AN ABSTRACT OF THE DISSERTATION OF

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Abstract approved:

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Thomas J. Roberts

Previous attempts to understand the factors affecting the energetic cost of locomotion have found a direct link between the energetic cost and the mechanical work done during periods when the limb is in contact with the ground. However, when the limb is not in contact with the ground during the swing phase, this link between mechanical work and energetic cost disappears. I examined the mechanics of swing to explore the possibility of passive mechanisms allowing for the performance of mechanical work with little to no energetic cost during swing. Previous studies have ruled out the possibility of a pendulum exchange of gravitational potential and kinetic energy during human locomotion because the swing frequencies are too high. I added the accelerations of the body during stance to the swinging lower limb to determine if the frequency where the pendulum-like exchange of energy occurs could be increased. These accelerations increased the frequency where energy exchange occurs and thereby reduced the work required to swing the human lower limb. The pendulum-like exchange of energy reduces the work required for swing, but some work is still required. To explore how the remaining work for swing was produced I examined two muscles potentially involved in producing an extension moment about the intertarsal joint of turkeys during swing. The only muscle providing force for intertarsal joint extension during swing was the lateral head of the gastrocnemius (LG). A comparison of the *in situ* length-tension curve and *in vivo* operating lengths during swing revealed the LG operated at long lengths on the descending limb of the length tension curve during swing. Finally I characterized the force-velocity curve of the LG and found the muscle to have mechanical properties within the range previously determined for other vertebrates. In conclusion, I determined a passive mechanism which could reduce the required mechanical work of swing and thereby explain part of the apparent lack of a link between mechanical work and energetic cost of swing. In addition, results from these studies suggest the remaining work necessary for swing may be provided by active contraction of muscle.

Energetics and Mechanics of Swing Phase during Terrestrial Locomotion

by
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APPROVED:

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Chair of the Department of Zoology

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Frank E. Nelson, Author

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CONTRIBUTION OF AUTHORS

Dr. Richard L. Marsh was involved with experimental design, data collection and writing of Chapter 3. Dr. Annette M. Gabaldón was involved with data collection and writing of Chapter 4.

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Chapter One:

General Introduction

The relationship between mechanical work and metabolic cost is established for animal locomotion – increased work requires greater muscle recruitment and this is reflected in higher metabolic costs. The presence of this relationship dictates that the manipulation of the variables affecting mechanical work during the support phase of locomotion should result in changes in energetic cost. During the support phase, the limb is in contact with the ground and provides vertical support for the center of mass. Furthermore, the limb decelerates the center of mass during the first half of support phase and accelerates the center of mass during the second half of support phase. The mechanical work performed by the limb during this period is therefore affected by the mass of the body (Heglund et al., 1982; Taylor et al., 1982) and the amplitude of the accelerations of the body (Cavagna and Kaneko, 1977; Cavagna et al., 1963; Cavagna et al., 1964; Cavagna et al., 1976). Taylor and coworkers (1980) showed that increasing the body mass of several species of mammal resulted in an increase in the energetic cost of locomotion. This supports the link between mechanical work and energetic cost. A later study (Kram and Taylor, 1990) showed the increase in energetic cost with increases in body weight and speed is a direct result of changes in the time over which muscles can produce force during stance. Although, the link between force production and energetic cost exists for support phase; this relationship between force production and energetic cost is tentative for swing phase when the limb is not in contact with the ground.

Muscular force production during swing phase is affected by the limb's inertia and angular acceleration (Cavagna and Kaneko, 1977; Fedak et al., 1982; Fenn, 1929). If a relationship between metabolic cost and force production exists during the swing phase, then variation of the variables affecting the amount of force required should also result in changes in the energetic cost. Taylor and coworkers (1974) tested the effects of these variables by comparing the cost of swing among three similarly sized species of mammal exhibiting different limb inertias. They found the energetic cost of swing did not differ with running speed or limb inertia, and concluded that the energetic cost of swinging the limbs was probably zero or very low compared to the total cost of locomotion. An alternative explanation is the natural variations in limb inertia were not large enough to increase the work required to swing the limbs and change the energetic cost associated with it.

It is also possible that the mechanical work required to swing the limb is overestimated, thereby obscuring the relationship between mechanical work and energetic cost. The mechanical work of swing could be overestimated if part of the work of swinging the limb was supplied from the accelerations of the body (Robertson and Winter, 1980). Using a ballistic model of human walking Mochon and McMahon (1980) showed that a transfer of energy between the body and limbs reduces the amount of work required from muscles and tendons to swing the lower limb. Willems and coworkers (1995) calculated that such a transfer would reduce the total work required to run by 10%. However, they concluded that such a transfer does not occur because the difference in amplitudes of the accelerations of the limbs is large enough in comparison to the accelerations of the body. A model such as Mochon and McMahon (1980) has not been constructed to test whether such a transfer of energy could occur during running or not. Even if this transfer of energy were present, it would not account for all of the work required during swing phase.

The remaining work not accounted for by the possible transfer of energy has to be performed by either the active contraction of muscle or the release of elastic energy from passive structures in muscles. Elastic energy can be stored in titin a molecule with spring-like properties found within the sarcomere (Lindstedt et al., 2002) and/ or the aponeurosis and tendon as in stance (Biewener et al., 2004; Roberts et al., 1997). These passive structures could store and release elastic energy in three different ways. First, passive muscle force is greater than active force at long muscle lengths (Gordon et al., 1966; Hill, 1953) due to stretching the titin molecule and other elastic structures (Lindstedt et al., 2002). If the muscle operated at these long lengths during swing than some or all of the force required for swing could be provided passively by these structures with little energetic cost. Second, some the aponeurosis and tendon could be stretched at any muscle fascicle length and store and release elastic energy. Third, the shortening velocity of the contractile element can be increased by the release of elastic energy from the passive structures of muscle, and thereby increase work output with little to no energetic cost. Previous studies have noted this possibility because the force a muscle produced at a given velocity was above that predicted by the contractile elements' mechanical properties alone (Askew and Marsh, 1997; Hill, 1938; Stevens, 1993).

I performed a series of four experiments to explore the possibility of energy transfer and the storage and release of elastic energy in explaining the low cost of producing work to swing the limbs. The first experiment was the construction of a model of the human lower limb. I based this model on the idea that energy could be transferred through the accelerations of the hip during walking (Mochon and McMahon, 1980;

Robertson and Winter, 1980), and used this model to test if energy transfer occurred between the body and the limbs during running.

The next experiment I performed, determined the muscles responsible for producing the force required to extend the limb at the intertarsal joint during swing in turkeys. Previous measurements from the peroneus longus and lateral head of the gastrocnemius suggested that the gastrocnemius muscle may be involved in producing the required force at this joint during swing (Gabaldon et al., 2004). I measured the forces produced by the lateral and medial head of the gastrocnemius muscle independently to determine each head's contribution to the force required for joint extension during swing.

Third, I compared the *in vivo* lengths the muscle operated at during running to the muscles *in situ* active and passive length-tension curve. All skeletal muscles have an active and passive length-tension curve which shows that maximum active force production occurs at intermediate lengths (Gordon et al., 1966; Hill, 1953). At longer lengths the amount of force produced actively decreases while the amount of force produced passively increases. If muscles operated at these long lengths, less energy would be required to produce a given amount of force. I determined the *in vivo* operating lengths of the lateral head of the gastrocnemius by comparing the muscle’s *in situ* active and passive length tension curves with the muscle’s *in vivo* lengths during force production in swing.

My fourth experiment was to characterize the force-velocity properties of the contractile element of the lateral head of the gastrocnemius (LG). Knowing the maximum force the active force producing structure can produce at any velocity allows for future comparisons with *in vivo* force and velocity values. Previous studies have noted the force a muscle produced at a given velocity could be above that predicted by the contractile elements’ mechanical properties alone due to the storage and release of elastic energy (Askew and Marsh, 1997; Hill, 1938; Stevens, 1993). Therefore, if the *in vivo* force-velocity values for the LG are higher than the maximum force-velocity predicted for the contractile element alone passive structures must be increasing the shortening velocity of the muscle. My determination of the force-velocity curve of the lateral head of the gastrocnemius makes such a comparison with future *in vivo* force-velocity values possible for the swing phase of locomotion in turkeys.

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Chapter Two:

A new passive mechanism for reducing the muscular work required for swing phase during running.

Abstract

If the human lower limb exchanged potential and kinetic energy like a pendulum during swing, when the limb is not in contact with the ground, the work required for the limb's movement would be near zero. However, the lower limb swings at a frequency higher than that where energy is exchanged optimally. The trunk's accelerations add kinetic energy to the swinging lower limb, thereby shifting the frequency where energy is exchanged optimally. This shift in the frequency where energy is exchanged optimally provides a passive mechanism for the reduction of the muscular work required during swing below that of the simple pendulum model at the same frequencies by 50-70%.

Introduction

Humans use various mechanisms to minimize the work required during locomotion (Dickinson et al., 2000). One possible energy saving mechanism is to swing the limb like a simple pendulum, exchanging gravitational potential and kinetic energy. During running the lower limb of humans appears to move like a pendulum, starting swing in a retracted position with a high gravitational potential energy (PE) and no kinetic energy (KE; Fig. 2.1, Panel A). PE is changed to KE as swing progresses and the lower limb rotates forward with the height of its center of mass decreasing and its velocity increasing. As the center of mass of the limb rises and its velocity decreases to zero in the second half of swing, this exchange is reversed. If the human lower limb functioned like this, as some researchers (Cavagna and Kaneko, 1977; Hildebrand, 1985) have suggested, the work required to swing the lower limb would be minimal only at a single frequency, called the natural frequency. However, the natural swing frequency of the human lower limb is lower than the swing frequencies used during walking and running (Grieve and Gear, 1966; Weyand et al., 2000).

We extended the simple pendulum model for swinging the lower limb to also include the accelerations of the body. The addition of accelerations to the point of rotation of the lower limb changes the simple pendulum model to a driven pendulum model. We used this driven pendulum model of the lower limb to determine whether the accelerations of the point of rotation of the lower limb can shift the frequency at which work is minimized, the minimal work frequency (MWF), to those observed during running. Mochon and McMahon (1980) showed such a shift in MWF occurs when the movements of the pelvis were included in the ballistic equations describing human walking. The shift determined for walking is due to a transfer of energy between the body and swinging lower limb and should also occur in our driven pendulum model for running. We hypothesized the MWF of the lower limb would increase when it was modeled as a driven pendulum, thereby minimizing the work of swing at the frequencies observed during running.

Materials and Methods

The swinging human lower limb was modeled as a simple and driven pendulum to determine the mechanical work required for swinging the lower limb without and with accelerations of the body (Working Model v. 8, MSC Software, Santa Ana, CA). The model included a body, swing limb, and the accelerations caused by the stance limb (Fig. 2.1, Panel A and B). The body was used as the mechanical point of attachment for the swing limb and accelerators. The swing limb represented the thigh, shank and foot. A swing limb length of 1 m and a total body mass of 70 kg along with the relative anthropometric data listed in Winter (2005) were used to determine the mass (13 kg), the position of the center of mass (0.45 m) from the point of rotation and the moment of inertia (1.4 kgm^2) of the straight swing limb. The effects of the stance limb on the point of rotation were modeled as vertical and horizontal accelerators (Fig. 2.1, Panel B).

Two different sine waves were used to approximate the vertical and horizontal accelerations of the center of mass of the body observed during running. The sine wave equations allowed us to change the magnitude and frequency of the accelerations while maintaining the relative amplitude and frequency of the vertical and horizontal accelerations constant (Fig. 2.1, Panel C). The pattern and relative magnitudes of the vertical and horizontal accelerations were based on previously measured values using force plates (Nilsson and Thorstensson, 1989). A range of amplitudes for the vertical accelerator were taken from values reported by Weyand and coworkers (2000) for a range of running speeds.

The MWF of the simple and driven pendulum was determined by calculating the work required to rotate the lower limb through a 50º arc at various frequencies. The range of swing frequencies used were taken from previous data on the duration of swing at the walk/run transition (Gatesy and Biewener, 1991) and across a range of running speeds (Weyand et al., 2000). The mechanical work was calculated by taking the integral of the absolute power required to rotate the lower limb through half a cycle (-25º to +25º) at each frequency (Igor v. 5.04B, Wavemetrics Inc., Lake Oswego, OR).

The amount of work transferred to the swing limb by the vertical and horizontal accelerations of the body was calculated in two steps. First, the mechanical work associated with swinging the lower limb at each frequency was determined for the simple pendulum model with no vertical or horizontal accelerations of the body. Second, the mechanical work associated with swinging the lower limb at each frequency was determined for the driven pendulum model with vertical and horizontal accelerations of the body. The percentage of work transferred (W_T) is a fraction of the work required to swing the limb at a single frequency in the simple pendulum model (W_S). The following equation was used to calculate W_T:

$$W_T = ((W_S - W_D) * W_S^{-1}) * 100$$

where W_D is the work required to swing the limb at a single frequency in the driven pendulum model.

Results

Energy transfer from the body to the lower limb occurred in the driven pendulum model and reduced the amount of work required to swing the lower limb. In the driven pendulum model, the MWF of the lower limb increased as the amplitude of the vertical and horizontal accelerations of the body increased (Fig. 2.2). With each increase in acceleration amplitude the MWF came closer to the frequencies used during running. The shift in MWF reduces the work required to swing the lower limb below that of the simple pendulum model (Fig. 2.3, Panel A). The observed shift in MWF is a result of a transfer of energy from the body to the swing limb (Fig. 2.3, Panel B).

Discussion

The results from the driven pendulum model show the accelerations of the body reduce the work required to swing the limb over the range of frequencies used during running below that of the simple pendulum. This is a result of the MWF being shifted to higher frequencies as the amplitude of the accelerations of the hip increases. The shift in the MWF was due to energy transferred from the body to the lower limb. The possibility of such a shift in the MWF of the lower limb illustrates the hip’s accelerations may be a mechanism to reduce the amount of work required from muscles during running. The possibility of this new mechanism for reducing the muscular work of swing may explain why the work required to swing the limbs is large (Cavagna and Kaneko, 1977) and not reflected in the energetic cost of locomotion (Taylor et al., 1974). In addition, the possibility of this new mechanism changes our thinking about stance and swing phase from two independent events to two events which are mechanically linked. We used a simplified model of the human lower limb, with no knee movement and a duty factor of 0.5 across all running speeds, to gain insights to the mechanics and energetics of swing during human running.

Our estimate of the work required to swing the human lower limb from the simple pendulum model is several times less than some previous estimates. We estimate the work to swing the lower limb at a frequency of 1.36 Hz in the simple pendulum model is 0.55 Jkg^{-1}. This estimate is several times less than previous calculations of the work required to swing the human lower limb at a similar frequency during running (2.86 Jkg^{-1}: Cavagna and Kaneko, 1977; 3.33: Jkg^{-1}: Chapman and Caldwell, 1983). Doke and coworkers (2005) calculated the mechanical work of swinging the human lower limb with an immobile knee through one half-cycle at 1 Hz to be 0.19 Jkg^{-1}. This estimate is similar to ours from the simple pendulum model at the same frequency (0.238 Jkg^{-1}). A possible reason our estimate is different from other previous estimates (Cavagna and Kaneko, 1977; Chapman and Caldwell, 1983) and similar to another estimate (Doke et al., 2005) is the presence or absence of knee movement. Another possible reason for the similarity or difference between our estimate and previous estimates is the method used to calculate the work required to swing the human lower limb. The work required to swing the limb in two previous studies was calculated from either the total change in kinetic energy of the limb (Cavagna and Kaneko, 1977) or the total change in kinetic and potential energy of the limb (Chapman and Caldwell, 1983). Our study along with Doke and coworkers (2005) calculated the work to swing the limb as the total change in kinetic energy after accounting for an exchange between potential and kinetic energy. This suggests the estimate of the work to swing the human lower limb from our simple pendulum model is realistic with the constraint of an immovable knee. In addition comparisons between our simple pendulum and driven pendulum models should result in realistic estimates of energy transfer due to the accelerations of the hips.

A comparison of the simple and driven pendulum models shows the accelerations of the hips during locomotion allows the transfer of energy between the body and the lower limb. The existence of such an energy transfer is contentious. Aleshinsky (1986) theoretical work on how energy fluctuations of each segment in a linked system effects the other segments, supports the idea of energy transfer between the body and lower limb. Other authors only support the idea of energy transfer within the swing limb (Willems et al., 1995) or also between the musculature of the hip and the swing limb (Robertson and Winter, 1980). Willems and coworkers state that such transfers could only reduce the work required to swing the limb by 10%. Willems and coworkers (1995) further state that the magnitude of the difference between the accelerations of the body and limb is not great enough to allow for energy transfer. Our driven pendulum model of swing shows 1.4% of the body's energy is transferred to the swing limb. This small amount of energy transferred from the body to the swing limb reduces the mechanical work of swinging the limb by up to 70% (Fig. 2.3, Panel B).

The transfer of energy from the body to the lower limb shifted the MWF of the lower limb closer to the frequency used during running, thereby reducing the work required for swing (Fig. 2.2). Hildebrand (1985) stated the work required to swing the lower limb would be minimal if it swings at its natural swing frequency. Although the human lower limb swings at frequencies above the natural swing frequency (Grieve and Gear, 1966), the accelerations and decelerations of the hips during walking reduce the amount of muscular work required for swing (Mochon and McMahon, 1980). Our driven pendulum model extended this finding to running, showing that as the amount of energy transferred to the lower limb increased across running speed the MWF of the lower limb also increased towards the frequencies used during swing (Fig. 2.3, Panel B).

Ideally the MWF would increase to the frequency used to swing the limbs at each running speed. Our results support the idea of a continual increase in the MWF of the swing limb with increases in the amplitude of hip acceleration. However, the increase in the MWF of the swing limb is always less than the increase in the frequency of swing for each running speed. Surprisingly this does not result in a higher amount of work required to swing the limb than if the MWF of the swing limb was the same as the swing frequency at each speed. For example, compare the amount of work required to swing the limb at 0.9 Hz with a 0.6 g (light triangles) and a 1.5 g (dark triangles) driver (Fig. 2.2). The larger driver (1.5 g) shifts the MWF of the lower limb to 0.9 Hz, but the amount of work required to swing the limb at that frequency is the same as with the smaller driver (0.6 g). This suggests there is a maximum amount of energy that can be transferred to the swing limb at each swing frequency. This example also shows that larger than normal magnitude accelerations of the hips do not result in additional energy savings for the swing limb, but do require additional energy to accelerate the hips. Therefore, the accelerations of the body may be tuned to reduce the work required to swing the lower limb, while keeping the total work required by the system low.

The possibility of tuned accelerations of the body to reduce the total work required by the system may have implications in understanding the freely chosen gait parameters and speed of human locomotion. Several studies (Cavagna and Franzetti, 1986; Minetti et al., 1995; Minetti and Saibene, 1992) have attempted to predict the preferred stride frequency for a given speed by locating the minimum sum of the work required to swing the upper and lower limbs, and accelerate and decelerate the body. Kuo (2001) tried to predict the preferred step-length for a given speed by four different measurements of swing work. Swing work was insufficient to predict the preferred step-length for a given speed (Kuo, 2001). One possible reason for these results is that Kuo (2001) examined the predictability of swing work in isolation of hip accelerations. Our model suggests the inclusion of hip accelerations in models of locomotion is essential to understanding the work required to swing the lower limb.

Our model's predictions of the accelerations of the hips reducing the work required to swing the lower limb also provides insight into the estimates of the cost of swing relative to the total cost of locomotion.

Previous estimates of the metabolic cost of swing phase in humans and other mammals, suggest it is minimal compared to the total cost of locomotion (Taylor et al., 1980; Taylor et al., 1974). A recent estimate of the cost of swinging the human lower limbs is 10% of the total cost of locomotion (Gottschall and Kram, 2005). This estimate of the cost to swing the limbs could be higher than previously thought because of the different techniques used. Taylor and coworkers used two different techniques to arrive at their conclusion: natural variation of limb length and mass distribution (Taylor et al., 1974) and the addition of weight to the body (Taylor et al., 1980). Gottschall and Kram (2005) calculated the cost of swing as the difference between the total metabolic costs while only pulling horizontally on the body, and while pulling horizontally on the body in addition to pulling on the lower limb. This is a direct manipulation of swing, but the control of pulling on the body may artificially increase the cost of swinging the limb. In normal locomotion the hips decelerate during the first half of swing and than accelerate during the second half of swing. The amplitude of these accelerations and decelerations are equal in magnitude. Pulling on the body with at a constant magnitude would increase the amplitude of the decelerations hips in the first half of swing and decrease the amplitude of the accelerations in the second half of swing. Our driven pendulum model suggests these manipulations of the amplitude of the horizontal accelerations of the hips would increase the cost of swing. This would be due to the availability of too much energy to the swing limb in the first half of swing and the availability of too little energy to the swing limb in the second half of swing. This alteration in the amplitude of the accelerations of the hips may result in an overestimation of the cost of swing in human locomotion.

