicate that it performs work-intensive shortening contractions to power the wing in the downstroke of flight (Dial and Biewener, 1993; Biewener et al., 1998a). By contrast, muscles that produce force while lengthening absorb net mechanical energy. Studies of *in vivo* and *in vitro* muscle function have produced

evidence for energy absorbing contractions in a cockroach leg muscle during running (Ahn and Full, 2002), and in muscles involved in steering during flight in blowflies (Tu and Dickinson, 1994). Finally, isometric contractions produce force, but neither absorb nor produce mechanical energy. These contractions might be expected during activities where the demands for force are high but little mechanical energy is required to maintain movement. Running and hopping on level ground require little mechanical energy to maintain steady speed movement, and some ankle extensors in running turkeys (Roberts et al., 1997) and hopping wallabies (Biewener et al., 1998b) have been shown to produce force with nearly-isometric contractions. These energy producing, energy absorbing, and isometric contractions described above represent fundamentally different mechanical functions, and the locomotor muscles that perform these functions have been conveniently referred to as motors, brakes and struts (Ahn and Full, 2002; Dickinson et al., 2000; Roberts et al., 1997).

Here, we investigate whether individual muscles can perform all three mechanical functions – motor, brake and strut – depending upon the demand for locomotor work. We measured force and work output during running in two ankle extensors, the lateral gastrocnemius and peroneus longus. Architecturally, these muscles include features that have been suggested as favorable for economic force production, including long tendons (Alexander, 1974; Biewener and Roberts, 2000), a short pinnate fiber architecture (Biewener and Roberts, 2000), and many of them cross two joints (van Ingen Schenau, 1989). Some studies suggest that these features, common to the distal limb muscles in general, may constrain them to a role as force producers, rather than work producers. For example, Alexander (1974) showed that jumping dogs increased the work output of proximal limb muscles to power jumping, but the ankle extensors produced high forces and low work outputs independent of the demand for work for different tasks. However, a previous study showed that the lateral gastrocnemius muscle in running turkeys altered mechanical function with running slope, acting as a strut during level running and as a motor when the turkeys ran uphill (Roberts et al., 1997).

We hypothesized that the mechanical work output of the lateral gastrocnemius (LG) and peroneus longus (PL) muscles in wild turkeys, *Meleagris gallopavo*, parallels the demand for mechanical work on the body during running. This hypothesis is based on the fact that both are stance phase muscles, and thus have the potential for altering the mechanical energy of the body. Hindlimb joint excursions in wild turkeys occur largely at the knee and ankle joints during running, further suggesting that the LG (a biarticular muscle that acts as a knee flexor and ankle extensor) and PL (an ankle extensor and third toe flexor) may play an important role in modulating work output of the whole body. We ran wild turkeys on level, inclined and declined treadmills to change the demand for mechanical work. Running on level ground at steady speed involves cyclical fluctuations in the energy of the body, but the net work required in each step is negligible. Running on an incline requires net mechanical energy production (positive work) with each step to increase the potential energy of the body, whereas decline running requires energy absorption (negative work) to decrease the energy of the body. Thus, we predicted that the LG and PL would produce force near-isometrically during level running, produce net mechanical energy during incline running, and absorb net mechanical energy during decline running.

Materials and methods

Animals and treadmill training

Adult female Eastern wild turkeys *Meleagris gallopavo* L. were obtained from a local breeder in Reedsport, Oregon, USA and housed in an outdoor enclosure. Food and water were provided *ad libitum*. Mean body mass was 3.51 ± 0.39 kg ($\pm$s.d.), N=6. Treadmill training consisted of running on a level, inclined ($+12°$) and declined ($-12°$) motor-driven treadmill (Keys Pro 2000 Series, Keys Fitness Products, Dallas, TX, USA) for 10–20 min day^{-1}, 4–5 days a week, for about 4–6 weeks. Birds ran on each slope on alternate days at speeds of 1–3 m s^{-1}. A wooden box with a Plexiglas window for video imaging, and an opening at the back for access to the bird, was placed around the edges of treadmill track. All animal use was approved by the Oregon State University Institutional Animal Care and Use Committee and in accordance with federal and institutional guidelines.

Surgery

Animals were induced and maintained on inhaled isoflurane anesthesia and a sterile environment was maintained for all surgical procedures. A pair of sonomicrometry crystals (Sonometrics, Inc., London, ON, Canada) 1 mm or 2 mm diameter in size (we began with the 1 mm size and found that the 2 mm worked better for eliminating level shifts) were implanted into small pockets made with a 16-gauge hypodermic needle within each muscle, to a depth of about 3 mm along the axis of a proximal fascicle. The crystals were aligned 8–12 mm apart, secured in place with a small drop of 3 mol l^{-1} Vet-bond glue and the wire leads were sutured to the muscle's fascia, the thin fascia associated with the fascicles, using 6-0 silk suture. Two bipolar, hooked electromyographic (EMG) electrodes, constructed of silver wire, with 1 mm of insulation removed from the tips, were implanted within each muscle near the sonomicrometry crystals using a 25-gauge hypodermic needle. The leads were sutured to the muscle's fascia using 6-0 silk suture. Two small strain gauges (Type FLK-1-11, Tokyo Sokki Kenkyujo Co., Ltd.) were glued to the superficial and deep aspects of the bony tendon of each muscle. The calcified tendons were prepared for gluing by gently scraping and then defatting the surface with chloroform. A thin layer of cyanoacrylate adhesive (Duro superglue, SUP-5; Loctite Corp., Avon, OH, USA) was applied to each strain gauge and it was pressed onto the tendon for 1 min for bonding. All transducer wires were
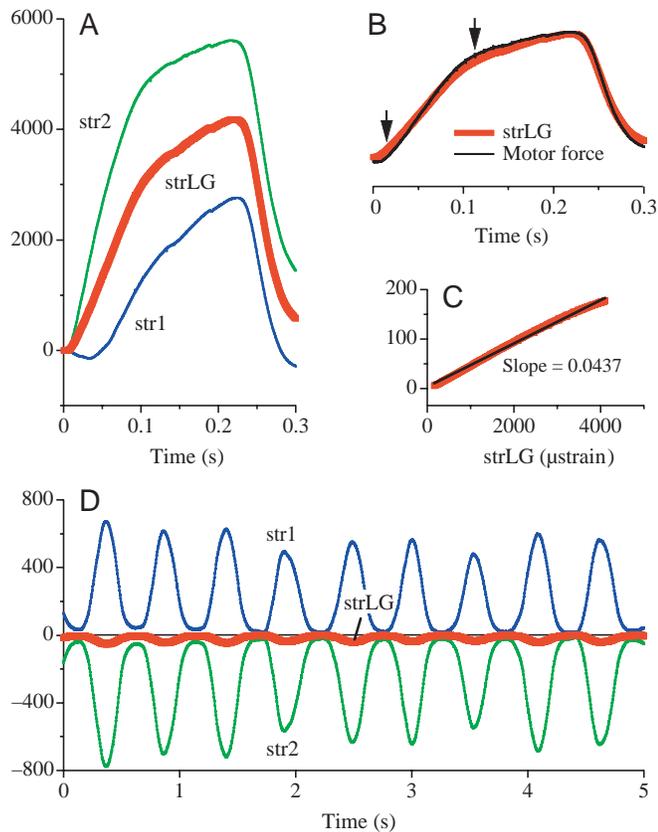
Fig. 1. *In situ* calibration of tendon strain to muscle force for the lateral gastrocnemius, LG, in one turkey. The sciatic nerve was electrically stimulated to generate a muscle contraction while tendon strain and muscle force were measured simultaneously. (A) Strain values for the deep (str1) and superficial (str2) aspects of the bony tendon were averaged (strLG) to account for bending. Muscle force was measured by attaching the free distal end of the tendon to a servomotor. (B) Tendon strain and muscle force during stimulation. The region where the muscle was developing force (indicated by the arrows) was the segment used for calibration. (C) Relationship of muscle force and tendon strain. The slope of the line was determined using linear regression analysis. (D) Pure bending of the bony tendon in the region where the strain gauges were glued. The deep (str1) and superficial (str2) tendon strains are averaged (strLG) to cancel most of the strain due to pure bending.

