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Muscle Activation and Strain in the Guinea Pig Hindlimb

by

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This paper was prepared
under the direction of
Professor Gary Gillis
for eight credits.

To my parents and brother, Walt:
Your love, support, and encouragement have kept me going all these years and inspire me to reach for my dreams. I could not have done it without you!

To Leia, Sarah, Ariel, and Stephanie:
For being my best friends.

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ABSTRACT

Recent work on muscle function during locomotion suggests that some limb muscles function similarly in divergent mammals, while others function quite differently. Specifically, work on the biceps femoris suggests that it plays the same role in all animals studied to date, actively shortening to produce energy for forward movement. In contrast, work on the vastus lateralis suggests that in larger animals, it functions largely in energy production, whereas in smaller animals it serves more to dissipate energy. The problem with such data is that these species are not closely related, which is why electromyography and sonomicrometry were used to study guinea pig hindlimb extensors in this study. Guinea pig data were compared to similar data from rats to determine if trends within rodents were similar to those across the larger quadrupeds. Results show that the guinea pig biceps shortened 7-18% L_0 during stance, whereas, the vastus underwent a stretch-shortening cycle, first lengthening 3-6% L_0 before shortening 4-8% L_0. Both muscles became active near the beginning of stance and were deactivated late in stance. Comparisons with rats reveal that the biceps functions similarly in both animals, however, the vastus undergoes different strain patterns in guinea pigs, which mirror differences in knee joint excursions.

INTRODUCTION

Muscles are remarkably versatile and are used to generate diverse types of locomotor movements, including running, hopping, flying, and swimming. The actions muscles undergo during these varied locomotory activities involve shortening, lengthening, and/or remaining isometric i.e., not changing length (Gillis and Biewener, 2003). Moreover, certain muscles even alter their length-change (strain) behavior during different types of locomotion. For example, rats' knee extensors undergo active shortening during swimming, but are stretched while active during running (Gillis and Blob, 2001).

It has really only been over the last 15 years or so that biologists have begun to quantify and explore muscle function *in vivo* during locomotion. During that span, two key generalizations have been made. First, muscles involved in the propulsive movements of swimming fish (Altringham and Johnston, 1990; Altringham et al., 1993; Johnson et al., 1994; Rome et al., 1993; Syme and Shadwick, 2000), flying birds (Biewener et al., 1998a), and jumping and swimming frogs (Gillis and Biewener, 2000; Lutz and Rome, 1996; Olson and Marsh, 1998) always exhibit active shortening, such that the muscle is activated and generating force during shortening to produce the power required for movement. Second, distal hindlimb muscles (i.e., ankle extensors) in terrestrial birds (Roberts et al., 1997) and mammals (Biewener et al., 1998b; Fukunaga et

al., 2001) appear to produce force isometrically, such that their fibers remain at a nearly constant length when active and generating force rather than shortening. While the activation and strain patterns of such distal muscles of mammalian limbs have been characterized relatively well during terrestrial locomotion, the role of more proximal thigh muscles is not as clear.

To date, only two thigh muscles, the biceps femoris (a hip extensor) and vastus lateralis (a knee extensor) have been studied *in vivo* during locomotion using electromyography (EMG) and sonomicrometry in more than one species. The biceps has been studied in both rats (Gillis and Biewener, 2001; 2002) and goats (Gillis et al., 2005), whereas vastus function has been explored in a much wider range of species: rats (Gillis and Biewener, 2001; 2002), goats (Gillis et al., 2005), horses (Hoyt et al., 2005; Wickler et al., 2005) and dogs (Carrier et al., 1998). These two muscles have been of interest because of their presumably important role in locomotion. Each is typically the largest muscle acting at the hip and knee joints, respectively, and thus, is likely to serve a critical role in support and propulsion during a stride (Gillis and Biewener, 2001). In addition, both muscles are superficially located, providing easy access during surgeries, and have fiber orientations that allow for straightforward implantations of electrodes and piezoelectric crystals. Specifically, the anterior biceps has parallel fibers, and originates from caudal sacral vertebrae and inserts onto the distal portion of the femur and proximal portions of the tibia, and acts largely in hip extension. The vastus has unipennate fibers and originates from the anteriolateral portion of the

proximal femur and inserts onto the patellar tendon; it serves as a knee extensor (Chiasson, 1980).

Actions of the biceps during locomotion appear very similar in the two species studied to date. In both rats and goats, while the hip extends throughout stance (when the foot is on the ground), the anterior biceps femoris actively shortens. Moreover, shortening amounts are comparable between species at around 20-30% of resting length (L_0). Finally, the timing of activation of the biceps is also very similar in both species and also in cats and dogs (Goslow Jr. et al., 1981; Rasmussen et al., 1978). The biceps begins activation slightly before the beginning of stance and ends activation in the last half of stance. Such data suggest that despite drastic differences in size and relatedness, the biceps functions remarkably similarly in a wide range of mammalian species.

In contrast, vastus function seems to be less consistent across the range of species that have been studied. For example, unlike with the biceps, rats and goats differ with respect to their vastus strain patterns. In both species, the knee joint flexes and then extends during the stance phase of locomotion when the vastus is active. Because the vastus is a knee extensor, it would be expected to stretch and then shorten in response to this knee flexion and extension. Although the vastus in both species does undergo such a stretch-shorten cycle, in rats, the vastus stretches much more than it shortens during stance (Gillis and Biewener, 2001; 2002). In goats, the reverse is true, and the vastus instead stretches much less than it shortens (Gillis et al., 2005). In both species, the timing of vastus

EMG activity is similar; it begins at or just before stance and is present over much of the stance phase. Given that actively shortening muscles generate energy but stretching an active muscle dissipates energy (Gillis and Biewener, 2003), data suggest that in rats the vastus functions more in energy dissipation (i.e., it stretches more than it shortens) whereas in goats the vastus generates more energy than it dissipates (i.e., shortens more than it stretches).

Strain recordings for the vastus in dogs (Carrier et al., 1998) and horses (Hoyt et al., 2005) provide more examples of how this muscle's function differs among different species. A dog's vastus undergoes patterns like those observed in goats, with slight stretching early in stance followed by more substantial shortening later in stance. In horses, the vastus undergoes almost all shortening during stance with little to no initial stretching. If one considers the data from all these species together, the general pattern seems to be that smaller animals exhibit more net vastus stretching and larger animals exhibit more net vastus shortening. In other words, the same muscle, i.e., the vastus, seems to function quite differently in these different animals.

It seems reasonable to consider body size as a potential factor underlying this pattern. It is well known that limb posture changes systematically with size such that smaller animals have more flexed limbs and larger animals have more upright limbs (Biewener, 1989; 1990). Perhaps the more flexed limb posture of rats leads to more knee flexion and vastus stretching during stance. In contrast, perhaps the more upright limbs of animals such as dogs and horses are not as

compliant and their knee joints flex considerably less (leading to less vastus stretching). The problem is that rats, goats, dogs and horses are so diverse (phylogenetically, ecologically) that there are many confounding factors that could underlie the functional differences in the vastus reported in the literature (Gillis et al., 2005). At this point it is hard to determine if size and limb posture are important or if there are more complicated differences in addition to size that might underlie muscle functional differences. To get at this, it is paramount to begin to study muscle strain patterns in more closely related species that differ in size.

In this study, the strain and activation patterns in the vastus lateralis and biceps femoris of guinea pigs will be studied and compared to similar data previously collected in rats. Guinea pigs are rodents, like rats, but are 3-4 times larger in body mass and exhibit slightly more upright limb posture as well (Rocha-Barbosa et al., 2005). The question to be addressed is whether muscles function similarly in more closely related animals that differ less dramatically in body size and limb posture?

Since the actions of the vastus and the biceps have been well characterized in rats at various speeds and grades, studying muscle function in a larger rodent, such as a guinea pig, seems an excellent way to begin to focus more explicitly on the effects of body size. In addition, recent studies using various imaging techniques have provided detailed and accurate descriptions of the hindlimb kinematics (movement patterns) of both rats (Fischer et al., 2002) and guinea pigs

(Rocha-Barbosa et al., 2005). Knowing the hindlimb joint angular excursions in these species during locomotion provides insight into what the underlying muscles might be doing.

In both species, the hip joint flexes during swing and extends during stance, and the hip excursions are comparable in both species between 50-60° (Fischer et al., 2002; Rocha-Barbosa et al., 2005). In rats, biceps strain patterns reflect those hip excursions; the biceps lengthens during the swing phase when the hip flexes and actively shortens during the stance phase when the hip extends, as would be expected of a hip extensor. The beginning of shortening and lengthening coincide quite closely with the beginning of stance and swing, respectively. Thus, given that the guinea pig hip also extends during stance and flexes during swing, it is predicted that biceps actions in guinea pigs should be similar to those seen in rats, i.e., shortening during stance when the muscle is active, followed by re-lengthening during swing when the muscle is inactive.

Similar to the biceps, the strain of the vastus closely follows the pattern of knee angular excursions in rats. The knee undergoes a cycle of flexion and extension during stance. Over the first half of the stance phase the knee flexes as the limb "yields" to the weight of the animal's body, and the knee then re-extends in the second half of stance as the animal pushes against the ground to propel itself forward. Imaging studies show that there is more flexion than extension of the rat knee during stance (Fischer et al., 2002), and this corresponds to substantial vastus stretching early in stance and less vastus shortening later in

stance (Gillis and Biewener, 2001). In comparison, while imaging studies show that the knees of guinea pigs also experience flexion and extension during stance, the trend is reversed relative to rats in that there is less knee flexion than extension (Rocha-Barbosa et al., 2005). Specifically, in rats during stance, the knee first flexes approximately 25° before re-extending about 10° (Fischer et al., 2002), in contrast, in guinea pigs, the knee initially flexes 14° before re-extending 20° (Rocha-Barbosa et al., 2005). Owing to this reversal in the trend of knee excursions during stance, it is predicted that the vastus of the guinea pig will also exhibit a reversal in its strain pattern, and will be stretched less than it subsequently shortens during stance.

Although the imaging studies only focused on limb kinematics during level locomotion and at just a few speeds, animals run on more diverse grades, and over a broad range of speeds in nature. Grade and speed have been shown to influence the function of limb muscles during locomotion. For example, in rats (Gillis and Biewener, 2002; Leon et al., 1994), cats (Pierotti et al., 1989), and horses (Hoyt et al., 2005; Wickler et al., 2005) the intensity of electromyographic activity of major limb extensor muscles (often used as an estimate of a muscle's recruitment level and thus number of fibers actually generating force (e.g., Biewener and Roberts, 2000), increases with speed and grade, such that the intensity is highest at fast speeds and/or on an incline. Similarly, *in vivo* recordings of muscle strain in a variety of species have shown that net amounts of active shortening in extensor muscles also increase with speed or on an incline

(Gillis and Biewener, 2001; 2002; Roberts et al., 1997; Wickler et al., 2005). Considering the EMG intensity and shortening trends with speed and grade, it seems likely that extensor muscles are producing more energy (i.e., more muscle is recruited, and it is shortening a greater distance) to move more quickly or across inclined surfaces. For that reason, it is predicted that guinea pigs will exhibit increased EMG intensities in the biceps and vastus as well as more shortening in these muscles as speed increases and on an incline.