Another study (Marsh et al., 2004) estimated the cost of swinging the lower limbs to be 25% of the total cost of locomotion. While these investigators did not externally dampen the NA accelerations of the body in their experiments (Marsh et al., 2004), their study animal, Guniea Fowl, may have a built in acceleration dampener. The consistently protracted femur of guinea fowl (Gatesy, 1999) is much different than the retracted and protracted position we model for the human lower limb (Fig. 2.1A). This difference in the posture of the lower limb could have major effects on the possibility of energy transfer shifting the MWF of the lower limb. Therefore the difference between Marsh and coworkers (2004) estimate of the cost of swing and Taylor and coworkers (1980) estimate may be entirely caused by the difference in limb posture.

A better understanding of the factors affecting the work required for swing is also important in designing lower limb prosthetics. Recent advances in lower limb prosthetics have focused on decreasing the total mass of the prosthetic to improve function (Trower, 2006). Several studies (Martin, 1985; Ropret et al., 1998; Royer and Martin, 2005; Soule and Goldman, 1969) examining the effects of added lower limb mass, support the idea that additional lower limb mass increases the cost of swing in humans. Data from another study of lower limb prosthetics (Lehmann et al., 1998) which varied the location of the lower limb center of mass show that its location has a greater effect than the total lower limb's mass in determining the cost of swing. If the lower limb operates like a driven pendulum as our model suggests than the latter conclusion is supported, since mass does not determine the natural frequency. The factors which affect the natural frequency and ultimately the cost to swing the lower limb are the position of the center of mass and how the mass of the entire limb is distributed around its center, the radius of gyration.

Several previous studies of swing phase have manipulated the mass and inertia of the limb to determine their effects. Mena and coworkers (1981) varied the inertia of each limb segment in a mathematical model. They (Mena et al., 1981) found the model required small muscular moments to approximate the previously measured kinematics of walking humans when the inertia of each limb segment was equivalent to that of a human limb's. This study supports our idea that matching the inertia of a prosthetic limb segment to its counter part in the human limb would minimize the energetic cost of swing. Two other studies (Mattes et al., 2000; Lin-Chan et al., 2004) which empirically manipulated the inertia of the limb do not support this idea. This is possibly because the limb inertia in these empirical studies was adjusted by discrete quantities of mass to change the inertia of the limbs. The primary problem with this experimental design is the position of the center of mass and radius of gyration of the prosthetic limb could never be equal to the actual limb. A more direct empirical test would be the creation of a prosthetic with the position of the center of mass and radius of gyration equal to the actual limb segment compared to prosthetics of other designs. Our model predicts the combination where the cost of swing will be minimized is when the position of the center of mass and the radius of gyration of the prosthetic lower limb are equal to the physical properties of the normal lower limb.

An understanding of the limb properties which minimize the cost of swing is important in designing legged robots. Recent studies of limbed robots used a rotation motor (Poulakakis et al., 2005) and spring (Hyon and Emura, 2004) at the hip to achieve maximum efficiencies of locomotion around 47%. One of the problems with these designs is the limited range of speeds these efficiencies occur over. The range of frequencies where high efficiencies occur could be expanded if energy was transferred between the body and swinging legs. Our study suggests near maximum energy transfer would occur when the position of the center of mass and the radius of gyration of the robots legs are tuned to the accelerations of the body. Our data suggest this tuning would result in the MWF of the robots legs increases as the amplitude of the body’s acceleration increases with speed. A continual shift in the MWF with increases in speed would lower the amount of work required from a rotational motor at the hip in robots and expand the range of speeds where high efficiencies of locomotion occur.

In conclusion our model provides evidence for a driven pendulum-like mechanism during running which reduces the work required to swing the lower limb and provides the basis for further exploration of how the work required for swing can be minimized in healthy animals, humans using prosthetic devices, and legged robots.

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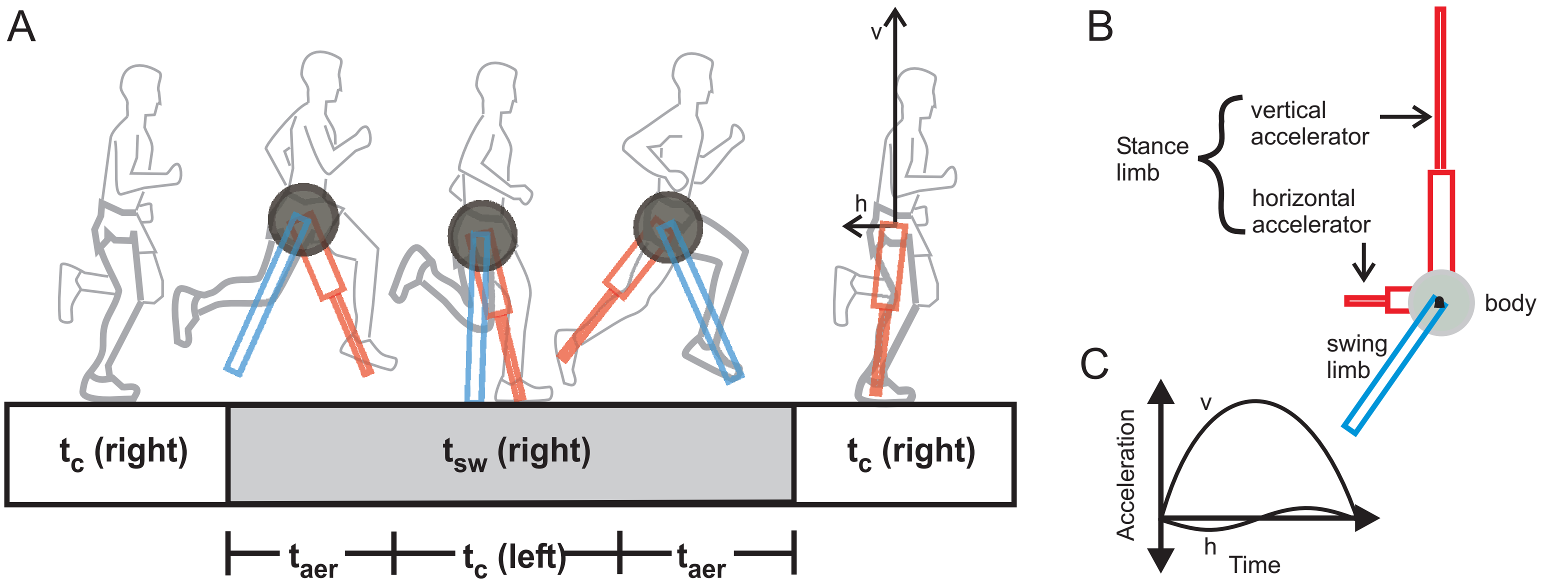
Figure 2.1. A schematic showing the footfall pattern and movement of the body's mass during running (A), the model used to calculate swing work (B), and the pattern and relative amplitude of the accelerations used for the vertical and horizontal accelerators (C). The swing limb was modeled as a rectangle (blue outline) rotated by a motor through a symmetric 50° arc centered on vertical. The rotational motor of the swing limb and the two accelerators were attached to a common mass (gray filled circle) simulating the mass of the body. The two accelerators (red outlined boxes) simulated the vertical and horizontal acceleration components of the stance limb. The swing time (t_{sw}) of the right leg was equal to the contact time of the left leg (t_c), so aerial time (t_{aer}) was zero for all speeds. Total stride time (t_{str}) and amplitude of the accelerations of the body changed with speed in our model to determine their effects on the cost of swing.



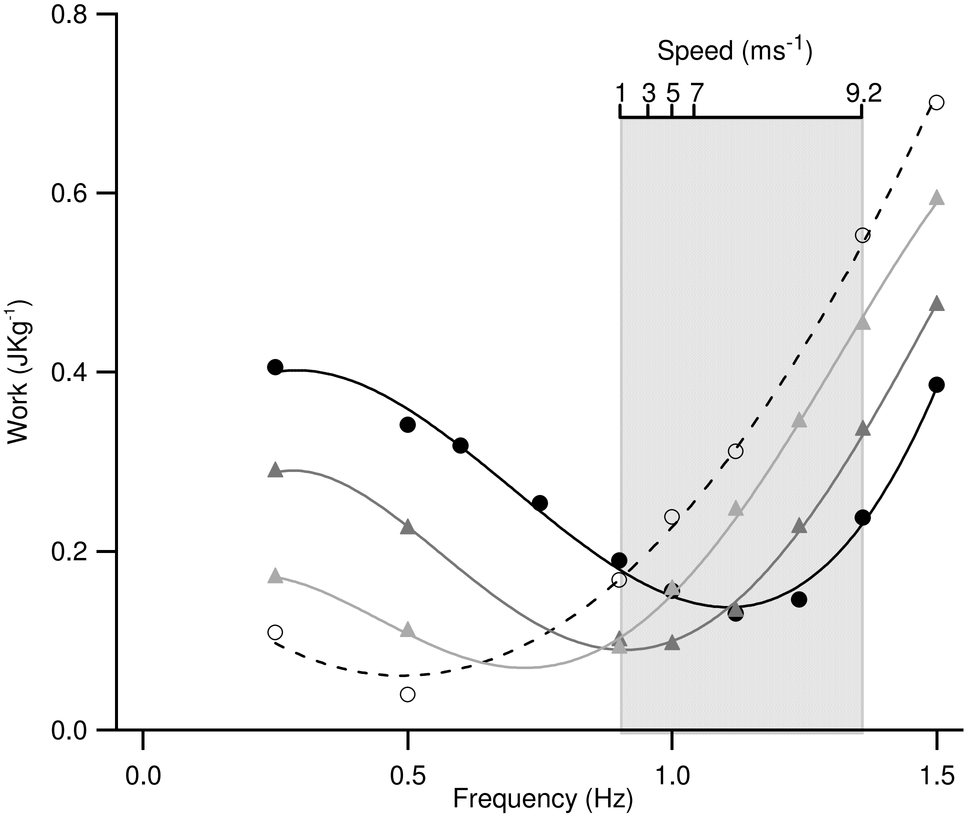


Figure 2.2 The minimum work frequency (MWF) increases as the magnitude of the driver increases. The MWF of the simple pendulum is the furthest to the left (open circles and dashed line). As the amplitude of the driver increased from 0.57g (light gray triangles) to 1.53g (dark gray triangles) to 2.5g (solid black circles) the MWF continues to shift to the right. The range of frequencies used during running (gray bar) are up to 3.5 times greater than the MWF of the simple pendulum, while close to the MWFs of the driven pendulums.

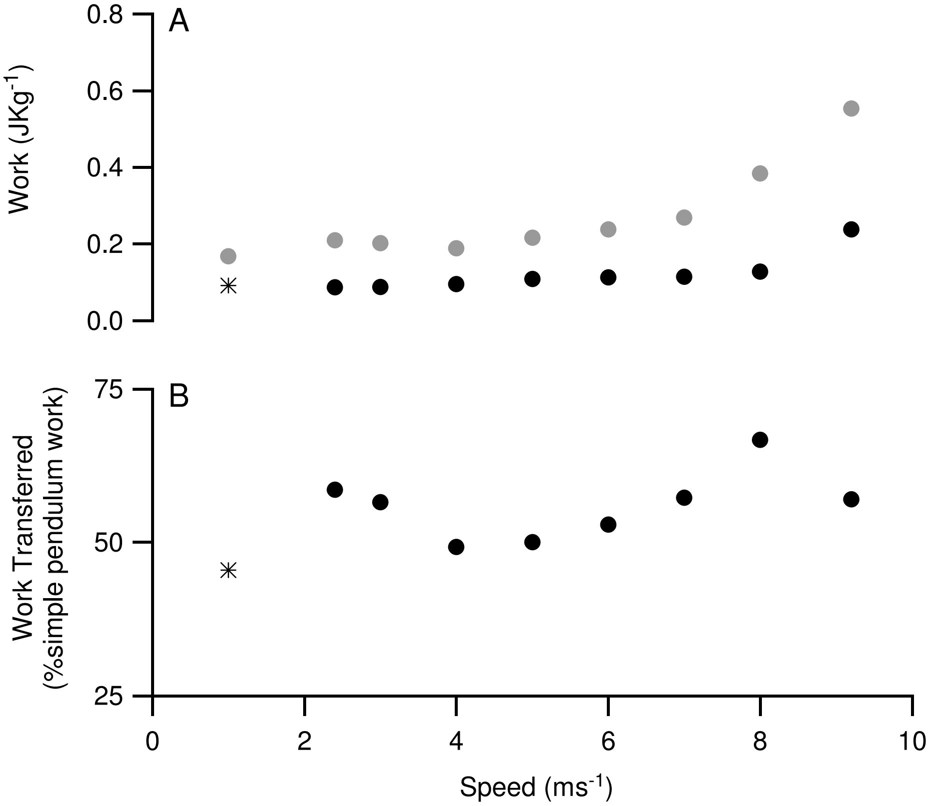


Figure 2.3 The work required to swing the limb was almost constant across running speed (A) and approximately 50% of the work required to swing the limb was done by the natural accelerations and decelerations of the body's mass (B). The swing frequencies and magnitudes of the body's accelerations and decelerations were taken from two published studies (asterisk, walk/run, Gatesy and Biewener, 1991; black circles, running, Weyand et al., 2000). The work required to swing the limb without a driver (gray circles, Panel A) was 45-66% higher than with a driver (black circles) at every speed.

Chapter Three:

How muscle synergists share extension force about the intertarsal joint during running in turkeys.

Abstract

The energetic cost of locomotion is proportional to the force produced when the foot is in contact with the ground. When the foot is not in contact with the ground during swing phase, this relationship between energy and force does not hold. The apparent lack of a relationship suggests force production during swing is provided by something other than active muscles allowing the storage and release of elastic energy from tendons as determined for stance. Optimization models to minimize force per cross-sectional area or fatigue of muscles show synergistic muscles share force production during stance. We hypothesized that the two heads of the gastrocnemius would share the production of the force necessary for joint extension in swing as muscle synergists. We measured the force produced by the lateral (LG) and medial (MG) heads of the gastrocnemius, and the extensor moment about the intertarsal joint of running turkeys (*Meleagris gallopavo*) during swing while varying limb inertia (by adding 30 and 60 g masses to the limb), and the speed of locomotion (1 to 3.5 ms^{-1}). We then compared these measured muscle forces with the forces calculated using inverse dynamics. The variations in limb inertia and

speed of locomotion should increase the force required to extend the limb, and further test how muscle synergists share force during swing. We found that the LG produced all of the force required for limb extension during swing. This pattern of force production in muscle synergists during swing suggests the different force and power requirements of swing and stance may act as a signal for the recruitment of muscles to produce force.

Introduction

If a relationship between metabolic cost and force production exists during locomotion, then manipulations of the variables affecting the force required should result in changes in the energetic cost. The force required during the non-support (i.e. swing) phase of locomotion is affected by the limb’s inertia and angular acceleration (Cavagna and Kaneko, 1977; Fedak et al., 1982; Fenn, 1929), and therefore should be affected by changes in the limb’s inertia and angular acceleration. Taylor and coworkers (1974) tested the effects of the limb’s inertia and angular acceleration on metabolic cost by comparing the cost of swing between three similarly sized species of mammals with presumably different limb inertias across a range of running speeds. Contrary to expectations they found that the energetic cost of swing did not differ between the species across the range of speeds examined, and concluded that the energetic cost of swinging the limbs was probably zero or very low compared to the total cost of locomotion. The mechanism allowing for variable amounts of force to be produced with no change in energetic cost is not known, but may involve the way muscles produce force during swing.

Insights into the mechanism allowing variable amounts of force to be produced with no change in energetic cost would be provided by an examination of how the required force during swing is produced. Most investigations about muscle function during locomotion have focused on the stance phase, when the foot is in contact with the ground and muscle forces are highest. In stance phase, optimization models of force sharing show synergistic muscles produce force to minimize the sum of muscle force per cross-sectional area (Pedotti et al., 1978) or muscle fatigue (Dul et al., 1984). Empirical measurements of force production in muscle synergists support these models (Akima et al., 2002; Biewener and Corning, 1991; Biewener and Baudinette, 1995; Fagg et al., 2002; Herzog and Leonard, 1991; Stokes and Gardner-Morse, 2001). However, the forces produced during swing phase are lower than during stance (Roberts et al., 1997) during the swing phase. The different levels of force production between swing and stance phase may allow muscle synergists to share force production differently as suggested previously (Raikova, 1992).

We explored how muscle synergists produced the force required during swing by measuring the production of the force required to balance the moment around a joint by two muscle synergists of turkeys (*Meleagris gallopavo*) during running. The lateral (LG) and medial (MG) heads of the gastrocnemius share a common soft tendon which inserts on the hypotarsus (George and Berger, 1966). This point of insertion allows both muscles to produce an extension moment about the intertarsal joint. We varied the mass of the limb and speed of locomotion to potentially change the magnitude of the extension moment. Measurements of the various forces required and produced in the two heads of the gastrocnemius allowed us to test the hypothesis that these synergistic muscles would share the production of the force necessary for joint extension in swing.

Materials and Methods

Animals and Treadmill Training

Six adult female Eastern wild turkeys (*Meleagris gallopavo*) were obtained from a breeder in Oregon and housed in an outdoor enclosure at Oregon State Univerisity. A diet of Game Bird Flight Conditioner (Purina-Mills, Inc.) and water were provided *ad libitum*. The mean body mass of the birds was 4.2±1.0 kg (±SD); and the mean mass of the lateral gastrocnemius (LG) muscle was 21.6±4.5 g (±SD); and the medial gastrocnemius (MG) muscle was 30.7±7.1 g (±SD).

Training protocol

The training protocol used was similar to that of Gabaldon and coworkers (2004). Turkeys ran on a level treadmill 10-20 min day^{-1}, 4-5 days a week, for about 4-6 weeks. On separate days, turkeys were subjected to one of three limb loading regimes: unloaded, 30 g, or 60 g weights. The 30 or 60 g weights were attached to the limbs just proximal to the intertarsal joint. All training and research was conducted in accordance with Oregon State University Institutional Animal Care and Use Committee and federal and institutional guidelines.

Surgery

Animals were induced and maintained on inhaled isoflurane anesthesia with a sterile environment maintained throughout all surgical procedures. A strain gauge (Type FLK-1-11,Tokyo Sokki Kenkyujo Co., Ltd.) was attached to both the superficial and deep aspect of the calcified tendon for both the lateral and medial gastrocnemius muscle after the tendons were scraped and degreased with chloroform. The strain gauge wires were routed subcutaneously from each muscle to a small skin incision near the middle of the synsacrum. The incision was closed and small electrical connectors (Microtech, Inc.) were secured to the skin with 3-O silk suture. Animals were allowed to recover from surgery for 24-48 h before treadmill running experiments.

Running Experiments

Gastrocnemius muscle force measurements were taken as the birds ran with and without limb weights on a level treadmill at speeds of 1-3.5 ms^{-1}. The birds were first run without limb weights to get them accustomed to running on the treadmill. The limbs were loaded with 30 and 60 g consecutively. The lead weights were secured to the limb segment just proximal to the intertarsal joint with tape. After completion the weights were removed and the bird ran at 2 ms^{-1} as a control for comparisons with the beginning of the experiment. Ten seconds of data were collected for each run. Birds remained on the treadmill at slow walking speeds between speed changes and were allowed to rest on a stopped treadmill as needed. As the birds ran on the treadmill data were collected at 4000 Hz with a Macintosh G3 computer using a 12-bit A/D converter (PCI-MIO-16-1, National Instruments) controlled by the software program IGOR Pro (WaveMetrics, Inc.). The tendon strain signals were amplified using a strain gauge conditioner (model 2120, Vishay Measurements Group) before being collected by the computer. Data were synchronized with high speed video which was recorded at 250 frames s^{-1} (Redlake Imaging MotionScope 1000S).

Kinematics

The timing of foot fall and the positions of the intertarsal and metatarsal-phalangeal joint were determined directly from video. The video recorded from the Redlake camera was transferred to a computer through ObjectImage software. This transferred video was then digitized in using a custom program (written by Ty Hedrick) written to operate in MatLab 7.0. All of the digitized coordinates for each bird were converted from pixels to meters by determining the pixel distance between the intertarsal and metatarsal-phalangeal joints and comparing this to the known distance in meters. After this conversion a smoothing spline (smoothing factor 1, S.D. 0.001-0.003 m) was fit to the data using the software IGOR Pro (Wavemetrics, Corp.) to remove random noise in the data. This smoothed data was used to locate the limb segment and also to calculate the joint angles. Joint angles were differentiated twice to obtain joint angular accelerations.

Inverse Dynamics

The muscular force required at a joint through time was determined by dividing the joint moment by the vector moment arm length for the muscle of interest. The sign convention was set such that a concentric contraction of the muscle resulted in an extension (negative) moment about the joint. The magnitude of the moment arm was obtained by the *in situ* method described below (see Methods, *Muscle Moment Arm*).

