

Muscle force–length dynamics during level *versus* incline locomotion: a comparison of *in vivo* performance of two guinea fowl ankle extensors

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Summary

For a terrestrial animal to move in the complex natural environment, the limb muscles must modulate force and work performance to meet changing mechanical requirements; however, it is not clear whether this is accomplished *via* a collective shift in function by all limb muscles, or a division of labor among limb muscles. Do muscles differ in their ability to modulate force–length contractile function to meet the mechanical demands of different locomotor tasks? We explore this question by examining the *in vivo* force–length performance of the guinea fowl *Numida meleagris* lateral gastrocnemius (LG) and digital flexor-IV (DF-IV), during level and incline locomotion. During level locomotion, the LG and DF-IV exhibit differing muscle fascicle strain patterns: the LG shortens by 10–15% while developing force, whereas the DF-IV undergoes a stretch–shorten cycle with large strain amplitudes and small net strains of 1–8%. Furthermore, the DF-IV operates at higher muscle stresses (92–130 kPa, compared to 23–39 kPa for LG) and possesses a longer

tendon, which allows the DF-IV tendon to recover greater elastic energy than the LG tendon. During incline locomotion, these muscles contribute only one-third of the energy expected for their mass, with the DF-IV exhibiting high stride-to-stride variability in work output. While the stretch–shorten cycle of the DF-IV muscle may allow more economic force production, it also leads to large changes in work output with small changes in the relative timing of force and strain. Thus, while the primary determinants of LG work are net strain and mean force, the primary determinant of DF-IV work is the phase relationship between force and strain. Our results suggest that, in addition to influencing a muscle's mechanical performance during steady level locomotion, morphology also affects its capacity and mechanism for altering work output for different locomotor tasks.

Key words: muscle, strain, phase, work, sonomicrometry, gastrocnemius, digital flexor, guinea fowl, *Numida meleagris*.

Introduction

Animal limb function must meet the varying mechanical demands of locomotion. In addition to generating force to support body weight, muscles must also produce or absorb energy when animals move over varying grades, change speed, jump or land. This raises two important questions. Do limb muscles accommodate changes in locomotor performance through a collective shift in muscle performance, or through muscles specialized for a particular mechanical role? If a muscle is specialized for a particular mechanical role, how is this reflected in its architecture? Many limb muscles might contract under near isometric conditions, performing relatively little work during steady terrestrial locomotion (Taylor, 1994), because force generation to support the body incurs most of the animal's metabolic cost (Kram and Taylor, 1990). Isometric muscle contractions generate force more economically than shortening ones, which probably reduces the cost of locomotion (Kram and Taylor, 1990; Roberts et al., 1997, 1998; Biewener and Roberts, 2000). However, measurements of external work done at proximal *versus* distal

limb joints of a variety of vertebrates suggest that muscle groups contribute differently to energy and force at each joint (Pandy et al., 1988; Gregersen et al., 1998; Belli et al., 2002). Further evidence of differential mechanical function among limb muscles during steady locomotion arises from *in vivo* measurements. For example, during steady bouncing gaits, ankle extensors of specialized cursors and hoppers produce force nearly isometrically (strain <6%: Roberts et al., 1997; Biewener et al., 1998), consistent with economic force production, whereas proximal limb extensors of dogs *Canis domesticus* and rats *Rattus norvegicus* strain substantially while the muscle is active and likely to be generating force (9–27% strain: Carrier et al., 1998; Gregersen et al., 1998; Gillis and Biewener, 2001).

These differences in mechanical roles between proximal and distal limb muscles may relate to muscle–tendon morphology. Proximal muscles generally have long parallel muscle fibers with little or no free tendon, suggesting the capacity to shorten or stretch over relatively long distances, which may favor a

role in work modulation. Distal muscles tend to have short, pennate fibers that transmit force *via* long free tendons, an architecture that favors force generating capacity per unit mass, and storage and release of elastic energy in the tendon (Ker et al., 1988; for reviews, see Biewener, 1998; Biewener and Roberts, 2000). Thus, a division of labor is likely to exist among limb muscles resulting from limb and muscle–tendon morphology.

In addition to performing diverse mechanical roles in a given locomotor task, muscles may differ in their ability to change force development and energy output across tasks. However, few studies have investigated how muscle performance changes to mediate behaviors with different force and energy demands. A clear example of the capability to distinctly shift mechanical function between tasks is demonstrated by the wild turkey *Meleagris gallopavo* lateral gastrocnemius (LG), which contracts with limited length change (strain <6%) during steady running, but provides significant positive work during incline running by shortening substantially (strain >10%) while generating force (Roberts et al., 1997). In addition, external power measurements during large accelerations in wild turkeys suggest power production by all limb muscles, which implies that most limb muscles must be capable of such a mechanical shift (Roberts and Scales, 2002). Yet, in contrast to the turkey LG, *in vivo* recordings of force–length behavior in the LG and plantaris of tammar wallabies *Macropus eugeni* reveal that the low energy production by these muscles does not change when the animals hop on an incline *versus* a level surface (McGowan and Biewener, 2002). Similarly, the mallard *Anas platyrhynchos* LG exhibits a limited ability to shift its mechanical performance when ducks swim *versus* when moving over ground. During both behaviors, the mallard LG shortens considerably (from 24% to 37% strain) while producing force to do substantial work (Biewener and Corning, 2001). Perhaps this is not surprising, since mallards are specialized for swimming in addition to terrestrial locomotion. Nonetheless, given these different findings, it remains unclear whether all limb muscles can adjust their mechanical performance to accommodate different locomotor behaviors.

Consequently, we seek to explore this issue by examining the *in vivo* force, length and muscle activity patterns of two ankle extensors of the guinea fowl *Numida meleagris*: the lateral gastrocnemius (LG) and the digital flexor to the lateral toe (DF-IV). Our general goal is to investigate the capacity of these two muscles for modulating force and work production to meet the mechanical demands of level *versus* incline locomotion. A similar recent study of the turkey peroneus (fibularis; Gabaldón and Roberts, 2002) has also been carried out. Guinea fowl are good runners, known to travel 30–50 km day⁻¹ while foraging (Crowe, 1994; Forshaw, 1998). Therefore, we expect the LG and DF-IV to contract with little length change during level running for greater force economy, but to shorten substantially during incline locomotion to increase energy production, similar to the turkey LG (Roberts et al., 1997). However, due to its smaller size, the guinea fowl may have a more compliant (flexed joint) gait than

the turkey (Gatesy and Biewener, 1991), which could affect the contractile behavior of its limb muscles. In contrast to turkeys and guinea fowl, mallards are specialized for swimming in addition to terrestrial locomotion but are approximately the same size as guinea fowl. Thus, study of the guinea fowl ankle extensors provides a comparison of two cursorial avian species of differing size, as well as two species of similar size but differing locomotor specialization.

Study of the guinea fowl LG and DF-IV also provides an opportunity to compare the contractile function of two agonist limb support muscles. These two muscles have short pennate muscle fibers and long free tendons (Baumel, 1993), which suggest that force economy is an important mechanical role of both (reviewed by Biewener and Roberts, 2000). However, whereas the gastrocnemius crosses the knee and ankle joints, the digital flexors cross the knee, ankle, tarso–metatarso–phalangeal (TMP) and all distal phalangeal joints (Baumel, 1993). Thus, the DF-IV crosses more joints and possesses a longer free tendon than the LG (Baumel, 1993), which may introduce greater compliance in-series with the muscle fibers and facilitate greater elastic energy savings. Thus, although the LG and DF-IV are architecturally similar, differences in tendon length and the joints that each muscle crosses may lead to differences both in their contractile function and how each is used to modulate changes in locomotor performance. By comparing energy production and modulation between these two muscles, we hope to gain greater insight into the relationship between morphology and the dynamics of muscle mechanical function.

Finally, the power and work output of a muscle over a contraction cycle is sensitive to the timing and amplitude of strain relative to force development (e.g. Josephson and Stokes, 1989; Askew and Marsh, 1997), which are strongly affected by the pattern of muscle stimulation as well as the interaction between the active muscles and the load upon which they act (see Josephson, 1999; Marsh, 1999). Consequently, muscle force and work output may be adjusted by altering the timing and intensity of muscle activation, the mechanical interaction between the limb and environment, or both (Gillis and Biewener, 2000). In this study, we examine how muscle activity, force development and energy output vary in the LG and DF-IV as a function of speed and incline. Because peak muscle force can be expected to increase with speed due to a decrease in duty factor (Gatesy and Biewener, 1991), whereas net muscle work must increase with incline to increase the potential energy (PE) of the center of mass of the body (COM), we can also compare muscle performance as a function of speed *versus* incline. To maintain force economy during level locomotion, increases in muscle force with speed should occur with minimal increase in muscle shortening work. During incline locomotion, net muscle work must increase. One possible way to accomplish this is for all muscles in the limb to contribute equally to increased energy production (i.e. changes in mass-specific work are similar among muscles). Alternatively, certain muscles may contribute more to work production by shortening more than others. We address these

two possibilities by comparing the mass-specific work performed by the guinea fowl LG and DF-IV when running on the level *versus* an incline, relative to the mass-specific work required to increase the PE of the COM during incline locomotion.

Materials and methods

Animals and training

Seven adult guinea fowl, 1.25 ± 0.02 kg body mass (mean \pm S.E.M.), were obtained from a local breeder and the primary feathers clipped to prevent them from flying. The birds were trained to locomote steadily on a motorized treadmill (Proform, Logan, USA, model PFTL 08040, belt: 0.4 m wide, 1.0 m long) over a range of walking and running speeds. The training period lasted only 7–10 days, as most birds could run steadily on the treadmill within a few days of training. During training and experiments, the birds ran voluntarily at 0.7, 1.3 and 2.0 m s^{-1} on the level but only at 0.7 and 1.3 m s^{-1} on the 16° incline. At 2.0 m s^{-1} on the incline, the birds frequently slipped due to inadequate traction, prohibiting data collection at this speed. Although we did not attempt to control gait, the lowest speed represents a walk while the higher two speeds represent running, based on the animal's duty factor and previous kinematic data on avian bipeds (Gatesy and Biewener, 1991; Gatesy, 1999a) that are suggestive of COM mechanics (Cavagna et al., 1977). Comparisons between level and incline locomotion were therefore made at 0.7 and 1.3 m s^{-1} , corresponding to what we interpret as moderate walking and moderate running.

Muscles

The two ankle extensor muscle groups of birds are the gastrocnemius, with large lateral and medial heads and one smaller intermediate head, and the digital flexors, which have superficial, middle and deep layers. Each digital flexor layer has heads that insert *via* tendons to all four toes (Baumel, 1993). Both muscle groups have relatively long free tendons, allowing direct measurement of muscle–tendon force for certain muscle components (Fig. 1).

Muscle activity and strain were recorded in the lateral head of the gastrocnemius (LG) and the superficial digital flexor to the lateral toe (DF-IV) by electromyography (EMG) and sonomicrometry (see below). These measurements were combined with recordings of muscle–tendon force for the common gastrocnemius tendon and the free tendon of DF-IV using tendon force buckles (Fig. 1). The DF-IV was chosen for ease of access and because its muscle belly inserts individually to a single tendon. It is impossible to relate muscle strain directly to force measurements for the other digital flexor muscle bellies because they originate and insert onto each other and have overlapping attachments to the tendons of digits II and III, making them mechanically interdependent.