In summary, this study was undertaken to characterize the actions of two major thigh muscles, the biceps femoris and vastus lateralis, in the guinea pig. These data will be used to compare thigh muscle function in the guinea pig to that in the rat, another rodent that is a quarter of its size. Given that rat muscle actions closely mimic what is happening at the underlying limb joints, it is predicted that the same will be true of guinea pigs. Since hip kinematics are similar between rats and guinea pigs, but knee kinematics are not, it is expected that biceps function will be similar between these species whereas vastus function will differ in ways mirroring the knee kinematic differences. The effects of speed and grade on thigh muscle function will also be explored, and it is predicted that increases in speed or transitions from level to inclined grades will lead to functional shifts that lead to more energy output from muscles (e.g., higher EMG intensities and more shortening strain during stance). Finally, data from larger but unrelated species including goats, dogs, and horses will be discussed to explore potential broader trends related to animal size and limb muscle function.

MATERIALS AND METHODS

Six female guinea pigs (735-1281 g, mean 923 g) were obtained from Harlan Laboratories and were housed singly in cages with a 12-hour light and 12-hour dark cycle. The room was kept at 22°C and the guinea pigs were fed Global 2041 HF Guinea Pig Diet (Harlan Teklad). All experiments were approved by Mount Holyoke College's IACUC Committee (BR-32-0902).

Guinea pigs were trained to exercise at various speeds on a rodent treadmill (Treadmill Simplex II, Columbus Instruments) several weeks prior to any experiments by running them for ten minute training sessions two to three times per week. Training sessions included speeds from 20-50 m/min and were important for familiarizing animals with the treadmill and increasing their stamina. Experiments were only conducted with animals that were able to maintain steady walking and trotting on the treadmill.

To estimate limb movement patterns (kinematics) during locomotion, the right hindlimb of two trained guinea pigs was shaved and the hip, knee, and ankle joints were marked using a black Sharpie pen. The animals were then filmed from a lateral perspective using a high-speed digital video system (125 frames/s, Photron Fastcam) during treadmill locomotion at various speeds. High-speed videos were used to determine "foot-up" and "foot-down" times for each stride in order to define the stance and swing phases (intervals when the right foot was on

or off the ground, respectively) and to determine how phase durations changed with speed. In addition, a customized digitizing program (Didge, Alistair Cullum, Creighton University) was used to digitize the two-dimensional coordinates of the joint markers during several strides at each speed. Coordinate (x, y) data were exported into Microsoft Excel and used to calculate the hindlimb joint angles at different points throughout the stride. Plots of joint angle versus percent of stance were used to determine when joints flexed and extended during this interval, and how the degree of flexion and extension changed with speed.

In preparation for each muscle function experiment, two offset hook (Loeb and Gans, 1986), fine-wire, silver (California Fine Wire, Inc.) electrodes were made, tested using a multimeter, and soldered into a female D-Sub connector (15 pins). The electrodes were used to measure electrical activity patterns in the muscles of interest, or electromyograms (EMGs), during experiments. Two pairs of sonomicrometry crystals (1.0 mm crystals, Sonometrics Inc.) were soldered into the same female connector as the electrodes. Sonomicrometry crystals were used to measure muscle length changes. Briefly, one sonomicrometry crystal emits sound waves and the second crystal receives the sound waves. Based on the velocity of sound through muscle, the distance between the crystals can be calculated using the time it takes for the second sonomicrometry crystal to receive the sound waves (Sonometrics Corporation, http://www.sonometrics.com/index-a.html). Slow-hardening epoxy was mixed and applied to the connector where electrodes and crystal wires (approximately 20

cm in length) were soldered in order to prevent their removal during experiments. The crystals and electrodes were soaked in isopropyl alcohol for several hours before implantation.

To implant the electrodes and sonomicrometry crystals, guinea pigs were first anesthetized with 30-40 mg/kg of sodium pentobarbital injected into the peritoneum and given 0.05 mg/kg of buprenorphine as an analgesic via subcutaneous injection. An additional 0.1-0.2 ml of sodium pentobarbital was given, if needed, during the surgical procedure to maintain an appropriate level of anesthesia. Following administration of anesthetic, the entire right hindlimb and dorsal surface of the guinea pig were shaved. The shaved areas were scrubbed with an iodine solution (Prepodyne) followed by isopropyl alcohol and this scrub was repeated three times.

The animal was then moved onto the surgical table and placed on a heating pad covered with a sterile surgical drape. Other sterile drapes were used to cover the animal and to create two surgical fields, one approximately midway along the animal's back, the other over the proximal region of the right hindlimb. A single 1 cm incision was made via scalpel along the midline of the animal's back and a 2-3 cm incision was made on the lateral surface of the thigh in line with the femur. Long blunt-tipped scissors were used to clear a subcutaneous tunnel between the two incisions, and the electrode and crystal wires were passed from the back incision through the tunnel to the hindlimb. The female connector

was then sutured using 4-0 silk to the back of the guinea pig just anterior to the back incision, which, in turn, was also sutured shut using 4-0 silk.

Electrodes and sonomicrometry crystals were implanted into two major thigh muscles, the vastus lateralis, a knee extensor, and the anterior biceps femoris, a hip extensor such that one electrode and one crystal pair were implanted into each muscle. The bared electrode tips were placed into the tip of a 21-gauge hypodermic needle and the electrode was folded back forming a hook. The needle was inserted into the muscle and then, slowly removed while the electrode wires were held in place using fine watchmaker's forceps. The electrode wires were gently tugged to ensure secure implantation into the muscle, then they were sutured to the muscle surface to help prevent dislodging of the electrode during movement.

Each sonomicrometry crystal was inserted into a small hole in the muscle formed by watchmaker's forceps and was maneuvered until the best signal was obtained on the oscilloscope. Each crystal pair was oriented parallel to the muscle fibers. Using 6-0 silk, the small holes into which crystals were placed were sutured closed and the crystals' wires were sutured to the muscle's surface to prevent inadvertent removal. After both sets of crystals and electrodes were implanted, 4-0 silk suture was used to close the hindlimb incision. Isopropyl alcohol was used to clean the suture sites on the hindlimb and around the connector. The animal was then returned to its cage and given 36-48 hours to recover.

The day after surgery, the guinea pig was placed on the treadmill to walk for 1-2 minutes at a low speed (15-20 m/min) in order to acclimate the animal to exercising with the back connector. Data were collected from several guinea pigs that did not have the opportunity to become accustomed to the connector and they often spun on the treadmill. Such spinning put tension on the cables connecting the amplifiers to the "backpack" connector and either caused the wires to break or the connector's sutures to break. When this occurred, the guinea pig was immediately removed from the treadmill and the cables were untwisted. If data could still be collected, the animal was placed back on the treadmill for additional data collection. When the sutures to the connector failed, no further data were collected as any tension could dislodge the electrodes and crystals from the muscle. The process of acclimatizing the animals to the treadmill with the sutured connector seemed to reduce the likelihood of the spinning behavior.

Two days after the surgery, the guinea pigs were placed on the treadmill for data collection. The female connector sutured onto the guinea pig's back was plugged into a male connector that transmitted muscle activity and length change signals to the EMG amplifiers (P511 Grass Amplifier), and sonomicrometer (Triton Technology); output from the sonomicrometer was also connected to an oscilloscope (Tektronix) for easy visualization of signal quality. The amplifiers' filters were set to minimize electrical noise outside the range of 100-3000 Hz, and a notch filter was implemented to minimize 60 Hz electrical noise associated with normal electrical activity in the building. Output from the sonomicrometer and

amplifiers was digitized through a Digidata 1322A Axon Instruments A/D card, and EMG and length-change (strain) signals were collected, saved, and analyzed on a personal computer.

During locomotor trials, the guinea pigs ran at various speeds so EMG and sonomicrometry data were recorded during walking, trotting, and sometimes galloping on a level, inclined, and declined surface (+/- 13°). There were 2-4 trials at each speed with speeds ranging from 20-80 m/min on the level, 20-60 m/min on the incline, and 20-60 m/min on the decline (see Table 1 for details). Between trials, the treadmill was kept at 15-20 m/min to keep the guinea pig slowly walking and deter it from spinning and twisting cables. After the level trials were completed and graded locomotion began, the treadmill was stopped between trials so the guinea pig could rest.

To correlate the stance and swing phases with the recorded muscle activation and strain, the guinea pigs were filmed using the same high-speed video system as was used in the kinematics recordings. A voltage pulse was used to stop video recordings, and that same pulse was recorded along with the EMG and sonomicrometry data so that one could back calculate and coordinate any video frame with an instant in time in the data set. After muscle activity and length change data were recorded, the guinea pigs were euthanized with an excess of sodium pentobarbital (Fatal Plus Solution). The hindlimb was dissected to verify that electrodes and crystals were in the correct muscle and in the proper orientation. Only one guinea pig had both EMG and strain signals from the

vastus and biceps (Table 1); all other guinea pigs were missing at least one signal. In addition, the range of speeds collected for each animal was variable and particularly narrow for decline trials (Table 1).

Table 1. EMG and strain signals obtained at various speeds and grades. The range of speeds for a particular signal and for a certain guinea pig were measured in m/min.

	Biceps			**Biceps**			**Vastus**			**Vastus**		
	EMG			**Strain**			**EMG**			**Strain**		
Individual	**Level**	**Decline**	**Incline**	**Level**	**Decline**	**Incline**	**Level**	**Decline**	**Incline**	**Level**	**Decline**	**Incline**
1	20-80	50	30-60				20-80	30-50	30-60			
2	20-60	20-50	20-40				20-40					
3	20-50	20-40	20-50	20-50	20-40	20-50	20-50	20-40	20-50			
4	20-40			20-40	20-30	20-40	20-40	20	20-40	20-40	20-30	20-40
5				20-70	20-60	20-50						
6				20-70						20-70		

To analyze data, the foot up and down frames were obtained from the high-speed video during each trial and were subsequently converted to time values. The stance, swing, and cycle times were calculated and standard deviations of the cycle times were calculated for every six strides. The six strides with the lowest standard deviation were used for muscle activation and strain analysis. These strides were viewed to ensure that no unsteady locomotion, such as acceleration or deceleration, occurred. Occasionally, there were fewer than six strides of steady locomotion available, however, the lowest number of strides ever

used was four. The duration of the stance phase was defined as the period of time when the foot from the experimental hindlimb was in contact with the ground and the swing phase was defined as the period of time when that foot was in the air. The cycle or stride time was the stance phase + swing phase and hence consisted of the period between when the foot contacted the ground in one stride to the when the foot hit the ground in the next stride. The stance, swing, and cycle durations were plotted as a function of speed for all animals. For two guinea pigs, the stance, swing, and cycle durations from before and after the limb implantation surgery were plotted to determine if the surgery had a serious effect on gross locomotor movements.