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The joint moment required to produce the observed motion of a joint through time was determined by balancing the external and internal forces at that joint following the methods described in Bresler and Frankel (1950) and Robertson and Winter (1980). Joint moments during swing are affected by an external gravitational force and two internal forces (translational and inertial) on the center of mass. The external gravitational force was summed with the vertical component of the internal translational force due to acceleration of the center of mass. Translational forces were required to accelerate the limb in the horizontal (x) or vertical (y) direction and acted directly on the segment's center of mass. The moment caused at the joint by the translational forces were determined by multiplying the translational force at the segment's center of mass times the distance from the center of mass to the joint in the x or y direction. The joint moment was expressed by the following equation:

$$M_{1p}=I_1*\alpha_1-(m_1a_{1x})*r_{1x}-(m_1a_{1y})*r_{1y} \tag{1}$$

where M_{1p} is the moment at the proximal joint (intertarsal joint), I_1 is the inertia of the tarsometatarsus and foot limb segment about the ankle, α_1 is the angular acceleration of the tarsusmetarsus and foot limb segment in radians, m_1 is the mass of the tarsometatarsus and foot limb segment, a_{1x} is the acceleration of the center of mass in the x direction, r_{1x} is the moment arm from the intertarsal joint to the center of mass in the x direction, a_{1y} is the acceleration of the center of mass in the y direction, and r_{1y} is the moment arm from the intertarsal joint to the center of mass in the y direction. A positive value was given to all moments in the counter-clockwise direction (Fig. 3.1).

The mass, position of the center of mass and inertia of the tarsometatarsus-foot limb segment were determined empirically. The tarsometatarsus-foot limb segment was severed from the rest of the limb at the intertarsal joint while frozen. The mass of this limb segment was then determined (52.37±14.29 g mean±S.D.). The center of mass (COM) for each limb segment was determined by balancing the segment on a plastic straight edge, marked and measured as described previously (Fedak et al., 1982). The average position of the COM was 7.78±0.54 (mean±S.D.) for six birds.

The moment of inertia about the centre of mass was determined by attaching the end of the tarsometatarsus-foot limb segment to a stiff steel rod with and without added weights. The segment was suspended from the rod in a manner that allowed it to pivot about the attachment and swung through a small arc around the pivot point five times. The time of those five swings was recorded. This procedure was repeated five times for each weight condition. The average of all five trials was determined and divided by five to obtain the average period of swing for each bird's tarsometatarsus-foot limb segement. This period was used to calculate the moment of inertia (I) about the pivot point in kg m^2 using the following equation derived from the basic mechanics of a physical pendulum:

$$I = (t^2 mdg)*(4\pi^2)^{-1} \tag{2}$$

where t is the period of one swing (s), m is the mass of the tarsometatarsus-foot limb segment (kg), d is the distance from the center of mass to the point of rotation (m), and g is the acceleration of gravity (m s^{-2}). The moment of inertia about the centre of mass (I_0) of the segment was calculated using the parallel axis theorem as:

$$I_0 = I - md^2. \tag{3}$$

All center of mass positions and accelerations in the x- and y-direction as well as moment arms during swing were also calculated from the smoothed joint position data. The position of the center of mass of the segment through time in the x-direction was solved for by the following equation:

$$COM_X = length_{COM} * length_s^{-1} * (X_M - X_I) + X_I \tag{4}$$

where COM_X is the position of the center of mass of the segment in the x-direction, $length_{COM}$ is the distance from the intertarsal joint to the center of mass of the segment, $length_s$ is the distance from the intertarsal joint to the metatarsal-phalangeal joint, X_M is the x-coordinate of the metatarsal-phalangeal joint and X_I is the x-coordinate of the intertarsal joint. The same equation was used to determine the position of the center of mass of the segment in the y-direction by substituting the y-coordinates for the x-coordinates. The x- and y-coordinate data was differentiated twice to obtain the accelerations of the segment's center of mass in the x- and y-directions. The moment arm for the x- or y-direction was calculated as the distance between the x- or y-coordinates of the center of mass of the segment and the intertarsal joint.

Predictions from Inverse Dynamics

The effect adding mass to the limb segment has on the moment required for joint extension was unclear, because the kinematics used during locomotion may change. If the kinematics of locomotion changed with added limb mass the effect additional mass has on the moment required for extension could be negligible. The direct effects of added mass on the moment required were determined by substituting values for the mass and inertia of the limb segment in equation (2). These substitutions used the measured mass and inertia of the tarsometatarsus-foot limb segment with 30g or 60g of mass added combined with the joint position data from the unweighted limbs at a single speed. The data from these substitutions for joint extension moments will be referred to as predicted maximum force ($F_{mx,p}$) and predicted impulse (I_p).

In situ calibration of muscle force

Tendon strains were calibrated to muscle force *in situ* at the end of running experiments according to techniques described by Gabaldon and coworkers (2004). The procedure involved electrically stimulating the muscle via the sciatic nerve while simultaneously measuring whole muscle force and tendon strain. The slope of a regression line fit to the linear portion of the tendon strain and muscle force data, between muscle forces of 0 and 200 N, was used to calibrate tendon strain to muscle force. The birds were kept under deep anesthesia with isoflurane gas during the experiments and body temperature was maintained at 38–40°C.

Muscle Cross Sectional Area

Muscle fiber length, angle of pennation, and muscle mass were measured post-mortem from excised muscle to determine the cross sectional area of the lateral and medial head of the gastrocnemius muscles. The fiber length was measured with a pair of calipers between the beginning of a fiber at the origin of the muscle and its insertion onto the muscle's superficial aponeurosis. The pennation angle was determined on longitudinally bisected muscles with a goniometer. Physiological cross-sectional area (PCSA) was calculated as:

$$\mathrm{PCSA} = (m_{muscle} * \cos(\theta_p)) * (l_{fiber} * \rho_{muscle})^{-1} \tag{5}$$

where m_{muscle} is the mass of the muscle, θ_p is the angle of pennation, l_{fiber} is the fiber length, and ρ_{muscle} is the density of muscle (Gans, 1982; Powell et al., 1984).

Muscle Moment Arm

The moment arm of the gastrocnemius muscle about the intertarsal joint was calculated in order to directly relate measured muscle force to the calculated joint moment. The moment arm of the gastrocnemius muscle about the intertarsal joint was determined indirectly by measuring muscle length change as a function of joint angle (Lutz and Rome, 1996) of a turkey gastrocnemius post-mortem.

The apparatus and technique used to make these measurements were modified versions of those used by Lutz and Rome (1996). We measured joint angles with a goniometer and the length changes of the muscle at given angle by movements of a string over a ruler. The joint angles were converted to radians before calculating the moment arm. The moment arm (r_j) of a muscle about a given joint is expressed as:

$$r_j = \Delta L_j \;/\; \Delta\theta_j \tag{6}$$

where ΔL_j is the length change of the muscle in meters and $\Delta\theta_j$ is the joint angle change in radians. The slope of a regression line fit to a plot of muscle length change vs. joint angle determined the moment arm of the gastrocnemius muscle about the intertarsal joint. Moment arm measurements using this technique corroborated earlier measurements using a more direct technique (Roberts et al., 1998).

Force Variables

The simple repeatable pattern of the intertarsal joint moment (Fig. 3.1) allowed the force required by the gastrocnemius for joint extension to be characterized by three variables: maximum force, time to maximum force and impulse. These three variables were determined from inverse dynamics for joint extension during swing only (Fig. 3.1) and are referred to as the required maximum force for extension ($F_{m,r}$), required impulse for extension (I_r), and time to required maximum force ($T_{m,r}$). Maximum force and impulse were used to compare the measured and the predicted required muscular forces when limb weights were added because the differences were in the magnitude of force production not timing. The predicted variables are referred to as: predicted required maximum force for extension ($F_{m,p}$) and predicted required impulse for extension (I_{p}). All three variables were used to compare muscular forces required and muscular forces produced for joint extension during swing, since the possibility of a timing difference between required and produced force could exist. The muscular force variables are referred to as: maximum force produced ($F_{m,LG}$ or $F_{m,MG}$), impulse produced (I_{LG} or I_{MG}), and time to maximum force produced ($T_{m, LG}$ or $T_{m, MG}$). We used the same variables to describe force production during stance in the lateral and medial head of the gastrocnemius with the addition of a variable for the time to the end of force production ($T_{e,LG}$ or $T_{e,MG}$).

Statistics

Balanced data sets suitable for analysis of variance (ANOVA) were obtained for six birds for joint forces, five birds for muscle forces from the lateral head of the gastrocnemius, and four birds for the muscle forces from the medial head of the gastrocnemius. Some descriptive statistics are provided for trials at speeds where all birds did not perform. All ANOVAs were restricted to speeds of 1 ms^{-1}, 1.5 ms^{-1}, and 2 ms^{-1} and three weighting conditions 0g, 30g, and 60g where all birds did perform. The measurements used in all ANOVAs were from 4 strides per individual per speed per weight. A three-way mixed model ANOVA for which speed (N=3) and weight (N=3) were fixed factors and individual (N=4) was a random factor were performed in the statistics program SPSS version 11.5 (Wilkinson, 1992). Multiple observations per individual were accounted for by calculating the F-ratio for the main effect of speed as the mean square for speed divided by the mean square for the speed x individual interaction term (Zar, 1999). Similarly, the F-ratio for the main effect of weight was the mean square for slope divided by the mean square for weight x individual interaction term. The F-ratio for the interaction effect of weight and speed was the mean square for weight x speed divided by the mean square for the speed x weight x individual interaction. The criterion for statistical significance was $p<0.05$.

All comparisons of force measurements were made in a series of t-tests. Predicted forces ($F_{m,p}$ and I_p) for each speed and weighting condition were compared with the required forces ($F_{m,r}$ and I_r) under the same conditions. Paired t-tests were used to compare the required forces ($F_{m,r}$, I_r, $T_{m,r}$) to the muscular force produced by the lateral ($F_{m,LG}$, I_{LG}, $T_{m,LG}$) and medial ($F_{m,MG}$, I_{MG}, $T_{m,MG}$) head of the gastrocnemius within the same stride.

The amount of force sharing between the two heads of the gastrocnemius was determined with reduced major axis regressions. Independent regressions were run for each of three variables characterizing force production during swing and stance. The data for these regressions were taken from the four strides for each speed and weight condition in the three birds we had simultaneous measurements of force in the lateral and medial head.

Measured and predicted results are presented as the mean±one standard error (SE). Unless stated otherwise, the mean values for each speed and weight combination presented for descriptive purposes were calculated so each individual was weighted equally. Depending on the particular speed and weight, mean values were from different numbers of individuals (Ni).

Results

Kinematics

The duration of swing and a stride as well as duty factor changed as expected with running speed and the addition of limb weights. Swing duration significantly decreased with locomotory speed ($p < 0.01$) and increased with added weight (Fig. 3.2). Stride duration showed a similar trend, decreasing with locomotory speed and increasing with weight. Duty factor still changed significantly with speed ($p>0.001$), but did not change with added weight ($p>0.05$). Duty factor ranged from 0.70 to 0.53 with increases in locomotory speeds.

Predicted vs. measured forces required at the intertarsal joint during extension

Predictions of how the force required for extension at the intertarsal joint would change with the addition of 30 and 60 g weights to the limbs and no change in kinematics were made. These predictions allowed testing of whether the forces increased as estimated from first principles and the effects changes in kinematics with added weights had on the forces required for joint extension. When 60 g weights were added to the limb, the predicted forces ($F_{m,p}$ and I_p) were significantly ($p<0.05$) higher than the required forces ($F_{m,r}$ and I_r) for all speeds. The $F_{m,p}$ and I_p were also significantly greater than the required forces ($F_{m,r}$ and I_r) for 2.5 ms^{-1} with 30g weights ($p<0.05$, Fig. 3.3). The $F_{m,p}$ and I_p were the same as $F_{m,r}$ and I_r for every other speed below 2.5 ms^{-1} with 30g added.

Intertarsal Joint Moments and Forces

$F_{m,r}$ and I_r significantly increased with speed ($p < 0.005$), but did not change ($p > 0.15$) with the addition of limb weights within a given speed (Fig. 3.4). $T_{m,r}$ did not change with running speed, but did increase with added mass ($p < 0.013$).

Muscle Forces

The lateral head of the gastrocnemius produced all of the extensor force required during swing phase across speed and weighting condition (Fig 3.1, Panel C). The magnitude of all three variables characterizing force production ($F_{m,LG}$, I_{LG}, $T_{m,LG}$) in the lateral head were not significantly different from the magnitude of the same three variables characterizing the force required for extension ($F_{m,r}$, I_r, $T_{m,r}$) at any speed or weight condition ($p > 0.31$, Fig. 3.4). However, both measurements of force produced in the medial head ($F_{m,MG}$, and I_{MG}) were less than the $F_{m,r}$ and I_r (Fig. 3.1, Panel C) and not significantly different from zero ($p<0.05$). Although the lateral head of the gastrocnemius is the sole producer of force during swing, force is produced similarly between the two heads during stance.

Force production changes by the same amount across speed in the two heads of the gastrocnemius during stance phase. Force production in neither head of the gastrocenmius was significantly affected by the addition of weights ($p>0.05$). The maximum force produced by these two heads increased significantly across speed ($p < 0.02$, Fig. 3.5). However, the impulse produced did not change in either head across speed ($p > 0.54$). The time to maximum force occurs later in stance for both heads with speed ($p<0.01$, Fig. 3.5), while only the lateral head produced force for a longer duration with increases in speed ($p < 0.01$).

The reduced major axis regressions were consistent with the two heads of the gastrocnemius producing different amounts of force during swing and similar amounts of force being during stance. The data for the reduced major axis regressions were pooled from each speed and weight condition and grouped into stance or swing. Maximum force from both heads is plotted against one another in Figure 3.6. A slope of 1 for swing or stance would indicate equal force production between the heads of the muscles. Alternatively a slope of 1.19 would indicate force was produced in proportion to the cross-sectional area of the two heads of the muscles to each other. The only reduced major axis regression for values measured during swing with a slope significantly greater than zero ($p<0.05$, slope=0.06) is for measurements of MG vs. LG maximum force. The other two reduced major axis regressions of MG vs. LG time to maximum force and impulse values during swing have non-significant slopes ($p>0.05$). Thus, the lateral and medial head do not show a consistent ratio of force production across speed or weighting condition during swing. In stance phase, reduced major axis regressions of MG vs. LG maximum force, time to maximum force, end of force and impulse values all have significant slopes ($p<0.001$) with magnitudes ranging from 0.87 to 1.36.

Discussion

Contrary to our expectation that the lateral and medial heads of the gastrocnemius would produce force similarly throughout a stride, our results show that the LG produces all of the force required to extend the tarsometatarsus-foot limb segment during swing. However, the two heads share force production during stance phase as predicted. The difference in the way force is shared between these muscles suggests other variables exist for how force is shared between muscle synergists during swing phase.

Changes in required muscular force with speed and added mass

As speed increased, we expected the angular acceleration of the segment to increase and therefore increase the force required to extend the tarsometatarsus-foot segment at the intertarsal joint. As expected, both variables describing the measured force required for swing ($F_{m,r}$ and I_r) increased with speed. These increases in $F_{m,r}$ and I_r are also consistent with previous measurements of increased work with speed (Cavagna and Kaneko, 1977; Fedak et al., 1982; Marsh et al., 2006; Steudel, 1990b), since work is the area under the curve of force times velocity. The changes $F_{m,r}$ and I_r with added limb mass, were not as straight forward.

The force required to rotate a limb segment at a joint is proportional to the segment inertia and angular acceleration. Assuming no changes in the kinematics of swing phase with added mass, the segment angular acceleration would not change while the segment inertia would increase. Therefore, we expected $F_{m,r}$ and I_r would increase in proportion to added limb weights. We tested these predictions by changing the limb mass in the inverse dynamic equations for each bird's running kinematics without weights. We observed an increase in both measurements of force ($F_{m,p}$ and I_p) as expected.

The forces required during swing when weights were added to the limbs ($F_{m,r}$ and I_r) were less than the forces predicted during swing when the mass and inertia of the limb segment were changed but kinematics were the same as without limb weights ($F_{m,p}$ and I_p). The only kinematic variables could cause a difference between $F_{m,r}$ and I_r and $F_{m,p}$ and I_p, since each bird was its own control. Changes in several kinematic variables may explain the lower values of $F_{m,r}$ and I_r than $F_{m,p}$ and I_p. Swing duration increased with the addition of limb weights at a given running speed (Fig. 3.2). The increase in the duration of swing affected the magnitude of joint acceleration. Joint acceleration is one of the variables with a direct affect on the force required at the joint, so decreases in its magnitude could counter any potential increases of joint moment with added limb weight. Other studies (Marsh et al., 2006; Martin, 1985; Ropret et al., 1998; Steudel, 1990a) have observed similar increases in swing duration with added limb mass. Other kinematic variables that could affect the forces required for joint extension are the amplitude of angular and linear movements.

Synergistic muscles share force production during stance

How synergistic muscles may share force during the stance phase of locomotion is predicted by theories based on minimizing either the total force produced by each muscle (Pedotti et al., 1978) or muscle fatigue (Dul et al., 1984). The total force theory predicts force to be shared between muscles depending on the muscles' size: larger muscles tend to contribute more force than do smaller muscles. The muscle fatigue theory refines this, stating that large muscles with slow oxidative fibers would be recruited first and as the force required increased, muscles with fast glycolytic fibers would share the force production.

The total force and muscle fatigue theory would predict that the two heads of the gastrocnemius muscle of turkeys share force production during stance across all speeds, because the cross-sectional area and fiber type composition is similar in both heads. The cross-sectional area is approximately the same in both heads with the MG being only 19% larger than the LG. Both heads of the gastrocnemius are presumed to also have the same fiber type compostition, since another study (Patak and Baldwin, 1993) determined this for another bird that also primarily moves on the ground, Emu (*Dromaius novaehollandiae*). Force production in both the LG and MG during the stance phase of locomotion (Fig. 3.6) agrees with the predictions from both of these theories that force is shared between the two heads. The sharing of force production between the LG and MG is also in agreement with other studies of these muscles in wallabies and ducks (Biewener and Baudinette, 1995; Biewener and Corning, 2001).

Synergistic muscles do not share force production during swing

Exclusive force production by the LG during swing supports neither the total force nor the muscle fatigue theory of force sharing between muscle synergists. Force production in the LG in the absence of force production from either the MG (this study: Figures 1 and 4) or peroneus longus (Gabaldon et al., 2004) during extension of the intertarsal joint is contrary to both the total force and muscle fatigue theories of force sharing between muscle synergists. In addition no other muscles seem to be producing force during intertarsal joint extension in swing, since the force produced by the LG is not different from the force required. This strongly suggests the force required for extension of the intertarsal joint is not shared between any muscles at all but produced exclusively by the LG.

This novel pattern of force production in swing could be the result of force being produced passively. The LG muscle undergoes a large stretch-shorten cycle during the swing phase of locomotion (Roberts et al., 1997). As the muscle lengthens during swing force starts to rise and then falls quickly as the muscle shortens at the end of swing. The coincidence of large stretch-shorten cycle and forces rising and then falling is consistent with the possibility of force being produced passively.

Another possibility is that the novel pattern of force production in swing is the result of the muscle synergists performing different tasks in stance and swing. Raikova (1992) suggested muscle forces are shared differently between muscle synergists depending on the task the muscle synergists perform at a joint. In stance phase, the LG and MG need to produce force and power to support body mass and move the tarsometatarsus-foot limb segment. Whereas in swing phase, the LG and MG need to produce force and power to move the tarsometatarsus-foot limb segment. One difference in these tasks is the amount of force required from these muscles. The force required during stance it is several times greater than during swing (Fig. 3.1). Another difference is the way these forces are used to power movement during stance and swing. In stance phase the contractile tissue of the muscles produce force but shorten little and therefore produce little power. Instead the contractile tissue works in series with its tendon to store and release elastic energy. This stored energy produces the power necessary for stance economically (Roberts et al., 1997). In swing phase the LG shortens considerably more (Gabaldon et al., 2004) and probably can not store the power required for movement as elastic strain energy in its tendon. Instead it may use a different mechanism to produce power at the intertarsal joint economically. The possible mechanism used during swing to increase the economy of power production is the transfer of power from the knee to the intertarsal joint. Between the LG and MG only the LG is capable of the task of transferring power.

Aleshinsky (1986) theorizes that a transfer of power from one joint to another through a muscle would decrease the amount of energy required for movement. Power transfer through a muscle can only occur when adjacent joints are both flexing or extending and the force required at the joint is already provided by a biarticular muscle. These criteria are met during the second part of swing phase at the intertarsal joint of turkeys. The LG is balancing all of the forces required at the intertarsal joint and the knee is extending at the same time the ankle is extending. The LG muscle in birds is a biarticular muscle (George and Berger, 1966) whereas the MG is a monoarticular muscle (Ellerby and Marsh, 2006). Therefore, the possibility of transferring power through the LG and reducing the amount of energy required for movement could be the underlying mechanism for solely recruiting the LG for intertarsal joint extension in swing phase. Future measurements of the power required and produced along with the knowledge of the fiber types and mechanical properties of the LG and MG will allow a better understanding of the shared production of force in these muscle synergists.