Surgical procedures

The birds were anesthetized for surgery using isoflurane delivered through a mask. After induction at 3%, the bird was prepared for sterile surgery. During the subsequent surgical procedures anesthesia was maintained at 1–2% isoflurane while monitoring the animal's breathing rate. Recording electrodes and transducers were passed subcutaneously to the shank from a 1–2 cm dorsal incision over the synsacrum. A second 4–5 cm incision was then made on the lateral side of the left shank, overlying the division between the anterior and posterior muscular compartments, to expose the LG and its tendon. Freeing the lateral border of the LG provided access to the deeper DF-IV. After exposing the DF-IV muscle and its

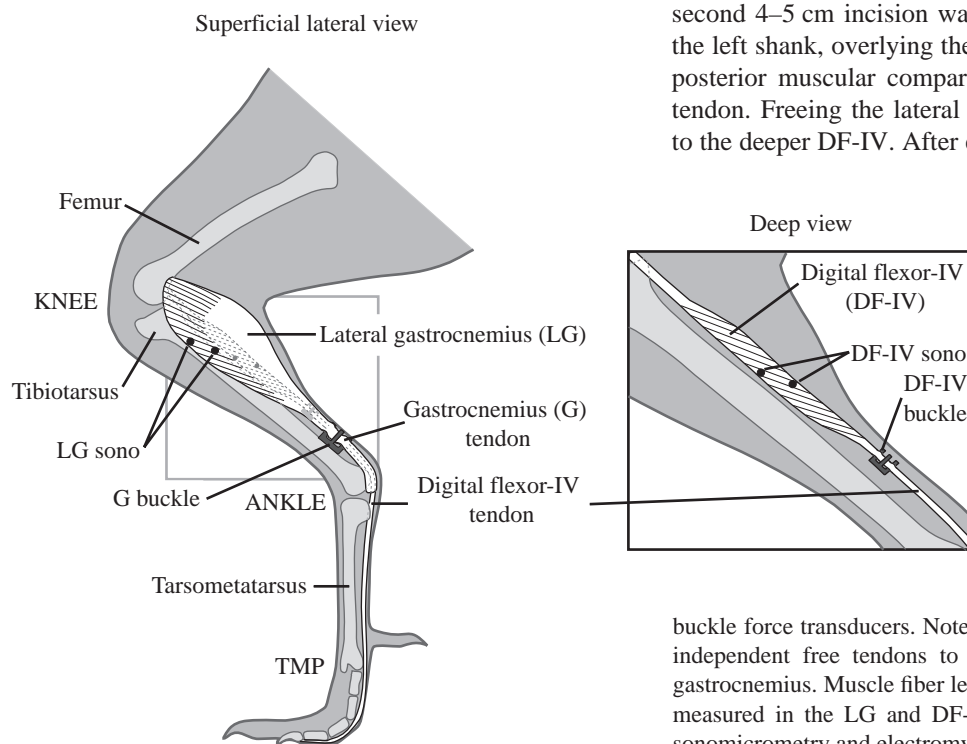


Fig. 1. Anatomy of the lateral gastrocnemius (LG) and digital flexor-IV (DF-IV) muscles in the guinea fowl hindlimb, illustrating the sites of transducer implantation. Forces were measured from the common gastrocnemius (G) tendon and the tendon to DF-IV (inset) *via* tendon

buckle force transducers. Note that the guinea fowl does not possess independent free tendons to the lateral and medial heads of the gastrocnemius. Muscle fiber length changes and muscle activity were measured in the LG and DF-IV (inset) of the left hindlimb using sonomicrometry and electromyography, respectively.

tendon, their identity was verified by gently pulling on the tendon to observe flexion of the lateral digit.

E-type stainless steel tendon buckle force transducers were used as described in previous studies (Biewener et al., 1998; Biewener and Corning, 2001). Because the lateral, intermediate and medial heads of the gastrocnemius do not have separate free tendons, a buckle placed on the common gastrocnemius tendon measured the total force exerted by all three heads. Measurement of force from the common tendon and muscle length change from the lateral head necessitated the assumption that all heads of the gastrocnemius contributed equally to mass-specific work, in order to estimate the work contributed by this muscle as a whole.

Sonomicrometry crystals (Sonometrics Inc., London, Canada) were implanted into small openings made with fine forceps within the muscle, approximately 3–4 mm deep along the axis of the fascicles. After verification of good alignment and signal quality, the crystals were secured using 5-0 silk suture to close the muscle opening. In the LG, 2.0 mm crystals were spaced approximately 10 mm apart in the middle third of the muscle belly. For the much smaller DF-IV, 1.0 mm crystals were implanted approximately 7 mm apart in the middle portion of the belly (Fig. 1).

Fine-wire (0.1 mm diameter, California Fine Wire, Inc., Grover Beach, USA) twisted, silver bipolar EMG hook electrodes (0.5 mm bared tips with 1 mm spacing) were implanted using a 23 gauge hypodermic needle immediately adjacent to each pair of sonomicrometry crystals and secured to the muscle's fascia using 5-0 silk suture. Skin incisions were sutured using 3-0 silk. The lead wires from all transducers were pre-soldered to a small epoxy-mounted, insulated connector (3×GM-6 Microtech, Inc., Boothwyn, USA). After closing the incision over the synsacrum, the connector was sutured to the skin of the back using 3-0 silk and covered with elastic surgical tape.

Each guinea fowl was allowed to recover for 24–36 h after surgery. All birds could walk and run the following day without apparent lameness. Experimental recordings took place over the subsequent 2 days. Once the experiments were completed, the guinea fowl were killed by an intravenous injection of sodium pentobarbital (100 mg kg⁻¹).

Muscle data and video recording

In vivo recordings of muscle strain, EMG and force were made *via* a lightweight 8 m shielded cable (Cooner Wire,

Chatsworth, USA) attached to the micro-connector on the bird's back. The cable connected at the other end to a Triton 120.2 sonomicrometry amplifier (Triton Technology Inc., San Diego, USA), a strain gauge bridge amplifier (Vishay 2120, Micromeritics, Raleigh, USA), and EMG amplifiers (Grass, P-511, West Warwick, USA). EMG signals were amplified 1000× and filtered (60 Hz notch, 100–3000 Hz bandpass) before sampling. The outputs of these amplifiers were sampled by an A/D converter (Axon Instruments, Union City, USA) at 2500 Hz and stored on a computer for subsequent analysis. Because the filters in the Triton sonomicrometry unit introduced a 5 ms phase delay, all length measurements were corrected for this offset before being related to muscle force, EMG and limb kinematics.

Digital high-speed video was recorded in lateral view at a rate of 250 frames s⁻¹ (Redlake Motionscope PCI 500, San Diego, USA). A post-triggered voltage pulse stopped the video recording and synchronized the video sequence to the *in vivo* muscle recordings of force, length and EMG activity.

Force buckle calibration

After completing all *in vivo* recordings, the tendon force buckles were calibrated *in situ post mortem* by cutting the proximal end of each tendon and tying it to a Kistler 9203 force transducer (Amherst, USA) 00 silk suture. The distal attachments of the tendons were left intact. Before applying tension, the sutured end of the tendon was secured by freezing it in a shallow dish of liquid nitrogen. Tension was applied cyclically to the tendon until the loads exceeded the maximum output recorded *in vivo* for 3–4 cycles. We obtained a dynamic calibration of each buckle using a least-squares linear regression fit to the rise and fall of the buckle output *versus* applied force measured from the force transducer. All buckle calibration regressions yielded $r^2 > 0.992$ with an average difference in the rise and fall of force of 3±1% (mean ± S.D.).

Morphological measurements

After the buckle calibrations were complete, we removed the buckles and inspected the tendons to verify lack of visible damage. Each muscle was then dissected free to confirm placement of sonomicrometry crystals and EMG electrodes, and to obtain measurements of wet muscle mass, mean fascicle length and pennation angle. This allowed us to calculate muscle physiological cross-sectional area A , assuming a

Table 1. Muscle and tendon data

	N	Mass (g)	L_f (mm)	L_t (mm)	α (degrees)	A_m (mm ²)	A_t (mm ²)
LG	6	7.2±1.1	17.3±1.2	98.9±13.6	20±2	888±115	7.6±1.9
DF-IV	7	2.1±0.4	17.1±5.7	184±18.7	21±9	114±44	1.5±0.2

LG, lateral gastrocnemius muscle; DF-IV, digital flexor-IV.

L , length; A , cross-sectional area; α , fascicle axis.

Subscripts m, t and f indicate muscle, tendon and fascicle, respectively.

Values are means ± S.E.M.

muscle density of 1060 kg m^{-3} (Table 1). The anatomical length L of each tendon was measured (equal to the muscle–tendon length minus mean fascicle length), and a measured section of free tendon was weighed to determine average tendon cross-sectional area, assuming a density of 1120 kg m^{-3} (Ker, 1981). For two guinea fowl, all hindlimb muscles were dissected and weighed ($80 \pm 4 \text{ g}$). Using these measurements, the total mass of the gastrocnemius and digital flexors ($24 \pm 3 \text{ g}$) comprised 30% of total hindlimb muscle mass.

EMG analysis

For each stride cycle analyzed, EMG recordings were baseline-corrected to zero offset and used to quantify onset phase, offset phase, duration and intensity. Intensity was measured as mean spike amplitude of the rectified signal, and reported as a fraction of the largest mean spike amplitude recorded from each bird while it ran at the highest speed (2.0 m s^{-1}). The relationship between activation intensity and mechanical force or work of a muscle is not necessarily constant because it is sensitive to numerous factors, including force–length and force–velocity effects, and the recent work history of the muscle (e.g. see Josephson, 1999; Marsh, 1999). To investigate whether the relationships between EMG intensity and mechanical force or work changed from level to incline locomotion for the LG and DF-IV, we used a two-way model I analysis of variance (ANOVA) described below.

Sonomicrometry

Sonomicrometry techniques and analysis followed those described by Biewener and Corning (2001). Signals were corrected for offset errors (underestimate of length) introduced by the greater velocity of sound through the epoxy coating of each crystal compared with the muscle. These were found to be 0.16 mm for the 1 mm crystals, and 0.82 mm for the 2 mm crystals, based on direct measurements made in a water bath with the crystals mounted on a digital caliper. Fractional length changes ($\Delta L_{\text{seg}}/L_0$) of the muscle's fascicles were calculated based on segment length changes measured between the crystals (L_{seg}) relative to the resting length (L_0), which was measured while the animal stood at rest. As a convention, shortening strains are negative, and lengthening strains are positive. Total fascicle length change was calculated as fractional length multiplied by the mean fascicle length of the muscle (L_f). Crystal alignment relative to the fascicle axis (α) was verified *post-mortem*, and found to be within $\pm 3^\circ$, indicating that errors due to crystal misalignment, equal to $(1 - \cos 3^\circ)$, were < 0.01 (1%).

Measurement of overall muscle length change based on one pair of sonomicrometry crystals assumes that fractional length changes measured between the crystals are representative of the fascicle as a whole and of all fascicles within the muscle. Because both muscles under investigation in this study have relatively short muscle fascicle lengths (LG, $17.3 \text{ mm} \pm 1.2 \text{ mm}$; DF, $17.1 \text{ mm} \pm 5.7 \text{ mm}$, mean \pm S.D.),

segment length measurements obtained from the crystals represent a substantial portion of whole fascicle length. Although regional differences in fascicle strain within a whole muscle may exist and deserve future study, they are not examined here.

