

The integration of lateral gastrocnemius muscle function and kinematics in running turkeys

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Abstract

Animals commonly move over a range of speeds, and encounter considerable variation in habitat structure, such as inclines. Hindlimb kinematics and muscle function in diverse groups of vertebrates are affected by these changes in behavior and habitat structure, providing a fruitful source of variation for studying the integration of kinematics and muscle function. While it has been observed in a variety of vertebrates that muscle length change can be minimal during locomotion, it is unclear how, and to what degree, *in vivo* muscle length change patterns are integrated with kinematics. We tested the hypothesis that the length of the turkey lateral gastrocnemius (LG), a biarticular muscle that has moments at the ankle and knee, is not solely affected by changes in joint kinematics. We recorded *in vivo* muscle length changes (using sonomicrometry) and hindlimb movements (using high-speed video) of wild turkeys running on various inclines, and at different speeds. We quantified the relationship between joint angle (knee and ankle separately) and muscle length in freshly euthanized specimens, and then applied an empirically derived correction for changes in pennation angle and tendon strain during locomotion to improve the accuracy of our predicted lengths. We estimated muscle length at four points during each stride and then compared these values with those measured directly. Other than during swing, the predicted changes in muscle length calculated from the changes in joint kinematics did not correspond with our measured values of LG length. Therefore, the lengths at which the LG operates in turkeys are not determined entirely by kinematics. In addition to strain in series elastic components, we hypothesize that heterogeneous strain within muscles, interactions between muscles and muscle pennation angle all contribute to the nonlinear relationship between muscle length changes and kinematics.

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Introduction

Animals commonly move at various speeds over surfaces with varying inclines in their natural habitat.

Incline (e.g., Roberts et al. 1997; Carlson-Kuhta et al. 1998; Gillis and Biewener 2002; Daley and Biewener 2003; Gabaldon et al. 2004; Higham and Jayne 2004; Gillis et al. 2005; Gregor et al. 2006; Rubenson et al. 2006; Roberts et al. 2007) and speed (e.g., Roberts et al. 1997; Gillis and Biewener 2001; Nelson and Jayne 2001; Daley and Biewener 2003; Hoyt et al. 2005; Higham et al. 2008)

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can have a considerable impact on *in vivo* limb muscle function in vertebrates. Detailed kinematics and changes in muscle function are commonly studied independently, though they are likely to be functionally coupled. For example, changes in kinematics can be associated with changes in the operating lengths of limb muscles (Nelson and Jayne 2001; Gillis et al. 2005; Wickler et al. 2005). However, large changes in the lengths at which muscles operate would lower their force generating performance since muscles produce maximal force over a narrow range of lengths (approximately 10% of resting length; Rome 1994). A key question is whether the lengths over which muscles produce force are constrained by limb joint position and, if not, what other factors are possibly responsible for relieving these constraints.

Changes in kinematics can predict the direction of changes in muscle length but not necessarily the magnitude. For example, Nelson and Jayne (2001) used a regression model to show which changes in kinematics caused the observed changes in muscle fascicle length. A more recent study indicates that exact changes in muscle fascicle length cannot be predicted from kinematics (Hoyt et al. 2005). One primary reason for this decoupling is that tendons, along with their aponeuroses, can change length with little or no change in muscle fascicle length (Roberts et al. 1997; Biewener 1998, 2002; Biewener et al. 1998; Ito et al. 1998; Herbert et al. 2002; Pappas et al. 2002; Roberts 2002; Kurokawa et al. 2003; Lichtwark and Wilson 2006). This permits muscles to maximize force while limiting energy expenditure. The amount of force that a muscle produces directly affects the amount to which the tendons connected to it are stretched (Alexander 1988; Griffiths 1991; Biewener and Roberts 2000). When tendons are stretched instead of muscle fascicles, the relationships between muscle strain and kinematics become uncoupled. Another source of decoupling between kinematics and overall muscle length is the dynamic changes in pennation angle during overall muscle length changes (Brainerd and Azizi 2005; Azizi and Brainerd 2007). Pennation angle can also vary along the length of a muscle, further complicating the relationship between kinematics and muscle length. Changes in pennation angle with muscle length alter the amount of length change in the fascicles in relation to the whole muscle. Such changes in the ratio of fascicle strain to whole muscle strain can change the point where the muscle fiber produces force on the force–velocity curve and therefore also affect the optimization of force and power production. Finally, muscles commonly exhibit spatial heterogeneity in strain (Pappas et al. 2002; Soman et al. 2005; Lichtwark et al. 2007; Higham et al. 2008; Higham and Biewener, 2008), which could contribute to the decoupling of muscle length change patterns and kinematics. Teasing apart the contributions of these factors to the decoupling of muscle strain and

kinematics will ultimately enable us to better predict how the muscle–tendon complex changes length with changes in kinematics.