Summary

We show that required muscular force does not increase as predicted with speed and weight, the LG produces all of the force required for joint extension during swing, and the LG shifts from producing force exclusively in swing to sharing force production with the MG in stance. The $F_{m,r}$ and I_r values are less than the $F_{m,p}$ and I_p values across speed and weight conditions. This difference is probably the result of changes in the limb kinematics. Regardless of the amount of force required for intertarsal joint extension during swing, it is exclusively produced by the LG. This pattern of force production is contrary to our expectations of force sharing between the LG and MG. However, the independent force production of the LG ends with the beginning of stance phase as the LG and MG produce force equally as expected for muscle synergists.

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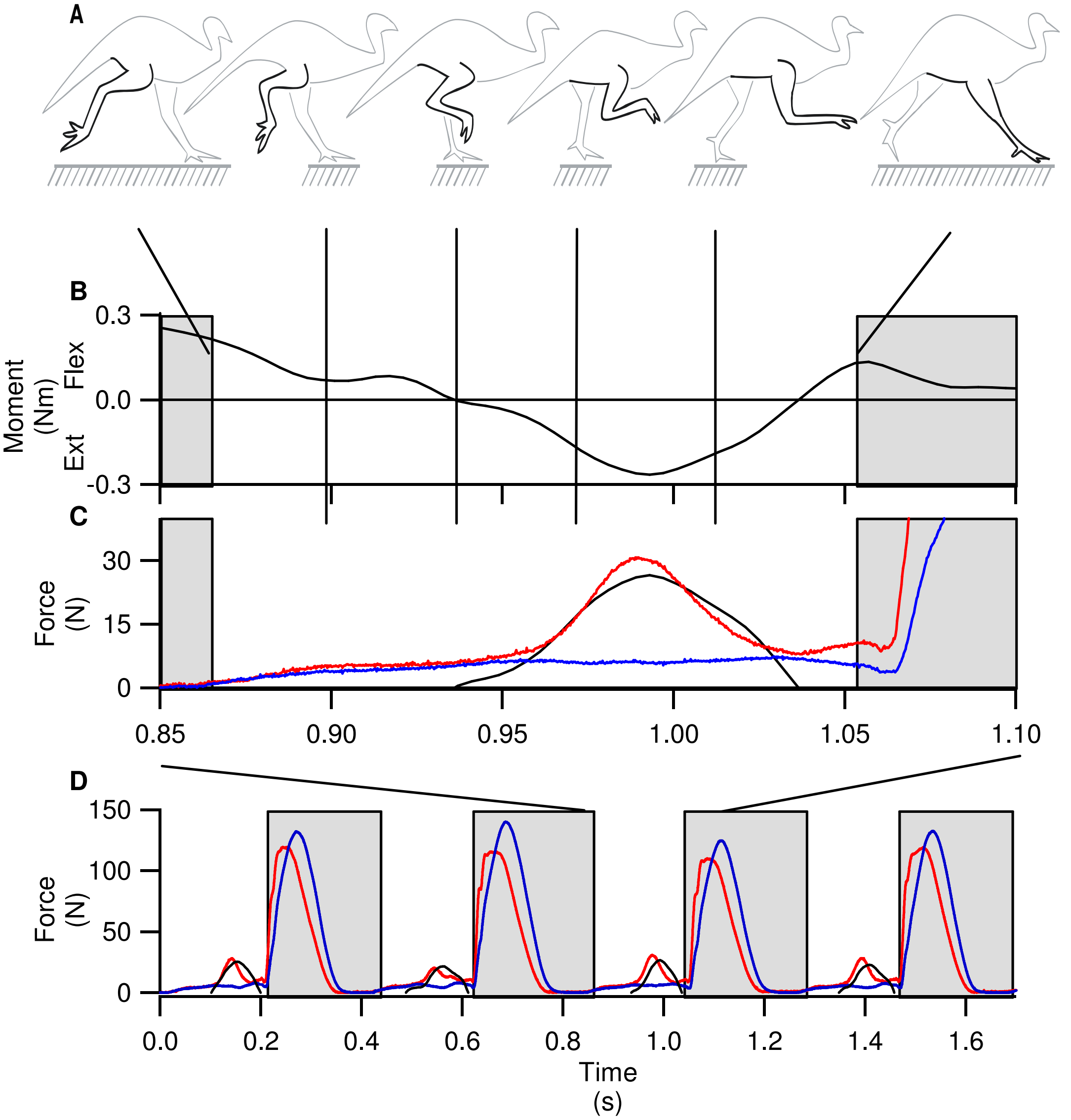


Figure 3.1 The kinematics (Panel A), joint moments (Panel B), and muscle forces (Panels C and D; required, black line; lateral gastrocnemius, red line; medial gastrocnemius, blue line) during swing and stance for the right limb of a turkey running at 2 ms^{-1}. A positive then negative joint moment (Panel B) is required at the intertarsal joint during swing as the joint flexes and extends. The required joint extension moment at the intertarsal joint was converted to the muscular force required from the gastrocnemius, by accounting for the distance and direction of the moment arm from the intertarsal joint to the insertion of the gastrocnemius (black line, Panel C). The muscular force required by the gastrocnemius for the joint flexion moment is closely matched by the force produced by the lateral head of the gastrocnemius (LG; red line, Panel C), while the medial head (MG; blue line, Panel C) produces almost no force. The exclusive force production by the LG ends at the beginning of stance (gray shaded region, panel D).

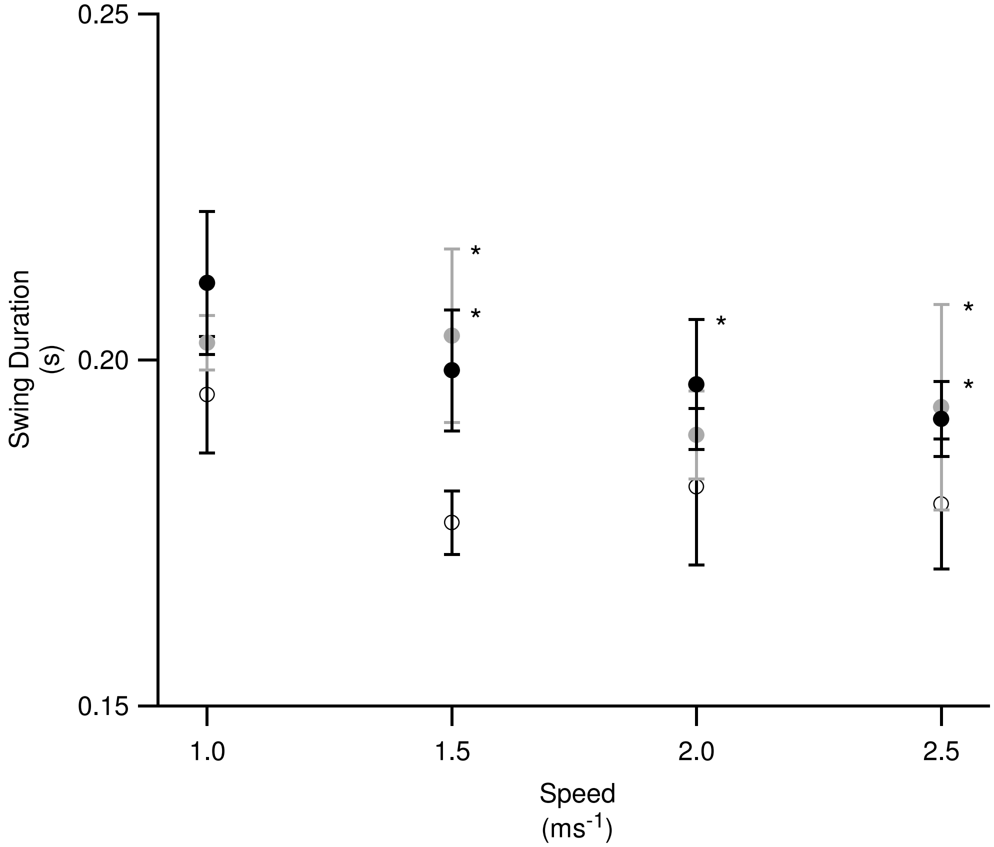


Figure 3.2 Swing duration for most speeds with 0 g of mass added (open circles) was significantly less (asterisks, $p<0.05$) than the durations with 30
(solid gray circles) and 60 g (solid black circles).

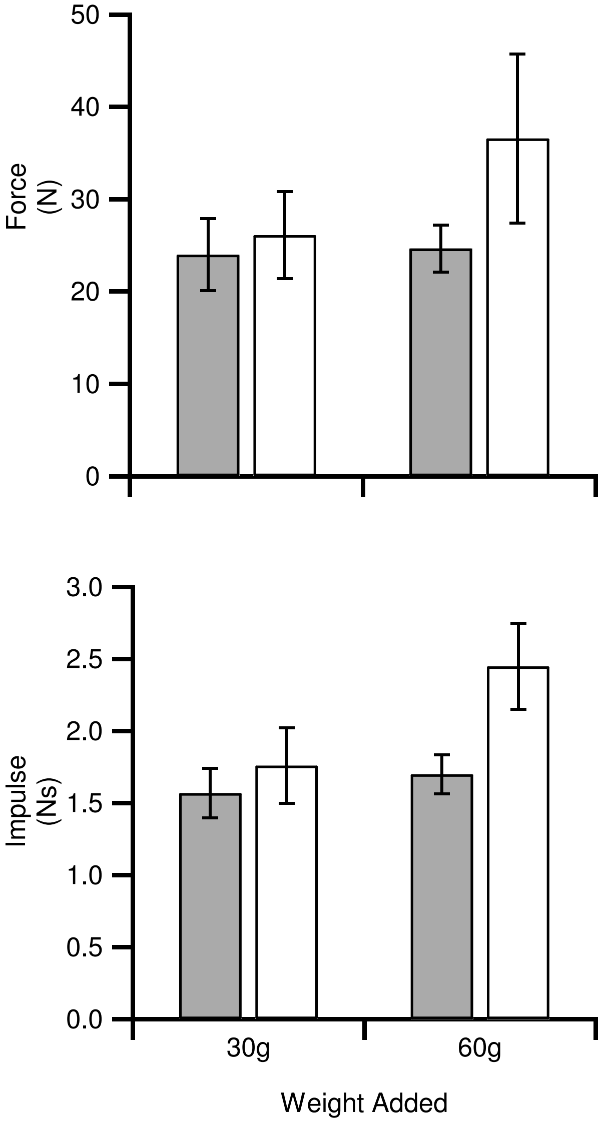


Figure 3.3 The predicted muscular force and impulse (white) required at the intertarsal joint with additional weight are significantly greater ($p<0.05$) than the actual calculated (gray) values from video of the animal running with added limb mass at 2.5 ms^{-1}. The predicted muscular forces and impulse were calculated using inverse dynamic equations based on running kinematics at 2.5 ms^{-1} with no limb weights and adding 30 or 60 g of mass to the mass of the limbs in the equations.

Figure 3.4 Values of peak force, time to peak force and impulse produced by the lateral head of the gastrocnemius (solid red bar) were not different from the values required to generate the flexion moment at the intertarsal joint (gray bar) across all speeds and weighting conditions. The force and impulse required for the flexion moment significantly increased ($p<0.05$) with speed and were equal or less ($p<0.05$) than the measured values produced by the LG.

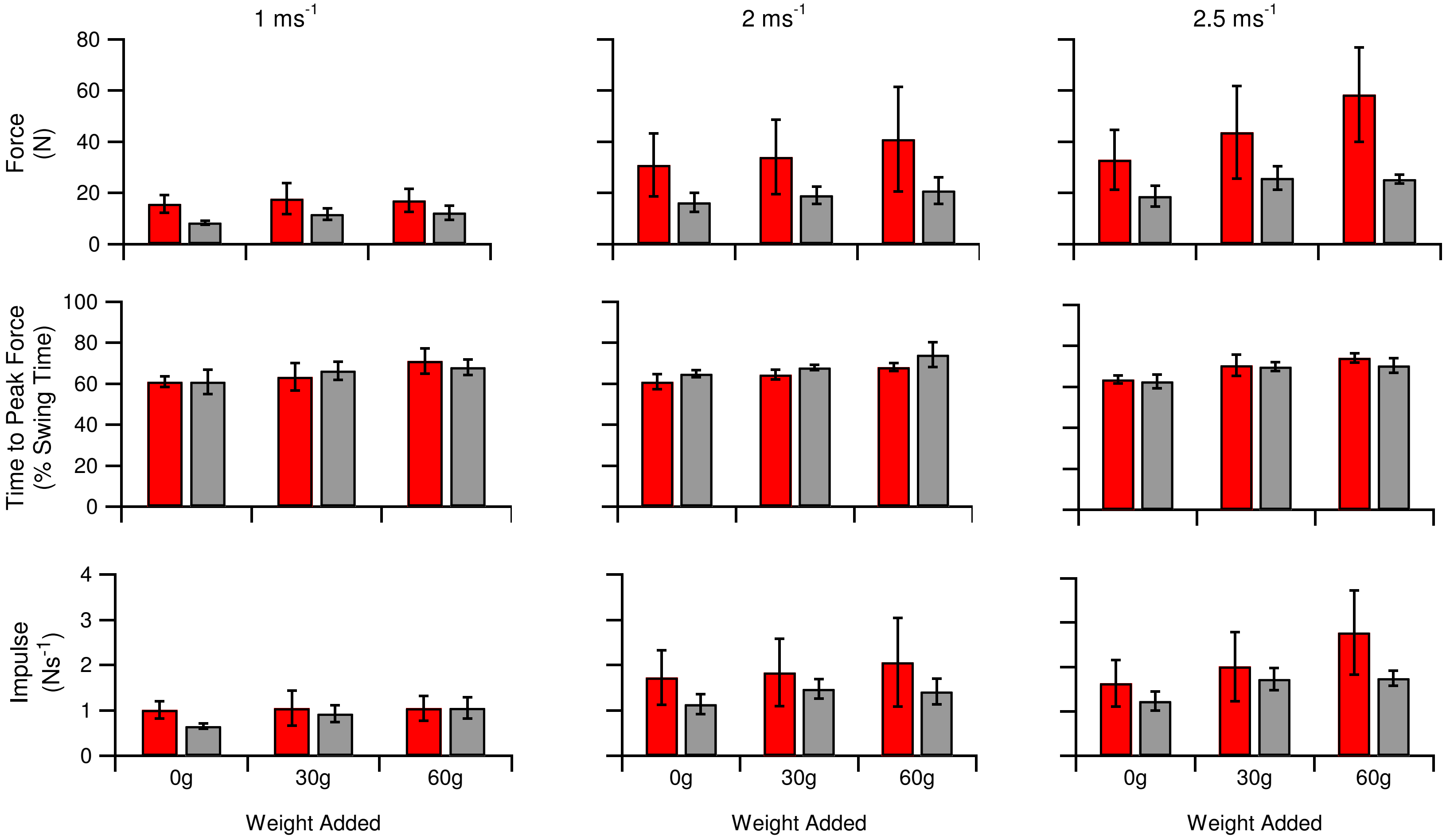
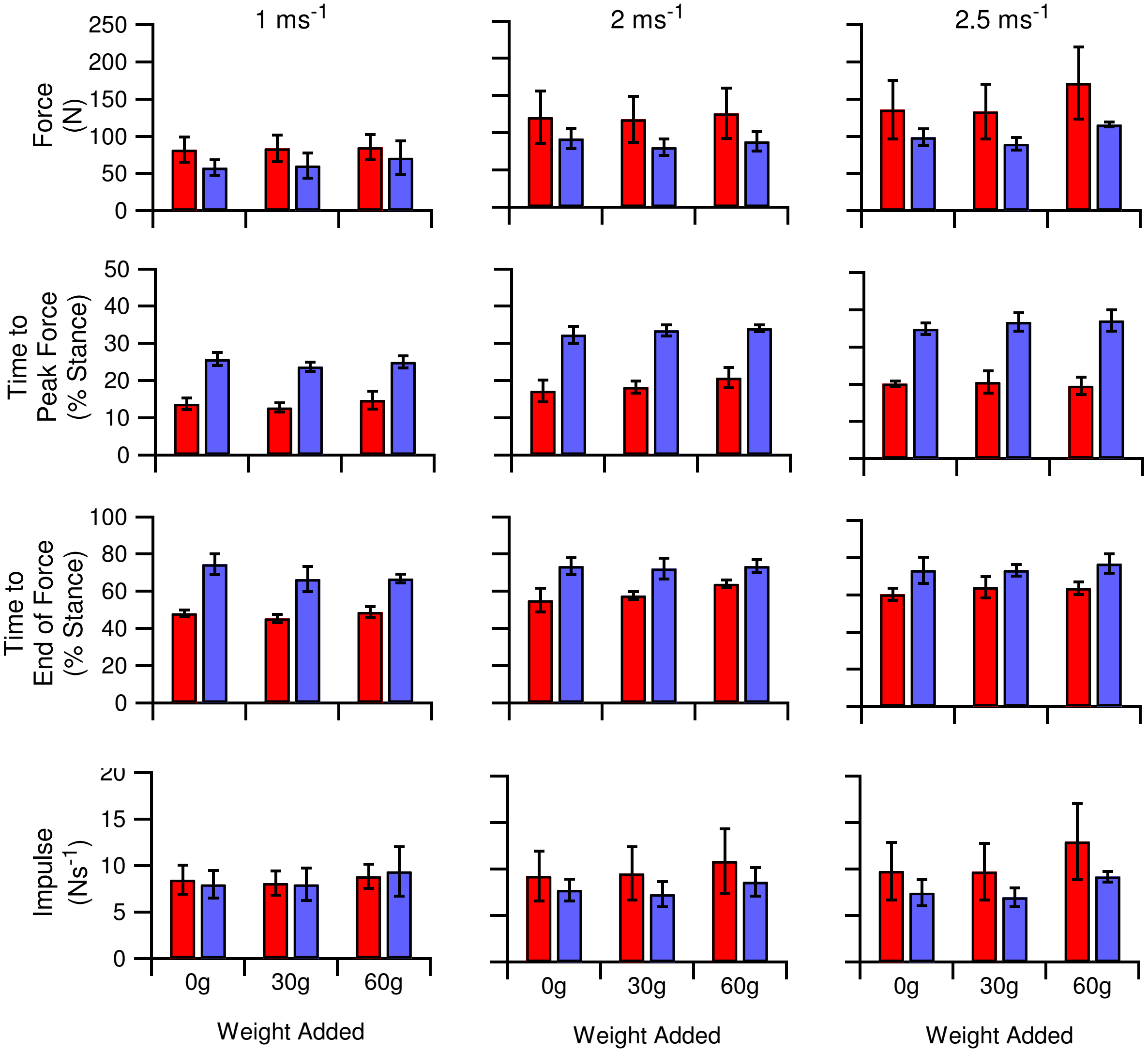


Figure 3.5 Values of peak force, time to peak force, time to end of force and impulse produced by the lateral (LG, solid red bar) and medial (solid blue bar) head of the gastrocnemius for three speeds at all weighting conditions during stance. Peak force significantly ($p<0.05$) increases in both the LG and MG with speed. The time of peak for and the end of force production always occur later in the MG than the LG across speeds and weighting conditions.



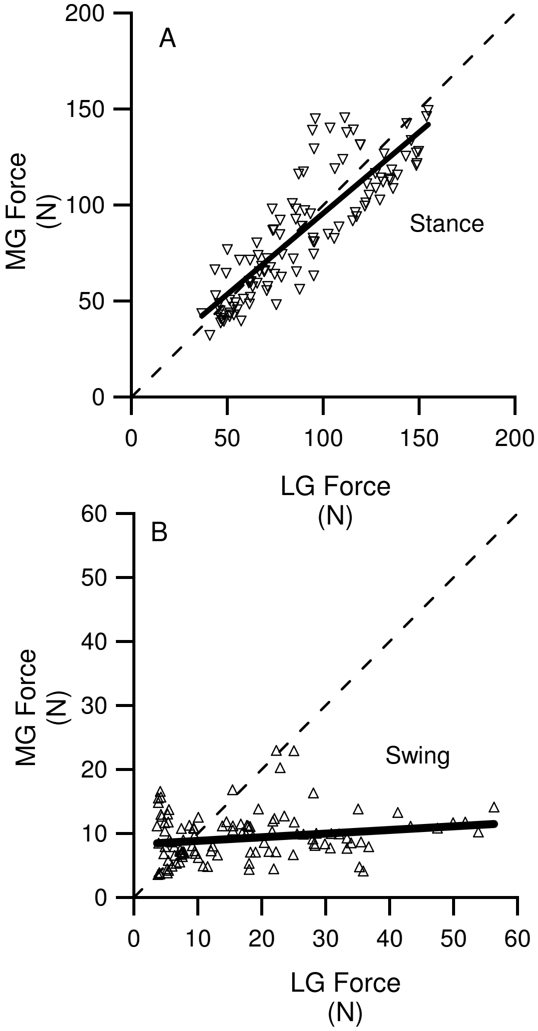


Figure 3.6 Peak force changes by the same amount in the LG and MG during stance phase. The values of peak force during stance are shown by a downward pointing triangle (Panel A) and values during swing are shown by an upward pointing triangle (Panel B). The slopes of both regression lines plotted through each of the clusters of points were significant ($p<0.05$). The dashed line from zero to the maximum value shows isometry between the two heads and is similar to the slope of regression line for stance forces (Panel A).

