

In vivo muscle function vs speed

I. Muscle strain in relation to length change of the muscle–tendon unit

Donald F. Hoyt^{1,*}, Steven J. Wickler², Andrew A. Biewener³, Edward A. Cogger² and Kristin L. De La Paz²

¹Biological Sciences Department and ²Equine Research Center, California State Polytechnic University, Pomona, CA 91768-4032, USA and ³Concord Field Station, Harvard University, Bedford, MA 01730, USA

*Author for correspondence (e-mail: dfhoyt@csupomona.edu)

Accepted 22 December 2004

Summary

The activity of muscles can be concentric (shortening), eccentric (lengthening) or isometric (constant length). When studying muscle function it is important to know what the muscle fascicles are actually doing because the performance of muscle is strongly influenced by the type of activity: force decreases as a function of shortening velocity during concentric contractions; force produced during eccentric contractions can be stronger than maximum isometric force, and force production is enhanced if a concentric contraction follows an eccentric phase. It is well known that length changes of muscle fascicles may be different from length changes of the overall muscle–tendon unit because of the compliance of the series elasticity. Consequently, fascicles of joint extensor muscles may not undergo eccentric activity even when the joint flexes, but the extent to which this occurs may vary with the compliance of the series elasticity and may differ between species: the vastus lateralis, a knee extensor, shortens when active during trotting in dogs and lengthens in rats. Previous studies of kinematics of trotting in horses have shown that during stance, the elbow extends nearly continuously with a brief period of flexion near mid-stance and the knee exhibits two phases of flexion followed by extension. The lateral triceps (an elbow extensor) has no external tendon but the vastus lateralis has a relatively long external tendon and the fascicles insert on an aponeurosis. Thus, one might expect the relation between fascicle strain and overall length change

of the muscle-tendon units to be quite different in these two muscles. In the present study in horses, fascicle length changes of the lateral triceps and vastus lateralis were measured with sonomicrometry and length changes of the muscle-tendon units were estimated from muscle architecture and joint kinematics for four horses trotting on a treadmill at nine speeds. Because the focus of this study was the relation between length changes of the muscle–tendon unit (estimated from kinematics) and length changes in the muscle fascicles, we divided the stance-phase sonomicrometry records into phases that corresponded to the alternating flexion and extension of the joint as indicated by the kinematic records. During its one eccentric phase, the triceps shortened by $0.7\pm 0.4\%$ despite a predicted lengthening of 1%. Similarly, the vastus shortened by $3.7\pm 1.9\%$ when kinematics predicted 3.2% lengthening. During their concentric phases the triceps shortened by 10.6% and the vastus shortened by 8.1%. Strain in the triceps did not change with speed but it did in the vastus. Strain rate increased with speed in both muscles as did the integrated EMG, indicating an increase in the volume of muscle recruited. Thus, despite differences in their architecture and the kinematic patterns of the associated joints, these two joint extensors exhibited similar activity.

Key words: Locomotion, quadruped, sonomicrometry, muscle.

Introduction

In addition to serving to attach muscles to bones and optimize the arrangement of fascicles within muscles (Gans and DeVree, 1987), the series elastic elements of muscles (tendons and aponeuroses) serve two important functions: elastic storage of energy and mechanical buffering (reducing eccentric activity). In recent years several elegant studies have quantified the storage and recovery of mechanical energy in stretched elastic structures and the nearly isometric activity of

the associated muscles that maximizes the energetic benefits of this function (Biewener et al., 1998; Prilutsky et al., 1996b; Roberts et al., 1997). Tendon compliance during concentric (shortening) contractions can enhance total power output of the muscle–tendon unit by uncoupling fascicle shortening and joint movement (Roberts, 2002). There is also an accumulating body of evidence detailing the elasticity of tendons and aponeuroses and the impact of this elasticity on strain in the

associated muscle fascicles. During 'isometric' contractions of the human gastrocnemius and tibialis anterior (Ito et al., 1998; Maganaris and Paul, 2000a,b; Muramatsu et al., 2002, 2001) the fascicles actually shorten and stretch the associated tendons and aponeuroses. Careful studies have compared the strain in different segments of the aponeuroses to detect heterogeneity, and have compared the amount of strain in the tendon with that observed in the aponeuroses (Maganaris and Paul, 2000a; Monti et al., 2003; Muramatsu et al., 2002, 2001). During slow pedaling, significant differences between the strain and strain rate of human knee extensors and the length changes of the muscle-tendon unit have also been found (Muraoka et al., 2001).

While these studies suggest that the direction and amount of strain in the muscle fascicles can be uncoupled from changes in the length of the muscle-tendon unit, there have been relatively few explicit examinations of this during running, trotting or hopping. In one of the first studies of muscle function using direct measurement of fascicle strain (sonomicrometry), Griffiths (1991) found that fascicles of the feline medial gastrocnemius shortened while the ankle flexed (and the overall muscle-tendon unit was lengthened) during stance – a phenomenon that Griffiths (1991) termed 'mechanical buffering'. A similar conclusion was reached for one of three cats, based on the kinematic analysis of the function of the gastrocnemius (Prilutsky et al., 1996b). This was a surprising demonstration because it had been assumed for years (Walmsley et al., 1978) that the cat gastrocnemius, an ankle extensor, was undergoing an eccentric (lengthening) contraction during this phase of the step cycle. Subsequent sonomicrometry studies have not explicitly addressed this issue but their data are relevant. In the studies that have quantified elastic storage in the tendons of ankle extensors (Biewener et al., 1998; Roberts et al., 1997), the tendons stretch because the ankle joints are flexing while the muscles shorten by small amounts. Ultrasonographic studies of human walking have shown that the fascicles of the gastrocnemius function nearly isometrically during lengthening of the muscle-tendon unit (Fukunaga et al., 2001) and a recent ultrasonographic study of eccentric activity in human tibialis anterior also showed that the fascicles behaved quasi-isometrically as the muscle-tendon unit lengthened during ankle movement (Reeves and Narici, 2003).