Electromyographic data were analyzed as follows. The onset and offset of the vastus and biceps EMG bursts were determined by eye from computer data records of each locomotor trial. From these values, the muscle activation and deactivation times were calculated with respect to the timing of the stance phase (i.e., when were muscles turned on and off relative to the stance phase). The intensity of the EMG bursts for each of the six strides was calculated by averaging the voltage spike amplitude over the duration of each burst signal (i.e., average signal amplitude was determined) and these were converted to relative values within each individual by dividing the average amplitude of each burst by the highest burst amplitude ever recorded from that guinea pig. Thus, relative EMG amplitudes range in value from 0-1. The EMG data from each guinea pig were compiled, all values from one speed were averaged, and the standard deviation

was calculated. A similar process was undertaken for all speeds and grades to allow for comparisons of how intensity changed with these variables.

Sonomicrometry data were analyzed as follows. The times and voltage values of the maximum and minimum points on the muscle strain signals were obtained for each stride. The voltage values were converted to distances between the crystals based on the speed of sound through muscle and these distances were converted to relative lengths by dividing by the average resting length of the muscle (determined in resting intervals during the exercise trials when the animals were standing still). These fractional lengths were used to determine the amount the muscle stretched or shortened based on the differences between maximum and minimum points on the signal traces. Since the high-speed video and the data acquisition program were synchronized, it was determined if the stretching or shortening occurred in the stance or swing phase. Similar to the EMG signals, the muscle length changes were averaged across all strides for a given trial at a particular speed. Because the biceps mainly shortened during stance, total shortening distance was calculated for all strides in this muscle. In the vastus, which stretched and shortened during stance, the stretching and shortening amounts were determined.

The number of EMG and strain signals obtained from each individual at each condition (i.e., speed/grade) was not conducive for doing meaningful statistical analyses. Ideally, at least four individuals per signal per condition would be needed, however, this number of individuals was never obtained for any

particular speed across all grades and was only obtained for a very limited range of speeds (20-40 m/min) on the level (Table 1). For this reason, means and standard deviations were used to identify general trends.

RESULTS

Kinematics

Each locomotor stride, or cycle, was composed of a stance phase, when the foot was in contact with the ground, and a swing phase, when the foot was in the air. The trends for cycle, stance, and swing duration were very similar on the level, decline, and incline surfaces (Figure 1). The cycle duration decreased as speed increased, from 400-500 ms during slow walking to approximately 200 ms at the highest running speeds obtained (Figure 1A). Stance phase duration decreased in a manner parallel to cycle duration (Figure 1B), whereas the swing phase duration remained relatively constant at 100-150 ms across all speeds (Figure 1C). Duty cycle was defined as the proportion of the stride that the stance phase occupied and was calculated as stance duration/cycle duration. Because stance duration decreased with speed while swing duration remained about the same, the duty cycle decreased as speed increased, meaning the stance phase took up less and less of the stride at higher speeds (Figure 1D).

To test whether surgical procedures had any substantial effect on basic locomotor kinematics, stance and swing duration data were collected from two individuals during level locomotion across a range of speeds before and after surgery. Figure 2 shows that the stance and swing times prior to and after surgery were very similar. In both cases, the stance phase duration decreased comparable

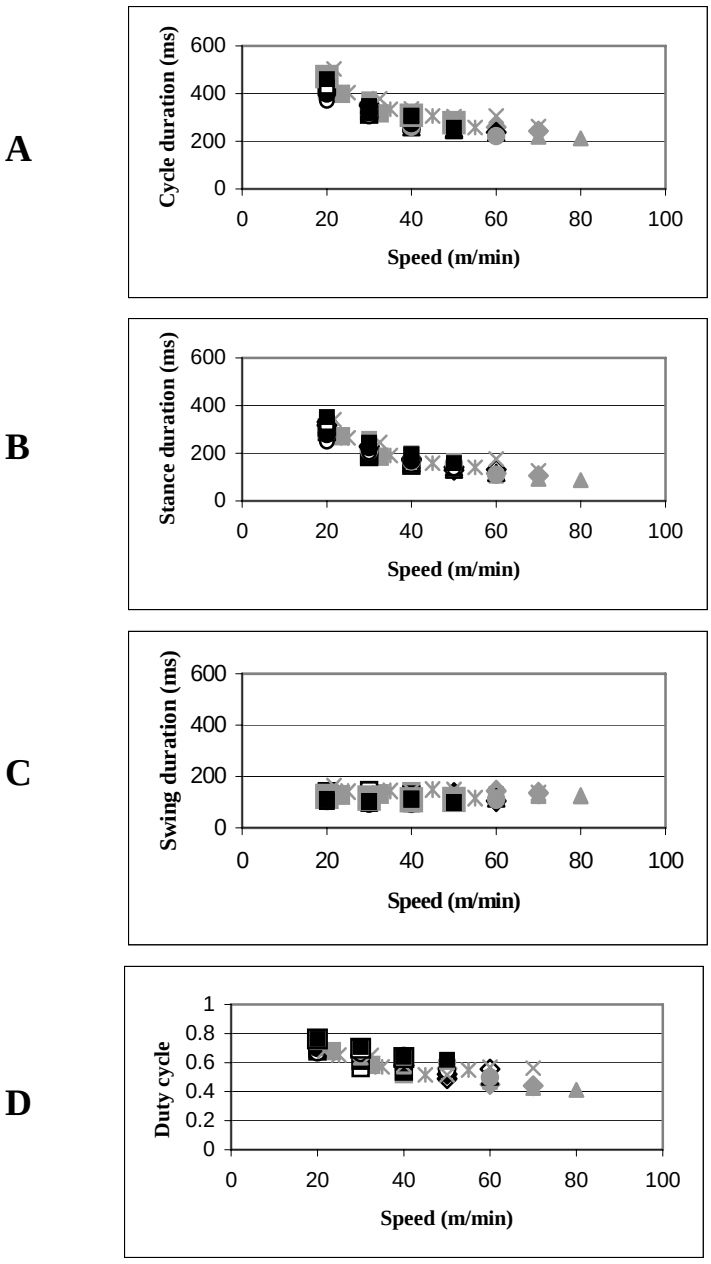


Figure 1. Cycle (A), stance (B), swing (C), and duty cycle (D) as a function of speed (n=7). Grey, white, and black colors represent level, declined, and inclined surfaces, respectively. Different symbol types represent different individuals. The duty cycle is the proportion of the stride comprising the stance phase (i.e., when the foot is on the ground). Note that the cycle duration (A), stance duration (B), and duty cycle (D) decreased with speed, however, the swing duration (C) did not change.

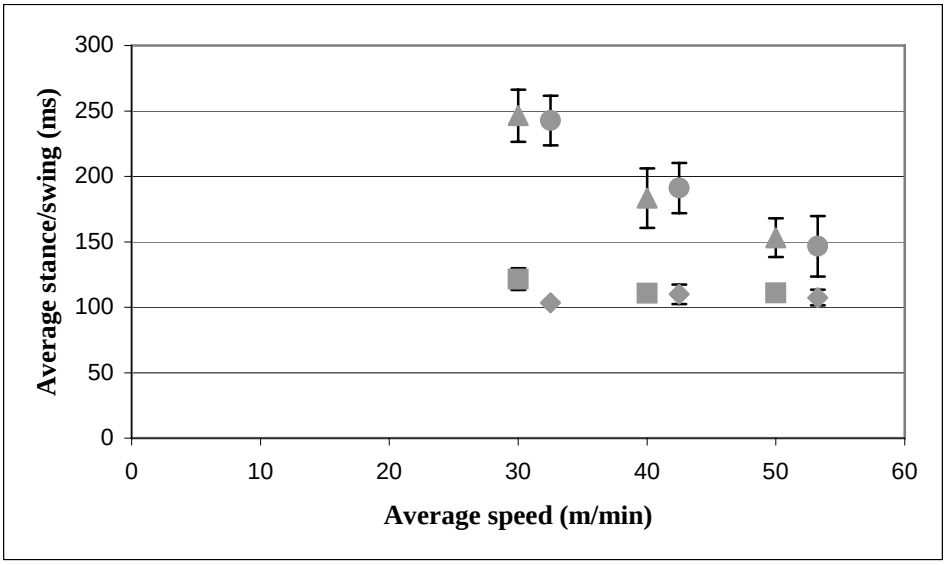
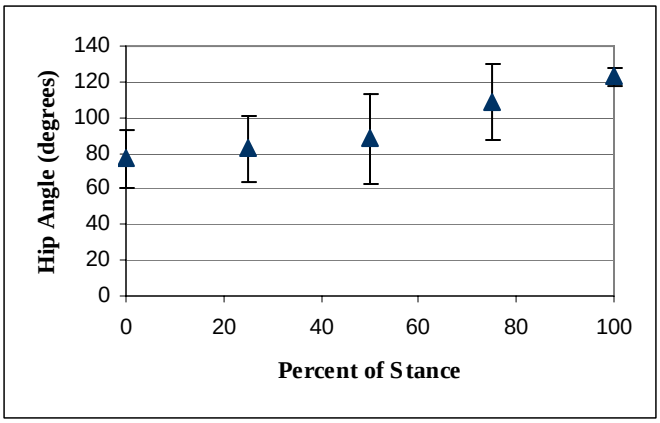


Figure 2. Average stance and swing durations prior to and after surgery at differing speeds on a level surface (n=2). Circles and triangles represent stance before surgery and after surgery, respectively. Diamonds and squares represent swing before and after surgery, respectively. Error bars represent standard deviations. Most swing error bars were too small to appear on the plot.

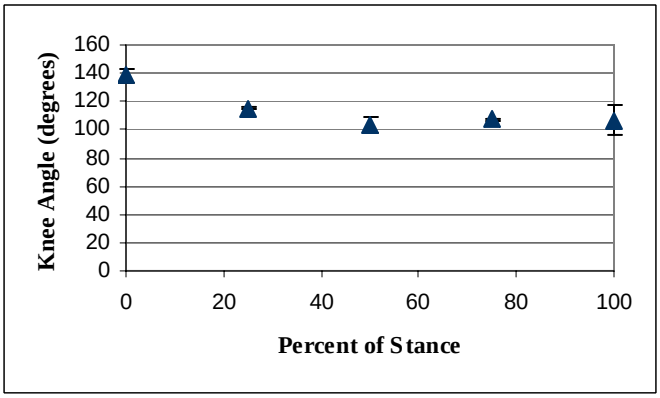
amounts and the swing phase duration remained approximately the same as speed increased.