routed subcutaneously from the muscle to a small skin incision near the middle of the synsacrum. The incision was closed and small electrical connectors (Microtech, Inc., Boothwyn, PA, USA) were secured to the skin with 3-0 silk suture. Animals were allowed to recover from surgery for 24–48 h before treadmill running experiments.

Running experiments

Measurements were taken as the birds ran on a level treadmill, followed by runs on an incline (+6° and +12°) and decline (–6° and –12°), at speeds of 1–3 m s^{-1}. Trials were generally started at 1 m s^{-1} and worked up to 3 m s^{-1} in 0.5 m s^{-1} speed increments. Ten seconds of data were collected for each run. Birds remained on the treadmill at

slow walking speeds between speed and slope changes and they were rested when needed. Fascicle lengths were recorded by sonomicrometry at a frequency of 992 Hz using the data acquisition software SonoLAB. Muscle EMG signals were amplified 1000× using a DAM50 differential preamplifier (World Precision Instruments, Sarasota, FL, USA) with high- and low-bandpass filters of 3 Hz and 10 kHz, respectively. The EMG signals were subsequently filtered in software with a custom-designed FIR filter (pass band 150–1000 Hz). Tendon strain signals were amplified using a strain gauge conditioner (model 2120, Vishay Measurements Group, Raleigh, NC, USA). Data were collected at a frequency of 4000 Hz to a Macintosh computer with a 12-bit A/D converter (PCI-MIO-16-1, National Instruments, Austin, TX, USA) using the software program IGOR Pro (WaveMetrics, Inc., Lake Oswego, OR, USA). High speed video was recorded at 250 frames s^{-1} with a Redlake Imaging MotionScope (model 1000S; Morgan Hill, CA, USA).

In situ *calibration of muscle force*

Tendon strains were calibrated to muscle force *in situ* at the end of running experiments. The procedure involved electrically stimulating the muscle *via* the sciatic nerve while simultaneously measuring whole muscle force and tendon strain. The birds were kept under deep anesthesia with isoflurane gas during the experiments and body temperature was maintained at 38–40°C. The sciatic nerve was isolated and severed at the proximal end, then placed across two silver wires in a nerve cuff. Mineral oil was poured around the nerve and the skin incision was sutured closed. The muscle origin was fixed in place by means of two bone screws inserted into the femur and attached to an aluminum frame. To calibrate muscle force, the tendon was cut free at its insertion and attached to an aluminum clamp connected to a servomotor (model 310B-LR, Aurora, Ontario, Canada), which is a calibrated force-measuring device. The sciatic nerve was stimulated with a Grass S48 stimulator (6–7 V supra-maximal stimulation voltage, 100 Hz frequency, and 250 ms train duration).

A representative calibration of muscle force for the LG in one bird is shown in Fig. 1A–C. Superficial and deep tendon strains were averaged and muscle force, measured using the servomotor, was plotted against average tendon strain. The period of time when the muscle was developing force was used for calibration. The slope of the line relating muscle force to average tendon strain was determined by linear regression analysis. Pearson's correlation coefficient values for LG and PL muscle calibrations were $r^2 \geq 0.96$. Our measurement of muscle force from tendon strain requires that measured strain is due to tensile stress applied to the tendon by the muscle, rather than tendon bending. Averaging the signals from the gauges on the superficial and deep aspects of the tendon should cancel out any strain due to bending, as pure bending imposes a tensile stress on one side of the tendon and a compressive stress on the other. To determine the effectiveness of this

cancellation, we manually imposed pure bending on the tendon following the force calibration (Fig. 1D). Averaging the two strain signals removed approximately 90% or more of tendon strain due to bending. Thus, even if significant bending occurs during running, the influence of bending on our calculated force values should be minimal.

Data analysis and statistics

All wave analyses were performed using the software program IGOR Pro (WaveMetrics, Inc.). Sonomicrometry signals were smoothed using the interpolation function (smoothing spline, with a smoothing factor of 1.0 and standard deviation of 0.01–0.025). Fascicle segment length (L), the distance between the two crystals, was differentiated to calculate instantaneous velocity. Fascicle segment length was expressed relative to the resting segment length (L_o), which was calculated by averaging the maximum and minimum lengths in swing. Segment velocity was also expressed in relative units of length ($L\,s^{-1}$). To determine muscle power, we first calculated total muscle fascicle velocity by multiplying the fascicle segment velocity by the ratio of total fascicle length to measured segment length. Power was calculated as the product of muscle force and fascicle velocity, and is expressed relative to muscle mass ($W\,kg^{-1}$). Net work ($J\,kg^{-1}$) was calculated by integrating power over time. We focused our analysis on changes in net work performed at each incline, because it is the net work performed by muscle that changes the body's energy. Though elastic tendons can cycle mechanical energy during running, they cannot perform or absorb *net* mechanical work, and therefore cannot contribute to net energy changes required at different inclines.