Many of these studies are of ankle extensors with relatively long tendons and short fascicles. Patterns of muscle strain in relation to joint angle change are less consistent for more proximal muscles. The vastus lateralis (a knee extensor) of the dog was found to shorten during the first half of stance when the knee flexed (see fig. 4A in Carrier et al., 1998). In contrast, in a study of the vastus lateralis and biceps femoris (hip extensor) of rats, Gillis and Biewener (2001) found a much closer correspondence between joint motion and muscle strain. In the present study we sought to extend these observations to the full range of normal trotting speeds in horses, with the goal of comparing two muscles (forelimb triceps and hindlimb

vastus lateralis) in which one might expect to find substantial differences in mechanical buffering.

During the stance phase of the stride in a trotting horse the elbow and the knee exhibit different patterns of joint angle change that would be expected to be the product of very different patterns of strain in their respective extensor muscles. During stance, the elbow extends about 25° with a brief period of flexion just before mid-stance (Back et al., 1995a). Thus, one would expect that elbow extensors would shorten throughout stance with, possibly, a brief period of eccentric activity near mid stance. The knee exhibits two phases during which flexion is followed by extension (Back et al., 1995b; Hoyt et al., 2002). The knee extensors are active during most of stance (Tokuriki and Aoki, 1995) as they must stabilize the knee in order to support the animal's body weight. Given these observations, there would seem to be at least two possible patterns of muscle strain in the vastus. If muscle strain closely follows the joint kinematics, then the muscle would undergo two cycles of eccentric activity followed by concentric activity. These 'stretch-shortening' cycles would be expected to increase force production (Ettema, 1996, 2001; Hof et al., 1983). Alternatively, if the muscle's series elasticity is sufficiently compliant, then during the apparently eccentric phases the muscle might remain isometric or even shorten, as Griffiths (1991) observed in the cat medial gastrocnemius. In the present study we measured muscle strain in the lateral triceps (an elbow extensor) and in the vastus lateralis (a knee extensor). Lacking specific information on compliance of their series elasticity, we hypothesized that muscle strain would track joint excursion in both muscles: the triceps shortening throughout stance and the vastus exhibiting two cycles of eccentric activity followed by concentric activity.

We were also interested in testing the hypothesis that the amount of strain in both muscles would increase with speed. We expected strain to increase because step length increases with speed (Farley et al., 1993; Hoyt et al., 2000) and increased step length should require increased range of motion in leg joints, a phenomenon previously observed in the equine knee (Hoyt et al., 2002). Because time of contact decreases with speed in a trot (Dutto et al., 2004b; Hoyt et al., 2000) we also expected to find that strain rate would increase with speed, even if the amount of strain did not. Previous studies of *in vivo* muscle function in quadrupeds (Carrier et al., 1998; Gillis and Biewener, 2001; Gregersen et al., 1998) have also studied changes in muscle function with speed. However, in these studies the increased speed was accompanied by changes in gait, which may confound the results from speed alone.

Muscle strain can be active or passive. In order to focus on strain when the muscle was active, EMG (electromyographic) activity was measured relative to the time the hoof was on the ground. These data also allowed us to determine the duty factor, the proportion of the stride during which the limb is in contact with ground. Duty factor is related to peak forces (Alexander et al., 1979) and, therefore to the total volume of muscle active (Taylor, 1994). Because we hypothesized that strain rate would increase with speed, we also predicted that

the volume of actively recruited muscle, as indicated by the integrated EMG (IEMG) would increase with speed.

Because a preliminary study revealed unexpected variability between individuals in the strain pattern of the vastus, we implanted sonomicrometry crystals in approximately the same locations within the vastus lateralis muscles of four horses in two consecutive summers and compared the patterns of strain observed throughout the stride cycle.

Materials and methods

Animals and training

Four Arabian horses *Equus caballus*, ranging in age from 4 to 7 years (5.1 ± 0.7 years, mean \pm S.E.M.) with a body mass of 433 ± 13 kg, were physically conditioned 5 days a week for at least 3 months prior to data collection. The 30 min conditioning protocol included a 3 min warm-up at a walk; a 5 min warm-up at a trot, approximately 15–20 min spent alternating between a trot and a canter, and a 5 min walk. These workout sessions were performed either on an equine treadmill (Säto I, jan.thoreson@satotreadmill.com; SÄTO® AB Lovisedalsvägen, 1S-741 30 Knivsta, Sweden) or over ground in an outdoor round-pen. All procedures involving animals were approved by the Cal Poly Pomona Animal Care and Use Committee.

Surgical procedures

Surgery was done on standing, sedated horses. On the morning of the surgery, each animal was catheterized using a 16-gauge catheter to facilitate the administration of drugs. An initial dose of xylazine (xylazine HCl; Fermenta Animal Health Co., Kansas City, MO, USA) was given (1.1 mg kg^{-1}) to tranquilize the horse. A combination of butorphanol tartrate (Fort Dodge Animal Health, Fort Dodge, IA, USA; 0.1 mg kg^{-1}) and detomidine hydrochloride (Pfizer Animal Health, Exton, PA, USA; $20\text{--}40 \mu\text{g kg}^{-1}$) was administered intravenously at the beginning of the surgery and throughout the surgery as needed to maintain sedation. The locations of the lateral triceps (M. triceps brachii caput laterale) and vastus (M. vastus lateralis) were determined by palpation in the right limbs. The approach to the lateral head of the triceps was identified by palpation of the lateral epicondyle, the deltoid tuberosity of the humerus, and the olecranon of the ulna. The vastus was identified using the landmarks of the greater and lesser trochanter and the lateral ridge of the trochlea of the femur. Anatomical locations were studied on several cadavers prior to surgery and anatomical validation of sonomicrometer crystal placement was done on three horses not part of this study that were euthanized for other medical conditions not related to musculoskeletal dysfunction.