Muscle work and tendon energy recovery per stride

Changes in instantaneous muscle length determined by sonomicrometry were differentiated to obtain muscle velocity and multiplied by instantaneous force to calculate muscle power. For pennate muscles, length changes measured along the fascicles overestimates whole muscle length change and thus, muscle work and power. However, because the pennation of the muscles averaged 20° (LG) and 21° (DF-IV) (Table 1), the resulting overestimate in muscle length change is $< 6.6\%$ [$= (1 - \cos \alpha) \times 100$], and being similar for both muscles, is unlikely to alter the interpretation of the results. Muscle power was integrated over each stride to provide a cumulative measure of work, in which the final value represents the net muscle work per stride. To characterize the effects of strain, force and their relative timing on the net work produced by each muscle, we measured the following variables for each stride, normalized as a fraction of stride duration: time of peak force, time of peak muscle length, the change in muscle shortening velocity during force production (Δ velocity), and the phase between peak force and peak muscle length (phase).

Elastic energy recovered from each tendon per stride was calculated based on peak tendon stresses, assuming a tendon stiffness of 0.34 GPa (Buchanan and Marsh, 2001) and a hysteresis of 7% (Ker, 1981).

Muscle work and center of mass work

To assess the shift in energy production by the muscles from level to incline running, we compared the total energy contribution by these two muscles as a group to the overall increase in potential energy of the animal's COM. We estimated the total work contributed by the gastrocnemius and digital flexors by assuming that all heads of each muscle contributed the same mass-specific work as the single head that was measured (LG and DF-IV). The work done during each step to increase the PE of the COM was calculated using the high-speed video to determine the animal's stride length (L_s). The PE increase is equal to $mg\Delta h$, where m is the bird's mass, g is the acceleration due to gravity and Δh is the change in height of the body during each stride cycle, which equals $L_s \sin(16^\circ)$.

Statistical analyses

Ten steady strides for each running condition (speed and grade) were analyzed. Strides were considered steady if the bird maintained fore–aft position on the treadmill belt. A three-way, mixed-model ANOVA was used to assess the effects of incline and speed, treating individual as a random effect and speed and incline as fixed effects. We tested whether speed and incline had a significant effect ($P < 0.05$) on mean

muscle–tendon force, net active muscle strain, net muscle work, EMG duration and intensity. To account for the number of simultaneous ANOVAs performed, the significance level for each test was adjusted using the sequential Bonferroni technique (Rice, 1989). To assess the effect of EMG intensity on mean force and net mechanical work within each condition, we used a two-way model I ANOVA, treating individual and EMG intensity as effects. To characterize the relative effects of net strain, mean force, phase and Δ velocity on work, a general linear model was used that included first order interaction effects. Student's paired *t*-tests were used for all paired comparisons. Statistical tests were performed using Systat (version 9.0 for the PC). Unless otherwise stated, average values given in the text are means \pm S.E.M.

Results

Force, length and activity patterns of LG and DF-IV

During moderate steady-speed running (1.3 m s^{-1}) on the level, the lateral gastrocnemius and digital flexor IV became active just before the beginning of the support phase of the stride, with LG EMG onset occurring slightly before foot-down, and DF-IV EMG onset occurring at foot-down (Fig. 2). Muscle activation continued until approximately mid-support for the LG, and slightly longer for the DF-IV. In both muscles force peaked around midstance, reaching an average of $27 \pm 4 \text{ N}$ for the LG and $12 \pm 1 \text{ N}$ for the DF-IV at 1.3 m s^{-1} (Table 2). Although peak force developed by the DF-IV was much lower than the LG, it consistently operated at higher muscle stress (force/area, kPa) than the LG. At 1.3 m s^{-1} , DF-IV muscle

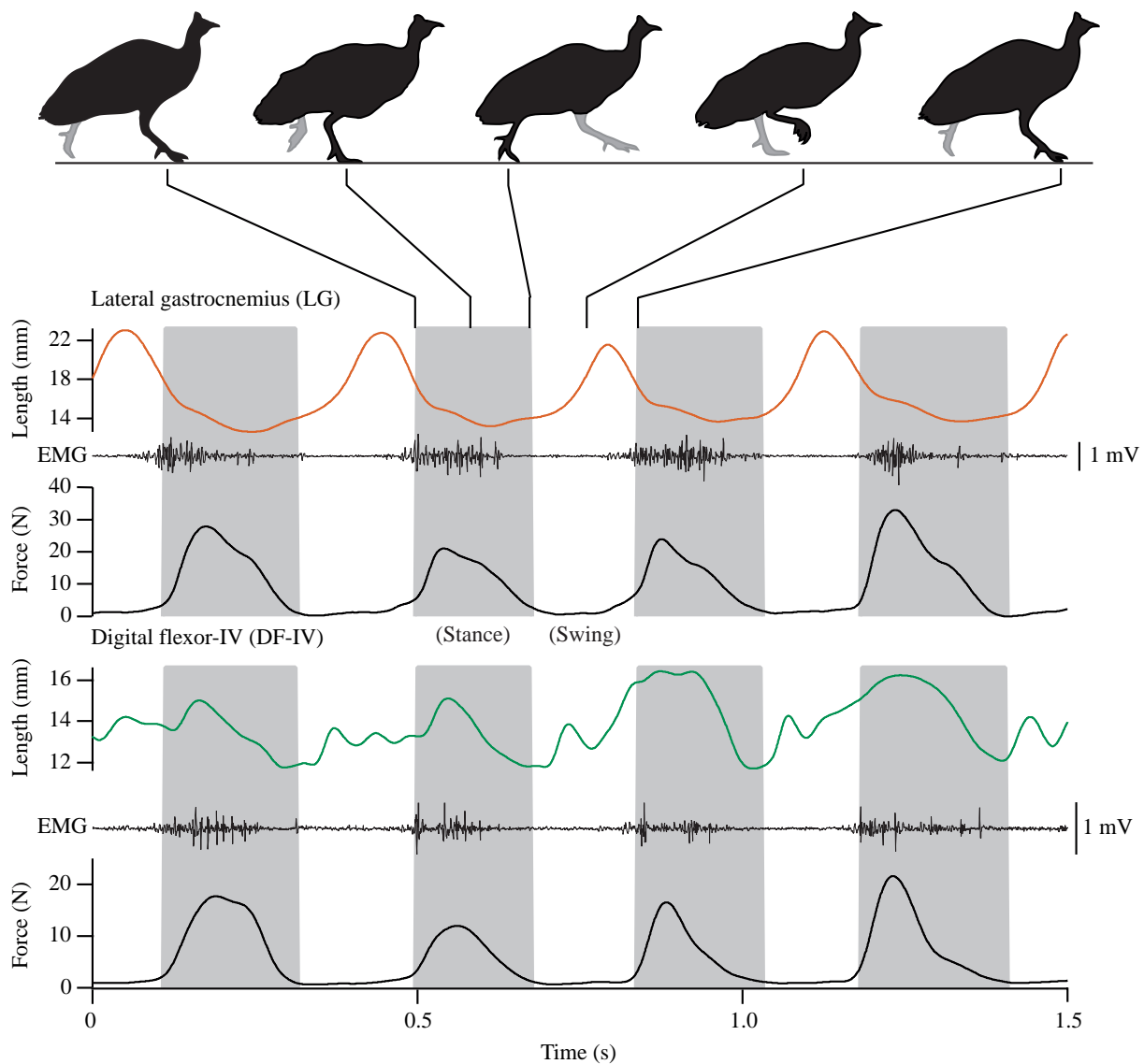


Fig. 2. Representative recordings of muscle force, length and electromyographic (EMG) activity for lateral gastrocnemius (LG, above) and digital flexor-4 (DF-IV, below) during level running at 1.3 m s^{-1} , from one individual (bird 5). Shaded bars indicate the support phase of the locomotor cycle. Top, silhouettes of the bird show the phases of the stride for one cycle, with the recorded limb in black and the contralateral limb in gray.

Table 2. Variables measured during level and incline running

Condition	Speed (m s ⁻¹)					
	0.7		1.3		2.0	
	Level	Incline	Level	Incline	Level	Incline
LG						
Peak force (N)	20.9±1.9	29.9±4.2	26.7±3.9	35.5±2.9	33.6±3.4	–
Peak stress (kPa)	23±2	34±6	31±4	39±5	37±4	–
Mean force (N)	10.3±1.2	15.3±2.2	14.0±2.2	20.2±1.9	18.3±1.9	–
Net strain (%)	-10.5±2.3	-18.0±2.5	-12.9±3.3	-20.4±2.9	-15.2±3.0	–
EMG intensity (%)	38±4	55±9	57±4	67±11	76±4	–
EMG duration (ms)	169±20	234±21	127±15	173±34	125±13	–
Tendon energy (J kg ⁻¹)	1.4±0.3	3.0±0.9	2.5±0.6	4.6±1.8	3.7±0.8	–
Net work (mJ)	32.7±6.3	79.6±11.8	56.0±12.2	92.2±14.4	84.6±15.7	–
Net work (J kg ⁻¹)	4.5±0.9	11.0±1.8	7.7±1.5	12.0±1.6	11.4±2.1	–
Δwork level <i>versus</i> incline (J kg ⁻¹)	6.5		4.3		–	
DF-IV						
Peak force (N)	9.3±1.1	11.4±1.2	11.9±1.1	13.3±1.6	13.6±1.4	–
Peak stress (kPa)	92±17	120±24	117±18	130±34	123±22	–
Mean force (N)	5.0±0.6	6.6±0.9	6.9±0.7	8.1±1.0	7.7±0.7	–
Net strain (%)	-7.8±5.7	-8.6±4.7	-1.1±6.0	-9.7±3.7	-3.3±6.1	–
EMG intensity (%)	41±6	57±6	55±7	65±7	64±6	–
EMG duration (ms)	250±9	260±19	172±20	197±7	145±9	–
Tendon energy (J kg ⁻¹)	11.5±4.5	15.5±4.0	14.2±2.3	21.1±8.8	20.0±6.7	–
Net work (mJ)	0.4±3.1	8.5±5.4	-1.9±5.3	9.8±6.7	-1.3±4.9	–
Net work (J kg ⁻¹)	0.7±1.6	4.7±3.4	-0.8±2.7	5.3±4.3	-0.3±2.7	–
Δwork level <i>versus</i> incline (J kg ⁻¹)	4.0		6.1		–	

Values are means + s.e.m.

LG, lateral gastrocnemius muscle; DF-IV, digital flexor-IV, EMG, electromyographic activity.

stress averaged 117±18 kPa, whereas LG muscle stress averaged 31±4 kPa (Table 2).

In addition to operating at different muscle stresses, the two muscles exhibited different length-change patterns. The LG shortened throughout most of force production, becoming relatively isometric during the latter half of support and then stretched slightly at the end of support. In contrast, the DF-IV typically lengthened until peak force, and shortened rapidly during force decline. Although active shortening strains were similar in magnitude for the LG and DF-IV, averaging -16±2% and -12±3% of L_0 , respectively, active stretch of the DF-IV averaged 11±3%, compared to only 3±1% of the LG (not shown). Thus, the average net strain over the support phase for the LG was -13±3% *versus* -1±6% for the DF-IV during level running at 1.3 m s⁻¹.