For each animal, 10 strides per run were averaged for analysis. We analyzed all values (net work, average force, average velocity and net fascicle strain) over the stance phase for the LG and PL during running at $2\,m\,s^{-1}$. We focused on muscle function during stance, when muscle work can act to increase or decrease the body's mechanical energy. Additional analyses were performed over the period of force production in stance for the LG, and over the two regions of the stretch–shorten cycle in stance for the PL. To determine the influence of running slope on the measured variables, we used the program Systat (version 7.0 for the PC) to perform a two-way mixed-model analysis of variance (ANOVA), with slope and individual as main effects. The F-ratio for the main effect of slope was calculated as the mean square for slope divided by the mean square for slope × individual interaction term (Zar, 1997). We also performed linear regressions on mean values, with slope as the independent variable. The criterion for statistical significance was $P \leq 0.05$. Summary data are presented as the mean ± S.E.M.
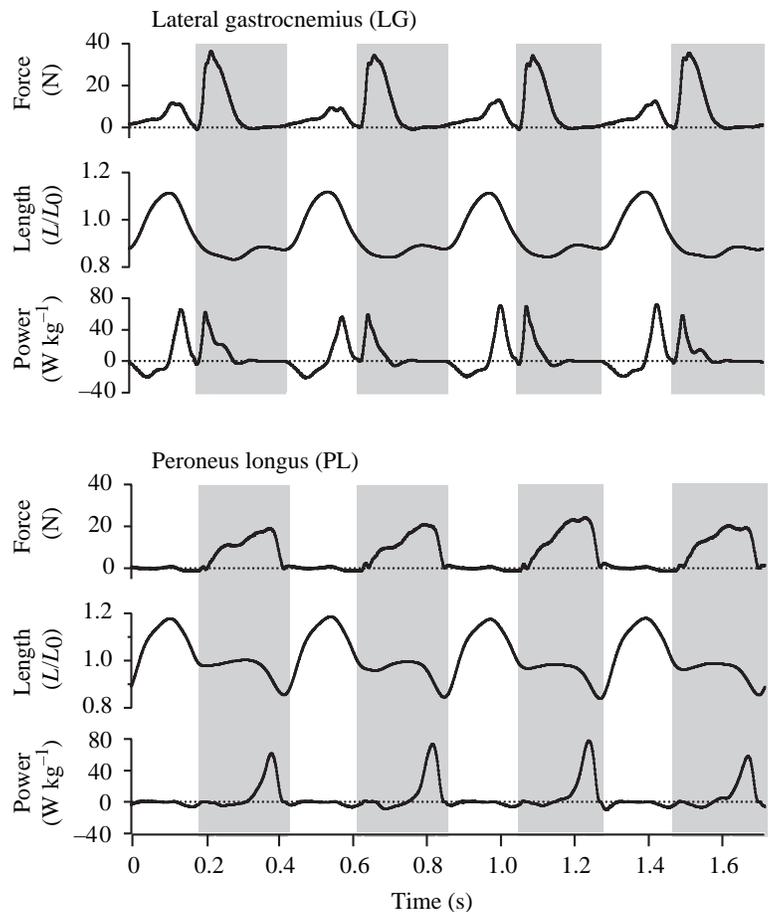

Fig. 2. Muscle force, fascicle length and power output for the lateral gastrocnemius, LG, and peroneus longus, PL, muscles in a turkey running at a steady speed of $2 \cdot m \cdot s^{-1}$ on level ground. Shaded areas are stance, when the foot is in contact with the ground; unshaded areas are swing, when the foot is off the ground and being repositioned for the next step. L, fascicle length; L_0, resting segment length.

Results

Force and length patterns of LG and PL during level running

Muscle force and fascicle length changes for the LG and PL in a turkey running at $2\,m\,s^{-1}$ on level ground are shown in Fig. 2. The LG and PL both actively produced force in stance, but the timing of force production was different for the two muscles. Peak force occurred earlier in stance for the LG, at $21.1 \pm 0.8\%$ of stance duration, and later in stance for the PL, at $69.7 \pm 1.6\%$ of stance duration. Fascicle length changes in stance were also quite different for the LG and PL. The LG actively shortened a little in early stance, by $4.6 \pm 1.8\%$ of resting length (Fig. 5), whereas the PL produced force across a stretch–shorten cycle from early to late stance. The stretch–shorten cycle was not symmetrical, however, and neither was the distribution of force across the cycle. The PL did more shortening *versus* lengthening and thus underwent a net shortening strain of $11.0 \pm 2.0\%$ of resting length (Fig. 5). In addition, PL produced a higher force during shortening *versus* lengthening across the stretch–shorten cycle.
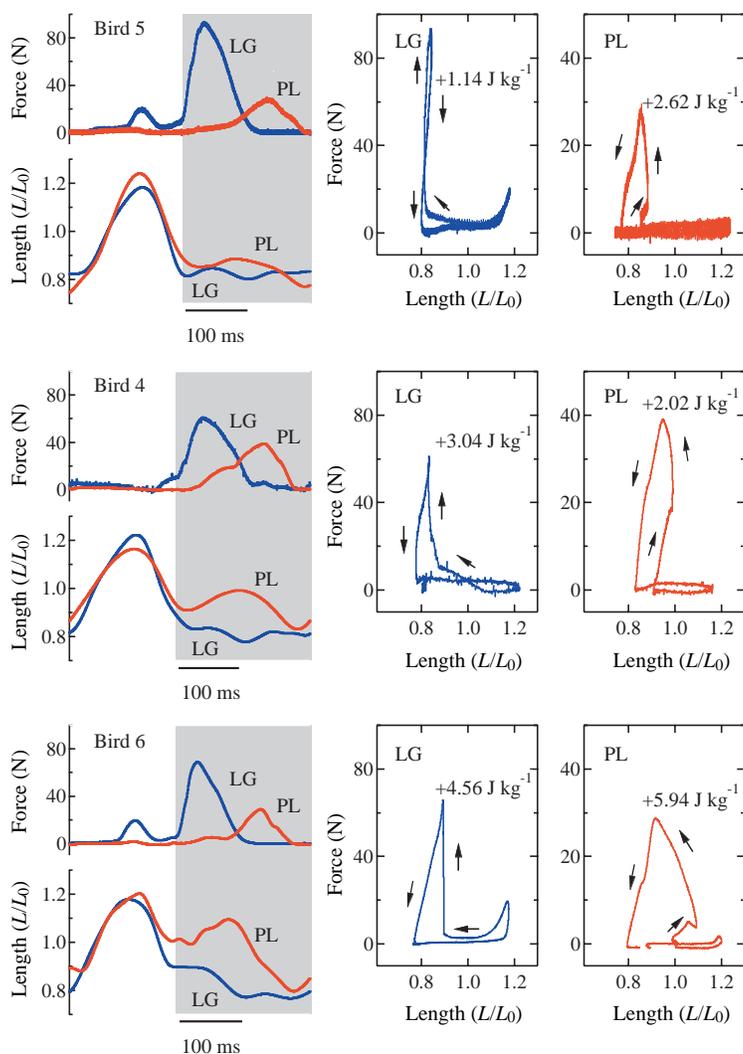
Fig. 3. Muscle force and fascicle length for the lateral gastrocnemius (LG) and peroneus longus (PL) muscles in three turkeys running at a steady speed of 2 m s^{-1} on level ground. Shaded areas are stance; unshaded areas are swing. Force–length relationships for the LG and PL over one complete stride (swing and stance) are also shown for each bird. Arrows in a counterclockwise direction indicate net positive work in the work loop. Arrows for PL at the start of stance indicate a small fraction of active lengthening performed in the stretch–shorten cycle. L, fascicle length; L_0, resting segment length.