Lidocaine HCl (Pro Labs Ltd., St Joseph, MO, USA) was administered subcutaneously at the incision sites and followed by a 7 cm long incision in the skin. The fascia was exposed by removing subcutaneous fat and, in the case of the triceps, incision through the omobrachialis muscle. Two small stab incisions were made into the fascia, approximately 10–15 mm

away from each other in a line parallel to the muscle fiber orientation. For each muscle, one pair of 2 mm omnidirectional, spherical, piezoelectric crystals (Tack crystals, Sonometrics Corporation, London, Ontario, Canada) was implanted to measure changes in muscle fiber length. Crystals were implanted 1 cm deep into the muscle using a polyethylene introducer. The crystals were anchored to muscle fascia using 0 silk suture, and a tension relief loop. The sonomicrometry output was sampled at 463 Hz using Sonometrics System Software and then output to the A/D card (PCI 1200, National Instruments, Austin, TX, USA), which acquired the data at 3704 Hz using LabVIEW® software (National Instruments, Austin, TX, USA) running on an IBM-compatible personal computer. The higher frequency was necessary for the EMG signals that were being acquired by the same A/D system, and the resulting multiple records of the sonomicrometry signal were removed during data processing.

Electromyography electrodes, made of multi-stranded, Teflon-coated, stainless steel wire (AS636, Cooner Wire, Chatsworth, CA, USA) were inserted by a sew-through technique (Carrier, 1996) 1 cm away from, and parallel to the sonomicrometry crystals. The modification made to the procedure of Carrier was the absence of a proximal silastic patch to anchor the EMG wire to the fascia. This reduced muscle tearing that might have resulted from the presence of two fixed anchors. Each of the two EMG electrodes had 7 mm of bared wire and the two segments were separated by 1.5 cm. The EMG signal was amplified (1000–10 000, depending on signal strength) and filtered (60 Hz notch and 100–1000 Hz bandpass) with a Grass model P511K preamplifier (Quincy, MA, USA), and sampled at 3704 Hz by the A/D system. A ground wire was implanted subcutaneously into the dorsal aspect of the horse's sacral region. Following implantation, all incisions were loosely sutured with 0 silk. Flunixin meglumine (Schering-Plough Animal Health Corp., Union, NJ, USA; $20\text{--}40 \mu\text{g kg}^{-1}$) was administered post-surgically to reduce inflammation and associated pain.

Data collection

After the surgery was completed, the animal recovered for at least 90 min and was allowed to drink water and graze freely. After this time period, a lameness examination was conducted by a veterinarian to ensure soundness in all four limbs and provide a subjective measure of any residual effects of sedation or surgery. An objective measure of surgical treatment and sedation was also used: measurements of heart rate and manually calculated stride frequencies during data collection were compared to pre-surgical measurements taken during exercise bouts.

A surcingle was placed around the horse slightly posterior to the scapula and was used to secure the wire connections from the implants and accelerometers. A biaxial accelerometer ($\pm 50 \text{ g}$, CXL25M2, Crossbow Technology, Incorporated, San Jose, CA, USA) was taped on the lateral aspect of the hoof of the right fore- and hindlimbs to record hoof contact and break-over (the end of stance when the hoof leaves the treadmill). All

accelerometer data were collected at 3704 Hz and analyzed using the LabVIEW software. The validity of the accelerometer as a measure of hoof contact and break-over was assessed by comparing accelerometer tracings with simultaneous synchronized high-speed video.

Each horse was run on a high-speed treadmill under two conditions: on the level and up a 10% incline. The data collected on the level are the focus of this paper and those collected on the incline are the focus of a companion paper (Wickler et al., 2005). Once on the treadmill, the horse was warmed up at a walk (1.7 m s^{-1}) for 3 min and a trot (3.0 m s^{-1}) for 5 min. Horses were run under each condition at speeds from 2.5 to 4.5 m s^{-1} in 0.25 m s^{-1} increments. The conditions and speeds were randomly ordered. Horses were brought up to speed, and after 45 s at speed, data were collected. All 18 experimental trials (nine of each speed at 0% and at 10% incline) were run in succession, with a 30 min break after the first nine. Following the last trial (defined as a combination of speed and condition), the horse was again sedated and sonomicrometry crystals and EMG wires were removed. Following this surgery, the animal received phenylbutazone (Pro Labs Ltd., St Joseph, MO, USA; $11\text{--}22 \text{ mg kg}^{-1}$) and was returned to an open paddock. The horses were hand-walked daily for 1 week, following which sutures were removed.

Kinematic data

For the study of knee and elbow kinematics, reflective markers (Peak Performance Technologies, Englewood, CO, USA) were glued to the skin on the lateral side of the right fore- and hindlimbs of the horses using standard, palpable positions for these joints (Back et al., 1993). The markers were 2.5 cm, lightweight, plastic spheres covered with corner-cubed reflective tape. The joint angles were calculated so that a decrease in joint angle indicates a flexing joint. The horses were filmed at 125 Hz using a Model PCI Motion Scope® camera (Redlake Camera Corp., Morgan Hill, CA, USA). The camera was placed approximately 8.5 m away from the treadmill. A linear calibration was performed daily. Five consecutive strides were recorded and digitized using Motus® software (Peak Performance Technologies, Englewood, CO, USA) during each sonomicrometry trial. The angular data were smoothed using a cubic spline filter, normalized for time using a cubic spline interpolation and five strides for each horse, speed and condition were averaged using the trial averaging feature of Motus. These data were used to interpret the muscle strain data (see below) and to determine mean joint angle of the knee and elbow at first hoof contact, break-over, mid-stance and maximum extension (elbow) or maximum flexion (knee).

Strain estimated from kinematics

The change in length of the muscle–tendon unit at 3.5 m s^{-1} was estimated from the kinematic data, the length and pennation angle of the muscle fibers, and the moment arm of the muscles. This length change was converted to strain by dividing by the observed length of the muscle fascicles and compared with the muscle strain observed during the same

period of time. Kinematic data from the elbow were not corrected for skin displacement because the errors are very small (Back et al., 1995a). Kinematic data for the knee were corrected for skin displacement (van Weeren et al., 1992) at all four points used in calculating knee angle. Individual horse's kinematic records were used to determine the range of motion of the elbow and knee during each of the phases used for analysis of muscle strain.