Hindlimb movements, or kinematics, can be described by quantifying the angular excursions of the different hindlimb joints. The hip, knee and ankle underwent cyclical excursions during every stride, and because patterns at each joint remained similar across speeds, representative joint angle plots from only one speed are shown (Figure 3). During stance, the hip joint extended between 23-46° (Figure 3A). Unlike the hip joint, which only extended during stance, the knee and ankle joints initially flexed early in stance before extending later in stance. Across the range of speeds examined, the knee joint flexed approximately 27-34° over the first half of stance, and during the second half of stance, the knee generally extended 3-9° (Figure 3B). The ankle flexed from 18-24° during the first quarter of stance and extended 28-40° during the last half to three quarters of stance (Figure 3C). Joint angles were obtained while guinea pigs ran on level surfaces.

A



B



C

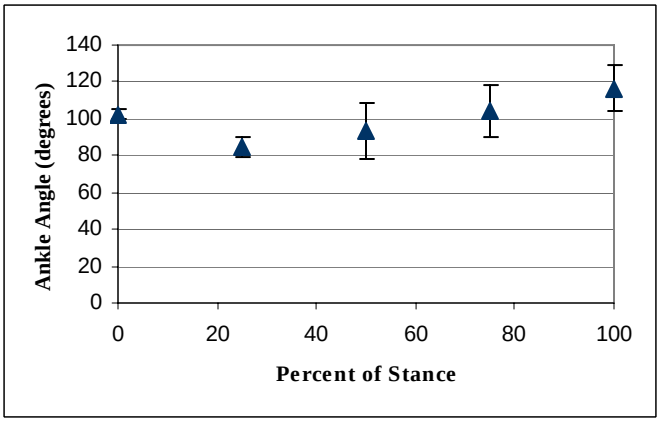


Figure 3. Average joint angles at different times during stance. Each point represents the average of two animals walking at 45.5 m/min. Error bars are standard deviations. A. Hip angle. Note that hip angle increases throughout stance, reflecting extension of the hip joint during this interval. B. Knee angle. The knee angle decreases for the first half of stance, which reflects knee flexion. Over the second half of stance, the knee angle increases as the knee extends. C. Ankle angle. The ankle angle decreases over the first quarter of stance, as the ankle flexes, and the ankle angle increases for the remainder of stance marking ankle extension.

Biceps

A representative plot of biceps EMG activity and strain (length change) is shown in figure 4. EMG activity in the biceps was consistent across all animals and was present in one discrete burst during each stride in the stance phase. On average, the biceps began activation shortly after the start of the stance phase and was deactivated about 75% of the way through stance (Figure 5). As speed increased, the onset of biceps activity approached the beginning of stance on all three grades (Figure 5). The duration of biceps activity generally decreased with speed on all surfaces (Figure 6A), while the biceps EMG duty cycle (EMG duration/stance duration) remained nearly constant at 0.6-0.85 (Figure 6B).

On the level surface, the relative EMG intensity increased with speed (Figure 7). This same trend was present on inclined and declined surfaces (Figure 8). In addition, the relative intensity at any given speed was highest on the incline and the lowest on the decline (Figure 8).

Biceps strain patterns were also consistent across individuals and consisted of a sinusoid-like waveform where the muscle mainly lengthened during swing and shortened during stance, when it was active (Figure 4). Across all individuals, speeds and grades, the biceps shortened between 7-18% of its resting length during stance. In two of four individuals, there was a tendency for the biceps to exhibit slightly increased amounts of shortening as speed increased (Figure 9). In addition, biceps strain was generally higher on an incline than on a

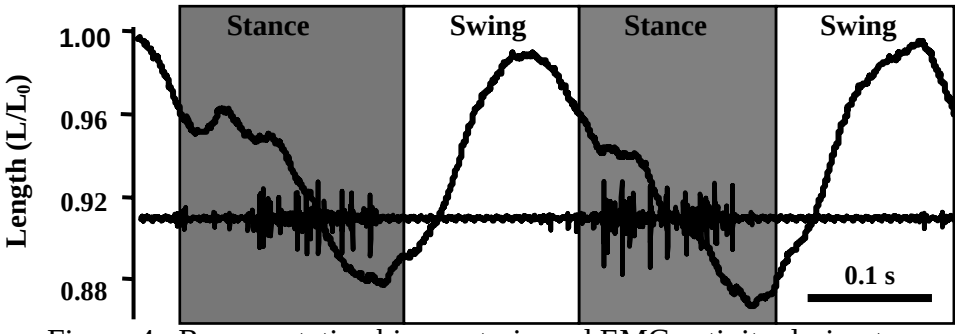


Figure 4. Representative biceps strain and EMG activity during two strides. Note how EMG signals are present during a large fraction of the stance phase and that the muscle is generally shortening during this interval.

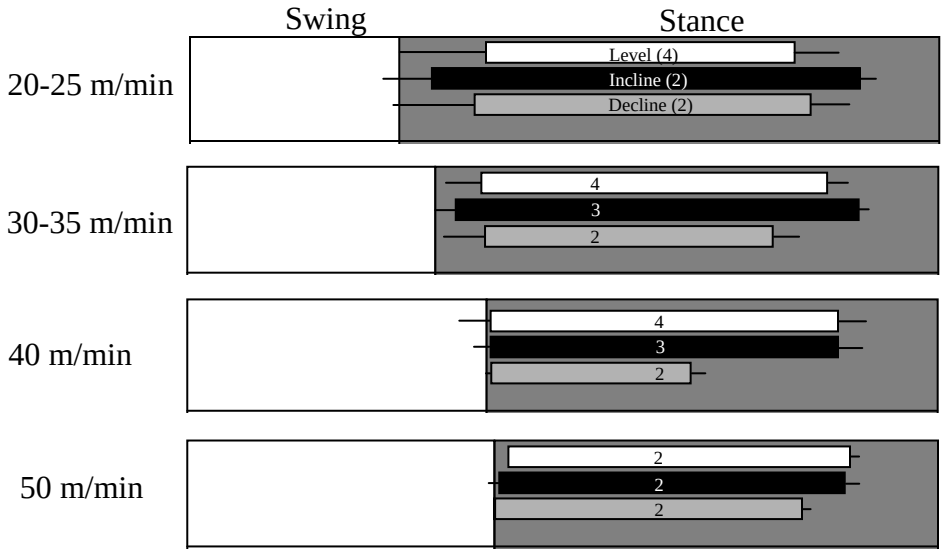


Figure 5. Average biceps EMG activation periods within one stride. White, black, and grey represent biceps activity on level, incline, and decline surfaces. The numbers within the muscle activation boxes were the number of individuals used during averaging. Error bars represent standard deviations. Note that the onset of biceps activity gets closer to the beginning of stance as speed increased.

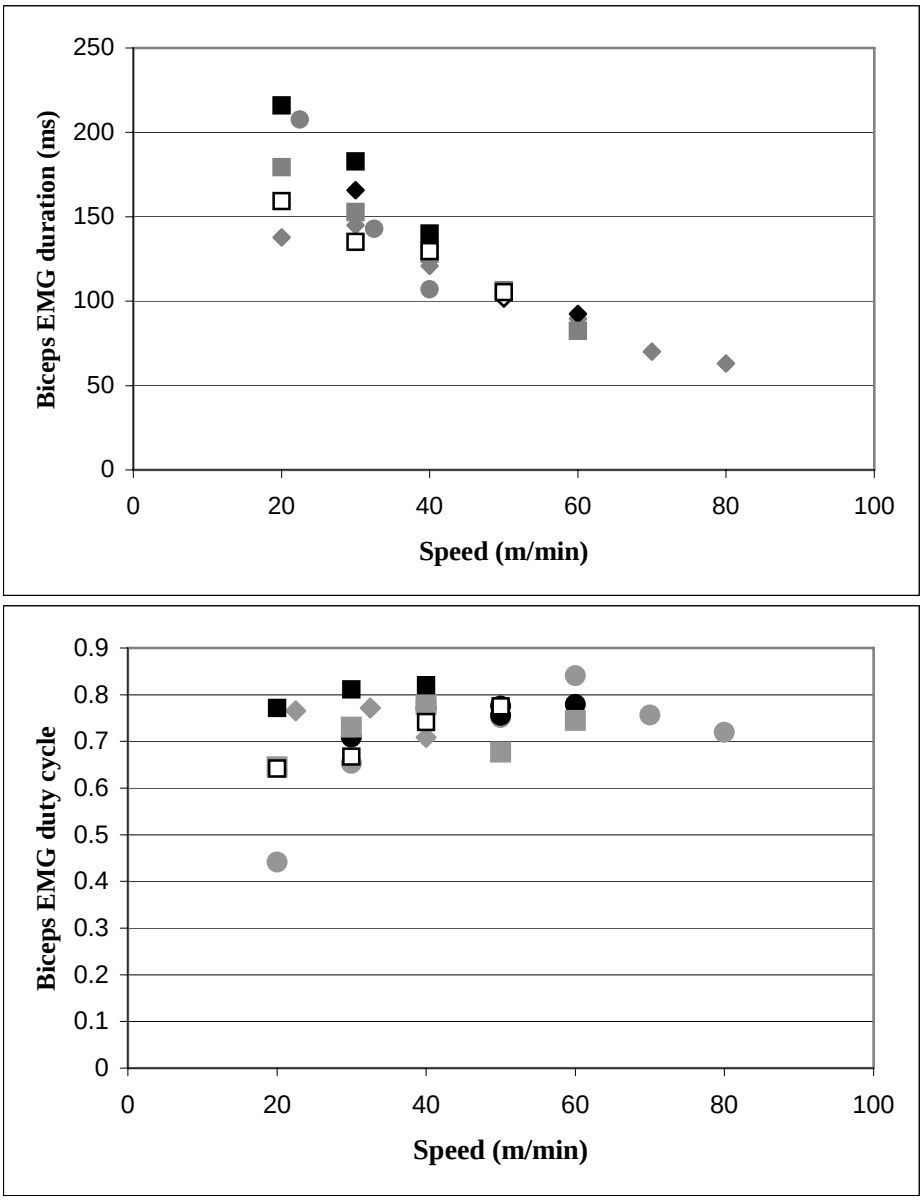


Figure 6. Biceps activity duration and biceps EMG duty cycle as speed increases (n=3). Different symbols represent different individuals. Grey, white, and black symbols represent level, decline, and incline surfaces, respectively. A. Biceps EMG duration decreased with speed. B. The biceps EMG duration was a nearly constant proportion of stance as speed increased (i.e., EMG duty cycle remained relatively constant across speeds).