Muscle power and work during level running

Instantaneous muscle power outputs for the LG and PL during level running were calculated from the force and velocity data and are shown for one bird in Fig. 2. Positive values represent mechanical energy production as force is produced during shortening and negative values represent mechanical energy absorption during lengthening contractions. The LG produced energy during late swing and in early stance. The PL absorbed some energy in early stance and produced energy in late stance as it actively stretched–shortened. Muscle net work (Fig. 5) was calculated by integrating power over time. Stance net positive work output for the LG averaged

2.0 ± 0.8 J kg^{-1} at a running speed of 2 m s^{-1}, but this value was not significantly different from a value of zero ($P>0.05$). The PL had a twofold higher stance net positive work output of 4.7 ± 1.6 J kg^{-1} at 2 m s^{-1} and work output was significantly higher than a value of zero ($P<0.05$). Stance net positive work for the PL reflected the sum of a little negative work (energy absorption) in early stance and much higher positive work (energy production) in late stance.

Muscle net work output for the LG and PL muscles showed some individual variation between birds. Force–length relationships for the LG and PL in three different turkeys running on level ground at a speed of 2 m s^{-1} are shown in Fig. 3 to illustrate the range of variation between individual birds. Work loops for the LG show that some birds produced force nearly isometrically and others produced force with some shortening, resulting in individual differences in stance net work output. Work loops for the PL show that force was produced across a stretch–shorten cycle for all birds during level running, but the shortening strain and/or force magnitude differed between individual birds to affect stance net work output. The brief fascicle lengthening at the start of stance indicates the small fraction of mechanical energy absorbed by the PL during lengthening in the stretch–shorten cycle. The individual variation in muscle length patterns is unlikely to be due to recording from functionally different sites within the muscles. The sonomicrometry crystals were always implanted into a proximal fascicle, just above the region where the aponeurosis forms an apex. This is a reliable anatomical landmark for implanting crystals. Instead, there may be subtle differences in running kinematics that account for the variation in muscle length patterns observed in different birds.

Muscle work output during incline and decline running

The LG and PL muscles increased stance net positive work output during 12° incline *versus* level running at speeds of 1–3 m s^{-1} (Fig. 4). During 12° decline running, both muscles shifted mechanical function and instead performed net negative work in stance (Fig. 4). The speed of 2 m s^{-1} was selected for more detailed analysis of muscle mechanical function during incline and decline running on 6° and 12° slopes (Fig. 5). At 2 m s^{-1}, stance net work output of the LG and PL muscles changed significantly in relation to running slope ($P<0.01$, LG and $P<0.05$, PL). When the birds ran on an incline, the LG and PL did increasingly greater amounts of net positive work on steeper slopes (Fig. 5). The LG increased net positive work output by 3.5-fold from level to 12° incline running (2.0 ± 0.8 *vs.* 7.0 ± 1.3 J kg^{-1}) and the PL increased net positive work output by 1.7-fold from level to 12° incline running (4.7 ± 1.6 *vs.* 8.1 ± 2.9 J kg^{-1}). When the birds ran on a decline, the LG and PL did increasingly greater amounts of net negative work
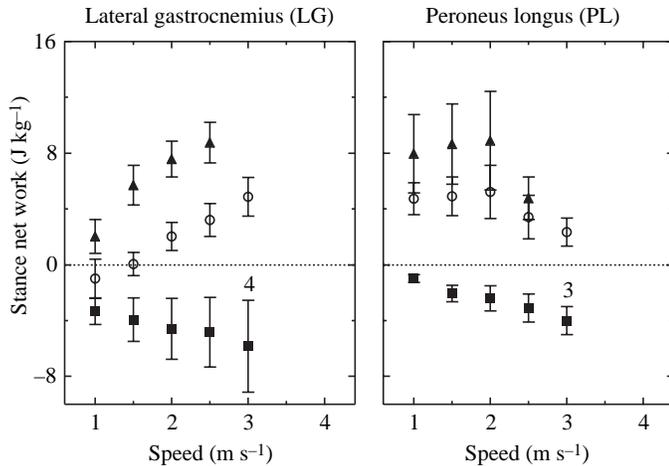
Fig. 4. Stance net mechanical work output for the lateral gastrocnemius (LG) and peroneus longus (PL) muscles during steady speed running at 1–3 m s⁻¹ on the level (open circles), 12° incline (filled triangles) and 12° decline (filled squares). Values are means ± S.E.M.; *N*=5 birds for the LG and *N*=4 for the PL; numbers above a symbol indicate a different number of birds at that speed. Across a range of running speeds, both the LG and PL performed greater net positive work in stance during incline *versus* level running, and they developed net negative work in stance during decline running.

on steeper slopes (Fig. 5). On the steepest decline of 12°, stance net negative work averaged 4.6±2.2 J kg⁻¹ for LG and 2.4±0.9 J kg⁻¹ for PL.

Muscle force, velocity and strain

Muscle work is influenced by four main variables: muscle force, fascicle strain and velocity, and the timing of force relative to the length trajectory. We calculated all four variables to determine the mechanisms for how the LG and PL muscles perform net positive work during incline running and net negative work during decline running. For this analysis, we focused on the 2 m s⁻¹ values for average muscle force, net fascicle strain, and average muscle velocity in stance (Fig. 5). Average muscle force did not change significantly with running slope for either the LG or PL (P>0.05, LG and PL). By contrast, net fascicle strain in stance changed significantly with the running slope (P<0.01, LG and PL). For LG, net fascicle strain in stance, expressed as a percentage length change relative to the resting fascicle length, increased from 4.6±1.8% shortening during level running to 15.6±2.0% shortening during 12° incline running, and changed to a net lengthening strain of 4.0±3.3% during 12° decline running. Net fascicle strain for the LG during stance force production only (shown by the open circles), was similar to net fascicle strain calculated over the entire period of stance. Net fascicle strain in stance for the PL changed from 11.0±2.0% shortening (level running) to 19.0±3.9% shortening (12° incline) and 7.1±1.5% lengthening (12° decline). Associated with the changes in net fascicle strain, average muscle velocities in stance changed significantly in relation to running slope (P<0.01, LG and PL). The LG and PL developed positive (shortening) velocities during incline
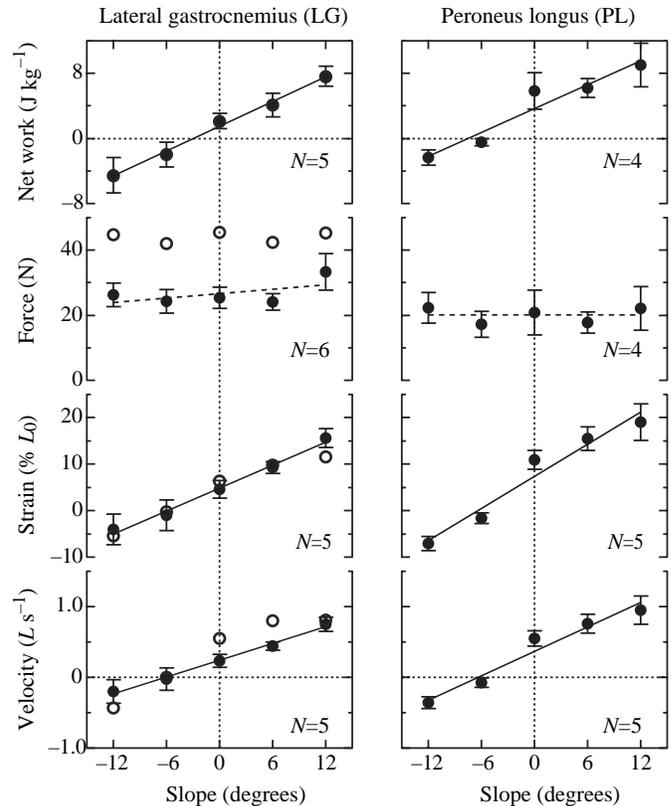