The moment arms of the equine triceps and vastus were determined from cadavers of four, similar-sized horses (based on body mass and linear dimensions of metacarpal and metatarsal bones). Muscles around the joints, other than the muscle of interest, were removed to permit the movement of the joint through the range of motion found in our kinematic studies. The distance along the line of action of the tendon was measured with the joint held at five different angles spanning the range of motion. Joint angles were measured to the nearest 0.5° with a protractor. For the forelimb, measurements were made from a pin placed into the most prominent palpable aspect of the deltoid tuberosity to a pin placed into the middle of the olecranon at a point approximately 4 cm from the posterior margin. For the hindlimb, measurements were made from a pin placed in the third trochanter on the midline of the femur to a pin placed in the center of the patella. The moment arm was calculated as the slope of a least-squares regression of the length of the muscle on the angle of the joint (in radians). Calculating moment arm in this manner means that a single average value was used at all joint angles.

The observed motion of the joint multiplied by the average moment arm was used to predict the length change of the muscle–tendon unit during each phase of muscle activity. This length change was converted to strain by dividing by the average length of the muscle fascicles observed in three cadavers. The equine vastus arises from the femur and inserts on the lateral margin of the rectus femoris where there is a thick aponeurosis that effectively serves as the tendon for the vastus. The angle the vastus muscle fibers made with this aponeurosis was measured on three cadavers with a protractor and averaged 60.3° . For each of the four individual horses used in the sonomicrometry measurements, the observed motion of the joint multiplied by the average moment arm from the cadavers was used to predict the length change of the tendon during each phase of muscle activity. The length change of the tendon, corrected for the pennation angle, yielded the predicted length change of the muscle fascicles (Gans and DeVree, 1987). This length change was converted to strain by dividing by the average length of the muscle fibers measured on the three cadavers.

Data processing

Initial hoof contact, break-over and subsequent hoof contact were determined using the record from the accelerometer, and from these were calculated duration of stance phase (t_c =time of contact) and duration of swing phase. All other stride parameters were derived from these measurements and speed. The EMG and sonomicrometry records were subdivided into

into individual strides using the simultaneously collected accelerometry record.

Muscle length changes (and velocities of shortening) were analyzed only for the time of contact because of its central role in determining metabolic cost (Kram and Taylor, 1990), although recent work identifies a significant energetic cost associated with the swing phase (Marsh et al., 2004). The voltage output of the sonomicrometer was converted to length using an empirically derived equation relating voltage to the actual distance between the crystals, measured in water with the crystals attached to a micrometer, similar to the procedure of Gillis and Biewener (2001). Muscle lengths from the sonomicrometry records of the stance phase were smoothed using a median pass filter that scanned through the data, substituting for each consecutive datum, the median of the seven consecutive values centered on that datum (the datum, the three preceding and three succeeding values). All records of muscle lengths were normalized by dividing each value by resting muscle length. The measurements of resting muscle lengths were taken when the animal was standing square on the treadmill (metacarpals and metatarsals perpendicular to the treadmill's surface). In order to calculate strain, the normalized muscle length records of ten consecutive individual strides were averaged after being temporally normalized to 100% of time of contact using a cubic spline interpolation.

Strain patterns were not simple monotonic changes (particularly for the vastus), so that a simple calculation of the strain based on the difference in muscle length between the start of stance and the end of muscle activity would obscure considerable information about the relation between muscle strain and limb motion. Therefore the average normalized muscle length data were divided into phases based upon the kinematics of the appropriate joint. This was possible because the joint kinematics were determined from five strides that were a sub-set of the ten strides averaged for muscle length. The net strain (change in muscle length, with shortening being negative) occurring during each phase was determined for each animal and trial. Strain rate (muscle lengths per second) during each phase was determined from the net strain and the duration of the phase in that trial.

Electromyography records were filtered using a second order low pass filter (1000 Hz), rectified and integrated, and analyzed for: (1) when the EMG started relative to hoof contact, (2) total duration of the EMG signal (including if it started before stance), (3) the length of the signal (only during stance) as a percentage of t_c , and (4) the integrated EMG during stance.

Reproducibility of sonomicrometry data

In an attempt to assess the reproducibility of inter-individual differences in sonomicrometry data, we compared records of changes in muscle length over the stride period from crystals implanted for a single day in the same horses in two consecutive summers. For this analysis we used a subset of the data reported here (limited to data collected on the level and four speeds) which were the only data available that

corresponded to a set of data obtained the previous summer from the same horses. All relevant details of the experiments were the same in both summers and the data were processed in the manner described above except that all muscle length data from both summers were normalized by dividing by the average length of the muscle during the stance phase while trotting at 3.0 m s^{-1} because we did not have standing square values for the first summer. Time was normalized by dividing by the stride period. Data were analyzed from three horses, trotting at four speeds (2.5, 3.0, 3.5, 4.0 m s^{-1}) on the level. Values for every 0.5% of the stride period were obtained by cubic spline interpolation. Reproducibility of the data was assessed by calculating the variance ratio (Hershler and Milner, 1978), which is the ratio of the average variance between corresponding points in different strides to the total variance of the entire data set.

Statistics

A two-way analysis of variance with repeated measures was run on all data using SuperANOVA[®] software (Abacus Concepts Inc., Berkeley, CA, USA) with significance set at $P < 0.05$. The two variables tested were speed and condition, and the four horses were used as the repeated measure. The data analyzed were mean values for each horse, based on five strides for the kinematic parameters and ten strides for all other parameters. All means are presented \pm S.E.M.