Variability in strain, force and work: LG versus DF-IV

Certain characteristics of the force–length behavior of the DF-IV were consistent across individuals; this muscle operated at higher muscle stresses than the LG, stretched during the beginning of the support phase of the stride and shortened during the later half of support. However, DF-IV force and strain performance varied significantly more from stride to stride than that of the LG (Fig. 3). The mean coefficient of variation (CV) for muscle strain was 19% for the LG and 30% for the DF-IV

($P<0.001$), and the mean CV for muscle force was 16% and 28%, respectively ($P<0.001$). Whereas the CV for LG muscle strain did not significantly differ between swing and stance phases of the stride ($P=0.2450$), the CV for DF-IV muscle strain significantly increased during the support phase of the stride ($P=0.0044$), averaging 14% during swing *versus* 46% during stance. Thus, DF-IV varied substantially more during force development, yielding greater variation in work production.

The stretch–shorten pattern of the DF-IV also yielded large changes in muscle fascicle velocity during force production, which caused work production to be sensitive to the relative timing of muscle force and strain (phase). As a consequence of the greater variability in force and strain of the DF-IV compared to the LG, as well as the increased dependence of work the timing between the two, energy production by the DF-IV varied widely, both stride-to-stride and among individuals (Fig. 4), even though the DF-IV averaged little net work per stride on the level. Both intra- and inter-individual variation in energy production per stride was greater in the DF-IV than in the LG (Fig. 4), and the variation in energy production by the DF-IV is greater than would be expected based on the variation in force and strain alone (Fig. 3).

Energy production by the LG and DF-IV

Net muscle work produced per stride is the area enclosed in

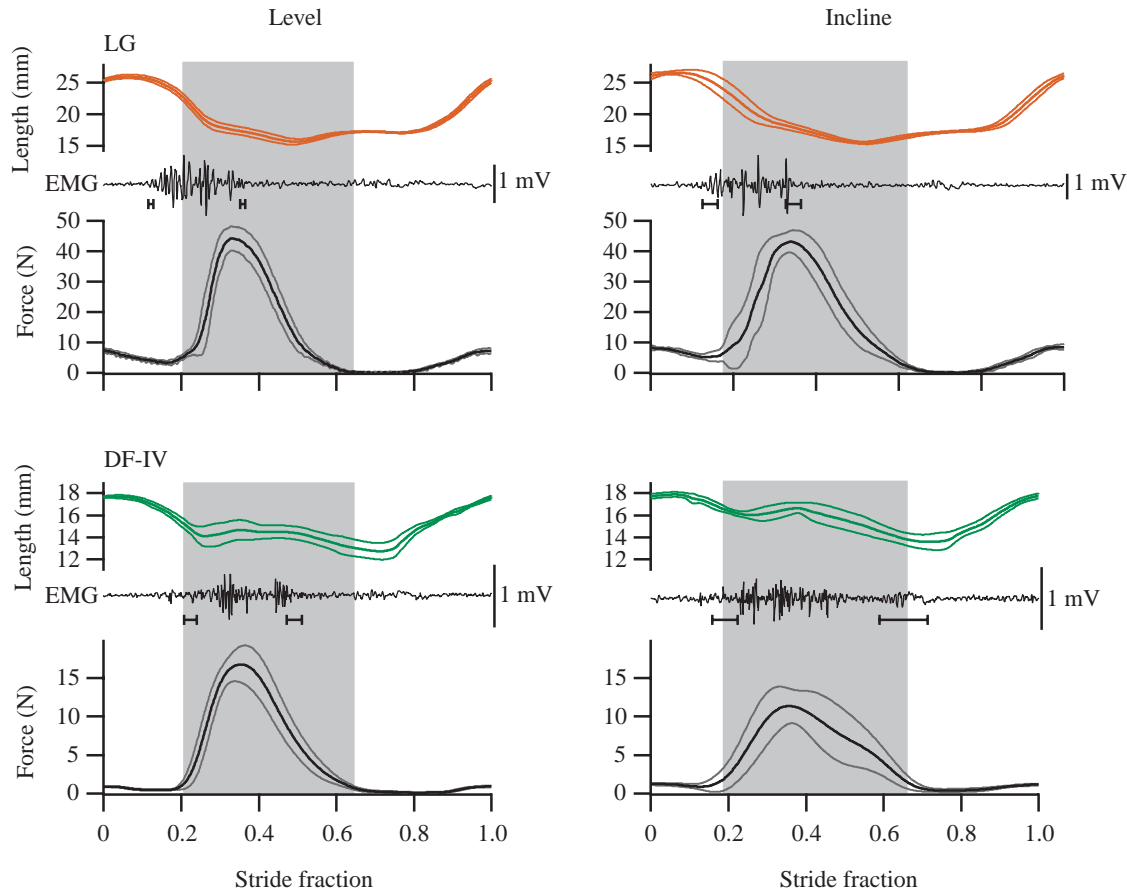


Fig. 3. Expanded and averaged recordings of muscle length, force and electromyographic (EMG) activity from the lateral gastrocnemius (LG, above) and digital flexor-4 (DF-IV, below), normalized to a single stride. The support phase of the stride is shaded in gray. Results are shown for bird 4, running at 1.3 m s^{-1} on the level (left), and at 16° incline (right). The traces of force and length are means \pm s.d. obtained from 10 strides, and a single EMG trace is shown for reference. The error bars below the EMG traces indicate the mean \pm s.d. for the timing of EMG activity (onset, offset).

a work loop, a graph of muscle force *versus* muscle length. To allow direct comparison of the two differently sized muscles, we plot muscle stress (kPa) against muscle strain ($\Delta L/L_0$), such that the area enclosed in the work loop represents the volume- or mass-specific work performed over the locomotor cycle (Fig. 5). The timing of EMG relative to force production did not change substantially with speed. Little variation existed among individuals in the general shape and direction of the work loop of the LG. This muscle often absorbed a small amount of energy during late swing phase as the muscle was passively stretched (Fig. 4). It then became active just before ground contact, shortened under relatively low muscle stress for most of the support phase of the stride, and stretched slightly near toe-off (Fig. 5). Under all conditions in this study, the LG generated a counter-clockwise work loop, indicating net energy production. The LG produced $7.7 \pm 1.5 \text{ J kg}^{-1}$ ($56 \pm 12.2 \text{ mJ}$) per stride during level, intermediate speed running (1.3 m s^{-1} , Table 2). In contrast to the positive work performed by the LG, the DF-IV averaged approximately zero net work during level running (Fig. 5). The DF-IV demonstrated a more spring-like force-length pattern during

the support phase of the stride, becoming active just before heel-strike, stretching until approximately peak muscle force, and subsequently shortening during force decline (Fig. 5). Overall, the DF-IV shortened and lengthened by similar amounts during force production, so that work produced by this muscle averaged only $-0.8 \pm 2.7 \text{ J kg}^{-1}$ ($-1.9 \pm 5.3 \text{ mJ}$) per stride during level running at 1.3 m s^{-1} (Table 2).

Changes in muscle performance with speed

As speed increased, general patterns of muscle activation, force production and muscle strain remained similar. However, peak force, mean force and EMG intensity increased with speed, while EMG duration decreased, for both muscles (Table 2). With increasing speed, LG peak force increased significantly ($P < 0.0001$) from $20.9 \pm 1.9 \text{ N}$ to $33.6 \pm 3.4 \text{ N}$, while DF-IV peak force increased significantly ($P < 0.0001$) from $9.3 \pm 1.1 \text{ N}$ to $13.6 \pm 1.4 \text{ N}$. Again, these forces correspond to higher peak muscle stresses for the DF-IV (Table 2, Fig. 6). In concordance with the increase in force, EMG intensity increased significantly with speed for both muscles (Table 3; LG, $P = 0.0068$; DF-IV, $P = 0.0095$). In contrast, EMG duration

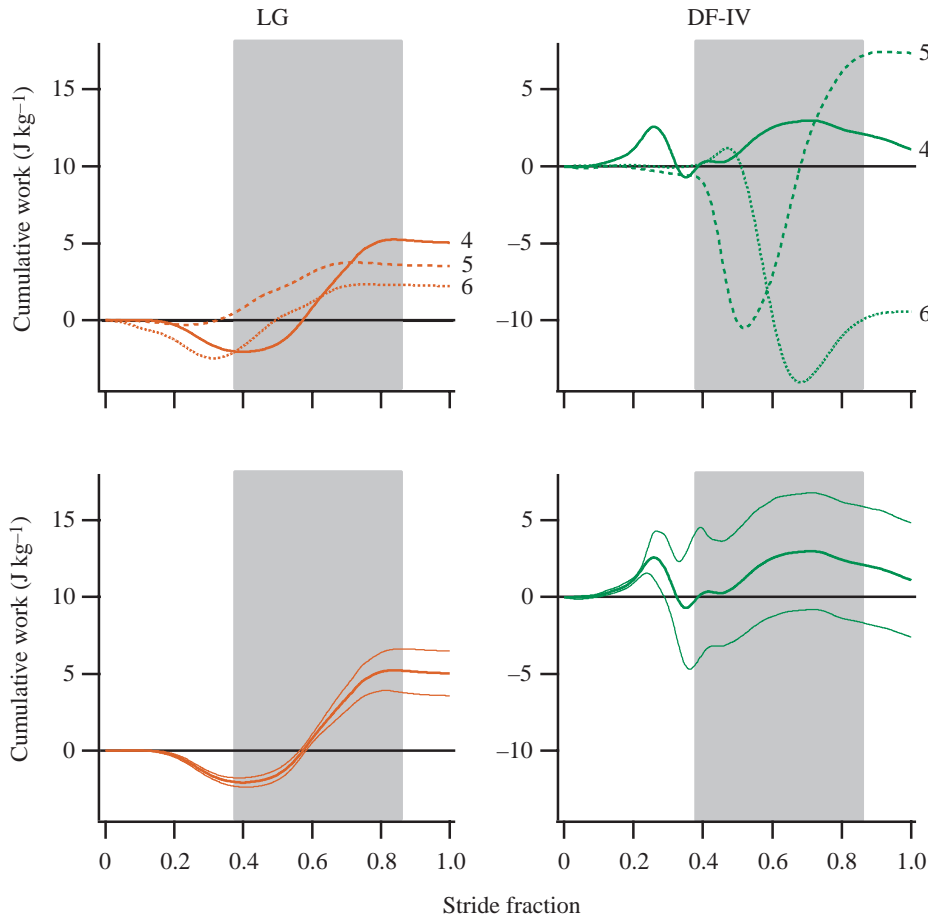


Fig. 4. Inter-individual (top) and intra-individual (bottom) variation in cumulative energy production by lateral gastrocnemius (LG, left) and digital flexor-4 (DG-IV, right) over the locomotor cycle, shown for level running at 1.3 m s^{-1} . The final value is the net work generated per stride. The traces of average cumulative work output shown across individuals (birds 4, 5 and 6, top) encompass the range of inter-individual variation for both muscles. The mean \pm S.D. of energy production over the normalized stride from one individual (bird 4, bottom) illustrates typical levels of stride-to-stride variation for each muscle.