It is imperative to establish the quantitative relationships between limb movements and *in vivo* muscle length change patterns given that muscle strain is commonly inferred from kinematics in a variety of vertebrate groups (Coughlin et al. 1996; Delp et al. 1996; Lutz and Rome 1996a,b; Prilutsky et al. 1996; Ettema 1997; Shadwick et al. 1998; Burkholder and Lieber 2001; Higham and Jayne 2004). Muscle models are commonly employed to estimate muscle length change patterns from kinematics, and these models typically receive inputs based on cadaveric studies (Hoyt et al. 1990; Delp and Loan 2000; Blemker et al. 2007). In addition, musculoskeletal models typically assume uniform strain among muscle fibers (Blemker et al. 2007). Thus, it is important to understand how various factors (e.g., pennation angle, muscle force, strain heterogeneity) contribute to the decoupling of kinematics and muscle function. Understanding how *in vivo* muscle fascicle length changes relate to changes in kinematics will drastically enhance the construction of musculoskeletal models.

Previous work has revealed that a muscle can remain relatively isometric during the force producing period of locomotion (Roberts et al. 1997; Biewener et al. 1998; Daley and Biewener 2003; Gabaldon et al. 2004). However, a muscle remaining isometric does not necessarily indicate a decoupling between muscle length change patterns and kinematics. For example, the lateral gastrocnemius (LG), a muscle commonly cited as remaining isometric during force production, is biarticular (acts at the ankle and knee). Thus, the ankle and knee angle could change in such a way that the muscle would be predicted to remain isometric. However, this has not been tested. To test the hypothesis that kinematics and muscle length are decoupled, we measured *in vivo* muscle fascicle strain (in the LG) and hindlimb kinematics of wild turkeys running at different speeds and on various inclines. We predicted the length changes of the proximal muscle fascicles by calculating moment arms at the ankle and knee from freshly euthanized specimens. If kinematics and muscle fascicle length were found to be decoupled, we hypothesized that tendon strain would account for these discrepancies.

Materials and methods

Animals and treadmill training

Four female Eastern wild turkeys (*Meleagris gallopavo* Linnaeus, 1758) were obtained from a breeder in

Oregon and housed in an outdoor enclosure at Oregon State University. The mean body mass of the birds was 4.06 ± 0.82 kg (\pm SD); and the mean mass of the LG was 21.59 ± 5.89 g (\pm SD). A diet of Game Bird Flight Conditioner (Purina Mills, St. Louis, MO, USA) and water were provided *ad libitum*.

Treadmill training consisted of running on a motor-driven treadmill at 0° , 6° , and 12° inclines (Keys Pro 2000 Series, Keys Fitness Products; Garland, TX, USA) for 10–20 min day⁻¹, 4–5 days a week, for 4–6 weeks. The treadmill was fitted with a cage made with a wood frame, a plywood side and top, Plexiglas windows for video imaging at the side and front, and an opening at the back for access to the bird. 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; Ont., Canada) 2 mm in diameter were implanted into 2-mm-deep pockets made along the long axis of a proximal fascicle of the LG. The crystals were aligned 9–12 mm apart and secured in place with a small drop of 3M 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. The error associated with sonomicrometry measurements was 4.6%. For two birds, two small strain gauges (Type FLK-1-11; Tokyo Sokki Kenkyujo Co., Ltd., Tokyo, Japan) 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 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., Cheshire, CT, 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 ($+6^\circ$ and $+12^\circ$) at speeds of 1–2 m s⁻¹. Ten seconds of data were collected for each run. Birds remained on the treadmill

at slow walking speeds between speed and slope changes and were allowed to rest on a stopped treadmill as needed. Fascicle lengths were recorded with a sonomicrometry system set to a frequency of 992 Hz using the data acquisition software SonoLAB. Tendon strain signals were amplified using a strain gauge conditioner (model 2120; Vishay Measurements Group, Malvern, PA, USA). Data were collected at a frequency of 4000 Hz using 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). Muscle length data were taken from the four best strides for each speed and incline combination. The best strides were those that exhibited steady locomotion, and were centered in the camera view. Maximum, minimum, and average muscle lengths were analyzed as well as the amount of shortening during the period of force production in swing and stance phase (Fig. 1). In swing phase, the muscle is always producing some force so muscle lengths throughout swing were analyzed in addition to the lengths during stance. The LG produces force only during the first half of stance phase. The amount of shortening, maximum, minimum, and average lengths were recorded from the first part of stance, a period of time previously determined to be the time when force is produced during stance (Gabaldon et al. 2004).

Calculating predicted values of strain

The change in muscle fascicle length between consecutive frames was predicted by combining measurements of knee and ankle angles during running, LG moment arms at the knee and ankle, and the effects of pennation angle on muscle length using the equation $l = (r_k \theta_k + r_a \theta_a) / \theta_m$, where r_k and r_a are the moment arms for the LG at the knee and ankle, respectively, θ_k and θ_a are the changes in knee and ankle angles, respectively, and θ_m is the correction factor for pennation angle (1.385). The moment arms at the ankle and knee were then divided by the resting muscle fiber length for each bird in this study. This was done so that changes in absolute length from the measured moment arms were converted to a similar length unit measured by the sonomicrometry crystals. This pennation angle correction represents the average pennation angle throughout a stride (M. Azizi, unpublished data). The predicted fascicle lengths were assumed to be equal to the length measured using sonomicrometry at the end of stance when force is zero given that this is when there is no strain on the tendon. Based on this assumption, the equation outlined above was used to calculate how muscle length would change through time for each stride.