Fig. 5. Mean±S.E.M. values of net work, average force, net fascicle strain, and average velocity for the lateral gastrocnemius (LG) and peroneus longus (PL) muscles in all turkeys running at a steady speed of 2·m·s⁻¹ on a level, incline, and decline. Filled symbols for the LG and PL are averages over the period of stance; open symbols for the LG are averages over the period of force production in stance. For clarity, S.E.M. values for open symbols are not shown; hidden open symbols for LG net work are the same as the stance values. Net work output for the LG and PL changed significantly with running slope (P<0.05; r²=0.99 for LG and r²=0.97 for PL). On steeper inclines, LG and PL did increasingly greater amounts of net positive work; on steeper declines, both muscles did increasingly greater amounts of net negative work. Average muscle force in stance remained constant across all slopes for both the LG and PL (P>0.05), whereas net fascicle strain, expressed as percentage length change relative to resting fascicle length, and average muscle velocity changed significantly with running slope, in parallel with the changes in net work output. For net fascicle strain, P<0.01; r²=0.99, LG and r²=0.98, PL; for average muscle velocity, P<0.01; r²=0.99, LG and r²=0.98, PL. L, fascicle length; L₀, resting segment length.

running and negative (lengthening) velocities during decline running.

Timing of peak force production and length pattern as mechanisms for shifting mechanical function

The LG and PL muscles used two fundamentally different mechanisms to shift mechanical function from net energy production during incline running to net energy absorption during decline running. Representative recordings of muscle force and fascicle length for a turkey running on a level,
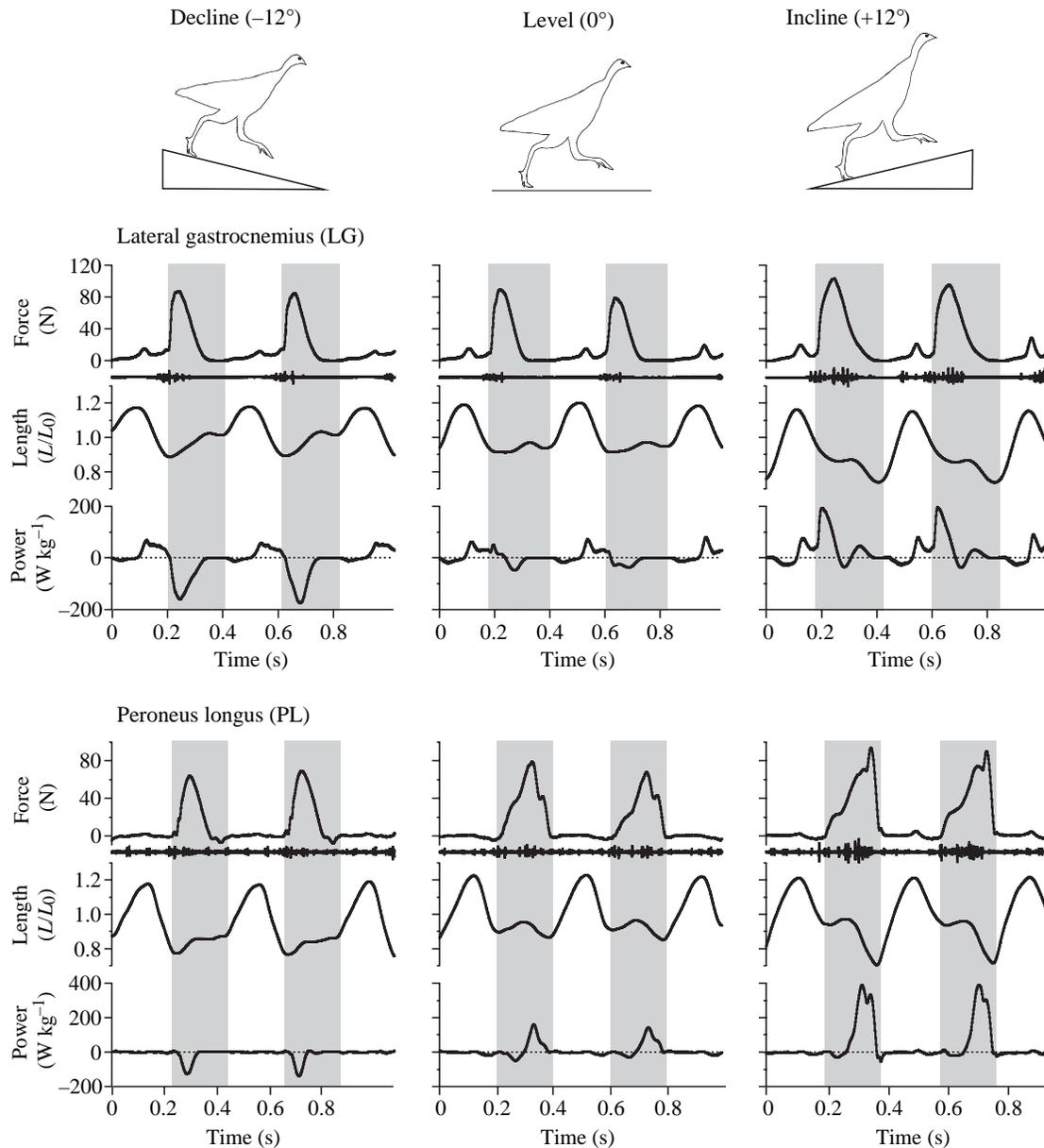
Fig. 6. Muscle force, electromyographic (EMG) activity, fascicle length, and power output for the lateral gastrocnemius, LG, and peroneus longus, PL, muscles in two different turkeys running at a steady speed of $2 \cdot m \cdot s^{-1}$ on a declined ($-12°$), level, and inclined ($+12°$) treadmill. Shaded areas are stance; unshaded areas are swing. Mechanical energy production (positive power) in stance increased during incline *versus* level running for both the LG and PL, as muscle shortening increased over their periods of force production. During decline running, both the LG and PL shifted mechanical function and instead absorbed mechanical energy in stance by forcefully lengthening. L, fascicle length; L_0, resting segment length.

inclined and declined treadmill illustrate the different mechanisms that LG and PL muscles used to change mechanical work output. The LG simply altered its length pattern in early stance to change mechanical work output. To increase net positive work output from level to incline running, the LG simply increased shortening, and to do net negative work during decline running, the LG instead actively lengthened. The timing of peak force production for the LG did not change significantly in relation to running slope ($P>0.05$). For all slopes, the timing of peak force production

for the LG occurred in early stance, at approximately 21% of stance duration (Fig. 7B).