Table 3. Effect of incline and speed

Variable	Incline			Speed		
	d.f.	<i>F</i>	<i>P</i>	d.f.	<i>F</i>	<i>P</i>
LG						
Net work	1, 5	36.79	0.0018*	1, 5	108.83	0.0001*
Mean force	1, 5	17.14	0.0090*	1, 5	347.54	<0.0001*
Net strain	1, 5	26.78	0.0035*	1, 5	53.04	0.0008*
Phase	1, 5	10.05	0.0248 [†]	1, 5	117.55	0.0001*
EMG intensity	1, 4	0.96	NS	1, 4	26.31	0.0068*
EMG duration	1, 4	5.96	NS	1, 4	8.73	0.0418*
DF-IV						
Net work	1, 5	7.84	0.0380 [†]	1, 5	0.59	NS
Mean force	1, 5	13.13	0.0152 [†]	1, 5	217.18	<0.0001*
Net strain	1, 5	9.21	0.0289 [†]	1, 5	1.92	NS
Phase	1, 5	6.03	NS	1, 5	43.64	0.0012*
EMG intensity	1, 4	2.62	NS	1, 4	21.86	0.0095*
EMG duration	1, 4	0.17	NS	1, 4	180.85	0.0002*

ANOVA; see Materials and methods for details.

NS, not significant.

*Significant at the 0.05 level after Bonferroni correction.

[†] $P < 0.05$, but not significant after Bonferroni correction.

LG, lateral gastrocnemius muscle; DF-IV, digital flexor-IV, EMG, electromyographic activity.

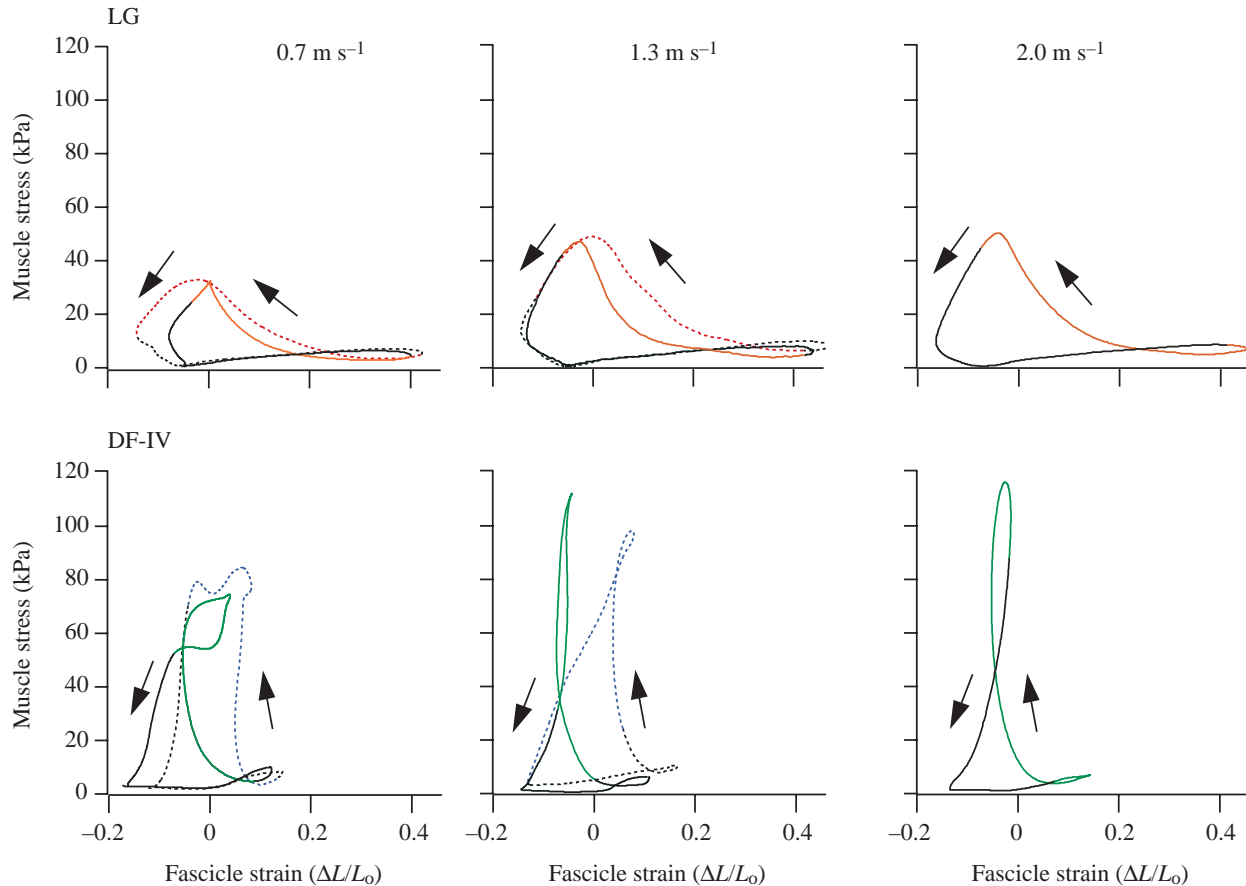


Fig. 5. Muscle stress plotted against fascicle strain, forming *in vivo* mass-specific work loops, shown for three level speeds (0.7, 1.3 and 2.0 m s⁻¹, left to right) and two 16° incline speeds (0.7 and 1.3 m s⁻¹, broken lines) for the lateral gastrocnemius (LG, top) and the digital flexor-4 (DF-IV, bottom) from one individual that was nearest the means for all birds (bird 4). The area enclosed in the loop is the net work performed per unit muscle mass per stride. Arrows indicate the direction of the work loop. Counter-clockwise loops indicate net positive energy, and clockwise loops indicate net energy absorption. Color indicates timing of electromyographic (EMG) activity relative to force and length change.

decreased significantly with speed for the DF-IV ($P=0.0002$), and similarly for the LG, although the trend was not significant after Bonferroni correction (Table 3).

Net shortening strain and net muscle work per cycle (Fig. 6) also increased significantly with speed for the LG. Net active shortening by this muscle increased from $10.5 \pm 2.3\%$ to $15.2 \pm 3.0\%$ ($P=0.0008$), whereas net energy produced per stride by the LG increased from 4.5 ± 0.9 to 11.4 ± 2.1 J kg⁻¹ ($P=0.0001$) from the slowest to the fastest speed on the level (Fig. 6). In contrast, for the DF-IV, net strain and net muscle work per stride did not differ significantly with speed (Table 3).

Tendon elastic energy storage

On a (muscle+tendon) mass-specific basis, the DF-IV recovered substantially more strain energy from its tendon than did the LG (Table 2, Fig. 6). For example, during level running at 1.3 m s⁻¹, elastic energy recovery in the LG tendon averaged only 2.5 ± 0.6 J kg⁻¹, whereas the DF-IV tendon recovered 14.2 ± 2.3 J kg⁻¹. In both tendons, the energy savings increased with speed and incline due to the significant increase in peak muscle-tendon force under these conditions. LG tendon

savings did not exceed 5 J kg⁻¹ and were less than half of the mass-specific net muscle work, whereas DF-IV tendon energy recovery reached 21.1 ± 8.8 J kg⁻¹, greatly exceeding DF-IV muscle shortening work (Table 2, Fig. 6). This difference in tendon energy storage reflects the longer length and higher operating stress of the DF-IV tendon. Given that the ratio of muscle to tendon cross-sectional area is lower in the DF-IV than the LG (Table 1), this difference is not a result of the DF-IV having a relatively thinner tendon.

Level versus incline locomotion

For both muscles, the net work generated per stride tended to increase when the birds moved up a 16° incline (Figs 5, 6). However, the change in muscle work from the level to an incline was not significant after Bonferroni correction for the DF-IV, due to its higher stride to stride variability (Table 3; LG, $P=0.0018$; DF-IV, $P=0.0380$). In fact, although the DF-IV averaged net energy production during incline running, it absorbed energy in 28% of strides on the incline (compared to 57% of strides on the level; $N=120$ and 170, respectively). On average, LG net work increased by 6.5 J kg⁻¹ and 4.3 J kg⁻¹ at 0.7 m s⁻¹ and 1.3 m s⁻¹,

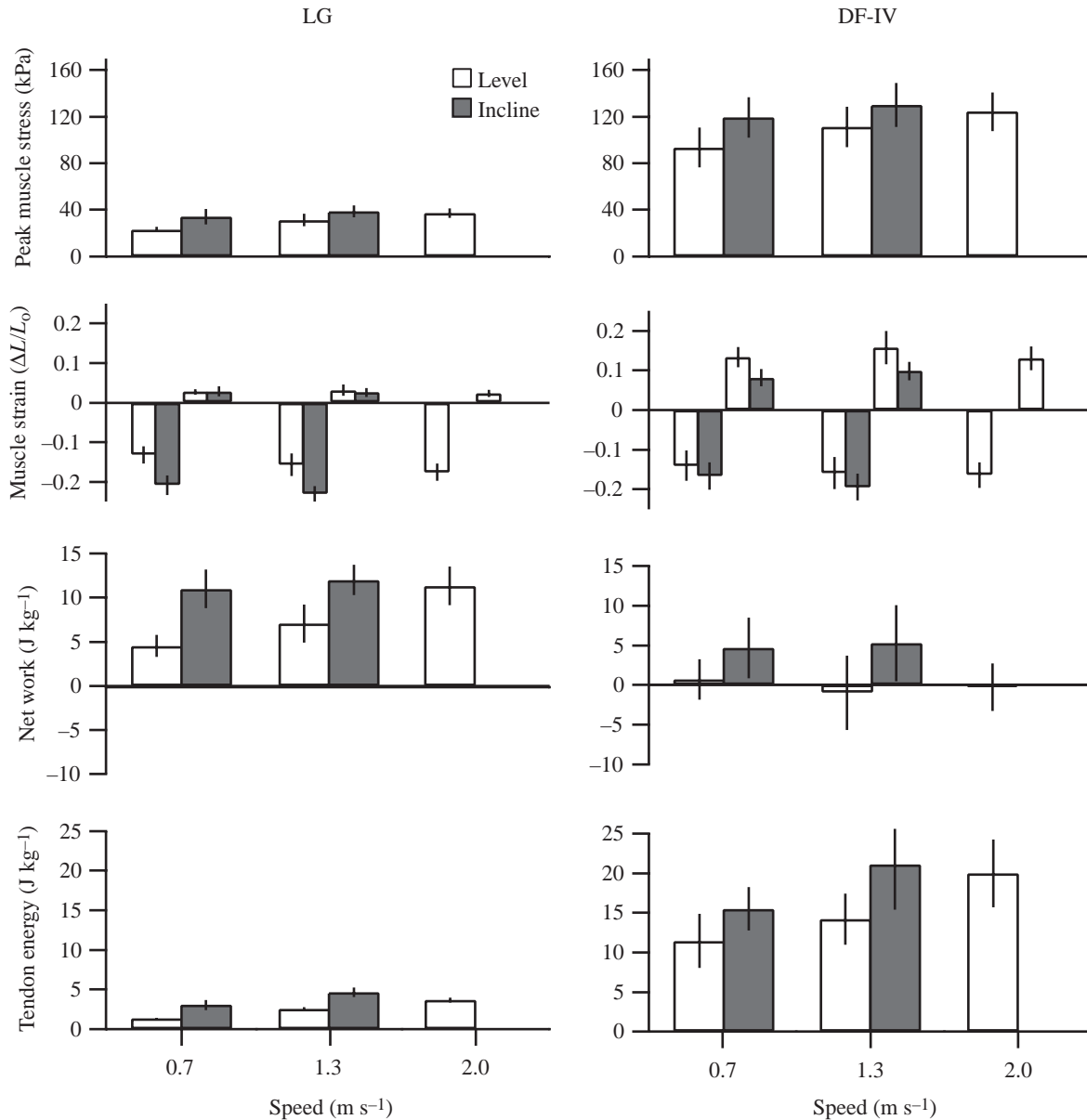


Fig. 6. Summary histograms of peak muscle stress, active fascicle strain, net work per stride and tendon elastic energy recovery per stride (means \pm S.E.M.) for the lateral gastrocnemius (LG, left) and digital flexor-4 (DF-IV, right) across speed for level (open bars) and incline (filled bars) locomotion. Shortening muscle strains are negative. See Table 3 for relevant sample sizes.

respectively (Table 2). The increase in energy production by the LG during incline locomotion occurred through a combination of increased mean force ($P=0.009$) and net shortening strain ($P=0.0035$) (Fig. 6). The DF-IV also showed trends for increasing mean force and net shortening strain on an incline (Table 2), with net active shortening increasing primarily through a decrease in active stretch (Fig. 6); however, these trends were again not significant after Bonferroni correction (Table 3, mean force, $P=0.0152$; net strain, $P=0.0289$).