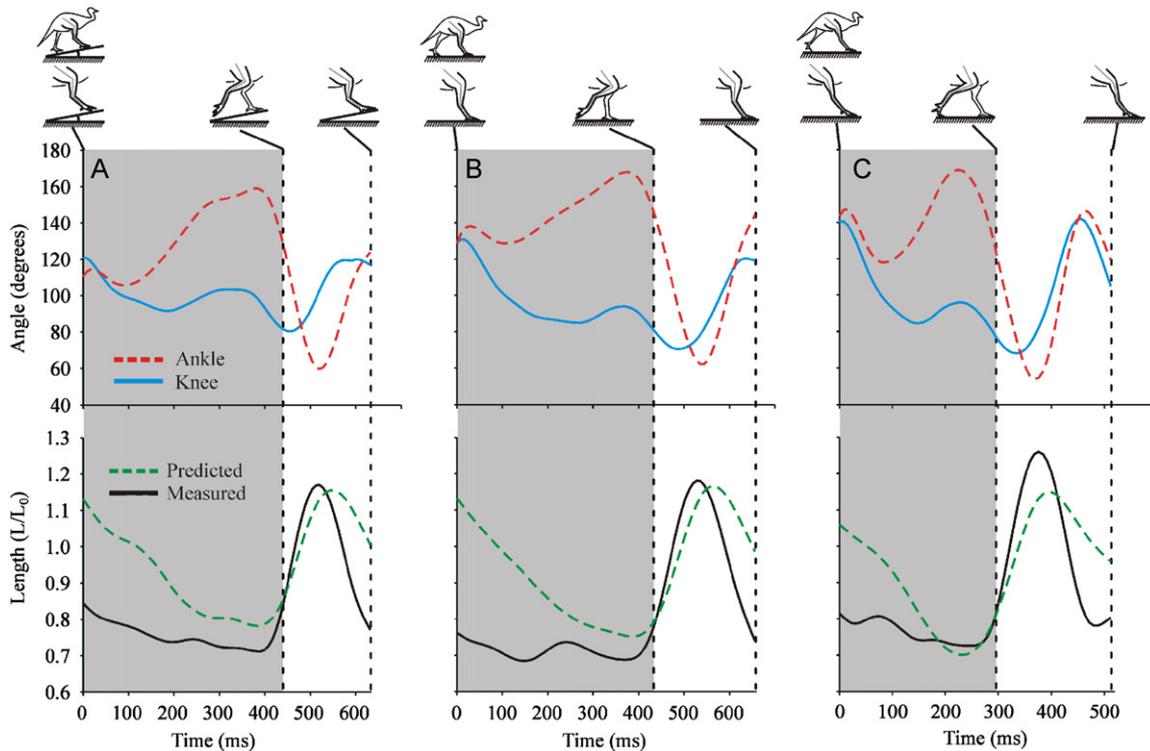


Fig. 1. A representative sequence of the ankle (dashed lines) and knee (solid lines) angles (top panels), and predicted (dashed lines) fascicle lengths and measured (solid lines) fascicle lengths (bottom panels) throughout one stride for a turkey running on a 12° inclined treadmill at a speed of 1.0 m s⁻¹ (A), on a level treadmill at a speed of 1.0 m s⁻¹ (B), and 2.0 m s⁻¹ (C). The shaded areas indicate the stance phase of the stride when the foot is in contact with the ground. Details of how the predicted strains were calculated are in the text.

Individuals were euthanized with a lethal injection of sodium pentobarbital. Moment arm measurements were made on dead turkeys the day after the experiment by measuring the length change of the LG muscle as the ankle and knee joint angle changed. The gastrocnemius muscle of these birds was isolated from the surrounding tissue and bisected in half with its site of insertion and origin still attached. One end of a piece of 3–0 silk suture was tightly tied to the free end of the bisected muscle. The other end of the suture was tied to a 50 g mass. The limb was aligned and the tibia–tarsus secured to a table, such that the suture ran from the end of the muscle parallel to the tibia–tarsus and over a pulley to the mass. The 50 g mass applied a constant force to the muscle and ensured muscle length change would only be affected by joint angle. A ruler was fixed below the suture just before the pulley and a drop of white paint was placed on the silk suture to track length changes. Joint angles were measured with a goniometer. The slope of a linear regression line fit to a plot of muscle length change versus joint angle data from these measurements determined the moment arm about the ankle and knee joints of the LG (ankle: slope = 0.86, $R^2 = 0.95$; knee: slope = -0.97 , $R^2 = 0.84$). Higher polynomial equations did not increase the R^2 value, so linear regressions were used.

Once the predicted values of muscle length were calculated, the greatest differences between the predicted and measured values were determined. This was assumed to be the maximum value of tendon strain. Tendon length (including the aponeurosis, bony part of the tendon and soft part of the tendon) was measured and the maximum difference between predicted and measured LG length was divided by the tendon length to get relative tendon strain.