Results

Muscle strain

Representative simultaneous recordings of accelerometer and EMG of the forelimb and triceps during one full stride cycle are shown in Fig. 1A, and a comparable graph for the hindlimb and vastus are shown in Fig. 1B. Our analysis of strain in the triceps was based upon the stance-phase kinematics of the elbow and the EMG of the triceps (Table 1). The elbow extended throughout stance (Fig. 2) except for a brief period of flexion (phase 2) that lasted for about 10% of stance. Phase 1 ended progressively later in stance as speed increased ($P = 0.001$), increasing from 21% to 32% of stance. Phase 2 lasted for 10% of stance, with the result that the termination of this phase of flexion was progressively delayed at higher speeds from 30 to 42% of stance ($P < 0.001$). Phase 3 lasted until about 81% of stance at all speeds. The triceps exhibited EMG activity (see below) during phases 1 and 2, but EMG activity stopped at an average of 40% of stance, shortly after the start of phase 3, so that force production by the triceps must have declined during phase 3. In phase 1, the elbow extended and there was EMG activity but the triceps lengthened 2.8%. Although we have no explanation for this anomalous observation, it was observed in all sono trials in all animals at all speeds, so we are confident that it is real. We suspect the elbow may undergo a very brief period of flexion immediately at hoof contact that, with the existing skin correction factors, does not appear in the kinematics. During phase 2, the elbow flexed but the muscle was active and

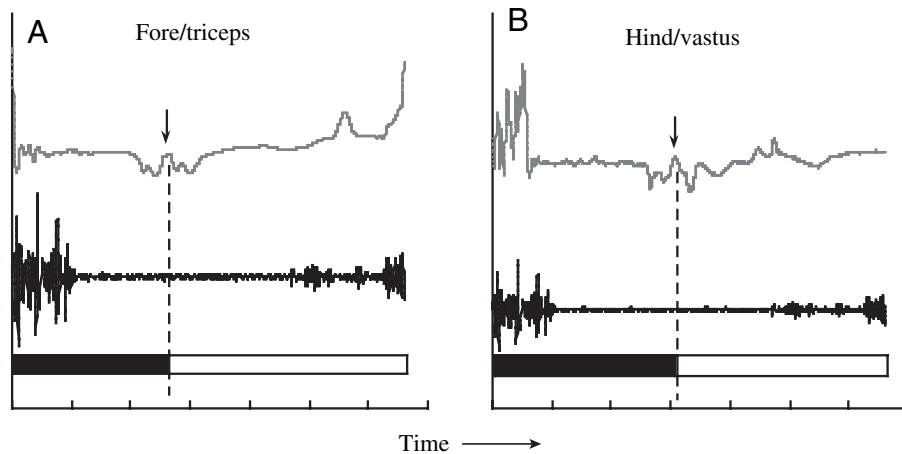


Fig. 1. Representative data for accelerometer and EMG of a forelimb (A) and hind limb (B) of a horse trotting on a level treadmill at 3.5 m s^{-1} . The graphs show the accelerometer used to identify stance phase, starting with hoof contact (axis origin) and lift off (denoted by the broken line). Stance is indicated by the solid bar at the bottom. EMG activity patterns, below the accelerometer record, show that both muscles are active early in stance.

essentially isometric (it shortened by $0.3 \pm 0.1\%$). The amount of strain during phase 2 did not change with speed ($P=0.247$). During phase 3, the elbow extended and the muscle shortened by $10.3 \pm 0.6\%$. However, the triceps EMG became inactive early in phase 3, so the muscle must have begun to relax, probably producing progressively less force as it shortened. The time delay between the end of EMG activity and the end of muscle shortening during phase 3 decreased from about 0.140 s at low speeds to about 0.110 s at high speeds and averaged 0.127 s. Because the muscle shortened and the elbow extended during phase 3, we assume this is a phase of active concentric activity. The amount of strain during phase 3 in the triceps (Fig. 3) did not change significantly with speed ($P=0.386$).

Our analysis of strain in the vastus (Fig. 3) was based upon the kinematics of the knee (Fig. 2) and the EMG and muscle length changes of the vastus (Table 2). The pattern of kinematics and muscle length in the vastus was more complex than the patterns recorded for the triceps. In part this was because there was a brief period of muscle lengthening (phase 2; Fig. 2Bi) in two of the animals that was not evident in the other two (Fig. 2Bii). The start of phase 2 was defined by the start of muscle lengthening and not kinematics, the only phase

in either muscle that was not defined exclusively on the basis of kinematics.

Phase 1 in the vastus lasted until 18% of stance at all speeds ($P=0.607$). The knee flexed but the muscle shortened by $10.0 \pm 0.3\%$. Because phase 2 was only observed at all speeds in one animal (where it averaged about 6% eccentric strain), we have omitted it from the analysis. On average, phase 3 lasted from 18% to 36% of stance. The end of this phase occurred progressively later ($P=0.041$) at higher speeds (increasing from 29% to 39%) so that the relative duration of phase 3 increased ($P=0.001$). However, owing to the decreased time of contact, there was no change ($P=0.52$) in the absolute temporal duration of this phase. Seen in all four horses, phase 3 was a period of concentric activity during which the knee extended and the vastus

shortened by an average of $3.0 \pm 0.6\%$. Strain increased ($P=0.016$) from 2.8% to 4.6% as speed increased from 2.5 to 4.5 m s^{-1} (Fig. 3). Phase 4 was a period of knee flexion that lasted, on average, from 36% until 61% of stance. The duration of phase 4 was relatively consistent ($\sim 15\%$ of stance) at all speeds because the end of the phase increased ($P<0.001$) with speed from 55% to 65% of stance. This was a period in which the muscle underwent minimal strain (Fig. 3), shortening by $2.0 \pm 0.1\%$. The EMG was active until 59% of stance, very near the end of phase 4. Phase 5 lasted until an average of 84% of stance and was another period of concentric activity when the knee extended and the muscle shortened by an average of $3.1 \pm 0.4\%$. Both the relative ($P=0.024$) and absolute ($P=0.002$) duration of phase 5 decreased with speed. During this phase, strain in the vastus (Fig. 3) increased from 1.6% to 4.1% with speed ($P=0.047$). Phase 5 occurred after the end of EMG activity and the average time from the end of EMG activity until the end of phase 5 was 0.069 s.