Relationship between EMG intensity and muscle work and force

During level locomotion, peak force showed a significant

linear relationship with EMG intensity for both muscles (Table 4, LG, $P<0.0001$; DF-IV, $P=0.0041$) (Fig. 7); however, the relationship was much stronger for the LG. Because the amplitude of EMG activity relative to peak muscle force can be expected to differ among individuals because of differences in electrode geometry and recording site (see Loeb and Gans, 1986), the slope of the relationship between EMG intensity and force (or work, see below) also can be expected to differ among individuals. Despite such differences, the pattern of change in EMG intensity relative to muscle force between level and incline was consistent among the individual animals sampled. Consequently, we report the averages of the slopes obtained among individuals for each treatment. The average slope of the

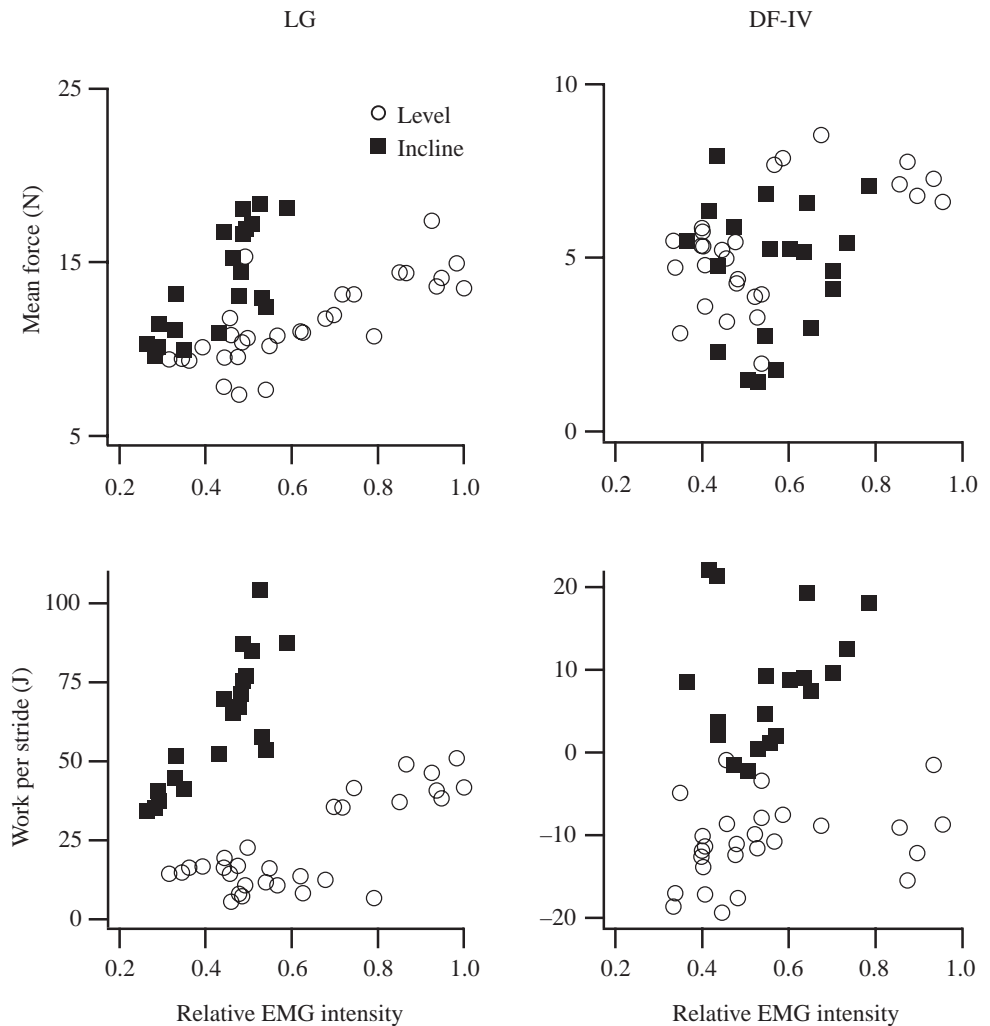


Fig. 7. Mean muscle force and net work per stride against relative electromyographic (EMG) intensity for lateral gastrocnemius (LG, left) and digital flexor-4 (DF-IV, right), during locomotion on the level (open circles) and incline (filled squares). For clarity, results are shown for one representative individual (bird 3); however, the pattern of change from level to incline was similar across individuals, as indicated by the mean slopes and r^2 values given in the text.

relationship (force over relative intensity, N) was 23 N for the LG (mean $r^2=0.65$) but only 10 N for the DF-IV (mean $r^2=0.33$). During incline running, LG muscle force still demonstrated a significant linear relationship with EMG intensity, with an average slope of 34 N (mean, $r^2=0.58$, $P<0.0001$), but the DF-IV did not ($P=0.6279$, Table 4, Fig. 7).

Similar to peak muscle force, LG muscle work (averaged across individuals) was also positively correlated with EMG intensity during both level (mean $r^2=0.55$) and incline (mean $r^2=0.50$) locomotion (Fig. 7, Table 4), with average slopes of 89 and 102 mJ, respectively. In contrast, DF-IV net muscle work showed no relationship with EMG intensity during level

Table 4. Effect of relative EMG intensity on net work per stride and mean force

	EMG intensity					
	Level			Incline		
	d.f.	F	P	d.f.	F	P
LG						
Net work	1, 137	90.07	<0.0001	1, 87	165.91	<0.0001
Peak force	1, 137	160.41	<0.0001	1, 87	80.76	<0.0001
DF-IV						
Net work	1, 147	1.09	NS	1, 101	4.45	0.0373
Peak force	1, 147	8.52	0.0041	1, 101	0.24	NS

ANOVA; see Materials and methods for details.

NS, not significant.

LG, lateral gastrocnemius muscle; DF-IV, digital flexor-IV, EMG, electromyographic activity.

running ($P=0.2991$), but exhibited a small significant positive trend during incline running with an average slope of 9 mJ (mean $r^2=0.16$, $P=0.0373$) (Table 4, Fig. 7).

Work output by LG and DF-IV in relation to strain, force and phase

Because muscle work is the product of muscle force and length change, the net work produced over the course of a stride depends on the relative timing of these two variables. If muscle fascicle velocity is constant during force production, the net work performed by a muscle over the course of a stride is simply the product of mean force and net fascicle length change. However, when fascicle velocity varies substantially during force production, the work generated also depends on the phase relationship between length change and force. We conducted a general linear model ANOVA to examine the effects of these factors (mean force, net strain, phase, Δ velocity) on the work produced by each muscle (Table 5). This analysis revealed that net strain and mean force alone explain 93% of variation in work for the guinea fowl LG, consistent with the relatively constant shortening velocity exhibited by this muscle during force production (Fig. 3). While all factors had a statistically significant effect on LG net work (Table 5), phase exerted only a minor influence on work because it did not vary substantially under the conditions in this study (Fig. 8). In contrast to the LG, net strain and mean force explained only 37% of the variation in DF-IV work, while phase and the interaction terms related to phase explained an additional 32%. Interactions among factors (force, strain and phase) exhibited a larger influence on DF-IV work than LG work (Table 5) because the DF-IV muscle fascicles undergo a dynamic stretch–shorten cycle. Note that although the bivariate plot of net work against net strain (Fig. 8) suggests a positive relationship between these two variables for the DF-IV, the effect of net strain on work is not significant for the DF-IV once the general lineal model accounts for interactions among variables, as reflected in the

statistical results in Table 5. Thus, the LG and DF-IV modulate force–length mechanical performance differently: the LG through changes in mean force and net strain, and the DF-IV through changes in their relative timing (Fig. 8).

Muscle work and COM work

Although the LG produced greater positive energy on the incline, neither muscle contributed energy proportional to its mass to move the bird's COM up the incline. The increase in PE of the COM per stride was 1400 mJ at 0.7 m s^{-1} and 1700 mJ at 1.3 m s^{-1} . We compared this energy to the total energy contributed by the gastrocnemius and digital flexors, assuming all heads of each muscle contributed equally to mass-specific work. During incline locomotion at 0.7 and 1.3 m s^{-1} , the gastrocnemius contributed 111 ± 26 and 89 ± 19 mJ (means \pm S.E.M.) greater average energy than during level running at the same speeds, whereas the digital flexors contributed 38 ± 25 and 63 ± 16 mJ (means \pm S.E.M.) greater average energy. Therefore, together, these muscles contribute approximately 9% (1.3 m s^{-1}) to 11% (0.7 m s^{-1}) of the additional energy required for incline running. However, because they represent 30% of the total hind limb muscle mass (see Materials and methods), they contribute only one-third of the energy that would otherwise be expected for their mass (Fig. 9).

Discussion

We investigated the extent of force and energy modulation by two hind limb support muscles of the guinea fowl in response to changes in mechanical demands associated with speed and incline. The results suggest that the guinea fowl gastrocnemius and digital flexors fulfill distinct mechanical roles during locomotion that relate to differences in their muscle–tendon morphology and, further, that the two muscles differ in their capacity and mechanism for altering work output. While the LG generated positive work on the level by shortening 11–15%, the DF-IV produced little work on

Table 5. Effect of net strain, mean force, phase and Δ velocity on work

Factor	LG			DF-IV		
	d.f.	<i>F</i>	<i>P</i>	d.f.	<i>F</i>	<i>P</i>
Phase (A)	1, 268	16.7	0.0001	1, 268	9.4	0.0024
Mean force (B)	1, 268	68.7	<0.0001	1, 268	7.9	0.0052
Net strain (C)	1, 268	9.2	0.0026	1, 268	1.3	NS
Δ velocity (D)	1, 268	18.1	<0.0001	1, 268	0.6	NS
A×B	1, 268	0.6	NS	1, 268	9.6	0.0022
A×C	1, 268	1.6	NS	1, 268	0.1	NS
A×D	1, 268	6	0.0150	1, 268	31.7	<0.0001
B×C	1, 268	4.3	0.0400	1, 268	0.1	NS
B×D	1, 268	0.9	NS	1, 268	9.4	0.0024
C×D	1, 268	0.2	NS	1, 268	0.4	NS

General linear model ANOVA; see Materials and methods for details.

× denotes interaction.

NS, not significant.

LG, lateral gastrocnemius muscle; DF-IV, digital flexor-IV.