***In situ* determination of series elastic element stress–strain properties**

The stress–strain properties of the series elastic element, the tendon and aponeurosis, were determined *in situ* at the end of running experiments for two of the four birds in this study (Nelson et al. 2004). We did this in order to account for the effects of force on the length of the muscle–tendon unit. The procedure involved electrically stimulating the muscle via the sciatic nerve while simultaneously measuring whole muscle force and fascicle strain from the sonomicrometry crystals. 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 LG's origin was fixed in place by means of two bone screws inserted into the femur and attached to an aluminum frame. The LG's tendon of insertion was cut free and attached to an aluminum clamp connected to a servomotor (model 310B-LR; Aurora, Ont., 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; Grass Technologies, West Warwick, RI, USA). The muscle tendon complex was held at a fixed length when stimulated so that any shortening in the muscle fascicles was due to stretching of the tendon and aponeurosis. The slope of the line relating muscle fascicle strain to muscle force therefore gives us the stress–strain properties of each bird's tendon and aponeurosis.

Kinematics

The positions of the ankle and knee joints were determined from high-speed (250 Hz) video recordings (Redlake Motionscope 1000; San Diego, CA, USA). Small reflective markers (3M; 70610WS) were applied to the skin at the ankle (intertarsal) joint. The knee center of rotation could not be determined via a marker because knee movement occurred independently of skin movement. To locate the position of the knee in the video, two markers were placed along the tibiotarsus at about a third and two-thirds the distance from the ankle to the knee. These markers were aligned such that the position of the knee center of rotation could be extrapolated from the position of the ankle marker and the known distance from the ankle to the center of rotation of the knee. These methods for determining joint centers of rotation have been validated previously using high-speed cineradiography, which indicated that the largest error in joint marking occurs at the most proximal joints and is less than 0.5 cm (Roberts et al. 1998). Video data were transferred to a Macintosh computer using a Scion LG-3 frame-grabbing card. Marker positions were digitized on a PC using IMAGE J version 1.33 (NIH, Washington, DC, USA). All of the digitized coordinates for each bird were converted from pixels to meters by determining the pixel distance between the joints and comparing this to the known distance in meters. After this conversion, joint position data were smoothed using a cubic spline interpolation (smoothing factor = 1, SD = 0.001) in IGOR Pro (WaveMetrics Inc., Lake Oswego, OR, USA) to remove noise. Two-dimensional joint angles were then calculated from digitized points using customized spreadsheets in Microsoft Excel.

Statistics

Analyses of variance (ANOVA) were used to determine how speed affected stride duration and duty factor, and to determine how incline and speed affected the hindlimb kinematics of turkeys. The independent variables for all ANOVAs were individual (random), incline (fixed), and speed (fixed). The two- and three-way interaction terms were also included in the analyses. To account for replication within individual, the denominator in the *F*-ratio was the two-way interaction term between the factor and individual (Zar 1996). Paired sample *t*-tests were used to determine whether the predicted values of muscle length were different from the actual values. SYSTAT version 9 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. The criteria for statistical significance was $P < 0.05$. Unless stated otherwise, all values are mean \pm standard error of the mean (S.E.M.).

Results

Stride duration ($P < 0.001$) and duty factor ($P < 0.001$) decreased significantly with an increase in running speed (Fig. 1B and C). For example, the average stride duration and duty factor decreased substantially from 1 m s^{-1} ($0.65 \pm 0.01 \text{ s}$ and $72.0 \pm 0.30\%$, respectively) to 2 m s^{-1} ($0.43 \pm 0.01 \text{ s}$ and $63.2 \pm 0.39\%$, respectively). Speed did not affect any of the kinematic variables other than the knee angle at the end of stance, which increased with an increase in speed ($P = 0.03$). Both knee and ankle angles were affected by incline, but at different points within a stride. At footfall, ankle angle ($P < 0.001$), but not knee angle ($P > 0.05$), decreased significantly with an increase in incline. In contrast, knee angle ($P < 0.01$), but not ankle angle ($P > 0.05$), increased with an increase in incline at the end of stance. For a given increase in ankle angle, the knee angle increased to a greater extent (Fig. 2).

The relative lengths of the LG at the four measured points during the stride were not significantly affected by incline or speed (e.g., Fig. 3). The shortest relative LG fascicle lengths occurred when force equaled zero during stance (Fig. 4). In contrast, the longest relative LG fascicle lengths occurred during swing. In general, the proximal LG fascicles remained relatively isometric during the stance phase of the stride and then underwent a large stretch-shorten cycle during the swing phase (Fig. 5A). The predicted values of LG length at footfall ($t = -13.9$, $P < 0.001$), when force equals zero during stance ($t = -8.0$, $P < 0.001$), and at maximum length during stance ($t = -12.7$, $P < 0.001$) were all significantly different from the actual lengths measured from sonomicrometry (Fig. 4). However, the predicted values