Muscle strain rate

In the triceps the strain rate (Table 1) during phase 2 averaged -0.112 ± 0.052 fascicle lengths s^{-1} and the effect of speed was nearly significant ($P=0.079$). During phase 3 in the

Table 1. Triceps muscle timing, strain and strain rate during the three phases of muscle activity

Phase	End of phase (% of stance)	Joint motion	Strain (% of fascicle length)	Strain rate (muscle lengths s^{-1})
1	$25 \pm 1.2^*$	Extension	$+2.8 \pm 0.3$	0.356 ± 0.041
2	$35 \pm 1.2^*$	Flexion	-0.3 ± 0.01	-0.112 ± 0.052
3	81 ± 0.5	Extension	-10.3 ± 0.6	-0.811 ± 0.100
EMG	40 ± 0.6	—	—	—

*Changed with speed

Values are means \pm S.E.M. averaged over all nine speeds; $N=4$.

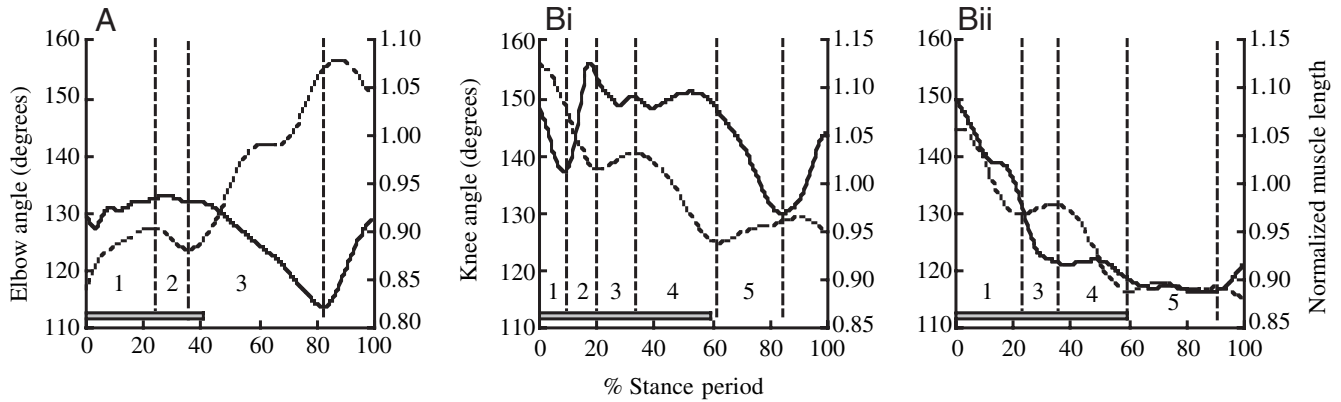


Fig. 2. (A) Representative patterns of normalized triceps muscle length (solid line) and elbow kinematics (dashed line) from one horse at 3.25 m s^{-1} . (Bi,ii) Representative patterns of normalized vastus muscle length and knee kinematics from two different horses at 3.25 m s^{-1} showing the two different strain patterns. Joint flexion is reflected in a decreasing angle. The dotted vertical lines, corresponding to inflections in the kinematics curve, show how the strain patterns were divided into phases. The numbers correspond to the phases described in the text. The vastus pattern (Bii), seen in two horses, lacked phase 2, seen in one horse at all speeds and in a second horse at low speeds. This phase is shown in Bi. Mean duration of EMG activity is indicated by horizontal, black bars.

triceps (Fig. 3) the strain rate increased with speed ($P=0.007$). In the vastus, strain rate (Fig. 3) remained constant (Table 2) in phases 1 ($P=0.306$) and 4 ($P=0.978$) and increased with speed during phases 3 ($P=0.034$) and 5 ($P=0.005$).

Electromyograms

The start of EMG activity in the triceps (Fig. 4A) preceded foot contact (termed 'phase advance') by an average of $0.036 \pm 0.001 \text{ s}$ and did not change with speed ($P=0.746$). In contrast, in the vastus, phase advance increased nearly linearly with speed ($P<0.0001$): at 2.5 m s^{-1} the onset of EMG activity coincided with the start of stance (phase advance = 0.003 ± 0.007) but at 4.5 m s^{-1} the EMGs started $0.069 \pm 0.018 \text{ s}$ before stance. The total duration of the EMG decreased with speed ($P<0.001$) in the triceps but stance duration also decreased with speed. Hence, the percentage of time the EMG was on during stance (Fig. 4B) did not change with speed in the triceps and averaged $40 \pm 1\%$ of stance. In the vastus, the total duration of the EMG did not change with speed ($P=0.274$). However, because of the increase in vastus phase advance, there was a decrease in the time during stance when the muscle was active that paralleled the decrease in time of stance. As a result, the percentage of time that the EMG was on during stance (Fig. 4B) also did not change with speed in the vastus ($P=0.084$) and averaged $59 \pm 2\%$. EMG duration in the triceps was shorter than in the vastus ($P=0.026$).

Integrated EMGs (Fig. 4C), normalized to the highest value for that muscle in each horse when trotting up an incline (Wickler et al., 2005), and including only the portion that occurred during stance when muscle force would be supporting the animal's weight, increased with speed in the triceps ($P=0.024$) and the vastus ($P=0.0001$). Average EMG phase advance was not different between the two muscles ($P=0.622$), averaging $0.036 \pm 0.001 \text{ s}$ in triceps and $0.042 \pm 0.005 \text{ s}$ in

vastus. However, this comparison was complicated by the fact that phase advance was nearly constant at 0.036 s in triceps and increased from 0 s to 0.069 s in the vastus.