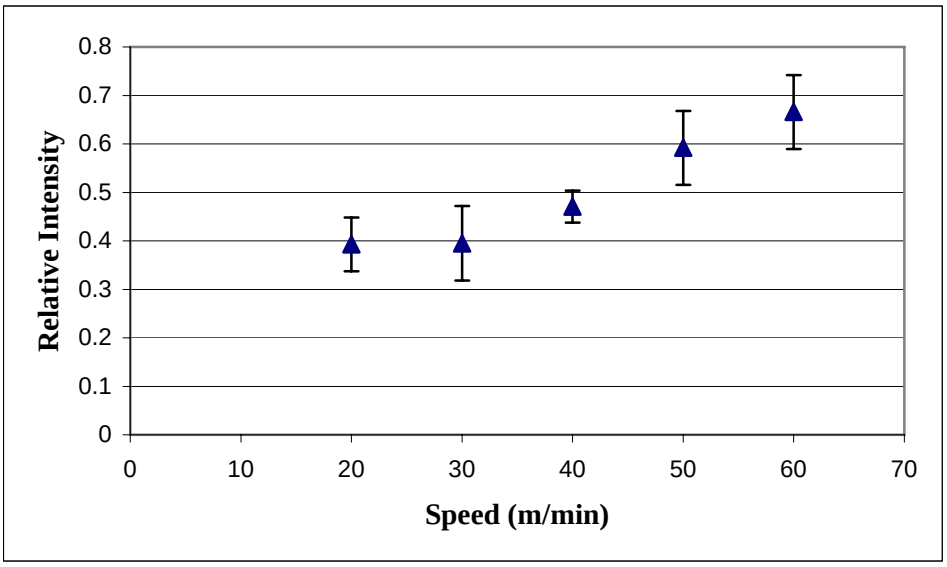


Figure 7. Relative biceps intensity on a level surface as speed varies (n=2). Error bars represent standard deviations. Note that the relative intensity increased with speed.

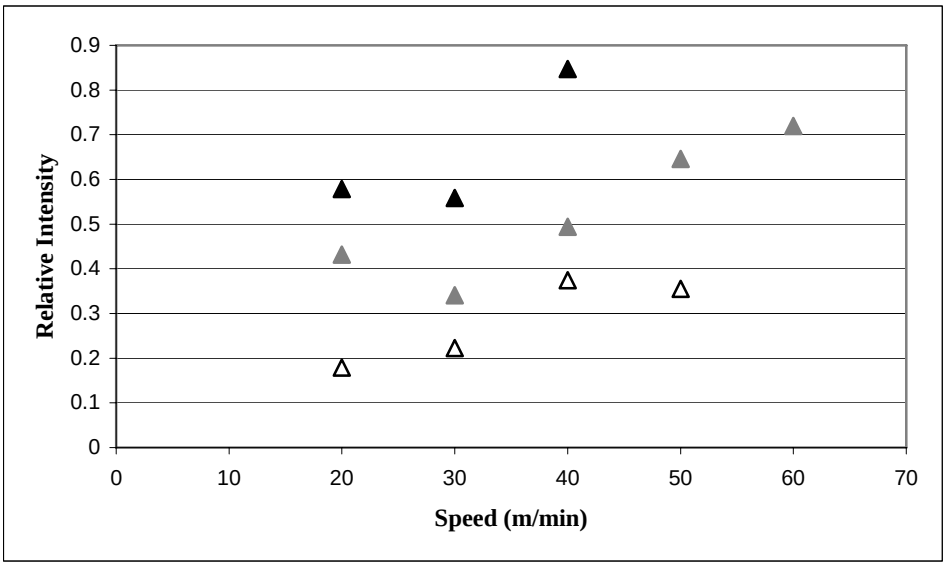


Figure 8. Relative EMG intensity of the biceps on a level (grey), declined (white), and inclined (black) surface as speed varies (n=1). Note that intensity tended to increase with speed, regardless of surface grade. In addition, for a given speed, intensity was greatest on an incline, intermediate on the level, and lowest on the decline.

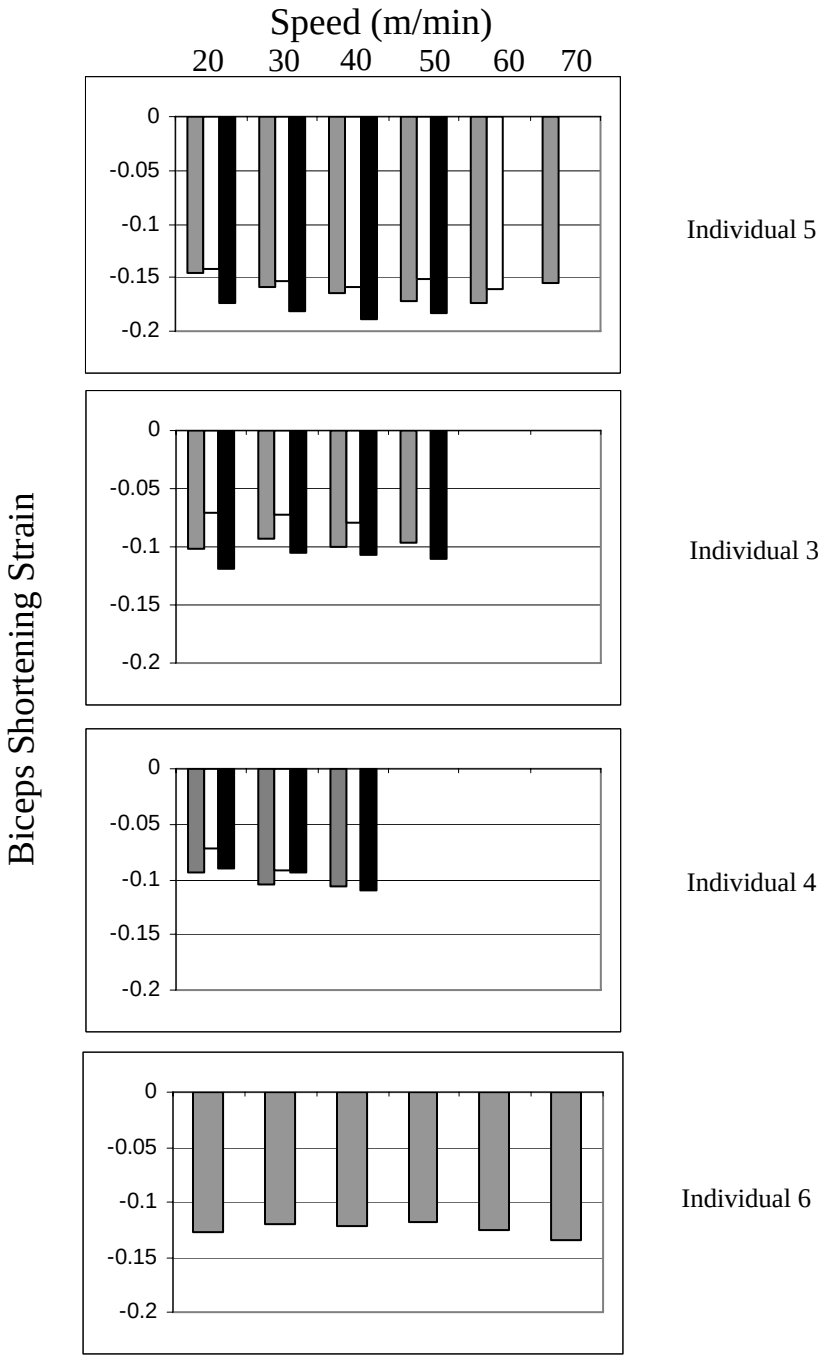


Figure 9. Biceps shortening strain during locomotion at various speeds and on different surfaces. The grey, black and white boxes represent the strain on level, inclined, and declined surfaces. Each plot represents one guinea pig.

decline (Figure 9). The biceps mainly shortened during stance so the beginning and end of shortening were essential for determining biceps strain trends as speed increased. The biceps began to shorten at or slightly before the beginning of stance and finished shortening at or slightly before the end of stance (Figure 10). Only one guinea pig had successful biceps strain recordings on all three grades (Figure 10B). In this animal, the beginning of shortening and end of shortening appeared to be at approximately the same time on inclined and declined surfaces as on the level surface (Figure 10).

If one compares the timing of biceps EMG activation and length change, the burst of EMG activity in the biceps generally corresponded to biceps shortening during stance. In some cases, the biceps continued to shorten a little after the biceps EMG activation ended (Figure 4).

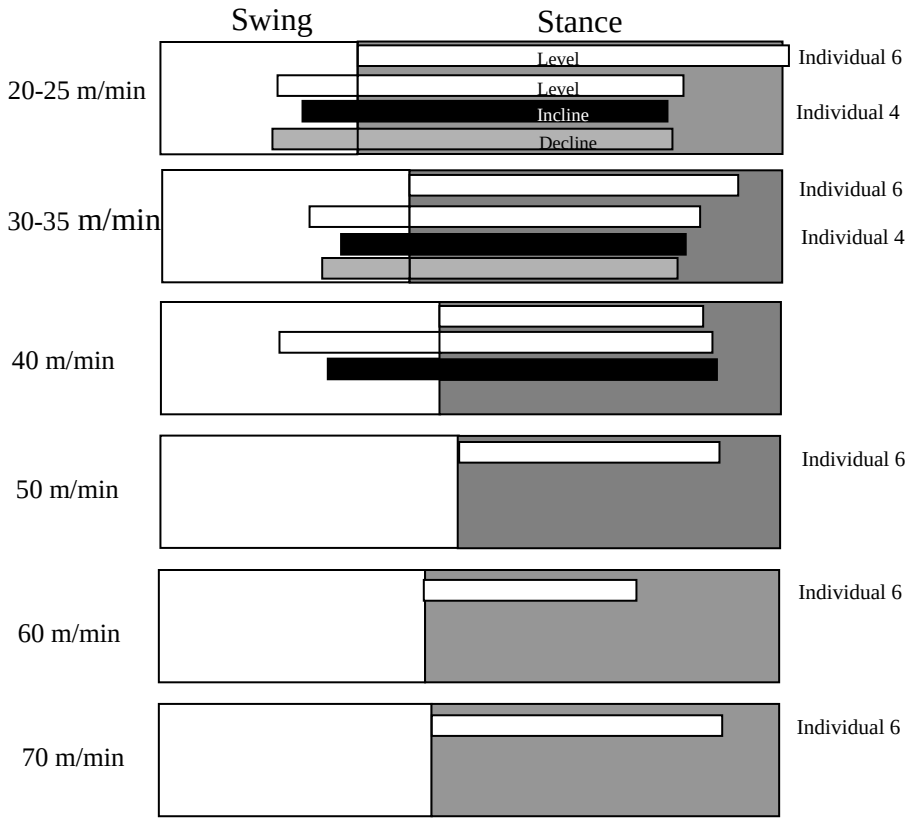


Figure 10. Timing of biceps shortening during stance as speed increases. Each box represents the beginning and end of biceps shortening with respect to the stance phase. The white, black, and grey boxes represent level, incline, and decline locomotion, respectively. Two individuals were used, however, only individual 4 had biceps times on all three grades.

Vastus

A representative plot of vastus EMG activity and strain is shown in figure 11. EMG activity in the vastus, as in the biceps, was present in one burst per stride, however, vastus activity generally began earlier than biceps activity. On all three grades, the vastus became active shortly before the beginning of stance and ended activity about 81% of the way through stance (Figure 12). The vastus activation duration decreased on all three grades as speed increased, but its duty cycle remained nearly constant between 0.8-1.1 (Figure 13). Thus, although the vastus EMG duration decreased, the stance phase duration decreased nearly proportionally, so that vastus duration remained a similar proportion of stance duration at all speeds. Similar to the biceps, the relative EMG intensity of the vastus increased with speed on level surfaces (Figure 14), and it exhibited its highest relative intensity on an incline and its lowest intensity on a decline as shown by a representative plot from one guinea pig (Figure 15).