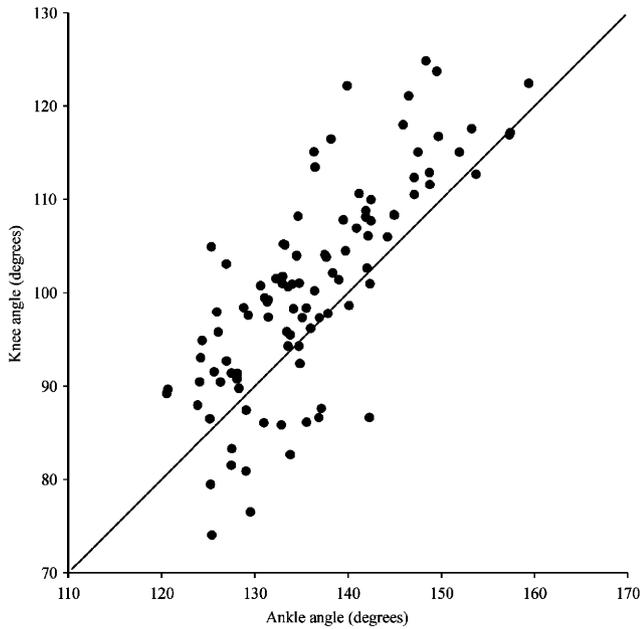


Fig. 2. Changes in knee angle versus changes in ankle angle when force equals zero during stance for all trials ($N = 101$). The solid line represents a condition where the slope equals 1. Note that as the knee angle increases, the angle of the ankle also increases ($R^2 = 0.59$; $P < 0.001$). However, the knee angle increases to a greater extent (i.e., slope greater than 1).

at the maximum length during swing were not significantly different ($t = 1.4$, $P = 0.18$) from the maximum lengths during stance that were measured from sonomicrometry (e.g., Fig. 1). If the maximum difference between predicted and measured LG length for each stride is used as an estimate of maximum tendon strain, we obtain values ranging from 0.3% to 4.8%,

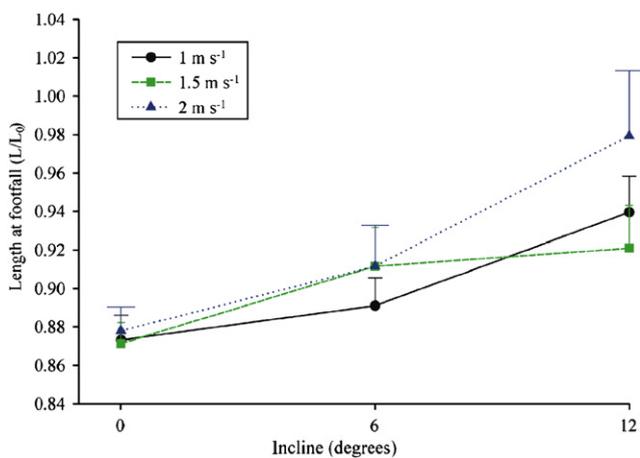


Fig. 3. The average length of the LG on inclines of 0° , 6° , and 12° while running at speeds of 1 m s^{-1} (circles), 1.5 m s^{-1} (squares), and 2 m s^{-1} (upward triangles). Note that the magnitude of measured length (relative to resting length) generally (but not significantly) increases with an increase in incline and speed. Values are means \pm S.E.M. for $N = 4$ birds and 101 trials.

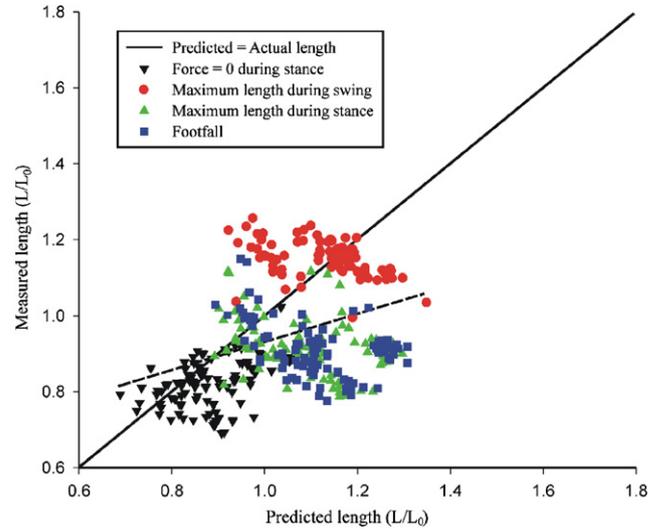


Fig. 4. Measured (using sonomicrometry) versus predicted length for the LG at four times within a stride including when force equals zero during stance (downward triangles), when the LG reaches its maximum length during swing (circles), when the LG reaches its maximum length during stance (upward triangles), and at footfall (squares). Note that these predicted values do not include a correction for pennation angle or tendon strain (see Fig. 5 for values including these corrections). The solid line represents the condition where the predicted length equals the actual length of the LG. The dashed line represents a linear regression performed on all of the data points ($R^2 = 0.14$, $P > 0.05$). Note that the predicted length is often larger in magnitude than the actual length.

which are likely not high enough to result in failure (Shadwick 1990).