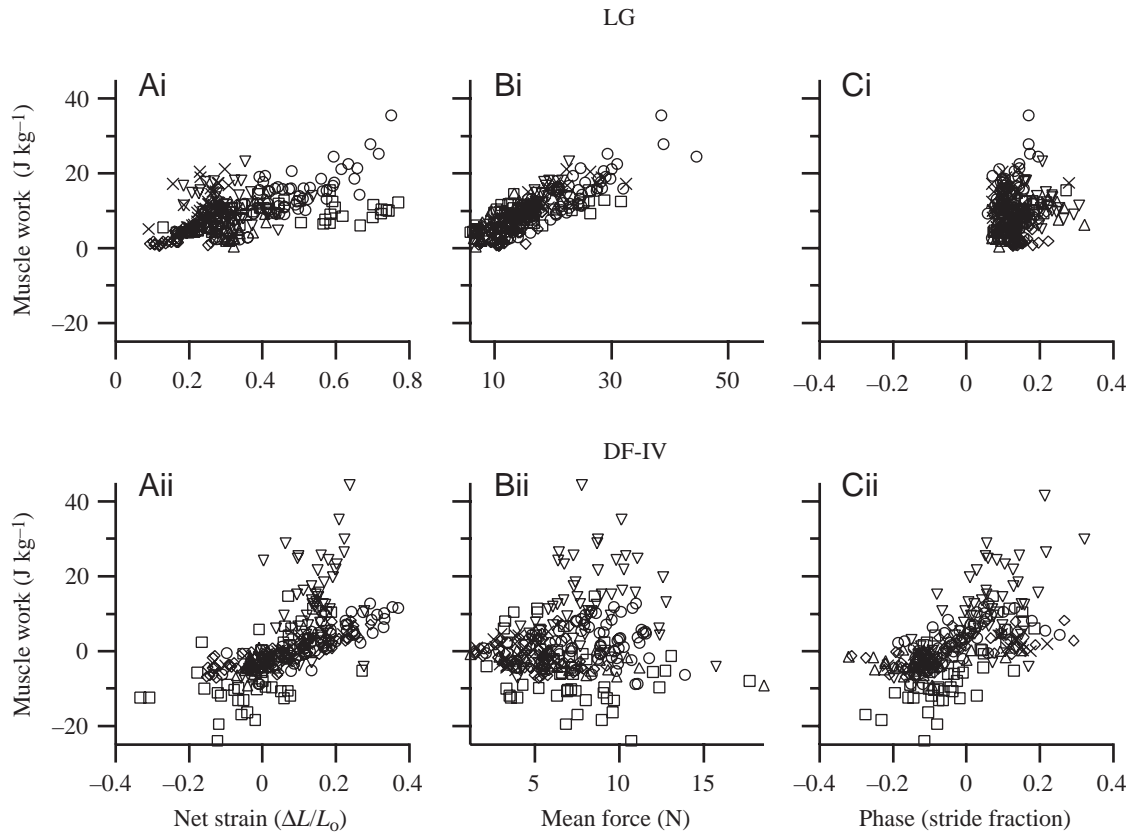


Fig. 8. Net mass-specific muscle work against (A) net strain, (B) mean force, and (C) phase relationship between force and strain for lateral gastrocnemius (LG, i) and digital flexor-4 (DF-IV, ii). Symbols indicate different individuals. Statistical results for the effect of each variable on work from a general linear model are shown in Table 5.

average, exhibiting a spring-like stretch-shorten cycle, with rather large strain amplitudes but little net strain (1–8%). These results suggest that the LG might incur a greater cost of force production than the DF-IV, because muscles generate less force at a greater energetic cost while shortening than while contracting isometrically or lengthening (reviewed by McMahon, 1984). Stretch-shorten contraction cycles, like

those observed in the guinea fowl DF-IV, lead to force enhancement that facilitates economic generation of force (e.g. Edman et al., 1978; Herzog and Leonard, 2000; Lee and Herzog, 2002). Due to its longer length and higher operating stresses, the DF-IV tendon recovered 4.5–8.5 times more energy per unit mass than did the LG tendon, resulting in 1.2–2.3 times greater total tendon energy savings, despite its smaller size. Neither muscle contributed as much energy as expected for its mass to increase the PE of the body COM during incline locomotion. This suggests that proximal muscles of guinea fowl may modulate work more than distal muscles. Furthermore, DF-IV work output is highly sensitive to stride-to-stride variation in the phase relationship between force and strain, possibly due to small changes in the mechanical interaction between the environment, limb and muscle-tendon complex (discussed below). This may imply a greater role for this muscle in mediating balance and stability for locomotion.

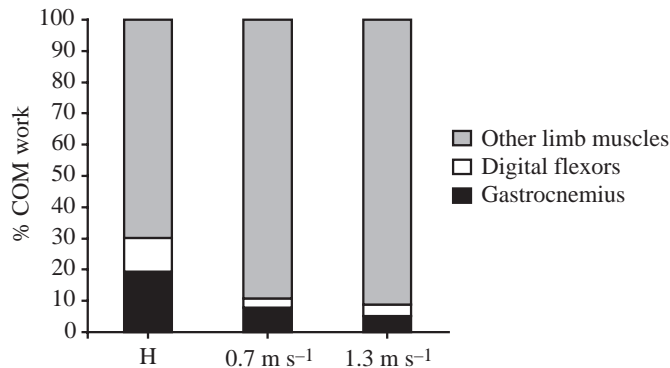


Fig. 9. Contribution of gastrocnemius (black portion) and digital flexors (white portion) to total center of mass (COM) work during incline running at 0.7 (middle) and 1.3 m s⁻¹ (right), compared to the contribution to be expected if all muscles in the limb contributed equally to mass-specific work for incline running (H; left).

Mechanical performance of the LG and DF-IV in relation to muscle-tendon architecture

The mechanical performance of the guinea fowl LG and DF-IV clearly differ in a manner that is consistent with differences in their morphology. Because the ratio of tendon to fascicle length is 10.8 in the DF-IV compared with 5.7 for the LG, the DF-IV tendon may be expected to provide relatively greater

elastic energy savings (for a given tendon stress). Indeed, according to the model of Ker et al. (1988), both muscles have a muscle–tendon morphology that favors elastic energy storage over muscular control of joint position. However, the peak muscle stresses developed during terrestrial locomotion by the guinea fowl LG and DF-IV differ substantially and average only 33 kPa in the LG and 116 kPa in the DF-IV. Under these peak stresses, the guinea fowl DF-IV tendon undergoes relatively large extensions, while the LG does not. As a result, the DF-IV contributes more to elastic energy savings. Whereas the gastrocnemius tendon stores only 1–5 J kg⁻¹ of energy, which amounts to 30–40% of shortening work by the muscle, the DF-IV tendon stores 12–21 J kg⁻¹ of elastic energy, exceeding DF-IV shortening work by 2.2- to 2.9-fold.

Although the guinea fowl is considered a cursorial species, it also uses its hind limbs for behaviors such as propulsion during jumping for flight take-off and energy absorption during landing. Consequently, the low muscle stresses in the LG and DF-IV may reflect the capacity to withstand much larger stresses during these behaviors. The peak muscle stresses measured in the LG are particularly low compared to those observed in other bipeds: 127 kPa in the gastrocnemius of the running mallard (Biewener and Corning, 2001) and 227–262 kPa in ankle extensors of hopping wallabies (Biewener and Baudinette, 1995). In an early study that measured *in vivo* muscle–tendon stresses, Biewener and Blickhan (1988) showed that the peak ankle extensor muscle stresses developed during hopping in kangaroo rats *Dipodomys spectabilis* were only one-third those experienced during jumping: 80 *versus* 297 kPa. Nevertheless, LG muscle stresses are surprisingly low compared with the stresses developed during steady locomotion observed in running mallards and hopping wallabies. In part, this may reflect a more limited range of performance in the case of mallards and the need to operate at high levels of stress for effective energy savings in the case of wallabies.

Diversity in LG force–length performance among avian bipeds

Although the pattern of LG muscle activity relative to limb movements, force development and muscle strain observed in a variety of avian bipeds during steady terrestrial locomotion is similar (Jacobson and Hollyday, 1982; Roberts et al., 1997; Gatesy, 1999b; Biewener and Corning, 2001), LG work performance differs substantially among mallards, turkeys and guinea fowl, due to varying degrees of shortening during force development. Fascicle shortening of the guinea fowl LG during force development is between that of the turkey LG, which contracts with limited shortening (1 to 6%), and the mallard LG, which shortens substantially (24–37%) (Roberts et al., 1997; Biewener and Corning, 2001). The large shortening strains of the mallard LG may not be surprising given that this bird is specialized for different locomotor modalities; however, both turkeys and guinea fowl are cursorial ground birds, known to be capable runners (Forshaw, 1998). The difference in size between the guinea fowl and turkey may account for some of the

observed difference in the contractile behavior of the LG. Smaller birds tend to run with more crouched postures (Gatesy and Biewener, 1991), which may reduce peak muscle–tendon stresses and elastic energy savings (McMahon, 1985; McMahon et al., 1987), and therefore require greater muscular work.

Different in-series compliance of muscles among species could explain different muscle strain amplitudes during stance, because a muscle with a more compliant tendon might shorten more to take up the stretch of the tendon; however, this does not appear to be the case. While tendon morphology differs substantially between species, the stretch of the tendon relative to muscle fiber lengths at the loads experienced during locomotion are similar. The gastrocnemius tendon of the guinea fowl is 5.7× longer than the muscle fascicles, while the mallard LG tendon is 1.4× longer than the muscle fascicles (Biewener and Corning, 2001), and the compliant element of the turkey gastrocnemius tendon is 3.2× longer than the muscle fascicles, because a large portion of the tendon is ossified (Roberts et al., 1997). These ratios suggest that the guinea fowl gastrocnemius tendon extends relatively more than the mallard and turkey tendons at a given load. However, the loads experienced by the tendons during locomotion differ between birds due to differences in peak muscle stress, which offsets these differences in tendon morphology. If the Fiber Length Factor (FLF), the ratio of muscle fiber length to tendon extension at peak load (Ker et al., 1988), is calculated for each bird using the peak forces measured during terrestrial locomotion, it is similar for the guinea fowl, turkey and mallard LG (12, 12 and 15, respectively, assuming the ratio of muscle:tendon cross-sectional area of the turkey is similar to the guinea fowl). Thus, when differences in tendon length and peak locomotor loads are considered simultaneously, the LG tendons of the turkey, mallard and guinea fowl function similarly to transmit muscle force with relatively little extension of the tendon. This suggests that further differences may exist among these species in muscle physiology, such as muscle contraction kinetics, that might explain the relatively greater shortening of the mallard and guinea fowl LG compared to the turkey LG.

The similarity in LG tendon compliance relative to the peak load experienced during locomotion results in similar elastic energy storage for the guinea fowl and turkey LG, in spite of the large differences in muscles stress and shortening work. As mentioned above, the guinea fowl gastrocnemius tendon stores 1–5 J kg⁻¹ of energy, which is 30–40% of shortening work of the LG muscle. In comparison, the turkey gastrocnemius tendon stores 1–4 J kg⁻¹, which is 2.5-fold greater than muscle shortening work (Roberts et al., 1997). The relatively low tendon compliance of the turkey gastrocnemius tendon, due to its ossification, offsets the effect of higher muscle stresses experienced during locomotion. If the guinea fowl gastrocnemius tendon were ossified in proportion similar to the turkey tendon, it would be less than half as long, and gastrocnemius elastic energy savings would be reduced to 1.7 J kg⁻¹ maximum. Likewise, the lower energy storage (2.5 J kg⁻¹) of the mallard gastrocnemius tendon can be

attributed primarily to its shorter length. It achieves half the mass-specific energy savings of the guinea fowl tendon because it is approximately half as long (56 mm compared to 99 mm for the guinea fowl tendon) (Biewener and Corning, 2001). In wallabies, which are specialized hoppers, tendon energy storage is 40–80 J kg⁻¹, which exceeds muscle shortening work by 20- to 36-fold (Biewener et al., 1998). Thus, while the ratio of tendon energy to muscle energy is lower in the guinea fowl LG than the turkey LG, the mass-specific energy recovered from these tendons is comparable. The guinea fowl and turkey LG tendons recover twofold greater energy than the tendon of the non-cursorial mallard, but much less energy than the ankle extensors of the wallaby. Thus, the level of elastic energy savings in the tendons of each species is consistent with differences in their locomotor specialization.