Chapter Four:

The operating lengths of the lateral gastrocnemius during running on level and sloped surfaces.

Abstract

The mechanical properties of skeletal muscle dictate that maximum force can only be generated at one optimum length. We measured muscle length in the lateral gastrocnemius muscle (LG) of running turkeys (*Meleagris gallopavo*) to determine where the muscle operates on the length-tension curve during stance and swing phase of running. Muscle length was measured by sonomicrometry as animals ran on a motorized treadmill at different speeds (1 to 3.5 ms^{-1}) and inclines (0º, 6º, and 12º). Length-tension properties of the LG were determined *in situ* from anesthetized animals following the running experiments. Muscles were supramaximally activated via the sciatic nerve and muscle force was recorded with a muscle ergometer attached to the LG tendon. The optimum length (L_0) for each muscle was determined by fitting a parabolic curve to the *in situ* length-tension data and calculating the length where maximum force occurred. In one group of birds, the operating length of the LG did not correspond to the length where maximum force is generated for the entire range of speeds and inclines. The LG operated over a range of lengths (range of lengths: 0.76 to 1.06 L/ L_0) during force production in

swing phase. The LG operated at shorter lengths during the period of force production in stance phase (range of lengths: 0.67 to 0.87 L/L_0) in one group of birds. The LG produced force mainly at lengths other than the length where maximum force is produced.

Introduction

In a single skeletal muscle there is only one length where maximum force can be generated, above and below this length force decreases. A number of studies (reviewed in: Burkholder and Lieber, 2001; Talbot and Morgan, 1998) estimated where a muscle's *in vivo* operating lengths occur in relation to the length where maximum force can be generated (L_0). Although joint movement allows some locomotory muscles to operate at lengths greater than ±5% of L_0 (reviewed in: Burkholder and Lieber, 2001), another study estimates that muscles operate *in vivo* over a narrower range of lengths ±5% of L_0 during locomotion (Rome, 1994). While average force production is highest for length ranges centered on L_0, the 20-30% range of lengths determined for other muscles during locomotion (Biewener et al., 1998; Nelson and Jayne, 2001) could cause muscle damage if they were centered on L_0 (Talbot and Morgan, 1998). Talbot and Morgan (1998) proposed the forces produced by the sarcomeres within the muscle are unequal and some sarcomeres may be pulled apart and damaged at these long lengths. We tested these predictions of how the length-tension curve relates to the *in vivo* lengths during force production for a muscle used during terrestrial locomotion.

We predicted muscles operate *in vivo* at lengths close to and including L_0 based on the energetics of locomotion. Kram and Taylor (1990) determined the cost of locomotion is linked to the force required to support body mass during stance. Roberts (1998) further showed that the energetic cost of locomotion is tied to the amount of muscle recruited to produce the force needed to support body mass during the stance phase of locomotion. The amount of muscle recruited to produce a given amount of force is minimal at L_0 and increases at longer and shorter lengths, because striated muscle produces maximum force at L_0 and sub-optimal force at longer and shorter lengths to the right and left of L_0 (Blix, 1894; Gordon et al., 1966; Huijing, 1985; Zajac, 1989).

We test the prediction*, in vivo*, that muscles produce force over a range of lengths center around L_0 during the stance phase of terrestrial locomotion, by using a direct measurement of contractile element length. We directly determine the *in vivo* operating lengths in an ankle extensor, by pairing a recent *in vivo* technique for measuring fiber length with previous methods for determining the whole muscle length-tension curve. We used the same device to determine the *in vivo* fiber length during force production and to measure the *in situ* fiber length during whole muscle stimulation. In this study, we determine the *in vivo* lengths of the lateral gastrocnemius muscle during force production across a range of speeds (1-3 ms^{-1}) and inclines (0-12º) to test the prediction that muscles produce force over a range of lengths centered on L_0.

Materials and Methods

Animals and Treadmill Training

Data were collected from two groups of birds. One group of birds consisted of four adult female Eastern wild turkeys (*Meleagris gallopavo*) obtained from a breeder in Oregon and housed in an outdoor enclosure at Oregon State Univerisity. The mean body mass of the birds was 3.56±0.21 kg (±SD); the mean mass of the lateral gastrocnemius (LG) muscle was 18.78±0.43 g (±SE). A diet of Game Bird Flight Conditioner (Purina-Mills, Inc.) and water were provided *ad libitum*. These birds will be referred to as the ORST birds. The second group of birds consisted of two adult female Eastern wild turkeys (*Meleagris gallopavo*) obtained from a breeder in Massachusetts and housed in a similar enclosure. The body and muscle mass of these birds was also similar. These birds will be referred to as the HRVD birds.

Treadmill training for the ORST and HRVD birds consisted of running on a level and inclined (+6º, +12º) motor-driven treadmill (Keys Pro 2000 Series) for 10-20 min day^{-1}, 4-5 days a week, for 4-6 weeks. Birds ran on each slope on alternate days at speeds of 1-3 m s^{-1}. A wooden box with a Plexiglas window for video imaging, and an opening at the back for access to the bird, was placed around the edges of treadmill track. All animal use was approved by the Oregon State University or Harvard University Institutional Animal Care and Use Committee and in accordance with federal and institutional guidelines.

Surgery

Animals were induced and maintained on inhaled isoflurane anesthesia and a sterile environment was maintained for all surgical procedures. A pair of sonomicrometry crystals (Sonometrics, Inc., London, ON, Canada) 2 mm in diameter were implanted into 2 mm deep pockets made along the long axis of a proximal fascicle. 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 using 6-O silk suture. Two small strain gauges (Type FLK-1-11,Tokyo Sokki Kenkyujo Co., Ltd.) were glued to the superficial and deep aspects of the bony tendon of each muscle. The calcified tendons were prepared for gluing by gently scraping and then defatting the surface with chloroform. A thin layer of cyanoacrylate adhesive (Duro superglue, SUP-5; Loctite Corp., Avon, OH, USA) was applied to each strain gauge and it was pressed onto the tendon for 1 min for bonding. All transducer wires were 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.) were secured to the skin with 3-O silk suture. Animals were allowed to recover from surgery for 24-48 h before treadmill running experiments. This same procedure was used for both groups of birds.

Running Experiments

The minor differences in the running experiment between the two groups of the birds are noted below. Measurements were taken as the birds ran on a level treadmill, followed by runs on an incline (+6º and +12º) at speeds of 1-3.5 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 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 for the ORST birds. A Triton (model 120) system was used to measure fascicle lengths in the HRVD birds. Tendon strain signals were amplified using a strain gauge conditioner (model 2120, Vishay Measurements Group). Data were collected at a frequency of 4000 Hz to a Macintosh computer with a 12-bit A/D converter (PCI-MIO-16-1, National Instruments) using the software program IGOR Pro (WaveMetrics, Inc.). High speed video was recorded at 250 frames s^{-1} with a Redlake Imaging MotionScope (model 1000S).

Muscle length data was taken from the four best strides for each speed by incline combination. We only analyzed the maximum, minimum, and average muscle length as well as the amount of shortening during the period of force production in swing and stance phase (Fig. 4.1). In swing phase the muscle is always producing some force so muscle lengths throughout swing were analyzed. The LG muscle produces force only during the first half of stance phase (Fig. 4.1). When force measurements were not available the amount of shortening, maximum, minimum, and average lengths were recorded from the first part of stance.

In situ preparation

The *in situ* preparation ensured that the blood flow to the muscle and the physiological temperature of the muscle (36-39 °C) were maintained throughout the experiment for both groups of birds. *In situ* experiments were performed while the animal was maintained under deep anesthesia with isoflurane gas. The sciatic nerve was isolated for electrical stimulation of the LG muscle. Thin connective tissue around the nerve was carefully removed and the nerve was severed just distal to its emergence from the pelvic girdle. The nerve was inserted into a bipolar electrode nerve cuff constructed of two silver wires and polyethelyne tubing (1 cm length; I.D. 5 mm). The pocket of tissue containing the severed nerve was filled with mineral oil to isolate it and to reduce voltage dissipation during nerve stimulation. The wire leads from the nerve cuff were connected to a Grass S48 stimulator for muscle stimulation.

We determined the force-length curve for the LG muscle of each ORST bird. We measured total muscle force using a servomotor (Aurora 310B-LR) and fascicle length changes using the same sonomicrometry system and crystals used for *in vivo* measurements. To ensure the servomotor accurately measured whole muscle force, compliance in the system was minimized by rigidly linking the bone of muscle origin (i.e., the femur) and the servomotor through a rigid aluminum frame. The rigid link was formed with an aluminum plate fastened to the femur with two machine screws and clamped to the frame holding the servomotor. After securing the bird to the frame, the distal tendon of the muscle was isolated and cut free. The freed tendon was then attached to the servomotor with a custom-built aluminum clamp weighing 21.78 g and a 1/16” aircraft cable. The sonomicrometry and servomotor signals were sent to a computer with a 12-bit A/D converter (National Instruments PCI-MIO-16-1). Data were collected at 1000 Hz using the software program Igor Pro (WaveMetrics Inc.).

We determined the force-length curve for the LG muscle of each HRVD bird in a similar way with two differences. In the HRVD birds the force measurements were made using the same strain gauges as used during running instead of an external force measuring device. The compliance in the system was removed in a slightly different way as well. In the HRVD birds compliance in the system was minimized by rigidly linking the bone of muscle origin (i.e., the femur) and the bone of muscle insertion (i.e., the tarsusmetatarsus) through a rigid aluminum frame. The rigid link was formed with an aluminum plate fastened to the femur with two machine screws, a second aluminum plate fastened to the tarsusmetatarsus with two additional machine screws and an aluminum cross-brace connecting these two plates. After the length tension data was collected, the distal soft tendon was freed. This soft tendon was attached to a Kistler (model 9203) force transducer to calibrate the strain values to force. The data were collected in a similar manner as for the ORST birds.

Contractile Property Measurements

Prior to constructing the force-length curves for each muscle, we determined the optimum stimulation voltage. This is critical to achieve maximal isometric force per cross-sectional area (F_0) during tetanic stimulation. The optimum stimulation voltage was determined in two steps. First, the voltage required to fully activate all available motor units was determined by increasing the voltage in one-volt increments beginning at 1 V and continuing until twitch force no longer increased. The voltage that produced maximum twitch force was then increased by one volt; this supra-maximal stimulation value (6-8 V for all preparations) was used for all subsequent muscle contractions within an experiment.

All isometric force-length measurements were taken from tetanically stimulated muscle. Smooth tetanic contractions were attained with a stimulation pulse duration of 0.2 ms at a frequency of 100 Hz. Contractions were obtained over a range of lengths and forces by adjusting the total length of the muscle and tendon unit. A representative recording of an isometric contraction is shown in Fig. 4.2. Total force and length values were calculated as the average over a 10 ms period of time, beginning shortly after force reached a plateau and ending before muscle stimulation ceased (Fig. 4.2, shaded bars). Whole muscle force was measured directly by the servomotor in the ORST birds and the strain gauges in the HVRD birds. The net force generated by the interaction between myosin and actin was calculated as the difference between the total force and the initial force before the contraction. Fascicle length (mm) was determined throughout a contraction using sonomicrometry (Fig. 4.2). To minimize possible effects of muscle fatigue or potentiation, a rest period of 5 min or greater was given between successive tetanic contractions.

Isometric contractions at a predetermined length were recorded at the beginning, middle, and end of a series of isometric contractions to check for potential changes in maximum isometric force in the ORST birds. Maximum isometric force (F_0) did not change during the experiment for any of the ORST birds, so no corrections for changes in muscle force due to fatigue were made.

The optimal length (L_0) for each muscle was determined mathematically from a curve fit to six to twelve measurements of force and length. Our first measurement of net isometric force was made at a predetermined length, close to an estimate of L_0 from previous experiments. Subsequent measurements of net isometric force and length were made after shortening the muscle tendon complex by 4 mm below our estimate of L_0. We continued to stimulate the muscle at shorter and shorter lengths every 4 mm until the net isometric force was ~80% of the maximum measured. Then the same procedure was used to characterize the force-length relationship at lengths longer than our estimate of L_0. After all of the measurements were made we fit a third-order polynomial to the data. We determined L_0 by finding the length value where maximum force was produced.

After characterizing the relationship between isometric force and length we determined the passive properties of the muscle for the ORST birds. To do this we recorded muscle force and length for a longer period of time (10 s). We started the passive stretch at a length with no passive force and increased the length in 2mm steps until force reached ~60 N. After each 2 mm change in length we let the muscle come to a near steady passive force. A third order polynomial was fit to this passive force-length relationship to mathematically determine the passive force at any muscle length.

Muscle fiber length, angle of pennation, and muscle mass were measured to standardized maximum isometric force between different muscles. We simultaneously measured the length of the muscle fibers and the distance between sonomicrometry crystals while the muscle was at a set length to determine the muscle fascicle length at L_0 from the sonomicrometer segment length. This was necessary because the sonomicrometer crystals measured only a segment of the fascicle. We used our measure of muscle fascicle length at L_0 to convert muscle length from absolute units of mm to relative units of muscle lengths (L / L_0). Physiological cross-sectional area was calculated as the product of muscle mass and the cosine of the angle of pennation, divided by the product of muscle density and fiber length (Gans, 1982; Powell et al., 1984). Maximum isometric force was standardized by dividing the measured force by the physiological cross-sectional area of the muscle.

Statistics and curve fitting.

We were able to obtain balanced data sets suitable for analysis of variance (ANOVA) for the four birds ORST birds and two HRVD birds. Some descriptive statistics are provided for trials at speeds where all birds did not perform; however, all ANOVAs were restricted to three speeds ranging from 0 to 2 ms^{-1}. The measurements used in all ANOVAs were from 4 strides per individual per speed per slope. We used SYSTAT version 5.0 (Wilkinson, 1992) to perform a three-way mixed model ANOVA for which speed (N=3) and slope (N=3) were fixed factors and individual (N=4) was a random factor. The F-ratio for the main effect of speed was the mean square for speed divided by the mean square for the speed x individual interaction term (Zar, 1999). Similarly, the F-ratio for the main effect of slope was the mean square for slope divided by the mean square for slope x individual interaction term. The F-ratio for the interaction effect of slope and speed was the mean square for slope x speed divided by the mean square for the speed x slope x individual interaction. The criterion for statistical significance was p<0.05.

Results are presented as the mean±1 standard error (SE). Unless stated otherwise, the mean values for each speed and slope combination presented for descriptive purposes were calculated so each individual was weighted equally. Depending on the particular speed and slope, mean values were from a different numbers of individuals (*N*i). The ANOVA results are the only tests of statistical significance since only these methods properly account for the repeated-measures experimental design. To clarify whether the effects of speed and slope on muscle length were similar among different individuals, we calculated linear least-squares regressions with speed at a given slope and slope at a given speed. Active and passive force-length curves were statistically fit using third-order polynomials in Systat (Systat Software, Inc.).

Results

The Lateral Gastrocnemius (LG) muscle's mechanical properties were consistent across birds. Peak isometric force per cross sectional area was 269±11 kPa for the ORST birds and 332±24 kPa for the HRVD. The composite fit of the data for all four ORST birds explained 82 % of the variance (R^2=0.82) and for the two HRVD birds explained 89 % of the variance (R^2=0.89) attesting to the similarity in length-tension properties between birds (Fig. 4.3). A composite fit to the pooled data sets only explained 56 % of the variance suggesting a major difference between the two groups. All data was kept separate between these two groups of birds since the composite fits explained the most variance when they were separate and L_0 is based on these composite fits.

We found the LG muscle operated at lengths to the right and left of L_0 (Fig. 4.4). The muscle operated from lengths 14% to the left of L_0 to 26% to the right of L_0 in swing phase (ORST: 0.76±0.02 to 1.10±0.04 L/L_0, HRVD: 0.96±0.01 to 1.26±0.04 L/L_0, Figures 4 and 5). There were no significant changes in swing operating lengths with speed, slope or slope by speed were found with ANOVAs. Individual variation and small sample size are the most likely reason these trends are not significant in our ANOVAs.

The LG produced force during stance to the right and left of L_0 (ORST: 0.67±0.03 to 0.93±0.01 L/L_0, HRVD: 0.91±0.01 to 1.08±0.02 L/L_0, Fig. 4.4 and Fig. 4.5). The only significant change in the lengths the LG operated at during stance was a decrease in the minimum length with speed ($F_{2,6} = 11.3$, $p < 0.01$, Fig. 4.5). Again individual variation and small sample size was most likely the reason these trends were not significant.

Discussion

We tested the hypothesis that muscles produce force at lengths on the plateau of the length-tension curve. Our determination of the operating lengths during stance and swing of the LG does not support this hypothesis. The two HRVD birds produced force during stance on the plateau of the length-tension curve, but the four ORST birds produced force on the ascending limb. Both groups of birds produced force during swing at lengths on the descending limb of the length-tension curve.

Differences between the groups of birds

The difference in operating lengths during stance between the two groups of birds was the result of significant offset in operating lengths during the whole stride. We choose to analyze these groups of birds separately because of the consistent 20% longer operating lengths in the HRVD birds. In addition to differences in the operating lengths, the shape of the LT curve was also different between the two groups of birds.

The different operating lengths and shape of the LT curve could result from the differences in the measurement technique and protocol used for the two groups of birds. One of the primary differences between the groups was how we measured force during the construction of the length-tension curve. We measured force in the ORST birds by attaching a servomotor to the distal end of the LG soft tendon. We measured force in the HRVD birds with the same strain gauges attached to the bony tendon as for the running part of the experiment. The other primary difference was the order of contractions used to obtain the length-tension curve. We started at a length close to L_0 in the ORST birds for the first tetanic contraction. We then proceeded to obtain force-length values at shorter lengths until tetanic force reached 80% of P_0 and then started a series of contractions at longer and longer lengths until we reached 80% of P_0 to complete the characterization of the curve. For the HRVD birds, we started at the longest length possible for the first tetanic contraction and then proceeded to shorter lengths to complete the characterization of the curve. In either case, an error in the determination of L_0 from the length-tension curve could cause both the observed difference in shape of the curve and the difference in operating lengths.

Alternatively, differences in muscle physiology or running kinematics might explain the differences between the groups. The training regime was slightly different between the ORST and HRVD groups of birds, they ran on different treadmills with different handlers. If one training regime was more intense than the other the tendon of the LG may have been stiffer in the group of birds with more intense training (Buchanan and Marsh, 2001). The amount a tendon stretches is dependent on the stiffness of the tendon and the magnitude of the change in force applied to it (Roberts, 2002). The magnitude of the change in force during swing and stance is the same in both groups of birds; therefore a stiffer tendon would undergo a smaller length change. A difference in the stiffness of the tendon could possibly explain the difference in lengths force was produced at in the two groups of birds. An alternative to a change in tendon stiffness between the birds is a difference in the kinematics of movement. The amount of length change the contractile tissue and tendon together undergo is dependant on the kinematics of locomotion and muscle moment arms (Holt et al., 2005). The muscle moment arms are most likely constant between the two groups of birds, so the amount of length change the contractile tissue and tendon undergo is the same if the two groups of bird moved with the same kinematics. If the HRVD birds had a more crouched posture the LG would operate at the observed longer operating lengths since the LG has a flexion moment arm at the knee and an extension moment arm at the intertarsal joint. One final possibility is ORST birds had longer fibers. If the muscle fibers of the ORST birds were longer than the relative length change would be less for the ORST birds than the HRVD birds. However, we are unable to explain the differences between these two groups of birds at this time.

Changes in operating length with speed and incline

We found that the operating length range during locomotion for the LG increased with speed and was constant across incline. Kinematics' studies have found total joint excursion changes across speed (Fieler and Jayne, 1998; Nilsson et al., 1985; Reilly, 2000) and incline (Carlson-Kuhta et al., 1998; Higham and Jayne, 2004) that suggests differences in operating length. Other studies (Daley and Biewener, 2003; Gabaldon et al., 2004; Gillis and Biewener, 2001; Nelson and Jayne, 2001) confirmed for the LG and digital flexor-IV in guinea fowl, LG and PL in turkey, Biceps Femoris and Vastus Lateralis in rat, and Caudofemoralis in desert iguana, that the range of muscle lengths changed as speed increased. Gillis and Biewener (2001) and Daley and Biewener (2003) further showed for the muscles in the rat and guinea fowl the range of muscle lengths changed with increases in slope. The disparity between our results and the results of predicted or measured changes in operating length with incline are the consequence of a difference in the points at which the measurements were taken.