Discussion

Hindlimb kinematics cannot be used to accurately estimate LG fascicle length changes during running in turkeys, evidenced by the significant differences between predicted (using kinematics) and measured LG lengths. Our original prediction was that, if kinematics and muscle length changes were decoupled, tendon strain could account for the differences between predicted and measured muscle fascicle length. While tendon strain did account for some of the uncoupling, it was not sufficient to explain all of the uncoupling. Muscle fiber pennation angle and heterogeneous strain are other key factors that could help explain the discrepancies between predicted and measured LG fascicle lengths.

Interactions between muscles and tendons

The interactions between muscles and tendons can lead to a decoupling of kinematics and muscle strain (e.g., Roberts and Marsh 2003). Tendons are elastic

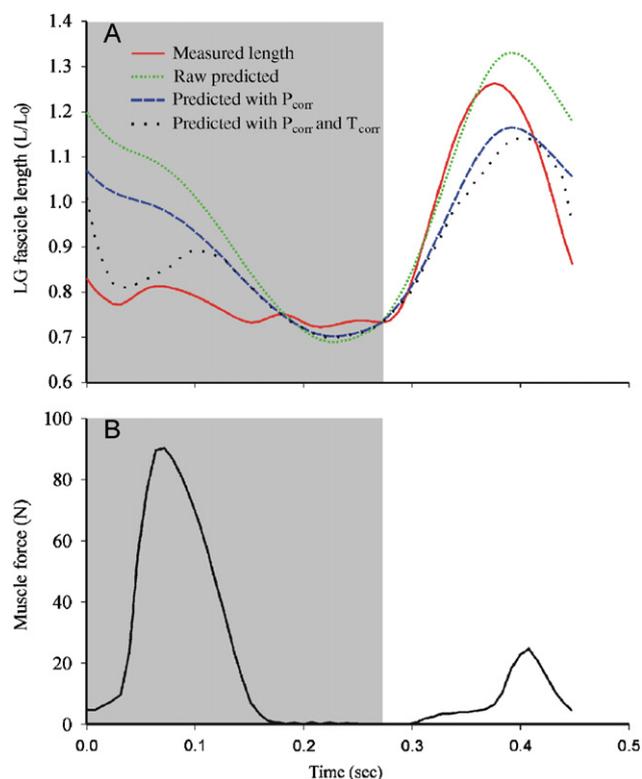


Fig. 5. (A) An example sequence showing the differences between predicted and measured lengths of the LG when using raw predicted values, using values with a pennation angle correction (P_{corr}), and when using values with a pennation angle correction and tendon strain correction (T_{corr}). Note that, during stance, the predicted values incorporating corrections for pennation angle and tendon strain most closely match the measured values. (B) For comparison: muscle force values during the same sequence. Note that these data are from the same sequence as shown in Fig. 1C, and are for a bird running on a level surface at 2 m s^{-1} . The shaded regions indicate the stance phase of the stride when the foot is in contact with the ground.

materials that can store potential and kinetic energy by lengthening when subjected to externally applied loads. Distal limb muscles insert via a tendon, and the morphology of distal tendons can vary considerably (e.g., McGowan et al. 2008). In addition to free tendons, distal limb muscles commonly have expansive aponeuroses continuous with the free tendon, defined together as the series elastic element that can also stretch and store elastic energy. A tendon with a smaller cross-sectional area requires a smaller magnitude of applied force for a given amount of stretch as defined by the elastic modulus of soft tendons (Alexander 1988; Shadwick 1990; Biewener 1998; McGowan et al. 2008). The stretching of the tendon allows muscles to contract isometrically or at a slow velocity, resulting in a preservation of maximal or near maximal force and a saving of energy (Biewener and Baudinette 1995; Roberts et al. 1997; Baudinette and Biewener 1998;

Biewener et al. 1998). For example, the LG of turkeys can contract very little while the muscle–tendon unit lengthens (Fig. 1). Thus, the decoupling of kinematics and muscle length in our study shows that stretching of the LG tendon is a primary mechanism (but not the only one) for maintaining muscle length. It is possible that the importance of tendon stretch in the decoupling of kinematics and muscle length depends on tendon anatomy, although future studies are needed to investigate this.

In order to explore the contribution of tendon strain to the decoupling of kinematics and muscle length changes, we isolated the LG from two of the birds in the study and performed *in situ* experiments to determine the force–length properties of the muscle (Fig. 6). For these two birds, *in vivo* force was also measured from the distal tendon during the experiments as in Gabaldon et al. (2004). We randomly selected five strides from the two birds and estimated tendon strain based on the forces. We then compared the estimated tendon strain with the maximum difference between predicted and measured LG strain during each of the 10 strides. Tendon strain alone can account for approximately 16% of the difference between measured and predicted