Muscle work modulation for incline running

In addition to investigating the relationship between muscle–tendon morphology and mechanical performance during level locomotion, we sought to compare how the guinea fowl LG and DF-IV modulate force–length performance for incline running. Only the LG significantly increased its net work output when the animals moved up a 16° incline compared with on a level (4.3–6.5 J kg⁻¹). This increase is similar to that observed in the turkey LG (5 J kg⁻¹ on a 12° incline; Roberts et al., 1997). However, the contribution of the guinea fowl ankle extensors to the increase in the animal's COM energy was only one-third of that expected for the muscles' mass (Fig. 9). This indicates that proximal limb muscles must contribute proportionately more work for incline running. Because direct measurements of force are difficult to obtain in proximal muscles, *in vivo* work has only been measured directly in distal limb muscles. However, sonomicrometry measurements show that proximal muscles of various species strain substantially during and immediately following muscle activation, suggesting that they generate work or absorb significant energy (Carrier et al., 1998; Gregersen et al., 1998; Gillis and Biewener, 2001). In addition, the biceps femoris and vastus lateralis of rats increase their active shortening when the animals run up an incline compared to on the level (Gillis and Biewener, 2002). Measurements of joint work also suggest that muscles at proximal joints may commonly contribute more work than those at distal joints (Pandy et al., 1988; Gregersen et al., 1998; Belli et al., 2002). Taken together, these results are consistent with the view that proximal limb muscles play a central role in modulation of limb mechanical work.

Although the guinea fowl LG performs significantly greater work during incline running, the qualitative shift in force–length contractile behavior is less dramatic than in the turkey LG. Unlike the turkey LG, which shifts from developing economic force on the level to generating work on an incline (Roberts et al., 1997), the guinea fowl LG functions similarly during both conditions to develop force and generate work (Fig. 5). The differing contractile behavior of these two

muscles results primarily from greater shortening (up to 15%) during level locomotion by the guinea fowl LG compared with the turkey LG (1–6%: Roberts et al., 1997). Thus, the work loop of the guinea fowl LG maintains a similar overall shape from level to incline locomotion, indicating little change in the basic contractile behavior of the muscle from level to incline locomotion. In contrast, the guinea fowl DF-IV shows a distinct shift in the shape of its work loop from level to incline locomotion (Fig. 5) that is qualitatively similar to the turkey LG, even though the net work increase on the incline for the DF-IV is not statistically significant.

Muscle activity in relation to force and work

The relatively uniform force–length contractile behavior of the guinea fowl LG compared to the DF-IV during level and incline locomotion are also mirrored by differences in the muscles' patterns of activation relative force and work performance. During incline running, changes in the timing of muscle activation relative to the limb cycle are generally small compared to the increase in EMG intensity (Roberts et al., 1997; Carlson-Kuhta et al., 1998; Gillis and Biewener, 2002), which is typically interpreted as reflecting an increase in muscle recruitment to meet the greater work requirements of incline locomotion. We found that during incline locomotion, guinea fowl LG EMG duration tended to be longer, while digital flexor-IV EMG intensity tended to be greater (Table 2). Although these changes were not statistically significant, they suggest different mechanisms of neuromodulation to mediate changes in mechanical performance for these two muscles. Furthermore, we found an interesting shift in the relationship between EMG intensity and mechanical output of the DF-IV, but not the LG. Because the mechanical output of a muscle is sensitive to numerous factors, including the pattern of activation, force–length and force–velocity effects, and the recent work history of the muscle (see Josephson, 1999; Marsh, 1999), the relationship between EMG intensity and mechanical output (force, work) is not necessarily constant. Yet, relative EMG intensity of the LG was strongly correlated with both mean force and net muscle work during level and incline running, whereas DF-IV EMG intensity was only significantly correlated with force during level running and with net work during incline running (Fig. 7, Table 4). These results are consistent with the functional shifts observed in the work loops for each muscle discussed above. Thus, it appears that the shift in force–length dynamics of the DF-IV from level to incline locomotion, shown by the work loops (Fig. 5), occurs in conjunction with a shift in the relationship between muscle recruitment and mechanical output of this muscle.

We observed that the DF-IV, and to a lesser extent the LG, operated at a significantly longer length at the onset of force when the animals moved on an incline (DF-IV: 13% longer, LG: 7% longer; Fig. 3). This is consistent with a shift to a more crouched (flexed joint) posture on an incline (Carlson-Kuhta et al., 1998), which suggests that the shift in DF-IV force–length dynamics may reflect effects associated with a postural change. By operating at a longer length, a muscle exhibits slower force

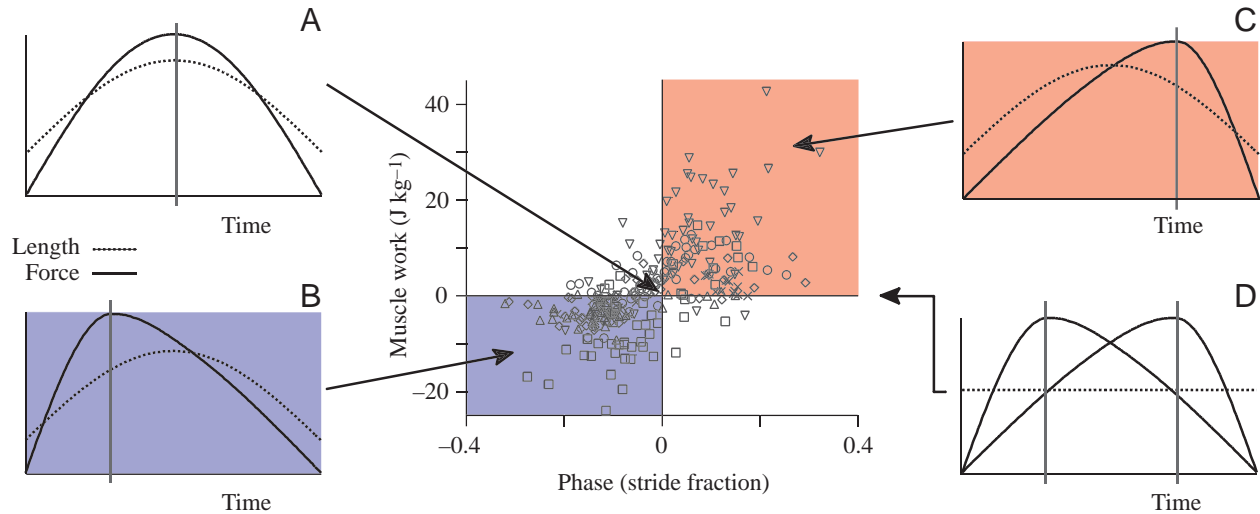


Fig. 10. Schematic illustration of the effect of phase (between length and force) and strain pattern on digital flexor-4 (DF-IV) muscle work. The center panel shows the relationship between phase and work for the DF-IV (Fig. 8Cii). If the muscle undergoes a stretch–shorten cycle (large Δ velocity) and muscle force and length are symmetrical, no net work results (A). However, if peak force precedes peak length, the muscle absorbs energy (B), and if peak force lags behind peak length, it produces energy (C). However, if the muscle contracts with constant velocity, whether positive, negative or isometric (as shown in D), phase has no impact on work. This explains why the interaction term, phase \times Δ velocity, is a larger factor underlying DF-IV work than phase alone (Table 5); a change in velocity during force production (Δ velocity) is required for phase to be an important factor in work. Vertical lines in A–D indicate peak force.

relaxation (Josephson and Stokes, 1989) and achieves greater and more prolonged lengthening force enhancement (Edman et al., 1978), both of which may favor increased work during the subsequent shortening phase of the muscle's contraction cycle. Interactions between muscle force and muscle length may play a large role in determining the mechanical performance of a muscle that undergoes a dynamic strain cycle. Consequently, small changes in limb mechanics, such as a change in posture from level to incline locomotion, may result in substantial changes in muscle mechanical performance, despite similar muscle activity patterns, as we observed for the DF-IV.

Determinants of work for the LG versus the DF-IV

Strain cycle dynamics relative to force development also affected the determinants of work output by these two muscles. Whereas the primary determinants of LG work were mean force and net strain, the primary determinant of DF-IV work was the phase relationship between force and strain (Table 5, Fig. 8). Because the LG shortened at a relatively constant rate during force production (Fig. 3), its work output generally reflects the product of mean muscle force and net length change. In contrast, the DF-IV underwent large changes in contractile velocity because it was stretched and then shortened (Fig. 3). Consequently, the relative timing between force and length change was a significant factor in its work output. This is because a given magnitude of mean force and net length change can result in positive, zero or negative work depending on the phase relationship between muscle force and length. Schematically, it can be thought of as follows: if force development and length change are symmetrical, no energy is

produced or absorbed, and the muscle–tendon system effectively operates as a simple spring (Fig. 10A). However, if peak force precedes peak length, energy is absorbed (Fig. 10B); whereas, if peak force lags behind peak length, energy is produced (Fig. 10C). Only when a muscle contracts with a constant velocity (whether zero or otherwise) is work independent of the timing of force development (Fig. 10D). In reality, the changes in relative timing of force and length change for most muscles are likely to be more complex than this. Even so, muscles that contract with dynamic changes in strain pattern, such as the guinea fowl DF-IV, can be expected to modulate work substantially through small changes in the timing of force relative to strain.

Stride-to-stride variation in work by the guinea fowl DF-IV (Fig. 4) probably results from small changes in the timing or intensity of muscle activation with respect to the mechanical interaction between the limb and environment, altering the phase relationship between muscle force and strain. Interestingly, Gabaldón and Roberts (2002) found that the peroneus (fibularis) muscle of the turkey uses a similar mechanism to modulate work for running on an incline *versus* on a level. Their previous results, combined with the stride-to-stride variation in work output in the guinea fowl DF-IV observed here, demonstrate that muscles can modulate work *in vivo* via the relative phase of muscle activation, length change and force. These findings parallel *in vitro* work–loop studies showing that relative phase is a critical determinant of a muscle's work output (Josephson, 1999).

In conclusion, the guinea fowl LG and DF-IV exhibit differing force–length dynamics during level and incline locomotion, which appear to have implications for their capacity

and mechanism of work modulation and, consequently, the mechanical roles they fulfill during locomotion. While the stretch-shorten strain cycle of the DF-IV muscle may facilitate more economic force generation, it also exhibits a more limited capacity to modulate work output to increase the energy of the body COM. Furthermore, the dynamic pattern of contractile function that we observed for the guinea fowl DF-IV suggests that this muscle may be more sensitive to small changes in the mechanical interaction of the foot and digits with the ground. Although this may lead to greater variability in its work output, it may also reflect a role in mediating stability and balance during locomotion, particularly when the animal moves over more variable terrain. This is an observation that we believe warrants future investigation.

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