The constant range of operating lengths across incline determined in this study is not different from previous studies showing a change in net change in length with incline. Previous studies examining the range of muscle lengths (Daley and Biewener, 2003; Gabaldon et al., 2004) focused on the net change in length from the beginning of force production to the end of force production, while another study examined the net change in length from the beginning to end of stance (Gillis and Biewener, 2001). The present study focused on the absolute maximum and minimum length during the period of force production, not net change in length.

Constraints of operating on the plateau of the length tension curve

Operating lengths above and below the plateau may be necessary to allow muscles to operate over a reasonable range of length. The plateau of the length tension curve only allows a 10% range of length. The LG muscle operated over a large range of length between 28-41% depending on the individual and locomotory condition. This large range of operating lengths is similar to previous ranges observed in other muscles used during locomotion (Biewener et al., 1998; Nelson and Jayne, 2000). These larger ranges of length could occur exclusively on the ascending or descending limb of the curve which has a 40% and 65% range of length, respectively, or any combination of the plateau and one or both of these limbs. Only a few studies have tried to determine where large muscle length ranges occur on the length tension curve.

The LG muscle operates over a large range of lengths, at L_0 and to the right and left of L_0 on the ascending and descending limbs of the length tension curve. Burkholder and Lieber (2001) reviewed a number of studies with similarly large operating ranges that showed a number of different muscles may also operate at L_0 and the right and left of L_0 during locomotion. However, only three of the studies included in this review were for operating ranges determined during locomotion. Two of these studies showed the biceps femoris, rectus femoris, lateral and medial gastrocnemius and tibialis anterior of rabbits (Dimery, 1985) as well as biceps femoris of cats (Chanaud et al., 1991) operated on both sides of L_0. The other study found the medial gastrocnemius of cats operated at lengths only to the right of L_0 (Griffiths, 1991). One other study of humans found the vastus lateralis and vastus medialis operated at lengths to the left of L_0 while the semimembranosus and rectus femoris operated on both sides of L_0 (Cutts, 1989). These studies show no clear pattern of where a large range of operating lengths must on the length tension curve during locomotion. This suggests there may not be a single criterion determining the length range a muscle operates over.

The economics of producing force on the ascending and descending limb of the LT curve

Our hypothesis of the lengths the muscle would produce force at was based on the knowledge that operating at lengths above or below the plateau of the length-tension curve reduces the force output from active fibers (Blix, 1894; Gordon et al., 1966; Huijing, 1985; Zajac, 1989) and thereby increases the necessary amount of recruited muscle volume to produce a given force. Several researchers have proposed (Kram and Taylor, 1990; Roberts, 1998) that the cost of locomotion is related to the recruited muscle volume. Other studies of muscle function during locomotion (Pennycuick and Rezende, 1984; Rome, 1994) similarly propose that a constraint for maximum muscular efficiency is operation on the plateau of the length–tension curve.

Isolated muscle energetic studies support the idea that the total cost of force production is higher on the ascending limb of the length-tension curve than L_0 for two reasons: force decreases while the ATPase rate is constant, and more fibers are activated to produce the same amount of force. Force per fiber is reduced on the ascending limb of the length-tension curve (Gordon et al. 1966). However, the ATPase activity is the same on the ascending limb as at L_0 (Stephenson, 2003). Therefore, the average force per fiber per ATP is lower on the ascending limb than at L_0. For example, the ORST birds operating at a length of 0.78 L/L_0 during stance produce only 78% of the force produced at L_0 for the same energetic cost. This means it costs 22% more energy to produce the same level of force at a length of 0.78 L/L_0 than at L_0. The only way the same level of force can be produced on the ascending limb as at L_0 is more fibers are activated on the ascending limb than at L_0. The activation of more fibers costs additional energy. The cost of activation ranges from 18-41% of the total cost of force production (Lou et al., 1997). If we assume the cost of activating the LG muscle fibers is 30% of the total cost of force production, then the cost of activating 22% more fibers is 7% above the cost of activation to produce the same amount of force at L_0. Together the total additional cost of producing force at a length of 0.78 L/L_0 is 29% above the cost of producing the same amount of force at L_0.

Isolated muscle experiments suggest the cost of force production on the descending limb is also higher than on the plateau because more fibers are activated to produce the same amount of force. Force per fiber is also reduced on the descending limb (Gordon et al. 1966). However, the ATPase activity decreases in parallel with the decrease in force production on the descending limb (Stephenson, 2003). Therefore, the average force per fiber per ATP is the same on the descending limb as at L_0 and no additional cost is associated with this ratio. Similar to force production on the ascending limb the only way the same amount of force is produced on the descending limb as at L_0 is the activation of more fibers. The activation of additional fibers costs energy. A muscle operating at a similar force decrement on the descending limb to the previous example on the ascending limb (22% less force) would cost 7% more energy to produce the same level of force as at L_0 because of activation costs.

Summary

The estimation of the turkey LG requiring an additional 29% more energy to produce force at $0.78L/L_0$, is not congruent with the idea of the musculo-skeletal system operating economically during locomotion. The consistent offset in operating lengths between the groups of birds and differences in the techniques used to make measurements do not allow us to make a conclusion. One of the primary differences causing concern is the 20% difference in operating lengths during stance between the HRVD and ORST birds. This difference in length could be the result of the ORST birds running with an abnormally upright posture or an error in the determination of L_0. Another major assumption is the *in situ* whole muscle length tension curve is representative of the whole muscle while only a subset of fibers are active during the stance phase of locomotion.

Provided the ORST birds were running normally and the *in situ* length tension curve is representative, the risk of muscle damage from operating at long lengths (Edman et al., 1978; Morgan, 1990) may be an additional design constraint acting on muscles used during locomotion.

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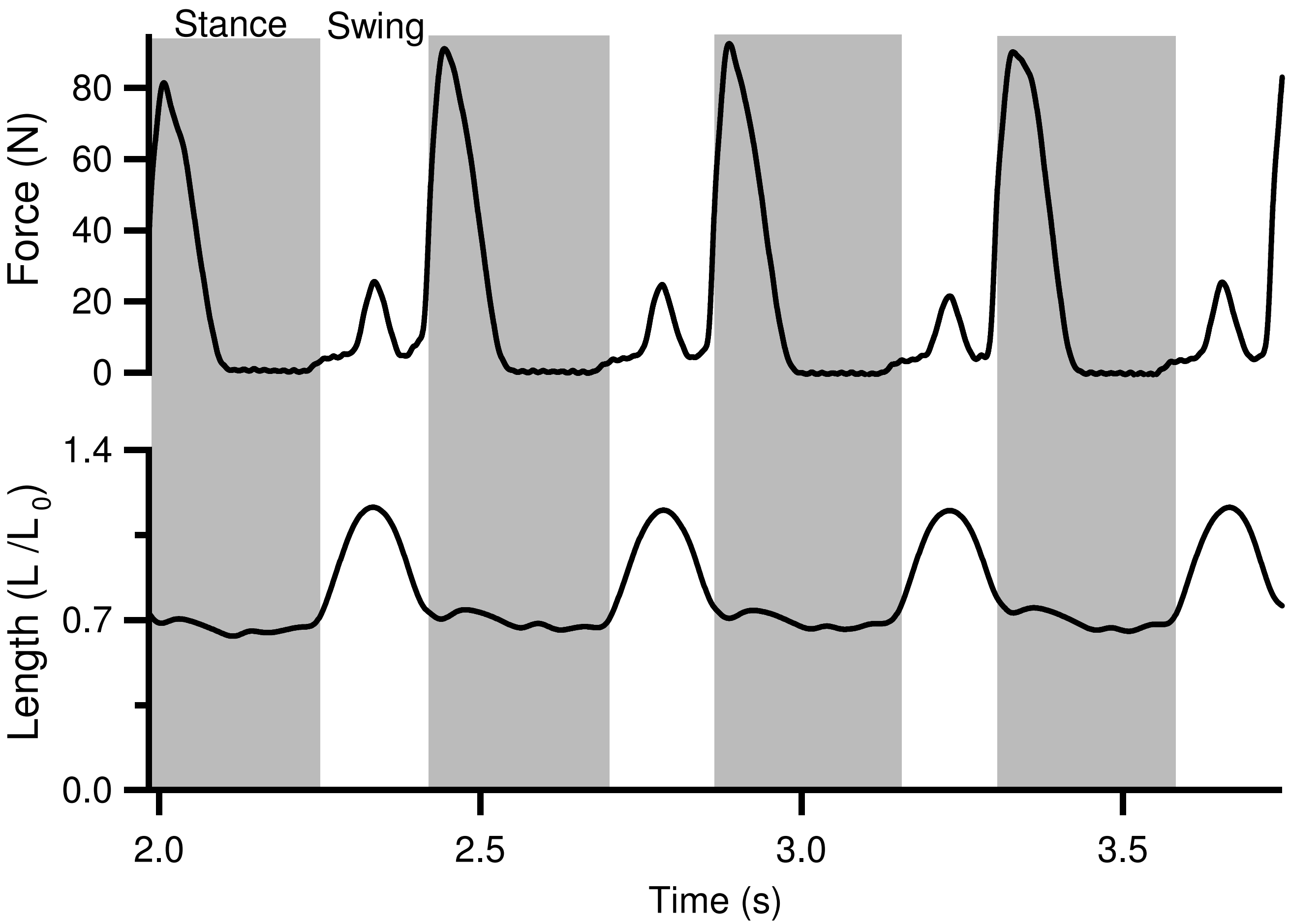

Figure 4.1 STEADY-SPEED LEVEL RUNNING. Representative force and length, for one turkey running at 2 ms^{-1}. During swing phase the LG accelerates the tarsometatarsus and foot from its most flexed position to the ground to begin stance. In swing phase the LG undergoes a large lengthening-shortening cycle while producing force. The shaded region indicates the period of a stride when the foot is in contact with the ground (stance).

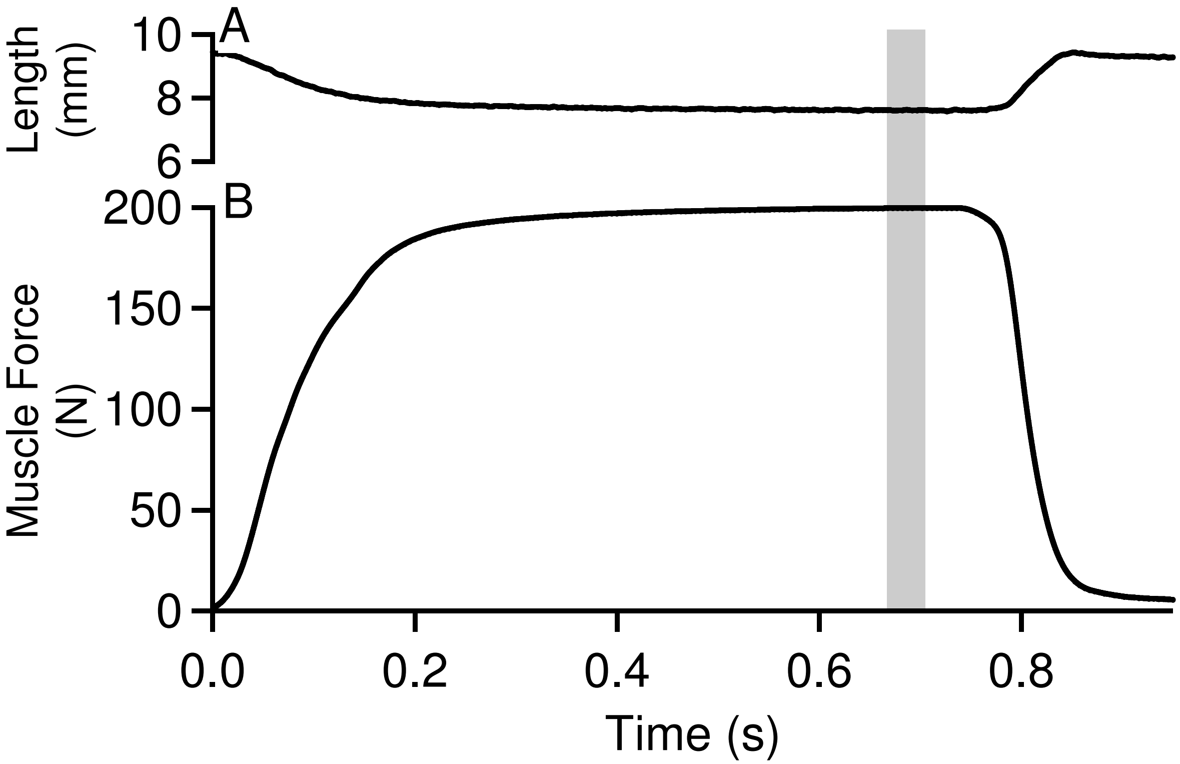

Figure 4.2 ISOMETRIC CONTRACTION. Representative isometric contraction with a 700 ms stimulation duration showing the muscle fascicles shortening as force rises to a plateau. Panel A shows the muscle length in mm measured by the sonomicrometry crystals. Panel B shows the muscle force in N measured by the servomotor. The gray region is representative of the areas where the values of average length and force we taken for the construction of a length-tension curve for each bird.

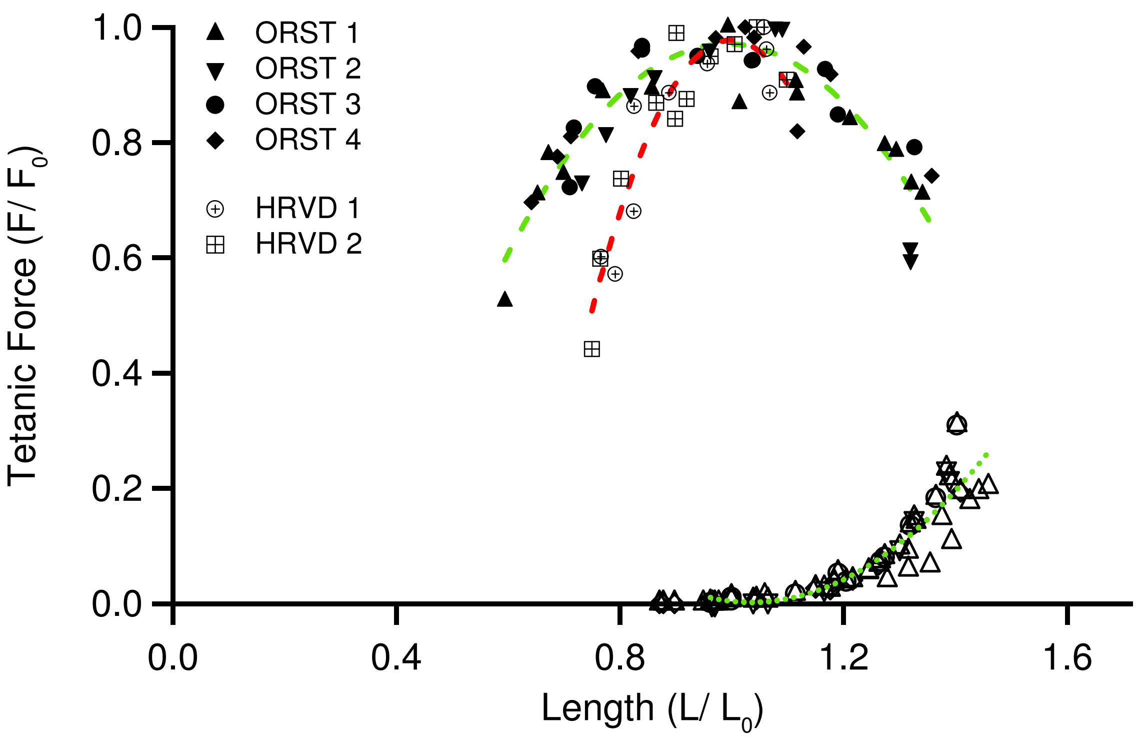

Figure 4.3 IN SITU LENGTH-TENSION CURVE. Tetanic and passive length-tension curves were measured for the same birds ran on the treadmill. All the individual data points for each of the bird's tetanic (filled symbols, ORST birds; hatched symbols, HVRD birds) and passive (open symbols. ORST birds) length-tension curves were fit with a second order polynomial. The composite fit (green dashed line) to the ORST set of tetanic length-tension data explains 82 percent of the variance in the data ($R^2 = 0.82$). The composite fit (red dashed line) to the HRVD set of tetanic length-tension data explains 89 percent of the variance in the data ($R^2 = 0.89$). The composite fit (green dotted line) to the ORST set of passive length-tension data explains 84 percent of the variance in the data ($R^2 = 0.84$).

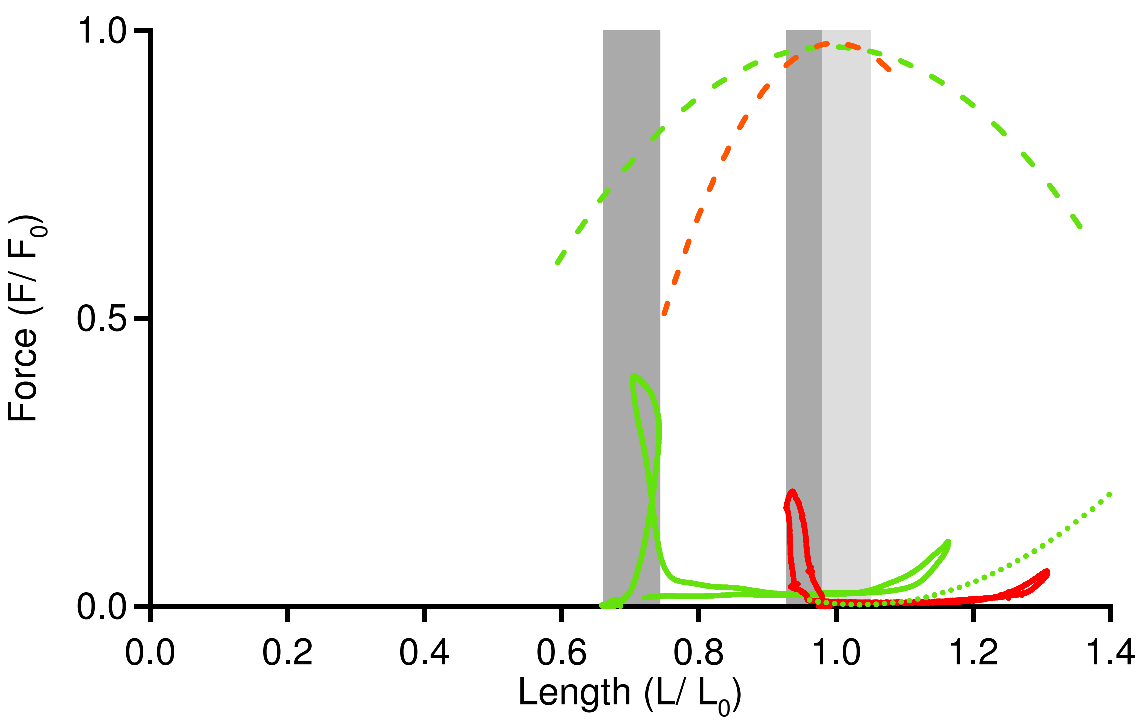

Figure 4.4 IN VIVO LENGTHS PLOTTED ON IN SITU LENGTH TENSION CURVE. During level running at a steady speed of 2 ms^{-1} the LG produces force over a range of lengths. Force-length data plotted are for ORST birds in green and HRVD birds in red. The dashed lines are the composite fit of the tetanic length-tension curve for the ORST birds (green) and for the HRVD birds (red). The dotted black line is the passive length-tension curve for the ORST birds. The range of lengths from 0.95 to 1.05 L/L0 is shown by the light gray bar. The darker gray bars show the lengths the LG produces force at during stance.