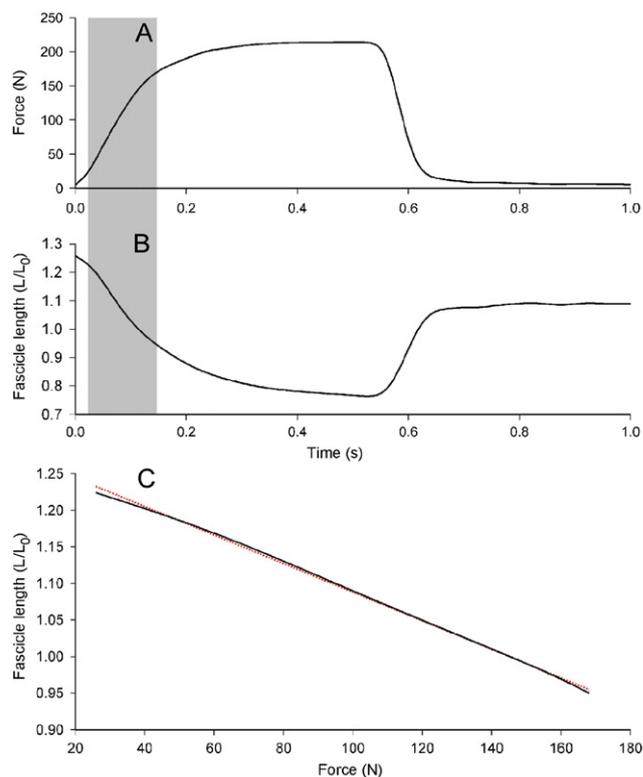


Fig. 6. An example sequence showing the tetanic force (A) and fascicle length (B) from an *in situ* preparation from one of the birds in our study. (C) Relationship between force and length during the initial shaded region from (A) and (B). The dashed line represents a linear regression fit to the data. See Materials and methods section for details regarding the preparation.

LG lengths at maximum length during stance (Fig. 5). Thus, a large source of the difference is from other factors, which are explained below.

Muscle force

The amount of force generated by a muscle will directly affect the stretch of the series elastic element (Griffiths 1991; Biewener and Roberts 2000; Roberts 2002). For example, if kinematics remain constant and force changes, the amount of the tendon and aponeurosis stretch also changes, resulting in an increase in muscle shortening. This occurs in the LG of turkeys, in which force generation is approximately four times greater in stance as compared to swing (Fig. 5; Roberts et al. 1997; Gabaldon et al. 2004). Ultimately, this will confound the predictions of muscle length from kinematics. For example, there are similar values of predicted LG lengths in early swing and early stance (Fig. 1B). In this example, the predicted values are both equal to 1.1, but the actual strain values (measured from sonomicrometry) differ by approximately 35% (0.75 during stance versus 1.15 during swing). Force generation can vary between strides and between species, further complicating predictions of muscle length from kinematics. It is also important to reiterate that tendon strain alone can only account for 16% of the difference between predicted and measured muscle length at maximum length during stance.

Pennation angle

Pennation angle varies considerably in different muscles, and varies dynamically during a contraction (e.g., Karamanidis et al. 2005). For example, the pennation angle of the gastrocnemius of humans increases as the muscle shortens during stance (Narici et al. 1996; Maganaris et al. 2001; Lichtwark and Wilson 2006). We adjusted our predicted values of length, which were parallel to the long axis of the muscle, in order to compare them to the measured values of LG length, which were made along the fascicle axis. When we accounted for pennation angle in our study, the predicted values for the most part more closely approximated the measured values of LG length. This is especially the case during the stance phase of the stride (Fig. 5). Although pennation angle and the stretch of a series elastic element are related, the former could play a larger role in decreasing or increasing the disparity between kinematics and muscle length. Future work which measures the dynamic variation in pennation angle will offer better insight into the role this has in minimizing length changes imposed by muscle moment arms. Given that distal limb muscles typically undergo a dynamic change in pennation angle (e.g., Karamanidis et al. 2005) whereas some proximal muscles (e.g., biceps brachii) do not (Murray et al. 2000), it would be insightful to determine whether this is correlated with

the amount of force produced by the muscle and/or the structure of the tendon and aponeurosis.

In our study, we applied a static correction to account for changes in pennation angle. In reality, pennation angle is dynamic during a muscle contraction (Karamanidis et al. 2005). To determine if a dynamic pennation angle correction could significantly reduce the discrepancy between predicted and measured LG lengths, we constructed an optimization algorithm in Matlab (The MathWorks Inc., Natick, MA, USA) that minimized the difference between predicted and measured lengths throughout a given stride. The algorithm did this by selecting a pennation angle between 24° and 43° that corresponded with a minimum difference between predicted and measured lengths. The optimization algorithm, after accounting for tendon strain, reduced the average maximum difference between predicted and measured fascicle length from 22% to 15%, which represents a modest decrease. Thus, the static correction for pennation angle is justified. While these realistic corrections for pennation did reduce the difference between measured and predicted muscle fascicle lengths, there was still a 15% difference between predicted and measured LG length at maximum length during stance unaccounted for by pennation angle and tendon strain.

Muscle heterogeneity

It is common to infer overall muscle function by studying a single location within a muscle, typically near the proximal or central region of the muscle belly. However, limb muscles can be heterogeneous and compartmentalized, and different parts of the muscle can exhibit different strain behaviors (Carrasco et al. 1999; Pappas et al. 2002; Ahn et al. 2003; Drost et al. 2003; Soman et al. 2005; Lichtwark et al. 2007; Higham et al. 2008; Higham and Biewener, 2008). This can be related to a number of different factors including morphological differences and patterns of fiber-type distribution. Our study reduces this complexity to a single average correction factor to account for measured differences between overall muscle and regional strain patterns. However, our study is not capable of determining the possible effects of regional force and strain due to spatial differences in fiber type or muscle architecture. Perhaps, a certain area of the muscle does show a coupling of length change with kinematics, while other areas show a decoupling.