While the EMG activity trends were similar for the biceps and vastus, the strain patterns differed considerably. Unlike the biceps, which shortened throughout stance, the vastus initially was stretched early in stance and then shortened later in stance (Figure 11). Vastus strain signals from only one guinea pig were obtained for all surfaces, and on all three grades there was more late-stance shortening than early-stance stretching (Figure 16). However, there was no obvious trend with respect to the amount of shortening or lengthening during

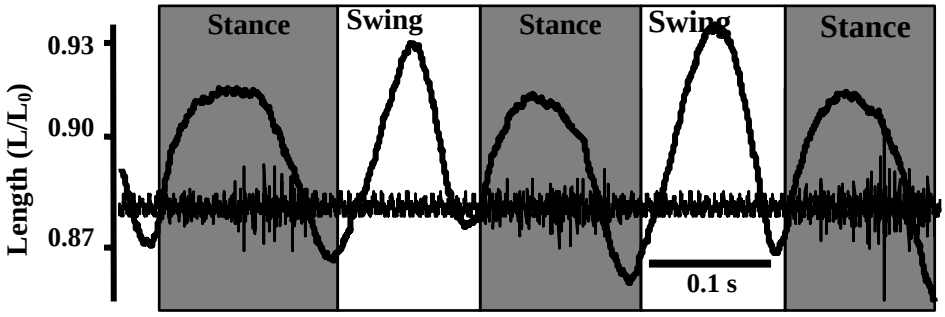


Figure 11. Representative vastus strain and EMG activity during 2.5 strides. Note that the vastus first stretches, then shortens during the stance phase when it is active.

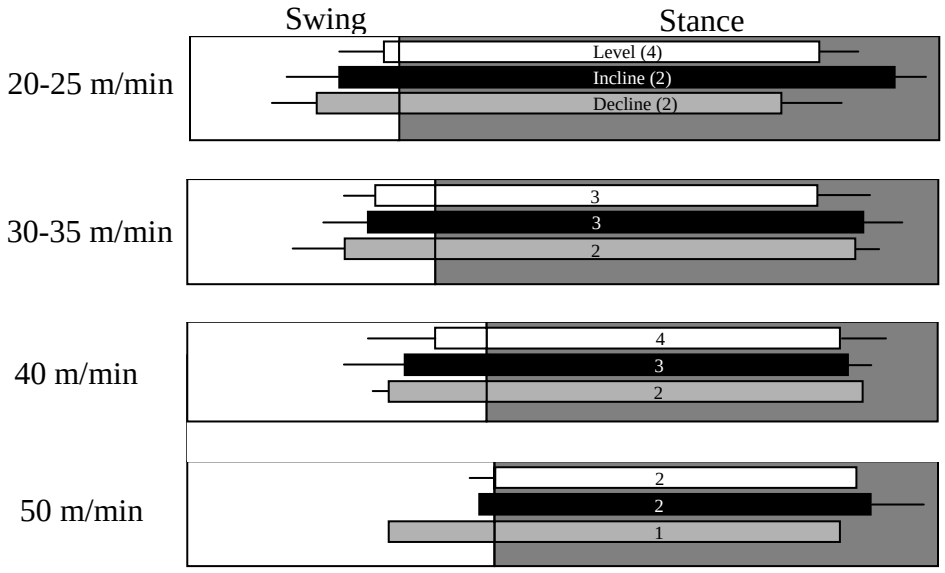
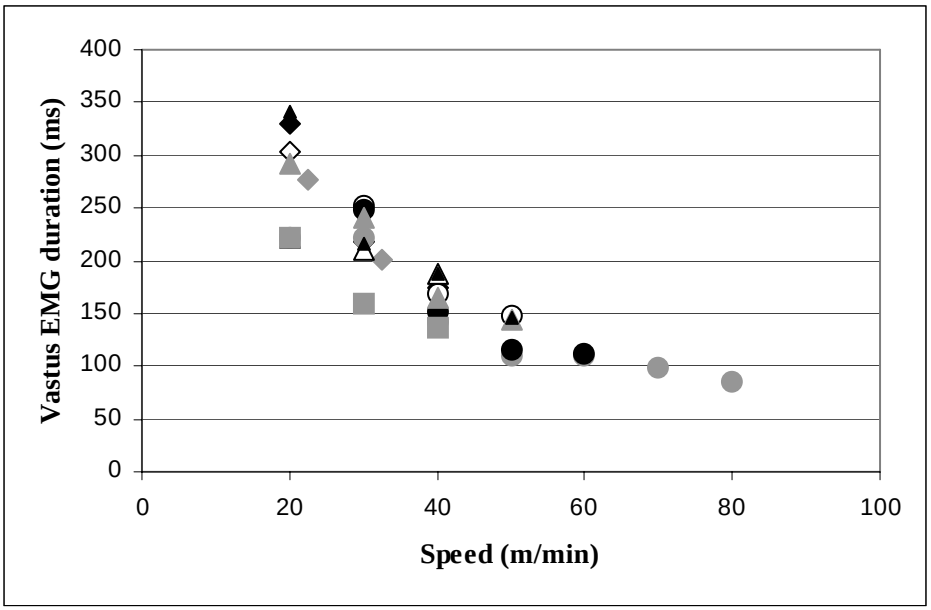


Figure 12. Average vastus EMG activation periods within one stride. The white, black, and grey EMG boxes represent level, inclined, and declined surfaces, respectively. The numbers within the muscle activation boxes were the number of individuals used during averaging. Error bars represent standard deviations. Note that on all grades and at all speeds activation periods generally started just before stance began and continued through approximately 80% of stance.

A



B

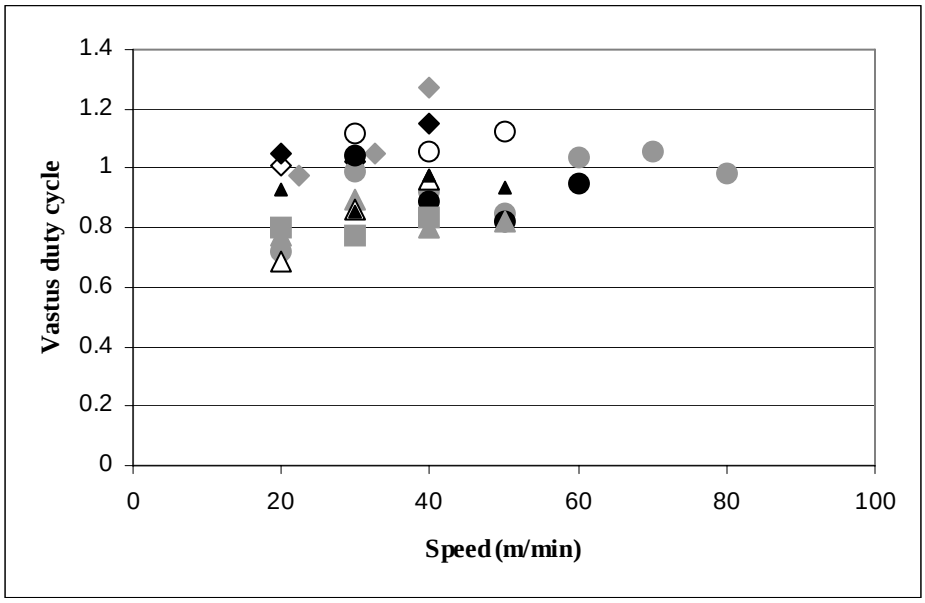


Figure 13. Vastus EMG duration and EMG duty cycle as speed increases (n=4). Different symbols represent different guinea pigs. The grey, white, and black symbols represent level, declined, and inclined surfaces, respectively. A. Vastus EMG duration decreased with speed. B. Vastus EMG duration remained a nearly constant proportion of stance.

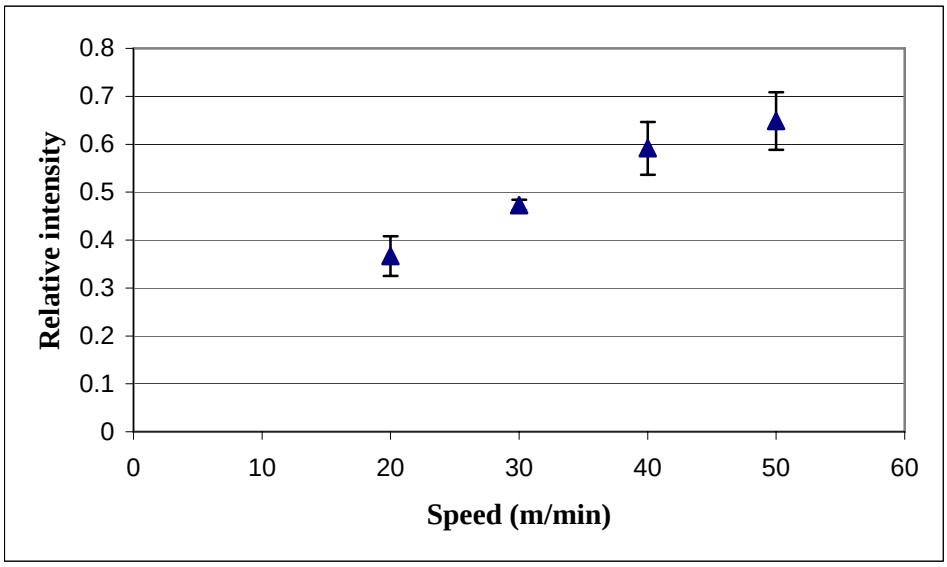


Figure 14. Relative vastus EMG intensity on a level surface with varying speeds (n=3). Error bars represent standard deviations. The relative intensity increased with speed.

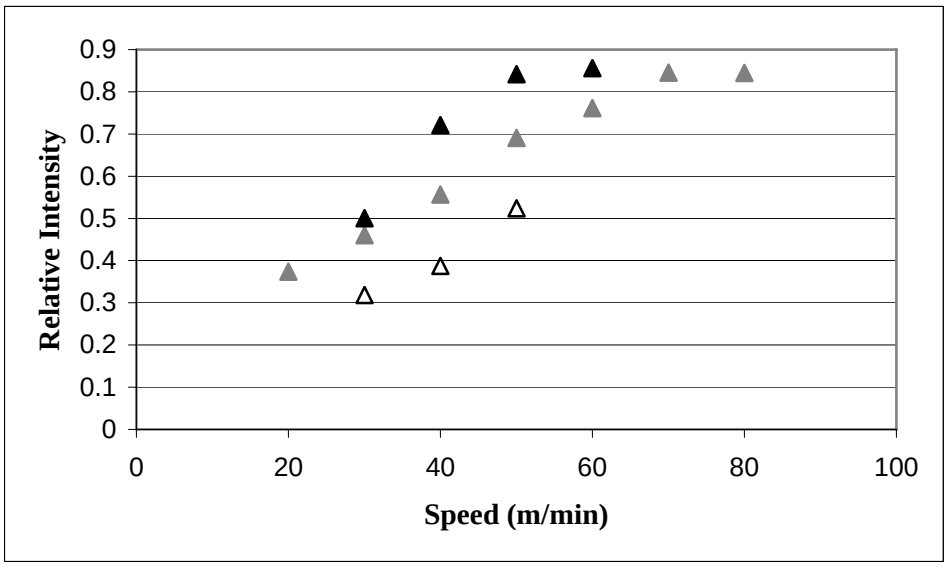


Figure 15. Relative EMG intensity of the vastus on a level (grey), declined (white), and inclined (black) surface as speed varies (n=1). Note that intensity tended to increase with speed, regardless of surface grade, and that for a given speed, intensity was greatest on an incline, intermediate on the level, and lowest on the decline.