Kinematics

The patterns and magnitudes of the joint kinematics of the fore- and hindlimbs during stance were similar to those reported by Back (1995a,b). The angle of the elbow at contact ($107 \pm 1.5^\circ$) did not vary with speed ($P=0.373$). The elbow extended until very near the end of stance ($\sim 97\%$) and the angle at maximum extension of the elbow ($146 \pm 1.2^\circ$) was not different with speed ($P=0.540$). Consequently, the range of motion of the elbow from contact to maximum extension ($39 \pm 0.8^\circ$) did not differ with speed ($P=0.754$). The timing of maximum extension of the elbow in the stance phase increased with speed ($P=0.0001$) from an average of $91.2 \pm 1.5\%$ of stance to $98.7 \pm 3.9\%$ of stance. Following maximum extension, the elbow flexed slightly but this was after the end of activity in the triceps.

The angle of the knee at contact ($153 \pm 1.0^\circ$) did not vary with speed ($P=0.837$). The knee underwent two cycles of flexion and extension during stance (see descriptions of the phases of muscle activity, above, for the timing of these events). The angle of the knee at the end of stance ($123 \pm 1.0^\circ$) was not affected by speed ($P=0.279$), and consequently, the range of motion of the knee from contact to end of stance ($30 \pm 0.5^\circ$) was not affected by speed ($P=0.384$). All of the angles reported above were not corrected for skin displacement errors so that they could be compared with data in a companion paper (Wickler et al., 2005) dealing with trotting up an incline – a condition for which corrections for skin displacement errors have not been reported. However, in general, correcting for skin displacement did not change the timing of kinematic events but decreased the range of motion of the knee by about 1.0° .

Stride parameters

Although stride period, stride frequency and stride length did not differ between the limbs, the two components of stride period (swing and stance) were different. As expected, stride period decreased with speed ($P=0.0001$, Fig. 5A), resulting in a linear increase in stride frequency with speed ($P=0.0001$). Stride length also increased with speed ($P=0.0001$). Whereas swing duration (Fig. 5B) did not vary with speed in the forelimb ($P=0.500$), it decreased monotonically with speed in the hindlimb ($P=0.0001$) and

averaged 10% shorter in the forelimb than in the hindlimb ($P=0.001$). Stance duration (Fig. 5C), also called time of contact (t_c), decreased with speed in both limbs ($P=0.0001$), but forelimb stance duration averaged 12% longer ($P=0.046$). Step length (L_c), the distance the body moves during time of contact (the product of stance duration and speed), increased with speed (Fig. 5D) in both limbs ($P=0.001$), but the longer time of contact in the forelimb produced a step length that was on average 11% larger ($P=0.046$). Because swing time did not decrease in the forelimb, time of contact decreased