The PL muscle changed mechanical work output by altering its length pattern and/or by shifting the timing of peak force production, depending upon the running slope. To increase net positive work output from level to incline running, the PL increased late stance shortening, while the timing of peak force production remained the same (Fig. 6). During decline running, the PL substantially reduced or eliminated late stance shortening in the stretch–shorten cycle, and shifted the timing

of peak force production from late stance into early stance to correlate with fascicle lengthening. The shift in timing of peak force production resulted in a marked increase in net energy absorption in early stance during decline running, as shown by the larger pulse of negative power (Fig. 6).

The shift in timing of peak force production for the PL muscle is illustrated for one bird in Fig. 7. The LG and PL muscle force and length patterns are superimposed, showing the earlier timing of peak force production for the LG *versus* PL muscle during level and incline running. During decline running, the shift in timing of peak force production for the PL more closely aligns LG and PL muscle forces in early stance, and aligns high PL muscle forces with lengthening in the stretch–shorten cycle, resulting in increased mechanical energy absorption.

Individual regions of the stretch–shorten cycle for the PL muscle were analyzed for average force, net fascicle strain and net work output (Fig. 8). Over the *stretch* region of the cycle (Fig. 8A), lengthening strain increased during decline running, but only by a small amount, from –6.2±1.9%, level running, to –9.1±0.6%, 12° decline running. A major factor leading to an increase in work absorption during decline running was the

threefold increase in average muscle force during muscle lengthening, reflecting the shift in timing of peak force production (Fig. 7). Muscle strain was an important determinant of muscle work output during incline running. Over the *shortening* region of the cycle (Fig. 8B), shortening strain increased to as much as 21.7±3.4% during 12° incline running.

Discussion

We investigated the mechanical function of two ankle extensors in wild turkeys during running. The lateral gastrocnemius (LG) and peroneus longus (PL) are of similar mass and fiber length, have a pennate fiber architecture, and transmit force *via* long compliant tendons. We ran the turkeys on different surface slopes to study muscle force and length patterns, and tested the hypothesis that mechanical work output of the individual muscles would change in parallel with the demand for work on the body. Our results support this hypothesis. When the birds ran on an incline, the LG and PL muscles increased net positive work output to function like motors; and when the birds ran on a decline, both muscles did
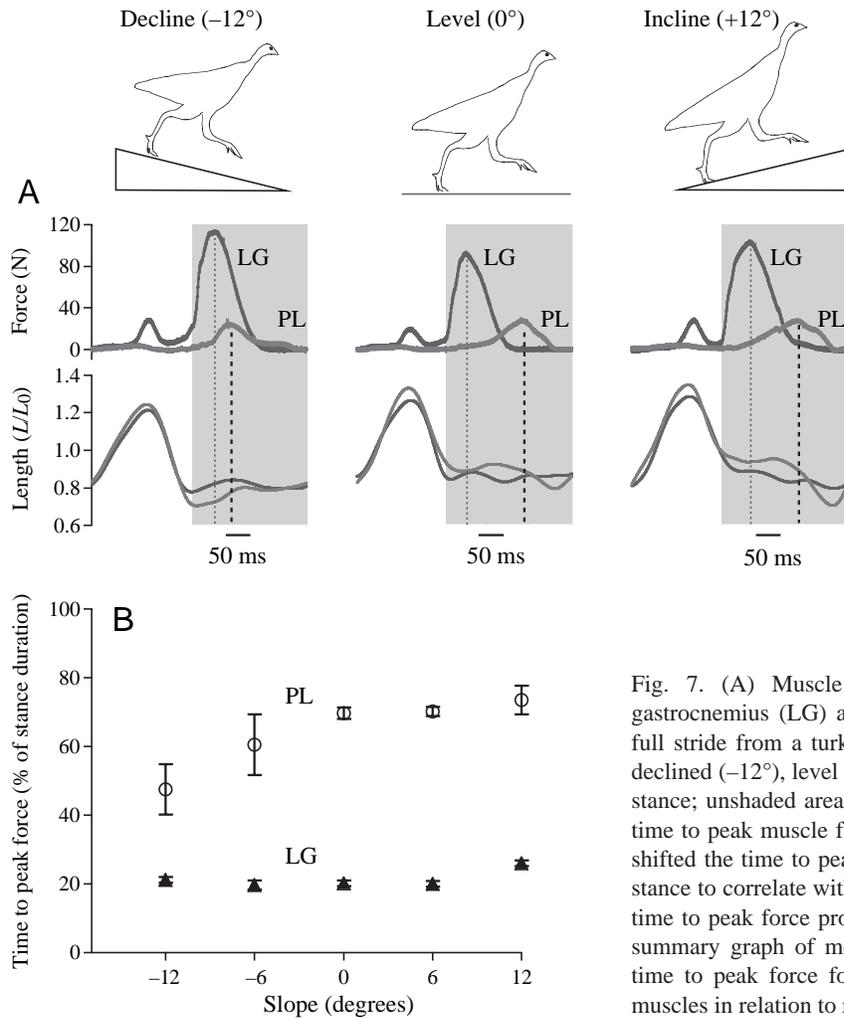

Fig. 7. (A) Muscle force and fascicle length for the lateral gastrocnemius (LG) and peroneus longus (PL) muscles during one full stride from a turkey running at a steady speed of 2·m·s⁻¹ on a declined (–12°), level and inclined (+12°) treadmill. Shaded areas are stance; unshaded areas are swing. Dashed vertical lines indicate the time to peak muscle force in stance. During decline running, the PL shifted the time to peak force production from late stance into early stance to correlate with muscle lengthening. The LG did not shift the time to peak force production in response to decline running. (B) A summary graph of mean ± S.E.M. values for all birds showing the time to peak force for LG (filled triangles) and PL (open circles) muscles in relation to running slope. *L*, fascicle length.

net negative work to function like brakes. To develop greater work output from level to incline running, the LG and PL used the same basic strategy. The muscles increased active shortening without increasing the magnitude of force or changing the timing of force production. However, the LG and PL had different strategies for changing mechanical function during decline running. The LG simply altered its length pattern and did active lengthening in early stance to absorb mechanical energy. The PL reduced late stance shortening and thus reduced the potential for positive work; in addition, the timing of force production shifted from late stance into early stance to align with muscle lengthening, resulting in net mechanical energy absorption. Together, these results show that the distal hindlimb muscles in wild turkeys change mechanical function to accommodate the mechanical energy demands of running.