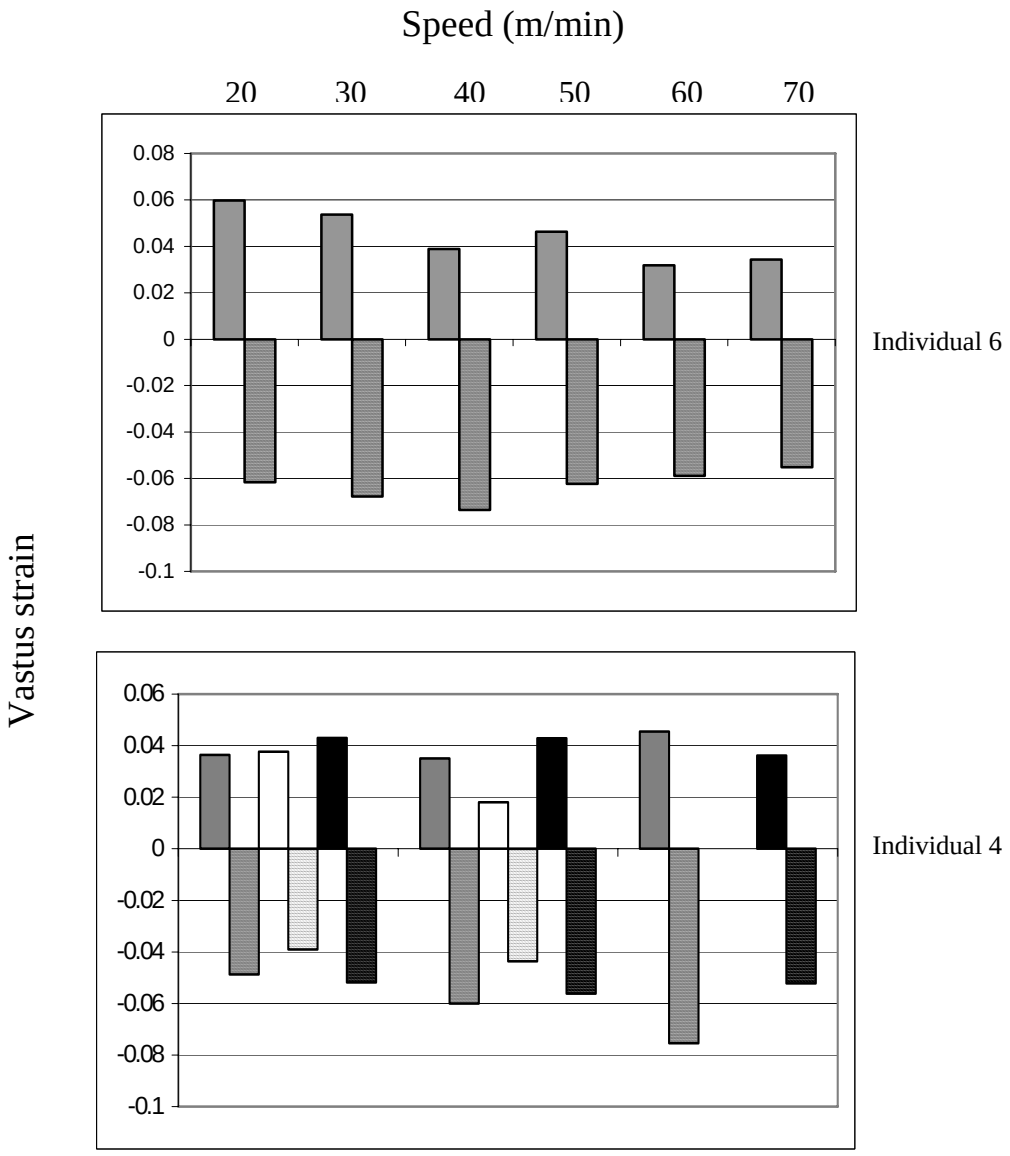


Figure 16. Vastus strain at various speeds and grades. The grey, white, and black boxes represent level, declined, and inclined surfaces, respectively. The boxes with hash marks represent vastus shortening, while boxes without hash marks represent stretching. Each plot corresponds to a different guinea pig. Only one guinea pig (bottom plot) had strain values on all three surfaces. Note that shortening strains were generally greater than stretching strains and that there were no obvious trends with speed or grade.

stance as speed increased (Figure 16). Across the two individuals for which vastus strain data were successfully collected, the muscle lengthened between 3-6% of its resting length early in stance and later shortened between 4-8% (Figure 16).

Vastus stretching occurred at the beginning of stance and this remained unchanged across speeds and grades (Figure 17). Only one individual had vastus strain values on all three grades (Figure 17B). The timing of the end of stretching changed with speed in one guinea pig, such that stretching occurred for a longer proportion of stance as speed increased (Figure 17A), but in the second guinea pig, the timing of the end of stretching did not appear to change (Figure 17B). The vastus also stopped shortening at the end of stance across all speeds and grades (Figure 17).

If one compares the timing of vastus EMG activation and length change, the burst of EMG activity in the vastus generally corresponded to the initial lengthening of the vastus during the stance as well as the majority of shortening during stance (Figure 11). Similar to the biceps, the vastus continued to shorten slightly after its EMG activity ended.

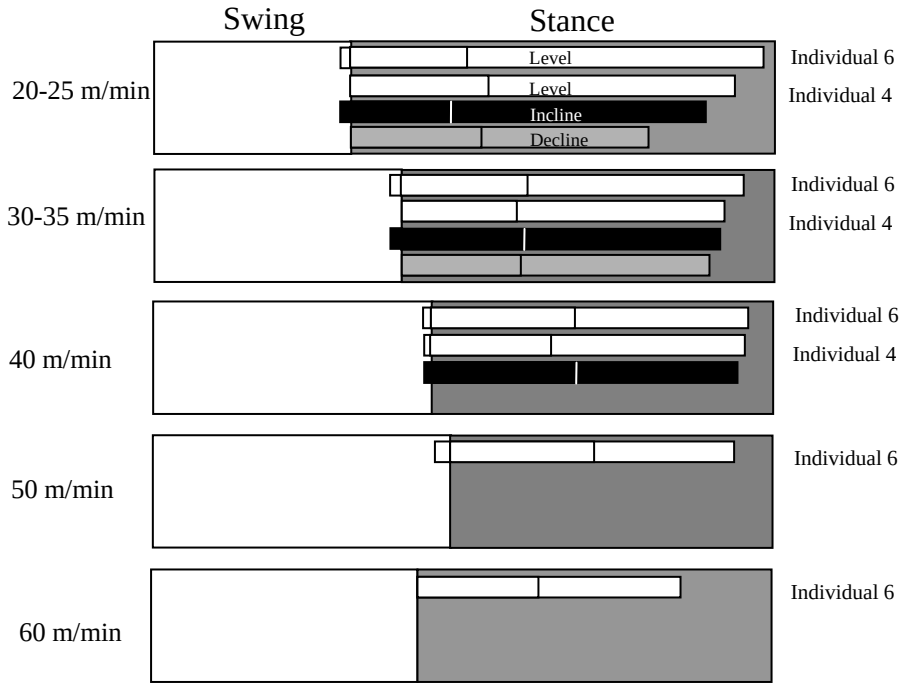


Figure 17. Timing of lengthening and shortening of vastus strain during stance. Each box represents the beginning of shortening, the end of shortening/beginning of lengthening, and the end of lengthening. The lines within the muscle timing boxes represent the end of shortening/beginning of lengthening. The white, black, and grey boxes represent level, incline, and decline locomotion, respectively. Two individuals were used, however, only individual 4 had vastus times on all three grades.

DISCUSSION

Kinematics

Trends for cycle, stance, and swing duration found in this study are similar to those found in a previous study of guinea pigs (Rocha-Barbosa et al., 2005), as well as in previous studies of various other mammalian quadrupeds (e.g., Gillis and Biewener, 2001; Gillis et al., 2005; Pierotti et al., 1989; Robert et al., 2000). In general, as speed increases, the stance phase duration decreases, while the swing duration remains about the same. As a result, the stance phase takes up less of the stride cycle as speed increases, implying that in order to increase speed, animals decrease the amount of time their feet spend in contact with the ground. This appears to be a very general result as even work with humans (Weyand et al., 2000) has shown that to run faster, sprinters decrease foot ground contact time (and increase the force applied during this interval), but swing their limbs at the same rate (i.e., constant swing duration). While the guinea pig (Rocha-Barbosa et al., 2005) and human (Weyand et al., 2000) studies were conducted on level surfaces, it is essential to our understanding of locomotion that we observe what is happening with the stride on inclined and declined surfaces as well.

In the rat (Gillis and Biewener, 2002), grade appears to impact the timing of the stride during slow walking, but not at faster speeds. Specifically, at slow speeds, the stride cycle duration is significantly higher on incline than decline

surfaces. Following this trend, both the stance and swing durations are longest on an incline and shortest on a decline at slow speeds. However, in guinea pigs, even during slow walking, there were no clear differences between the timing of stride parameters on inclined and declined surfaces.

Since studies of *in vivo* muscle function during locomotion involve surgical procedures, there is a chance that the damage sustained during surgery has a serious effect on how the experimental subjects actually move. However, most such studies have just assumed that the surgical procedures involved in assessing muscle function did not have a significant impact on the locomotor stride of the animal. To test if this was true in this study, the stance and swing phase durations from two individuals were compared before and after surgery (Figure 2). Results suggested that surgery had little effect on the durations**.** Work with other animals is required to see how widespread this result is, however in guinea pigs, at least, gross limb movement patterns appear to be undisturbed by the surgical implantation of muscle function transducers.