The average maximum predicted strain during stance in the LG, after accounting for tendon strain and pennation angle, is 15% higher than that measured with sonomicrometry. It is important to note that this is a maximum value, which means the predicted and measured values more closely match for the rest of the stride (see Figs. 1 and 5). We predict that this difference can be attributed to differences in fascicle shortening

along the length of the muscle and/or experimental error. Based on our results, we predict that the distal regions of the LG undergo more shortening than the proximal regions. This prediction is supported by a study of the human LG in which the distal regions shortened more than the proximal regions during walking (Lichtwark et al. 2007). However, future studies that examine the spatial strain patterns of the turkey LG are warranted.

We propose four possible sources of heterogeneous strain within the LG. First, the distal regions of the LG may have a different fiber type distribution than the proximal regions. Second, the timing of activation of motor units in the distal region of the LG might differ from the proximal region. Third, the relative concentration of connective tissue might vary along the length of the muscle, resulting in variable amounts of strain (Higham et al. 2008). Fourth, the angle and length of the fibers might differ between the proximal and distal regions of the muscle. If the fibers were relatively parallel in the distal regions, a greater amount of strain would be expected relative to proximal fibers that have a significant pennation angle. Future work that teases apart the relative contributions of these factors will provide considerable insight into the dynamic function of muscles.

The distal limb of vertebrates comprises more than one muscle. Thus, it is quite possible that other muscles in the hind limb will undergo patterns of length change that more closely match changes in kinematics. In addition, the action of another muscle might facilitate the decoupling of LG function and kinematics. For example, our predicted changes in LG length were based on moment arm calculations for the LG in isolation and therefore do not account for any interaction between the LG and the other muscles around it. Thus, the actual changes in LG length under *in vivo* conditions could be constrained by other muscles that are also generating force before, or at the same time as, the LG. Future experiments that examine the relationship between kinematics and muscle length change patterns in multiple hindlimb muscles will provide insight into the interactions between muscle function and skeletal movement.

Differences between joints

The LG of turkeys is biarticular, acting to extend the ankle and flex the knee. The knee joint of turkeys, however, appears to contribute more to LG length change given that the knee angle changes more for a given change in ankle angle at different points during stance (Fig. 2). For example, a given increase in ankle angle will result in a greater increase in knee angle. Thus, the angular excursion of the knee likely contributes

more to the disparity between kinematics and muscle length since the moment arms are similar between the two joints. Put another way, the angular excursion of the knee likely contributes more to series elastic strain than does the ankle. It would be interesting to determine if the knee also contributes more to length changes in a proximal biarticular muscle compared with the hip.

Conclusions

Musculoskeletal models are pervasive throughout studies of animal locomotion. The challenge, as is the case with any model of a biological system, is incorporating a substantial number of realistic inputs. The muscles in these models are typically described by their geometry and force-generating properties (Delp and Loan 2000). While these variables provide a dynamic model, they are typically extracted from *in situ* or *in vitro* studies. It is clear that observations under these controlled conditions frequently fail to represent the dynamic contractile behavior of a muscle under *in vivo* conditions (Marsh 1999). Our data confirm that moment arm measurements taken from dead specimens are not sufficient for predicting *in vivo* behavior. However, our data provide a relationship between *in vivo* muscle behavior and limb movements, and thus provide insight for future musculoskeletal models. Constructing increasingly sophisticated musculoskeletal models will provide the framework for studying the diversity and evolution of musculoskeletal systems in a wide variety of vertebrates.

In the present study, we show another musculoskeletal system exhibiting an uncoupling between changes in kinematics and muscle operating lengths. This is similar to the previous findings of Hoyt et al. (2005) for the vastus lateralis muscle in the hindlimb of horses. However, the LG is a biarticular muscle of the distal hindlimb whereas the vastus lateralis is a monarticular muscle of the proximal hindlimb. Unlike the study by Hoyt et al. (2005), we quantified how much of the difference between the predicted lengths and measured lengths can be attributed to the force produced by the muscle. We predict this is due to the stretching of the free tendon and aponeurosis along with changes in pennation angle. However, future studies are required to test our predictions and explore other possible factors. Until we better understand the uncoupling between changes in kinematics and muscle length, inferring muscle length changes from changes in kinematics should be avoided (e.g., Higham and Jayne 2004). However, similarly rigorous studies examining changes in kinematics and muscle lengths in fishes have shown these variables to be completely coupled (Coughlin et al. 1996; Shadwick et al. 1998). The muscle architecture and

arrangement of the tendons of the fish studied is very different from the LG of turkeys or the vastus lateralis of horses. The muscle fibers studied in these fishes were parallel in structure with short tendons of a relatively high cross-sectional area. Future studies examining the integration of muscle lengths and kinematics using muscles (and tendons) that vary considerably in architecture will increase our understanding of how architecture influences the interaction between joint movement and muscle function.

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