Mechanical function of LG and PL muscles during level running

We predicted that stance net mechanical work output of the LG and PL muscles would be close to zero during steady speed level running, since negligible net work is required to move the body under these conditions. Work output of the LG was low as predicted (2.0 ± 0.8 J kg^{-1}). However, work output of the PL was about 2.5-fold higher than for the LG. Our results for the LG are consistent with previous work showing that this ankle extensor muscle does little mechanical work during level running (Roberts et al., 1997). Work output for the LG was low because during most of force production the muscle operated nearly isometrically. Isometric contractions also characterize the function of some ankle extensor muscles, the lateral gastrocnemius and plantaris, in tammar wallabies during steady speed hopping on level ground (Biewener et al., 1998). It has been proposed that isometric contractions in the leg muscles of runners and hoppers may reduce the energy cost of locomotion (Taylor, 1985, 1994; Roberts et al., 1997). Stretch–shorten cycles can also potentially result in force production with zero net mechanical work, depending upon the timing of force production relative to the stretch–shorten cycle, and the specific strain pattern (rate and amount) of each segment within the cycle. We found that the turkey PL muscle actively produced force across a stretch–shorten cycle during level running. However, net mechanical work output was positive (4.7 ± 1.6 J kg^{-1}) because the timing of peak force production correlated with muscle shortening rather than lengthening, and because shortening strain exceeded lengthening strain. Stretch–shorten cycles have also been measured by sonomicrometry in a rat knee extensor, the vastus lateralis (Gillis and Biewener, 2002), and a guinea fowl digital flexor (Daley and Biewener, 2003) during steady speed level
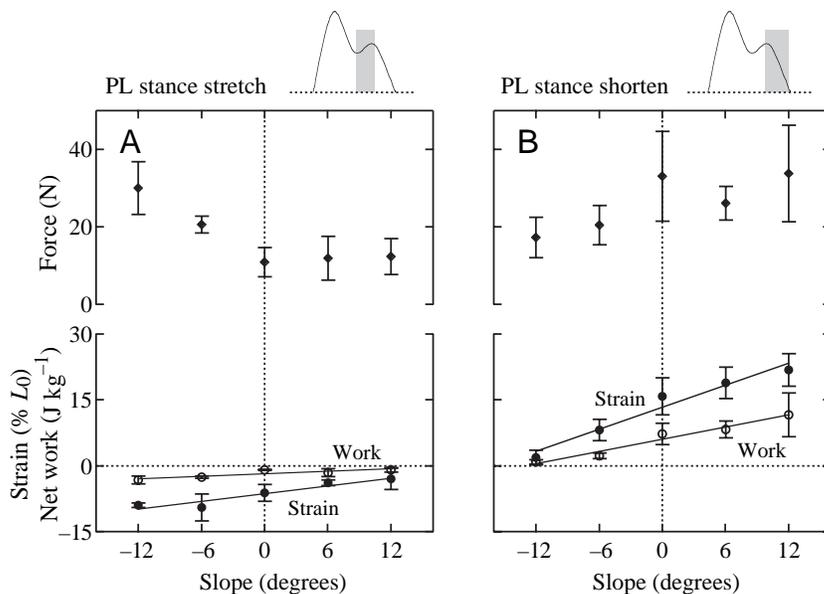

Fig. 8. Average muscle force, net fascicle strain and net work output for the peroneus longus (PL) analyzed over individual segments of the stretch–shorten cycle during steady speed running at $2 \cdot \text{m} \cdot \text{s}^{-1}$ on a level, inclined and declined treadmill. (A) The lengthening region of the stretch–shorten cycle and (B) the shortening region of the stretch–shorten cycle (analyzed as the region in stance following lengthening). L_0, resting segment length.

running. Recent work on the guinea fowl digital flexor (DF-IV) shows that the muscle undergoes significant strain amplitudes in the stretch–shorten cycle, yet the muscle averages approximately zero net work during level running at 1.3 m s^{-1} because it lengthens and shortens by similar amounts during force production (Daley and Biewener, 2003). In lizards, the caudofemoralis muscle also appears to actively stretch–shorten during locomotion on level ground over a range of slow to fast speeds (Nelson and Jayne, 2001). Still other measurements of muscle length patterns during level walking and running in a variety of animals indicate that some muscles exclusively shorten when active (Ahn and Full, 2002; Biewener and Corning, 2001; Carrier et al., 1998; Daley and Biewener, 2003; Gillis and Biewener, 2002; Prilutsky et al., 1996), and there is at least one observation of a muscle that exclusively lengthens (Ahn and Full, 2002). In all, there appears to be a great diversity of length patterns in muscles during level running. The present results demonstrate that strain patterns can differ even among muscle agonists. The implication of this variation in length pattern for locomotor energetics requires further study.

The turkey LG and PL muscles have different origins and insertions that may partly explain their different length patterns observed during level running. The LG muscle originates on the distal end of the femur and proximal end of the tibiotarsus, and distally has a single long tendon that joins with the medial gastrocnemius tendon before crossing the ankle to insert on the tarsometatarsus for ankle extension (Raikow, 1985). The PL muscle originates on the proximal

end of the tibiotarsus and has two tendon insertions. A short branch of the PL tendon inserts on the tibial cartilage for ankle extension, while a longer branch inserts on the tendon of flexor perforatus et digiti III muscle to assist in flexion of the third toe (Raikow, 1985). Muscle shortening and work output of the turkey LG muscle are minimized by elastic stretch and recoil of the tendon-aponeurosis (Roberts et al., 1997), and this is probably an important mechanism influencing the PL length pattern and mechanical work output as well. Nevertheless, our findings indicate that PL fascicles undergo greater length changes and do more net positive work compared to LG fascicles during level running. Mechanical work performed by the PL in late stance may be associated with ankle extension and flexion of the third toe to lift and reaccelerate the body at the end of each step. The difference in timing of peak force production between the LG and PL during level running (Fig. 2) may also be related to the PL's function as a toe flexor in late stance.

Mechanical work during incline and decline running

By varying the slope of the running surface, we varied the demand for work, to determine if LG and PL muscle work outputs parallel the demand for work on the body. The major finding was that both muscles changed mechanical function and acted as energy producing motors during incline running and as energy absorbing brakes during decline running (Fig. 5). The maximum capacity of the muscles for energy production and energy absorption was not determined in this study; work outputs at steeper slopes may be greater than measured here. For the slopes investigated, nevertheless we found a significant linear relationship between muscle work and running slope (Fig. 5), which is parallel to the demand for work on the body. This is consistent with our hypothesis that mechanical work output of these muscles parallels the demand for work on the body.