While studying stance and swing phase durations provides information on the fundamental timing of limb movements during the stride, studying limb joint angular excursions provides insight into the actual limb movements during locomotion. In general, quadrupeds studied to date have similar hip excursions during the stride, where the hip extends during stance and flexes during swing (Fischer et al., 2002; Gillis et al., 2005; Goslow Jr. et al., 1981; Rasmussen et al., 1978). Knee excursion patterns are also broadly comparable across mammals and

involve a period of flexion followed by extension during both the stance and swing phases (Fischer et al., 2002; Gillis et al., 2005; Goslow Jr. et al., 1981; Rasmussen et al., 1978). Using skin markers to identify joints, it was found in this study that guinea pigs follow these broad trends with the hip extending during stance, and the knee exhibiting flexion prior to extension during stance. Broadly similar joint angle excursions were also found in a recent cinefluorographic study of limb kinematics in guinea pigs (Rocha-Barbosa et al., 2005), however the details of limb movements differ substantially between the cinefluorographic study and this one. Table 2 compares the joint angles found by Rocha-Barbosa et al. (2005) and in this study. While hip extension patterns are comparable, if slightly lower in this study, knee excursions are markedly different. Skin over the knee tends to be particularly loose in rodents such that the underlying knee joint is able to move relative to any surface marking. Hence it is not surprising that knee joint kinematics, in particular, are seemingly inaccurate when using skin markers. Cinefluorography, which allows direct visualization of the underlying limb skeletal elements surely provides a better estimate of joint angular excursions during locomotion, especially at the knee joint.

Table 2. Joint angle comparisons within guinea pigs using cinefluorography and joint markers on skin.

	Hip	Knee	
	Extension	Flexion	Extension
Cinefluorography*	57°	14°	20°
Joint Markers	23-46°	27-34°	3-9°

*(Rocha-Barbosa et al., 2005)

Comparisons between rat and guinea pig biceps and vastus EMG and strain

Electromyography and sonomicrometry reveal when muscles are active and what those muscles are doing with respect to their length changes during locomotion. Electromyographic activity patterns in both the biceps and vastus of rats (Gillis and Biewener, 2001; 2002) and guinea pigs are very similar. Both muscles become active near the start of the stance phase in both species, and both end activation within the second half of stance, generally about 75% of the way through. In fact, this timing of activation of major extensors seems to be true of most quadrupeds studied to date, and has lead previous researchers to suggest that there has been conservation of the neural control system governing locomotion in mammals over evolutionary time (Peters and Goslow, 1983).

If one turns from electrical activity patterns to length change patterns, both similarities and differences are present when comparing guinea pigs and rats. In both species (Gillis and Biewener, 2001; 2002), the biceps strain closely mirrors the hip angular excursions. For example, when the hip extends during stance, the biceps shortens and when the hip flexes during stance, the biceps lengthens. The amount the biceps shortens is slightly lower, on average, in guinea pigs, 7-18% L_0, than rats, 23-27% L_0, but the general trend is the same. Specifically, the biceps is actively shortening during stance, such that the muscle is producing rather than absorbing energy.

Vastus strain also mirrors knee angular excursions in both rats (Gillis and Biewener, 2001; 2002) and guinea pigs. In both species, the knee joint undergoes

flexion followed by extension during stance. However, rats exhibit more knee flexion than extension and the opposite is true of guinea pigs. In line with this, the vastus of the guinea pig stretches less than it shortens during stance, whereas the reverse is true of rats, in which the vastus stretches more than it shortens. The higher degree of vastus shortening in guinea pigs corresponds with the greater amount of knee extension observed in the cinefluorographic study of limb kinematics in these animals. Similarly, the higher degree of vastus stretching in rats corresponds with the greater amount of initial knee flexion observed in kinematic studies of these animals. In short, imaging studies have shown that rats and guinea pigs have different knee joint kinematics during locomotion, and these differences are reflected in the different vastus actions in the two species. Why the two species exhibit different knee joint kinematics is unclear; perhaps the slightly more flexed limb of the rat is more compliant, and this leads to a greater degree of knee flexion during stance (i.e., more vastus stretching). In any case, this study suggests that the vastus in guinea pigs likely functions differently than that in rats, generating more energy than it dissipates since it shortens more than it is stretched.

Effects of Speed and Grade

Animals come into contact with a broad array of grades during natural locomotion, which is why muscle activity and strain need to be characterized on different inclines to gain insight into how limb muscles accommodate different locomotor surfaces. As on the level surface, the EMG intensity of both the biceps

and vastus increases with speed on inclined and declined grades in rats (Gillis and Biewener, 2001; 2002) and guinea pigs. In addition, EMG intensities at a given speed are generally highest on an incline, lowest on a decline and intermediate on the level in both rats (Gillis and Biewener, 2002) and guinea pigs. These results are consistent with the idea that more energy is required for higher speeds and/or uphill movement (e.g., Raab et al., 1976; Armstrong et al., 1983) because the greater EMG intensities likely indicate more fibers being recruited to generate force, and using more fibers uses more energy.

In rats and guinea pigs, the EMG durations of the vastus and biceps decrease with speed on all grades, thereby demonstrating that both muscles are active for shorter, but more intense periods with increases in speed. However the EMG duty cycle remains constant across speed in the biceps and vastus, indicating that the muscle activation duration is somehow linked to the stance duration, a result previously demonstrated in a number of different animals (e.g., Pierotti et al., 1989). Similarities in muscle function in thigh muscles of rats and guinea pigs do not stop with similar EMG activity patterns, but continue with biceps strain patterns.

Biceps strain responds similarly to speed as well as to inclines and declines in both rats and guinea pigs. In particular, the biceps shortens more during stance as speed and grade increase in rats (Gillis and Biewener, 2002), and in guinea pigs, two of four individuals exhibited increased biceps shortening with speed increases. Moreover, in all three individuals for which decline and incline

data were collected for the biceps, shortening strains tended to be greater on the incline than on the decline. Data from more individuals are needed to determine if this trend holds true for guinea pigs, however, it would appear that in addition to exhibiting higher recruitment levels (EMG intensities), the biceps also shortens a greater distance as speed and grade increase, presumably increasing its energy output in doing so.

Unlike in the biceps, the vastus strain pattern did not obviously change in response to speed or grade in guinea pigs. Regardless of condition, the stretch-shorten cycle of the vastus appeared similar, with stretching values remaining consistent between 3-6% Lo and shortening values staying consistent between 4-8% Lo. In rats, vastus strain varies more with speed and grade than in the guinea pig. For example, vastus shortening amounts in the second half of stance were greater at high speeds (galloping) and on an incline (Gillis and Biewener, 2002). Unfortunately, vastus strain data were only collected successfully in two guinea pigs in this study. Neither animal reached speeds high enough to elicit galloping and only one animal successfully moved on graded surfaces as well as on the level. Thus, sample sizes for vastus strain are low, particularly in the context of grade and high speeds, hence more data are required before it can be concluded that this muscle responds differently to these conditions in a guinea pig than in a rat.

Comparisons across taxa

Do the trends within rodents hold true across a wide range of quadrupeds? The biceps and vastus EMG activity timing is very similar between rats (Gillis and Biewener, 2001; 2002), guinea pigs, cats (Rasmussen et al., 1978), and goats (Gillis et al., 2005). In dogs (Carrier et al., 1998) and horses (Hoyt et al., 2005), only the vastus was studied, however, the vastus EMG timings in these animals are similar to that in rats, guinea pigs, cats, and goats. The general trend is for EMG activity to begin slightly before stance begins and end within the second half of stance. In addition, all animals studied to date exhibit increasing EMG intensity but constant EMG duty cycle in response to increases in speed and grade (de Leon et al., 1994; Gillis and Biewener, 2001; 2002; Gillis et al., 2005; Hoyt et al., 2005).

The hip joint and biceps also appear to function similarly in all quadrupeds studied so far. The hip joints of rats (Fischer et al., 2002), guinea pigs (Rocha-Barbosa et al., 2005), cats (Rasmussen et al., 1978), dogs (Carrier et al., 1998) and goats (Gillis et al., 2005) extend throughout stance and in the animals for which it has been studied, the biceps actively shortens during this time. In fact, the biceps strain patterns closely follow the hip excursions in these species, such that the onset of hip extension closely coincides with the onset of biceps shortening while the onset of hip flexion coincides with the start of biceps lengthening.

However, while the vastus' length changes tend to reflect the knee joint excursions within rodents (i.e., rats and guinea pigs), they appear to diverge from

knee joint actions in larger animals. For example, the knees of dogs undergo approximately equivalent amounts of flexing and extending during stance, and thus comparable amounts of vastus stretching and shortening would be expected. In reality, there is much more late-stance shortening than early-stance stretching (Carrier et al., 1998). In goats (Gillis et al., 2005), there are again similar amounts of knee flexion and extension in stance, but the vastus' actions are similar to those of dogs, with much less stretching than shortening. Finally, in horses, vastus strain is exactly the opposite of what would be expected from the knee excursion (Hoyt et al., 2005). The knee flexes throughout stance, but the vastus shortens throughout this period. Why does the vastus follow knee excursions in rodents, but not in larger quadrupeds?

Initially, it was thought that the flexed limbs of rodents might be more compliant than the more upright limbs of larger animals, such as goats and horses, and that these differences might help explain differences in vastus strain patterns in these animals. However, upon closer examination of limb joint excursions, it is now clear that the knees of large quadrupeds flex considerably at the beginning of stance, indicating that their limbs are also compliant. So, major differences in limb compliance appear to be unlikely across animals of different size and thus, cannot explain differences in vastus strain patterns. Instead, it is possible that muscle/tendon anatomy may be a potential source of these differences. Larger animals generally have more tendinous material attached to, investing, and surrounding the vastus than smaller animals. Since tendinous material is

somewhat elastic, perhaps such material stretches as the knee flexes early in stance, rather than the muscle fibers themselves, allowing the muscle to shorten considerably even as the knee is flexing (Gillis et al., 2005; Gary Gillis, personal communication). Since actively shortening muscles produce energy and actively lengthening muscles absorb or dissipate energy (Biewener and Roberts, 2000), the vastus muscle functions to absorb energy in small animals, like rats, where it is stretched through most of stance, but in larger animals, it appears that the vastus tendons may absorb energy as they stretch, while the muscle itself can generate energy as it actively shortens throughout much of stance. The differences within the vastus may also be due to differences within the pelvic structure of various animals and therefore, the morphological differences between mammalian quadrupeds must be studied more to determine what is causing the change in vastus function.

An interesting question is whether length changes in the vastus of larger rodents (i.e., 10-40 kg) follow knee excursions, such as in rats (Gillis and Biewener, 2001) and guinea pigs, or if the strain diverges from knee excursions, such as in dogs (Carrier et al., 1998), goats (Gillis et al., 2005), and horses (Hoyt et al., 2005). Dissections of such animals would reveal the extent to which their vastus is invested with and surrounded by tendinous material. Predictions could be made regarding vastus actions based on the musculotendinous anatomy and then *in vivo* studies of muscle function would support or refute those predictions. Thus, studies of muscle function during locomotion in larger rodents would

definitely add to our understanding of the function of knee extensors in mammalian quadrupeds and perhaps, help to elucidate and explain the trends seen in smaller rodents versus larger quadrupeds.

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