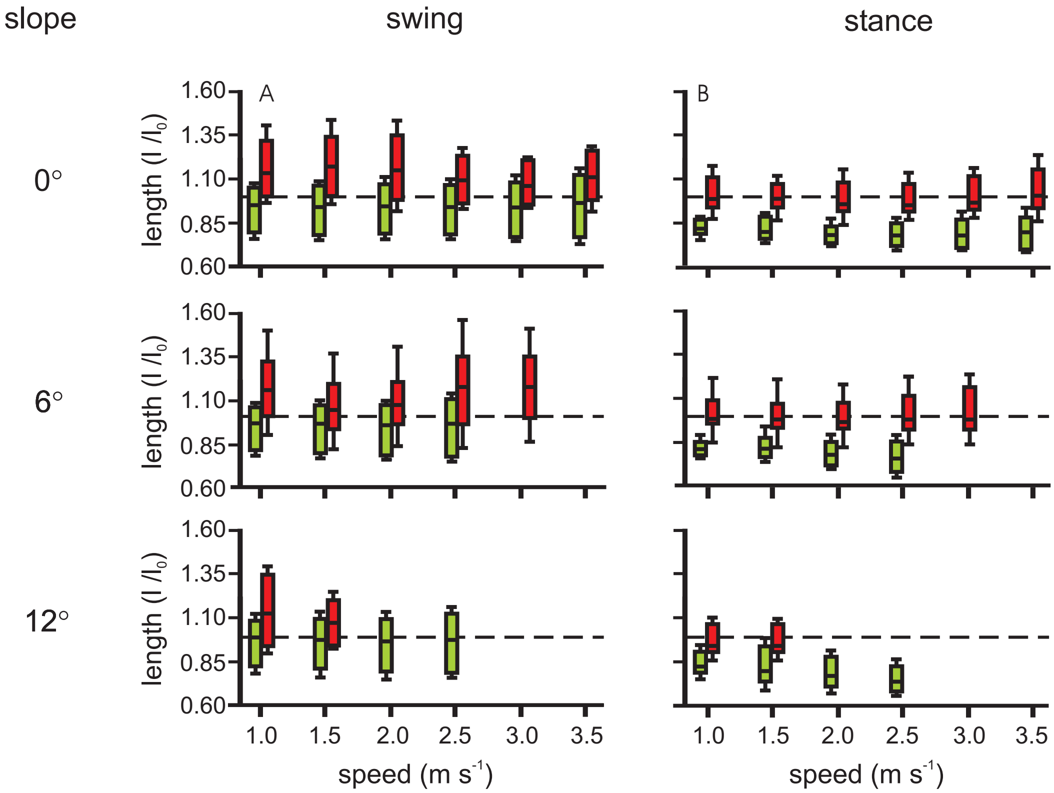

Figure 4.5 RELATIVE IN VIVO LENGTHS ON DIFFERENT INCLINES. LG muscle fascicle length during force production in swing (Panel A) and stance (Panel B) phase. The green bars are for data from the ORST birds and the red bars are for data from the HRVD birds. The top and bottom of the bars represent the average maximum and minimum length with standard error bars for each combination of speed and incline. The line in the middle of bar represents the average length during swing or stance for that speed and incline combination. The dashed line indicates L_0. The number of individuals for the ORST data is at 3.5 ms^{-1} on the level is N_i=3 and for 2.5ms^{-1} on a 12 incline N_i=2.

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Force-Velocity Properties of Two Avian Hindlimb Muscles.

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Chapter Five:

Force-Velocity Properties of Two Avian Hindlimb Muscles.

Abstract

Recent work has provided measurements of power output in avian skeletal muscles during running and flying, but little is known about the contractile properties of avian skeletal muscle. We used an in situ preparation to characterize the force-velocity properties of two hind limb muscles, the lateral gastrocnemius (LG) and peroneus longus (PL), in wild turkeys (Meleagris gallopavo). A servomotor measured shortening velocity for at least six different loads over the plateau region of the length-tension curve. The Hill equation was fit to the data to determine maximum shortening velocity and peak instantaneous power. Maximum unloaded shortening velocity was 13.0±1.6 L s^{-1} for the LG muscle and 14.8±1.0 L s^{-1} for the PL muscle (mean±S.E.). These velocities are within the range of values published for reptilian and mammalian muscles. Values recorded for maximum isometric force per cross-sectional area, 271±28 kPa for the LG and 257±30.5 kPa for the PL, and peak instantaneous power output, 341.7±36.4 W kg^{-1} for the LG and 319.4±42.5 W kg^{-1} for the PL, were also within the range of published values for vertebrate muscle. The force-velocity properties of turkey LG and PL muscle do not reveal any extreme differences in the mechanical potential between avian and other vertebrate muscle.

Introduction

Avian skeletal muscles operate over a wide range of power outputs during terrestrial and aerial locomotion. Recent *in vivo* measurements of muscle power output during locomotion suggest power outputs are relatively low during level steady-speed movement in comparison to take-off and terrestrial acceleration (Dial and Biewener 1993, Roberts et al. 1997, Dial et al. 1997). For example, Dial and Biewener (1993) determined the power output over an entire locomotor cycle (sustained power) for the pigeon pectoralis muscle of the same bird during takeoff was 119 W kg^{-1} and for steady level flight was 51 W kg^{-1}. The highest value for avian power output (390 W kg^{-1}) was calculated during an aerial acceleration (Askew et al. 2001). This value not only stands out from other estimates of avian muscle power output, but it is to our knowledge the greatest power output ever reported for a vertebrate muscle contraction during cyclical movement.

The relatively low power outputs measured during steady locomotion suggest muscles are operating well below their capacity for power production during these activities. It is more difficult to state whether the high power outputs measured during takeoff and acceleration are near maximal because the maximum power output of avian muscle is not known. Maximum shortening velocity and force per cross-sectional area have been measured in chicken muscle fibers at temperatures below physiological (15-25ºC, Alway, 1995; Reiser et al., 1982; Reiser et al., 1988; Reiser et al. 1996), but there is no measurement of power. The conserved contractile properties of vertebrate skeletal muscle (Josephson 1993) suggest measurements of power from mammal and lizard muscle might provide a reasonable estimate of the power producing ability of avian muscle. However, the remarkably high average power measured for quail muscle *in vivo* (390 W kg^{-1}, Askew et al. 2001) is approximately twice the highest measured for other vertebrates (150 W kg^{-1} for lizard muscle, Swoap et al., 1993; 196.7 W kg^{-1} for mouse muscle, Askew and Marsh 1997). Though many factors determine average power during movement, one possible explanation for the remarkably high power outputs determined during quail flight is that the maximum power-producing capacity of avian muscle is greater than that of other vertebrates.

Here we use a unique *in situ* preparation to characterize the force-velocity relationship in turkey lateral gastrocnemius muscle and peroneus longus muscle to determine their intrinsic power-generating capacity. Both muscles are examples of avian fast-twitch muscle (Patak and Baldwin 1993), similar to the pectoralis muscle (Khan, 1978). These measurements provide the first estimate of the force-velocity relationship and peak instantaneous power of avian muscle at physiological temperature. This study was motivated in part by our long-term goal to directly compare muscle contractions *in vivo* with muscle contractile properties. In addition, this study allows us to evaluate whether avian skeletal muscle has an unusually high power producing capacity when compared to other vertebrates.

Materials and Methods

Animals

Data were collected from 6 adult female Eastern wild turkeys (*Meleagris gallopavo*) obtained from a local breeder. The mean body mass of the birds was 4.0±0.4 kg (±SD); the mean masses of the lateral gastrocnemius (LG) and peroneus longus (PL) muscles were 21.8±0.5 and 20.5±1.8 g (±SE), respectively. The animals were housed in a large indoor enclosure and maintained at 20-22 ºC on a 12:12-h light-dark cycle (lights on at 0600, off at 1800). A diet of Game Bird Flight Conditioner (Purina-Mills, Inc.) and water were provided *ad libitum*. All animal use was approved by the Oregon State University Institutional Animal Care and Use Committee and in accordance with federal and institutional guidelines.

In situ preparation

The *in situ* preparation ensured that the muscle received blood flow throughout the experiment and that muscle temperature was maintained within a physiological range (36-39°C). Surgery and *in situ* experiments were performed while the animal was maintained under deep anesthesia with isoflurane gas. The sciatic nerve was isolated for electrical stimulation of the LG and PL muscles. Thin connective tissue around the nerve was carefully removed and the nerve was severed just distal to its emergence from the pelvic girdle. The nerve was inserted into a bipolar electrode nerve cuff constructed of two silver wires and polyethelyne tubing (1 cm length; I.D. 5 mm). The pocket of tissue containing the severed nerve was filled with mineral oil to isolate it and to reduce voltage dissipation during nerve stimulation. The wire leads from the nerve cuff were connected to a Grass S48 stimulator for muscle stimulation. To determine muscle fascicle length during isotonic contractions, two 1 mm piezo-electric crystals (Sonometrics, Inc.) were implanted into the proximal region of the LG or PL muscle. A skin incision was made below the knee and crystals were implanted parallel to muscle fascicles at a distance of 8-12 mm apart. A small drop of Vet-bond skin adhesive secured the crystals in place and 6-O suture secured the crystals' wire leads to muscle connective tissue.

For construction of the force-velocity curve for each muscle, we measured total muscle force and muscle length changes using a servomotor (Aurora 310B-LR). To ensure the servomotor accurately measured whole muscle length changes, compliance in the system was minimized by rigidly linking the bone of muscle origin (i.e., the femur) and the servomotor through a rigid aluminum frame. The rigid link was formed with an aluminum plate fastened to the femur with two machine screws and clamped to the frame holding the servomotor. After securing the bird to the frame, the distal tendon of the muscle was isolated and cut free. The freed tendon was then attached to the servomotor with a custom-built aluminum clamp weighing 21.78 g and a 1/16” aircraft cable. The sonomicrometry and servomotor signals were sent to a computer with a 12-bit A/D converter (National Instruments PCI-MIO-16-1). Data were collected at 1000 Hz using the software program Igor Pro (WaveMetrics Inc.).

Contractile property measurements

Prior to constructing the force-velocity curves for each muscle, we determined the optimum stimulation voltage and optimum muscle length. These are the two main criteria necessary to achieve maximal isometric force per cross-sectional area (F_0) during tetanic stimulation. The optimum stimulation voltage was determined in two steps. First, the voltage required to fully activate all available motor units was determined by increasing the voltage in one-volt increments beginning at 1 V and continuing until twitch force no longer increased. The voltage that produced maximum twitch force was then increased by one volt; this supra-maximal stimulation value (6-8 V for all preparations) was used for all subsequent muscle contractions within an experiment. To determine the optimum muscle length (L_0), we constructed a twitch length-tension curve and determined the muscle length producing the greatest force during stimulation. This was done using the fascicle length measurements from sonomicrometry system. We constructed a length tension curve using twitch contractions instead of tetanic to minimize potential muscle fatigue prior to a force-velocity measurements.

All isotonic force-velocity measurements were taken from tetanically stimulated muscle over the plateau of the twitch length-tension curve, when the muscle length was at ±5% L_0. Smooth tetanic contractions were attained with a stimulation pulse duration of 0.2 ms at a frequency of 100 Hz. Contractions were obtained over a range of forces and velocities by adjusting the maximum motor force and stimulation train duration (60-700 ms). Representative recordings of two isotonic contractions are shown in Fig. 5.1. Force and velocity values were calculated as the average over a 10 to 30 ms period of time, beginning shortly after force reached a plateau and ending before muscle stimulation ceased (Fig. 5.1, shaded bars). Contraction velocity (mm s^{-1}) was determined by differentiating muscle length measured by the servomotor (Fig. 5.1). Whole muscle force was also measured directly by the servomotor. Our measurements are accurate estimates of the force-velocity properties because force and velocity reached a constant value before muscle activation ceases as shown in the representative recordings of an isotonic contraction at a low load (Fig. 5.1B). A constant force and velocity indicate the muscle was fully active when we made our measurements. To minimize possible effects of muscle fatigue or potentiation, a rest period of 5 min or greater was given between successive tetanic contractions. Isometric contractions were recorded at the beginning, middle, and end of a series of isotonic shortening contractions to check for potential changes in maximum isometric force. Maximum isometric force (P_0) did not change during the experiment for any of the birds, so no corrections for changes in muscle force due to fatigue were made.

The large series elastic element present in our preparation creates some challenges with making measurements of the force-velocity properties and muscle fiber length of the muscle during contractions. We measured force and velocity, from the servomotor, during the region of constant force when elastic elements are not changing length to ensure force-velocity properties are not enhanced by elastic elements (Askew and Marsh, 1997; Stevens, 1993). The large series elastic element makes ensuring the muscle shortens over the plateau of the length tension curve, when the muscle length is at ±5% L_0, challenging because muscles shorten against the series elastic compliance during the early (rising force) portion of each contraction (Hill, 1938; Roberts, 2002). By including measurements from sonomicrometer crystals placed along the proximal muscle fascicles, we were able to account for muscle fascicle shortening during the early non-isotonic period of the contraction and ensure that all force-velocity measurements were taken over the plateau of the twitch length tension curve.

We measured muscle fiber length, angle of pennation, and muscle mass to standardize the muscle contractile properties (maximum shortening velocity, maximum isometric force, and peak instantaneous power) between different muscles. To determine the muscle fascicle length at L_0 we simultaneously measured the length of the muscle fibers and the distance between sonomicrometry crystals while the muscle was at a set length. This was necessary because the sonomicrometer crystals measured only a segment of the fascicle. We used our measure of muscle fascicle length at L_0 to convert muscle velocity from absolute units of mm s^{-1}, measured from the muscle motor, to relative units of muscle lengths per second (L s^{-1}). We did not use any measurement of muscle fiber pennation angle to correct our determination of muscle velocity from the motor, since effects of pennation angle are small. Physiological cross-sectional area was calculated as the product of muscle mass and the cosine of the angle of pennation, divided by the product of muscle density and fiber length (Gans, 1982; Powell et al., 1984). Maximum isometric force was standardized, by dividing the measured force by the physiological cross-sectional area of the muscle.

The theoretical unloaded maximum shortening velocity (V_{max}), the curvature of the force-velocity relationship (a/F_0), and peak instantaneous power (P_{max}) were determined by fitting the Hill equation (1938) to our data. Hill's equation is a rectangular hyperbola:

$$(F + a) \cdot (V + b) = (F_0 + a) \cdot b;$$

F_0 is the maximum isometric force of the muscle, a and b are constants that vary between muscles, F is the force of a given contraction, and V is the velocity of the same contraction. The ratio a/F_0 is a conventional measure of the curvature of the relationship between force and velocity. V_{max} is calculated from this equation by setting F equal to zero and solving for V. Power is the product of force and velocity. The force (F_{opt}) where peak power is developed was determined from the equation given by Woledge and coworkers (1985):

$$F_{opt} = (a^2 + a \bullet F_0)^{1/2} - a.$$

By substituting F_{opt} into Hill's equation, we calculated the shortening velocity at maximal power output, V_{opt}. Peak instantaneous power was calculated as the product of F_{opt} and V_{opt}.

Statistics and curve fitting.

Results are presented as mean±one standard error (SE). Comparisons of contractile properties between the LG, PL, and the values from the literature were made using student's T-test. Force-velocity curves were statistically fit using the Hill equation (1938) in Systat (Systat Software, Inc.).

Results

The three parameters that define the force-velocity curve (F_0, a/F_0 and V_{max}) were not significantly different between the lateral gastrocnemius (LG) and peroneus longus (PL) muscle ($P > 0.05$). The statistically fit composite force-velocity curve for the LG and PL explained 99% of the variance in the data (R^2). Fig. 5.2 shows the individual data points for all birds and a composite force velocity curve based on the mean values V_{max} and a/F_0. Mean maximum isometric tension per cross-sectional area (F_0) was 271±28 kPa for the LG and 239.8±30.1 kPa for the PL. The theoretical unloaded maximum shortening velocity of the LG was 13.0±1.6 L s^{-1} and PL was 14.8±1.0 L s^{-1}. The shape of the force-velocity curves, as indicated by a/F_0, was not statistically different for the two muscles (0.39±0.10, LG and 0.26±0.02, PL). Peak instantaneous power of the LG and PL was also similar (340.8±48.2 W kg^{-1}, LG and 319.4±43.8 W kg^{-1}, PL).

Discussion

The V_{max} and P_{max} presented here are the first, to our knowledge, reported for whole avian muscle at physiological temperature. Our measurements allow a comparison of avian contractile properties (V_{max}, F_0, a/F_0, P_{max}) with those of other vertebrates to determine if avian muscle contratile properties are unique. Three sources of information on the contractile characteristics of fast vertebrate muscles are available for comparison. Below we present each of these and discuss their potential limitations. First, we compare turkey muscle properties with measurements made from chicken muscle fibers at non-physiological temperatures. Second, we calculate predicted V_{max} values for turkey muscles from scaling equations for non-avian vertebrates. Finally, we compare turkey muscle properties with the limited number of values for reptilian and mammalian V_{max} and P_{max} available from the literature.

To our knowledge, the only published values of avian muscle contractile properties are for isolated fibers (Reiser et al., 1982; Reiser et al., 1988; Reiser et al. 1996) or whole muscles (Alway, 1995) at 15-25°C from chicken latisimus dorsi and pectoralis major muscles. Only two of the mechanical properties we determined for turkey muscles (V_{max} and F_0) are reported in these studies of chicken muscle. Reiser and coworkers' (1996) measurements of fast fibers from the pectoralis major muscle yielded a V_{max} of 4.66 ± 0.78 L s^{-1} at 15°C. To calculate a value for chicken V_{max} at physiologic temperature (~35ºC), we used published Q_{10} values for rat muscle [$Q_{10} = 2.0$ for 15-25 ºC; $Q_{10} = 1.8$ for 25-35 ºC (Ranatunga, 1984)], because Q_{10} values for birds are not known. With this correction, a V_{max} of 4.66 ± 0.78 L s^{-1} at 15°C (Reiser et al., 1996) corresponds to a V_{max} of 16.8 ± 2.8 L s^{-1} at 35°C (Table 2). A comparison of chicken and turkey F_0 values also requires corrections to skinned fiber measurements to estimate physiological values. Fiber swelling tends to increase the measured value of cross-sectional area in skinned fibers, therefore decreasing the estimate of peak muscle stress (Godt and Maughan, 1981). Godt and Maughan (1981) found the diameter of skinned fibers typically swelled by ~20%, which increased the cross-sectional area by

~44%. This correction for fiber swelling raises Reiser et al.'s (1996) measured F_0 of 165±21 kPa to 238±30 kPa. Thus, though these estimates of V_{max} and F_0 require unproven assumptions, they are comparable to V_{max} and F_0 for turkey muscles.

Allometric scaling equations for V_{max} can be used to predict values for turkey muscles. One published equation is based on whole muscle measurements at ~35 ºC, close to the temperature for our turkey measurements (McMahon 1975). We modified McMahon's (1975) scaling equation to predict V_{max} in L s^{-1} by dividing by the average sarcomere length of 3 µm reported for these studies (Close 1969; Close and Hoh 1967). The modified equation ($V_{max} = 11.8M_b^{-0.13}$, M_b in kg) for mammalian fast fibers (for original data see Close 1965; Close and Hoh 1967; Close 1969), predicts a V_{max} of 9.85 L s^{-1} for a 4 kg animal. Though these predictions require a questionable extrapolation beyond the size range of the original data (~0.02-3 kg, McMahon 1975), the calculated values are similar to measured V_{max} values for turkey muscles.

Two scaling equations for V_{max} have been determined from mammalian skinned fibers (Rome et al. 1990, Seow and Ford, 1991). These studies included values for V_{max} across a wider range of body masses than for the McMahon (1975) study given above, but experiments were carried out below physiological temperature (Rome et al. 1990, Seow and Ford, 1991). Rome and coworkers' (1990) scaling equation ($V_{max} = 5.01M_b^{-0.073}$), predicts a V_{max} of 4.53 L s^{-1} for muscle fibers at 15 ºC for a 4 kg mammal (for original data see Eddinger et al., 1986; Moss, 1986; Rome et al. 1990). Soew and Ford's (1991) scaling equation, modified here by dividing by their measured half-sarcomere length to get V_{max} in L s^{-1} ($V_{max} = 1.24M_b^{-0.126}$), predicts a V_{max} of 1.04 L s^{-1} for muscle fibers at 5 ºC for a 4 kg mammal. We used Ranatunga's (1984) measurements for rats to correct both predictions for temperature, assuming the Q_{10} from 5 to 10 ºC was the same as measured for 10 to 15 ºC (Q_{10}=2.7). With these Q_{10}s Rome et al.'s (1990) and Soew and Ford's (1991) predicted V_{max} is converted to 16.31 L s^{-1} and 10.12 L s^{-1}, respectively, for muscle fibers at 35 ºC for a 4 kg animal. Though it is problematic to assume rat Q_{10}s apply to all mammals and extrapolate outside the measured Q_{10} range for rats (5 to 10 ºC), these predictions are reasonably close to our measurements for turkeys.

To make a direct comparison between our contractile measurements and those of other vertebrates, we collected published values of V_{max} and P_{max}. We used values from studies which reported the following: measurements made near avian physiological temperature (35–40 ºC), measurements of body mass, and measurements of relative maximal shortening velocity, in L s^{-1}, or measurements of fiber length to allow calculation of relative shortening velocity (Table 2, Fig. 5.3). A comprehensive search of the literature revealed relatively few contractile measurements that met these criteria (n=9; body mass and resting fiber lengths for Sréter et al. 1975 and Luff 1975 were obtained by personal communication from T. Luff). Additionally we calculated P_{max} when the mass of the muscle and a/F_0 were published (Table 2, Fig. 5.3). Our measurements of turkey F_0, a/F_0, V_{max}, and P_{max} fall within the range reported for other vertebrates (Table 2).