Changes in ankle excursion with incline (Fig. 9) illustrate how changes in muscle strain might be related to changes in joint kinematics: there was a significant positive linear relationship between running slope and ankle excursion in stance. Birds landed with the leg more extended during decline *versus* incline running; by contrast, they lifted the foot off the ground at the end of stance with the ankle more flexed during decline *versus* incline running. Thus, ankle net excursion in stance, the difference between toe-off and toe-on ankle angles, was negative for decline running, indicating net flexion, and positive for incline running, indicating net extension. These changes in ankle excursion parallel the work outputs for the LG and PL muscles on different running slopes. We did not analyze joint kinematics at other joints, though muscle fiber strains in these muscles are also influenced by the excursions of the knee (LG) and toes (PL). Duty factor (stance time/stride time) was relatively unchanged with running slope (0.48 ± 0.01 for 12° decline, 0.49 ± 0.01 for level, and 0.53 ± 0.01 for 12° incline) and stride frequency was also unchanged (strides s^{-1}: 2.4 ± 0.04 for 12° decline, 2.4 ± 0.05 for level, and 2.5 ± 0.08 for 12° incline).
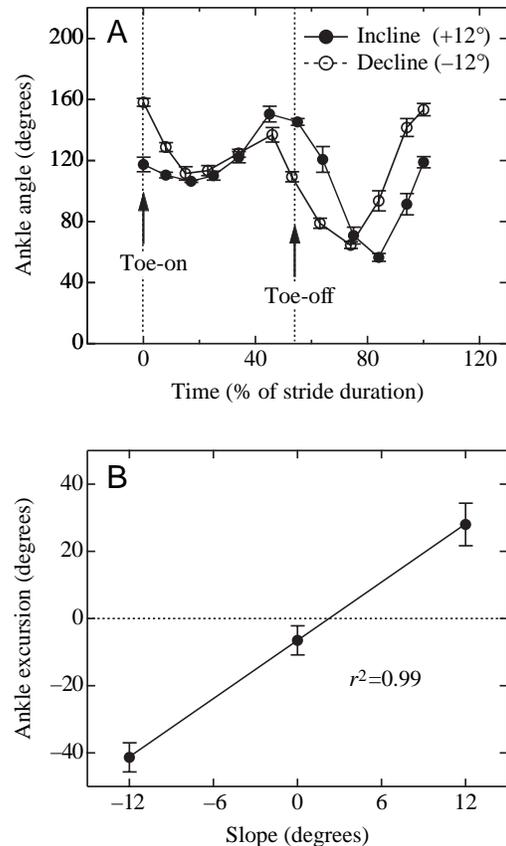

Fig. 9. Effects of running slope on (A) ankle joint angle changes during a single stride at a running speed of 2 m s^{-1} and (B) ankle net excursion in stance, calculated as the difference in ankle angle between toe-off, the end of stance, and toe-on, the beginning of stance. Values are means $\pm$ S.E.M. for $N=6$ birds.

How does the work output of the LG and PL compare quantitatively with the demand for work on the body? For 12° incline running at a steady speed of 2 m s^{-1}, the average mechanical power required to increase the body's potential energy is 4.1 W kg^{-1} body mass. The average stride frequency for turkeys running on this slope was 2.52 strides s^{-1}, so the body mass specific work required per stride was on average $4.1/2.52=1.6$ J kg^{-1} stride^{-1}, or 0.8 J kg^{-1} step^{-1}. The hindlimb musculature of turkeys is equivalent to approximately 7.7% of body mass for one limb (Roberts and Scales, 2002). The mechanical work required on average in each step from each kg of hindlimb muscle is then $0.8/0.077=10.5$ J kg^{-1}. The work outputs of both the LG, 7.0 J kg^{-1}, and the PL, 8.1 J kg^{-1}, were not substantially different than the 10.5 J kg^{-1} that must be developed, on average, by the hindlimb musculature. This suggests that the LG and PL operate as effective motors during incline running. The same calculations used above yield a net energy absorption required per unit muscle of 11.3 J kg^{-1} during 12° decline running (slightly different from incline running due to a slight difference in stride frequency, i.e. 2.35 *versus* 2.52). The energy absorption values of 4.6 J kg^{-1} for the LG and 2.4 J kg^{-1} for the PL during 12° decline running

indicate that some muscles must operate as much more effective brakes than the LG or PL during decline running.

How single muscles change mechanical function

Two fundamentally different mechanisms to change muscle work output were apparent in the mechanical behavior of the LG and PL. Changes in muscle strain explained virtually all of the change in positive work output from level to incline running for both muscles. Both the LG and PL did more shortening in stance from level to incline running, while the timing of peak force and average force in stance were unchanged. For the LG, changes in strain also explained the change in mechanical work from incline to decline running, as the muscle switched from forceful shortening to forceful lengthening. However, the change in function of the PL reveals a mechanism for altering muscle work output independent of changes in muscle strain. The timing of peak force shifted significantly from incline to decline running, as peak force was produced in the second half of stance for incline running and in the first half of stance for decline running. This change in timing of peak force significantly altered muscle work output because the muscle typically lengthened early in stance and shortened late in stance. The change in timing of peak force production meant that during decline running more force was developed during the lengthening period of the stretch–shorten cycle while during incline running more force was developed during the shortening period. As a result, the muscle did net negative work for decline running and net positive work for incline running. This mechanism explains in part how the PL increased net negative work for decline running with virtually no change in the magnitude of lengthening strain (Daley and Biewener, 2003). Modulation of the timing of activation and force production may be a particularly important mechanism for altering muscle work output in muscles that undergo stretch–shorten cycles.

Strategies for changing whole body work output

Our results show that the changing demands for mechanical work of the body are met in part by a change in work output of individual muscles, but other studies suggest that this is not the only mechanism used to alter whole body work output. Recent studies of muscle EMG activities in cats walking on different surface slopes show that some muscles are active on some slopes but inactive on others (Carlson-Kuhta et al., 1998; Smith et al., 1998). In the cat proximal hind limb, for example, the biceps femoris and semimembranosus (two hip extensors) are highly active in stance during incline walking but show almost no EMG activity during decline walking (Carlson-Kuhta et al., 1998; Smith et al., 1998). In the cat distal hindlimb, the plantaris and flexor hallucis longus (two ankle extensors and digit flexors) are active during incline walking but inactive during decline walking (Carlson-Kuhta et al., 1998; Smith et al., 1998). These observations suggest that, in addition to changing the mechanical function of individual muscles, whole-body work output can be modified by selective muscle recruitment. For example, incline running might be powered by increased recruitment of muscles that typically shorten, and derecruitment of muscles that typically lengthen, to result in an increase in net mechanical work output for the whole limb. This strategy appears to be reflected in the function of the rat biceps femoris during running on different surface slopes. The biceps femoris exclusively shortens on level, inclined and declined slopes during steady speed walking and running (Gillis and Biewener, 2002). On level and inclined slopes, the muscle is electrically active while shortening in stance, but on declined slopes EMG activity is absent or very low (Gillis and Biewener, 2002). Because biceps femoris does not actively lengthen in stance during decline running, it does not absorb mechanical energy; however, because it is switched off, it no longer contributes any positive work.

Conclusion

The demands for mechanical work in running vary widely, from net energy absorption for decline running and decelerations, to net energy production for incline running and accelerations. The present results show that the changing demands for whole body work are met, at least in part, by an ability of single muscles to change from net energy producers to net energy absorbers. The observation that the LG and PL act as effective work-producing motors is not consistent with the idea that the spring-like function of distal limb extensors limits their ability to perform mechanical work. Both changes in net muscle strain (for the LG and PL) and timing of force production (for the PL) appear to be important mechanisms for altering muscle mechanical work output with demand.

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