To determine whether some of the variability in literature values for V_{max} and P_{max} was due to body mass dependence of contractile properties, we plotted V_{max} and P_{max} as a function of body mass (Fig. 5.3). The slope of a linear regression of log V_{max} or P_{max} on body mass was non-significant ($p>0.05$, Fig. 5.3) for our collected literature values. The lack of a body-mass related change in V_{max} is surprising, given that commonly cited scaling equations for fast muscle V_{max} vary between studies but all indicate a generally small decrease in V_{max} with increasing body mass (fish fast fibers, $M_b^{-0.11}$, (James et al., 1998); lizard fast fibers, $M_b^{-0.084}$, (Marsh, 1988); frog fast fibers, $M_b^{-0.094}$, Marsh 1994; mammalian fast whole fibers, $M_b^{-0.13}$, McMahon 1975; mammalian skinned fibers, $M_b^{-0.073}$, Rome et al. 1990 and $M_b^{-0.13}$, Soew and Ford 1991). The non-significant slope could be a result of natural and experimental differences in the muscle preparation (reviewed in Askew and Marsh 1997) along with our small sample size. The mean value of V_{max} and P_{max} from our collection of literature values is, 14.5 L s^{-1} and 400.8 W kg^{-1}. Neither of these values is different from our measurements of turkey V_{max} and P_{max} ($P > 0.05$).

Avian muscle P_{max} compared with in vivo performance

The estimates of chicken fiber V_{max} and F_0 along with the predictions of V_{max} from scaling equations and the collected values from the literature all indicate turkey muscle P_0, V_{max}, a/F_0 and P_{max} are not unusual. Our measurements of peak instantaneous power also provide an estimate of the power producing capacity of avian muscle for comparison with measurements in biomechanical studies of locomotion (Fig. 5.3). These comparisons between our measurements of the power producing capacity of the LG and PL in turkeys with different muscles in turkeys or avian muscle in general should be made cautiously. The primary problem with making comparisons across muscles or species of birds is the possibility of differences in fiber type distribution. Large variation in the fiber type distribution would result in differences in whole muscle contractile properties (Ranatunga and Thomas, 1990).

The fiber type distribution in all of the bird muscles studied <u>in</u> <u>vivo</u> is not know, but avian muscle is known to posses the three classic vertebrate fiber types; slow oxidative (SO), fast oxidative glycolytic (FOG), and fast glycolytic (FG) as defined by Peter and coworkers (1972). Patak and Baldwin (1993) showed that the gastrocnemius muscle group in emus was composed of FOG and FG muscle fibers and the LG possessed them in almost equal proportion.

The digital flexor muscles of emus and the LG and illiotibialis cranialis muscle of pigeons, coots, mallard ducks, and gulls posses all three fiber types; with FOG and FG fibers composing 60% or more of the cross-sectional area (Torrella et al., 1993; Torrella et al., 1995; Torrella et al., 1998; Torrella et al., 1998; Leon-Velarde, 1993). Another muscle commonly studied, the pectoralis major, is reported to solely posses FOG or a combination of FOG and FG fiber types in common coots, gulls, mallard ducks, and pigeons (Kahn, 1978; Torrella et al., 1996; Yoshitaka et al., 1998). We do not know the fiber type composition of the LG and PL in turkeys but assume they are similar to those in the emu because of a similar life history. Given this assumption, general predictions from our measurements of the LG and PL to the capacity for power production in other commonly studied avian muscles can be made.

The highest possible power output from a muscle's contractile element at any one instant during the propulsive phase of locomotion is determined by the muscles capacity for peak instantaneous power, P_{max}. Roberts and Scales' (2002) conservative estimate of the peak instantaneous power required from the hindlimb muscles of turkeys during the power stroke of a running acceleration (400 W kg^{-1} muscle) is greater than our measurement of peak instantaneous power available for isolated turkey muscle (Fig. 5.3, Panel B). This supports their conclusion that muscular power from the contractile element may be enhanced by elastic energy storage.

Most measurements of power during locomotion are for the average power over an entire cycle (sustained power), which is always lower than peak instantaneous power (Caiozzo and Baldwin 1997, Josephson 1993). The average power during a single contraction (stroke power) is lower than peak instantaneous power because power is reduced during periods of muscle activation and deactivation, and because muscle velocity and force vary during a contraction. Sustained power is further reduced, by including the period of time when the muscle is lengthening and providing no positive work. For example, Swoap and coworkers (1993) found that the maximum sustained power the iliofibularis muscle of Desert iguanas could generate over an entire cycle was 150 W kg^{-1}, about one third of the muscle's peak instantaneous power of 460 W kg^{-1} (Marsh and Bennet 1985). Other studies have found this approximate three-fold difference to exist between peak instantaneous power and maximum power produced during a sinusoidal lengthening and shortening contractions (2.8, Askew and Marsh 1997; 4.4, Marsh and Olson 1994, 3.4, Swoap et al. 1997; 3, Stevens 1993). This difference in power is the primary reason why the measurements of sustained power taken from the literature in Fig. 5.3 for takeoff and level flight are below the average peak instantaneous power from isolated muscle. For example, the high powers measured during pigeon takeoff (119 W kg^{-1}) are well below our measurement of peak instantaneous power (372 W kg^{-1}). Given the three-fold difference between sustainable power and peak instantaneous power for some muscles, the 119 W kg^{-1} developed by pigeon pectoralis major muscle suggests it is operating at its capacity for power production.

In contrast to most of the measurements of sustained power, the calculated sustained power (average power over a single cycle) during take-off in quail (390 W kg^{-1}) is above our measured peak instantaneous power of 372 W kg^{-1} (Askew et al. 2001). In fact, the typical three-fold difference between sustained and peak instantaneous power would suggest that quail muscle is capable of a peak instantaneous power output of ~1200 W kg^{-1}. This predicted value is not only higher than our measured peak power for turkeys, but well above any measured power output for vertebrate muscle (472 W kg^{-1} for rats, Close 1969; 505 W kg^{-1} for lizards, Marsh and Bennet 1985). A likely explanation for the very high sustained power outputs in quail is the reduction of this three-fold difference between peak instantaneous power and average power (Marsh and Olson, 1994; Swoap et al., 1997; Stevens, 1993), to approximately a two-fold difference(Askew and Marsh, 1997). This reduction from a three-fold to two-fold difference between peak and average power is possible by a change in the amount of time spent shortening in a cycle from 50% to 75% (Askew and Marsh, 1997). Askew and Marsh (2001) have shown quail use a 70% shortening cycle and gain a further 16% more power from their muscle by varying the velocity during lengthening and shortening phase of contraction. Assuming a 1.7-fold difference between peak and average power output reduces the estimated peak instantaneous power for quail pectoralis to ~663 W kg^{-1}. This estimate from quail is not outside of the range of peak instantaneous power measured for other vertebrates. This estimate of quail pectoralis major power and our measurements of peak instantaneous power in turkey muscle suggests avain muscle properties are generally not different from other vertebrates.

Summary

Our measurements of turkey LG and PL force-velocity properties are the first of their type for avian muscle. These measurements are useful for comparison with other vertebrate muscle and as a first estimate of the capacity of avian muscle to produce power. Similar measurements of different avian muscles would help confirm our predictions of the generality of these measurements and provide a better perspective for determining how muscles work during avian locomotion.

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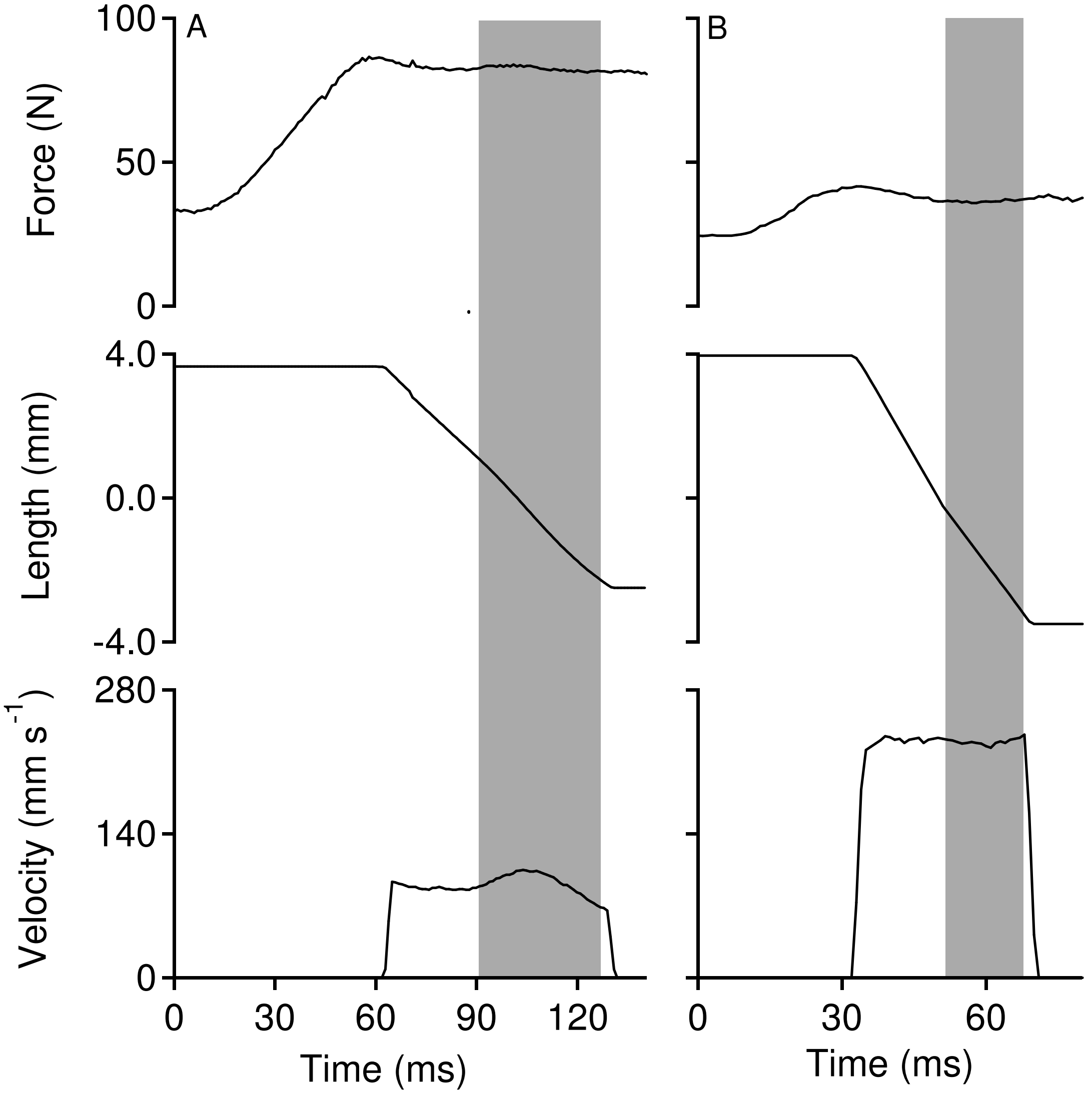

Figure 5.1 Representative measurements for two individual contractions of the lateral gastrocnemius muscle. The top two panels show measurements of whole muscle force and length. The bottom panel shows the whole muscle velocity calculated by differentiating the length measurements. The muscle was stimulated during the entire period shown. Force and velocity values were calculated as the average over a 10 to 30 ms period of time, beginning shortly after force reached a plateau and ending before muscle stimulation ceased (shaded region). Graph A shows a contraction of moderate force and shortening velocity; graph B shows a low force high velocity contraction.

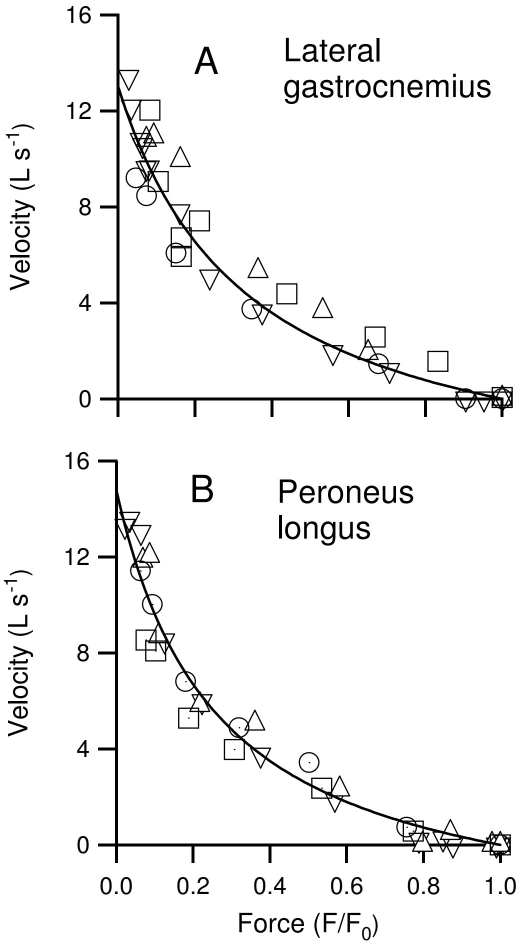

Figure 5.2 The composite force-velocity curve for the lateral gastrocnemius (A) and peroneus longus (B) muscles based on the mean values Vmax and a/F_0, along with the individual data points for all birds is shown. Data for each individual bird are shown with different symbols: Bird1 (up triangle), Bird2 (down triangle), Bird3 (circle), Bird4 (square), Bird5 (circle dot), and Bird6 (square dot).

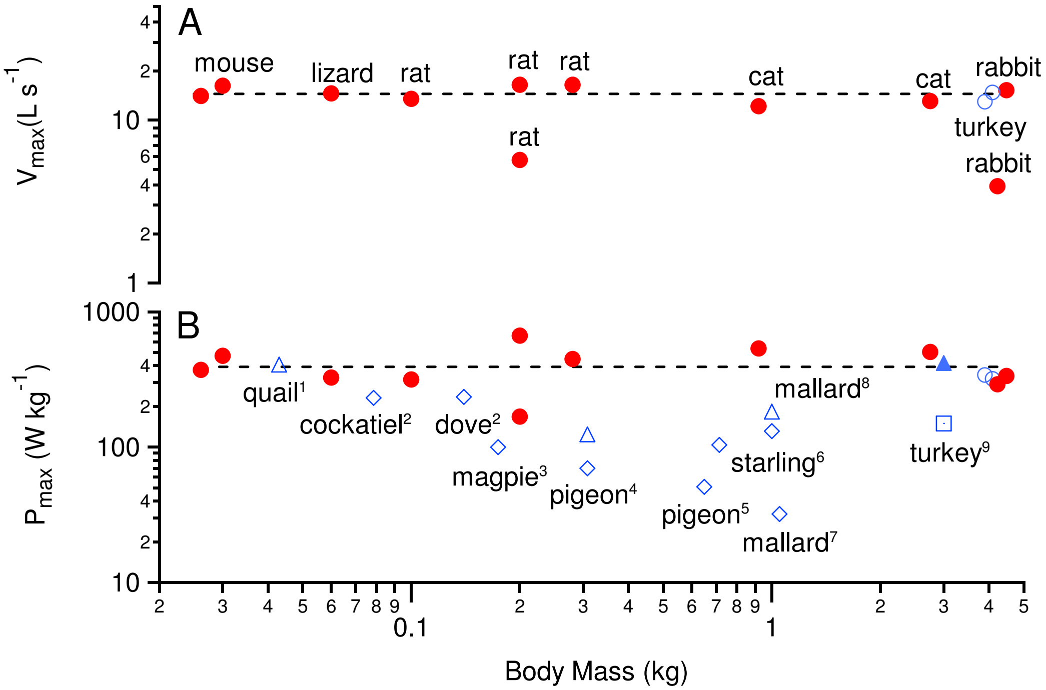

Figure 5.3 Maximum muscle shortening velocities (V_{max}, panel A), peak instantaneous powers (P_{max}, panel B) and power outputs during locomotion (panel B) across a range of body masses collected from the literature along with our own measurements are shown. The V_{max} and P_{max} values from the literature (listed in table 1) are indicated by the filled circles and our values are indicated by open circles. The slopes of both V_{max} and P_{max} vs. body mass were non-significant, so the lines in panels A and B are the means of all values. In panel B published values of the average muscle power output measured over an entire locomotor cycle are plotted against body mass for steady speed locomotion (open diamonds) and takeoff or terrestrial accelerations (open triangles). The filled triangle shows peak instantaneous power measured during terrestrial accelerations in turkeys. The muscles and reference for the in vivo power outputs are: 1) pectoralis: Askew et al., 2001; 2) pectoralis: Tobalske et al., 2003; 3) pectoralis: Dial and Biewener, 1993; 4) pectoralis: Dial et al., 1997; 5) pectoralis: Biewener et al., 1998; 6) pectoralis: Biewener et al., 1992; 7) lateral gastrocnemius: Biewener and Corning, 2001; 8) pectoralis: Williamson et al., 2001; 9) lateral gastrocnemius: Roberts and Scales, 2002.

Chapter Six:

General Conclusions

In this dissertation, I examined possible reasons why there appears to be no link between the energetic cost and mechanical work of swing. I pursued this goal in three ways: by modeling the mechanical work of running, determining which muscles are involved in extension of the intertarsal joint during the swing phase in turkeys, and the mechanical properties of these muscles.

The construction of simple models, such as the driven pendulum model of human running, is an approach which offers insight to the function of the musclo-skeletal system during locomotion. I used this simplified model of the human lower limb to under stand the mechanics and energetics of swing during human running. The driven pendulum model showed that accelerations of the body shift the frequency at which the work required to swing the human lower limb is minimal (MWF) and thereby reduces the work required to swing the limb. The shift in the MWF of the lower limb illustrates how the body's accelerations may be a passive mechanism for the minimization of work during running. This passive mechanism provides a new perspective for why the work to swing the limbs is large (Cavagna and Kaneko, 1977) but not reflected in the energetic cost of locomotion (Taylor et al., 1974).

This model has the added potential of being applied towards discovering methods for rehabilitation in patients with gait disorders. By increasing the sophistication of the current model to account for multiple joint kinematics, it would be possible to asses how manipulations of locomotor variables (e.g. ground reaction forces and joint accelerations) affect energetic cost. For instance if a person had an abnormal gait where their body accelerated very little in vertical and horizontal direction while swinging their limbs quickly, the cost of swing may be very high. A model with inputs for this person's behavior would offer considerable insight to possible rehabilitation for more efficient locomotion.

In my second study, I determined the muscles involved in extension of the intertarsal joint during swing. I found force was not shared between the two heads of the gastrocnemius muscle as predicted from force sharing models based on stance (Dul et al., 1984; Pedotti et al., 1978). It is likely that the differences in measured patterns of force production in the lateral head (LG) and medial head (MG) of the gastrocnemius in turkeys are due to the muscles performing different tasks during stance and swing as suggested by Raikova (1992).

Power transfer between joints, as proposed by Robertson and Winter (1980), could cause this shift in force sharing during swing. The LG, in turkeys, unlike the MG crosses two joints and produces all of the required force for extension. Therefore, the LG could transfer power from the knee to the intertarsal joint during extension in swing. Future measurements of the power required and produced, along with knowledge of the fiber types and mechanical properties of the LG and MG will allow a better understanding of the shared production of force in these muscle synergists.

The LG produced force on the descending limb during swing, but not at lengths long enough for passive force to contribute to swing work. One possible reason the LG did not operate at longer lengths is in case of a potential fall. The LG not only produces force during swing but also at the beginning of stance (Gabaldon et al., 2004), so if the bird tripped the LG would probably be the first muscle to produce force to brake the fall. Provided the turkeys were running normally and the *in situ* length tension curve is representative of the fibers producing force, the prevention of muscle damage by operating at shorter lengths (Edman et al., 1978; Morgan, 1990) may be an additional design constraint acting on muscles used during locomotion. Future studies of the muscle lengths and forces used during tripping in birds along with *in situ* muscle mechanics experiments can further explore this possibility.

In my last study, I measured the force-velocity properties of the LG in turkeys for comparison with other vertebrates and with future comparisons with measurements of the *in vivo* force-velocity values during running. I found the LG of turkeys has a maximum shortening velocity and peak instantaneous power output which is not different from the range of values published for other vertebrates (Josephson, 1993). Future studies of the force-velocity values during running can be compared with this curve to determine if passive elements increase the shortening velocity during swing.

Altogether, the research described in this dissertation furthers our understanding of the link between the mechanics and energetics of swing during legged locomotion, and lays the ground work for many future studies. With modeling, *in vivo* force measurements, inverse dynamics, and *in situ* muscle physiology studies, I have shown the work required to swing the limb is reduced by a passive mechanism and some of the remaining work is provided by the LG during joint extension.

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