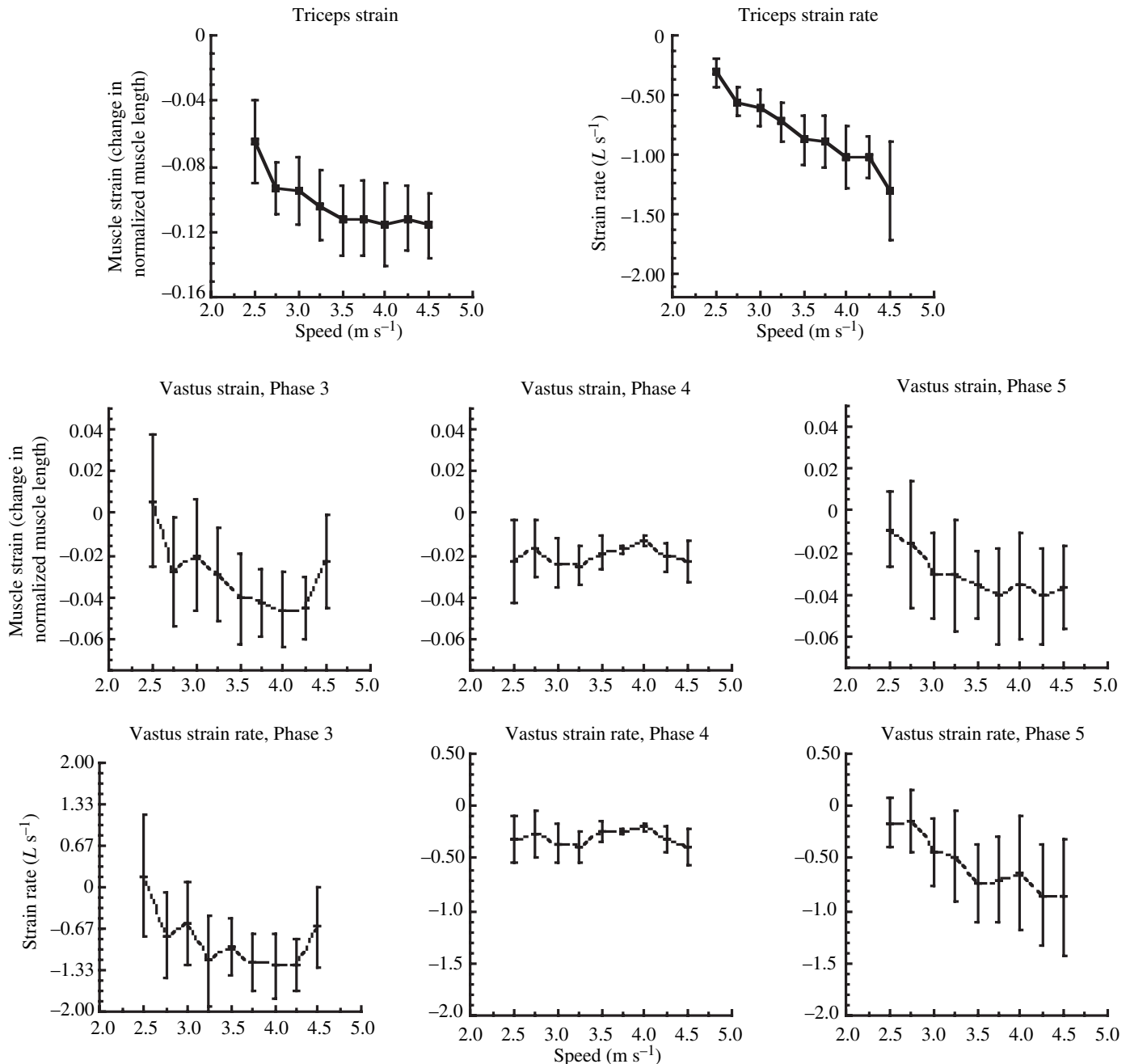


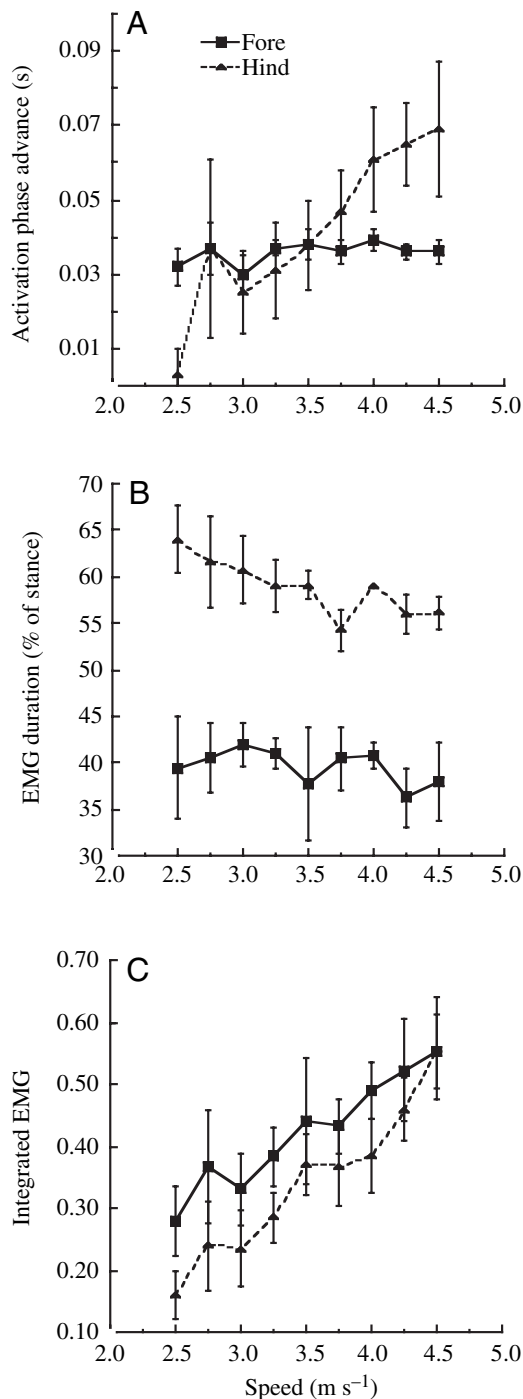
Fig. 3. Strain in triceps did not change as a function of speed during phase 3 but the strain rate was faster (more negative) at higher speeds. Values are means \pm S.E.M. from the four horses used in the study. In the vastus, strain increased with speed during phases 3 and 5, the two concentric phases, but not during phase 4. Also during both concentric phases in the vastus the strain rate was faster at higher speeds.

Table 2. *Vastus muscle timing, strain and strain rate during the four phases of muscle activity*

Phase	End of phase (% of stance)	Joint motion	Strain (% of fascicle length)	Strain rate (muscle lengths s ⁻¹)
1	18±0.3	Flexion	-10.0±0.3	-2.170±0.084
3	36±1.0*	Extension	-3.0±0.6*	-0.850±0.158*
4	61±1.4*	Flexion	-2.0±0.1	-0.300±0.022
5	84±0.7	Extension	-3.1±0.4*	-0.556±0.091*
EMG	59±1.0	-	-	-

*Changed with speed.

Values are means ± S.E.M. averaged over all nine speeds; N=4.



more with speed than in the hindlimb and the difference in time of contact between the two limbs decreased with speed ($P=0.001$) from an average of 0.048 s at 2.5 m s⁻¹ to 0.021 s at 4.5 m s⁻¹. The inverse of time of contact ($1/t_c$) increased linearly with speed (Fig. 5E) in both limbs ($P=0.0001$) but it was 10% lower in the forelimb ($P=0.044$). Duty factor, the proportion of time the foot is in contact with the ground during the entire stride (duration of stance divided by stride period), decreased with speed (Fig. 5F) in both limbs ($P=0.0001$), but the combination of a longer time of contact for the forelimb with identical stride periods produced a 12% larger duty factor ($P=0.015$).

Strain estimated from kinematics

The estimates of muscle moment arms (taken as the slope of the linear regression of muscle length on joint angle in radians) seem fairly reliable because the two variables were highly correlated (the average r^2 was 0.98 and the lowest was 0.96). For each of the phases, the length change of the fascicles at 3.5 m s⁻¹ was predicted from the product of the observed range of motion, as determined from the kinematics, and the empirically determined moment arm (triceps: 5.39±0.26 cm; vastus: 6.46±0.24 cm). This was converted to strain by dividing by the observed length of the muscle fascicles (triceps: 21.7±0.6 cm; vastus: 20.0±0.8 cm) and compared with the values observed by sonomicrometry (triceps, Table 3; vastus, Table 4). In the triceps the observed length change is the opposite of the prediction during phases 1 and 2. The difference during phase 1 is inconsistent: prediction of -6.4% (shortening because the joint is extending) compared with +3.0% (lengthening) observed. During phase 2 the differences are not nearly as large (e.g. +2.0% predicted because the joint is flexing and -0.7% observed). During phase 3 the observed and predicted

Fig. 4. EMG activity as a function of speed in the triceps (filled squares) vastus (filled triangles). (A) Activation phase advance did not change with speed in the triceps but it did in the vastus. (B) EMG burst duration, as a percentage of stance duration, did not change with speed in either muscle, averaging ~40% in the triceps and ~60% in the vastus. (C) Integrated EMG increased with speed in both muscles, indicating an increase in the volume of active muscle. Integrated EMG was normalized to the highest value measured in the same animal trotting up an incline (Wickler et al., 2005).

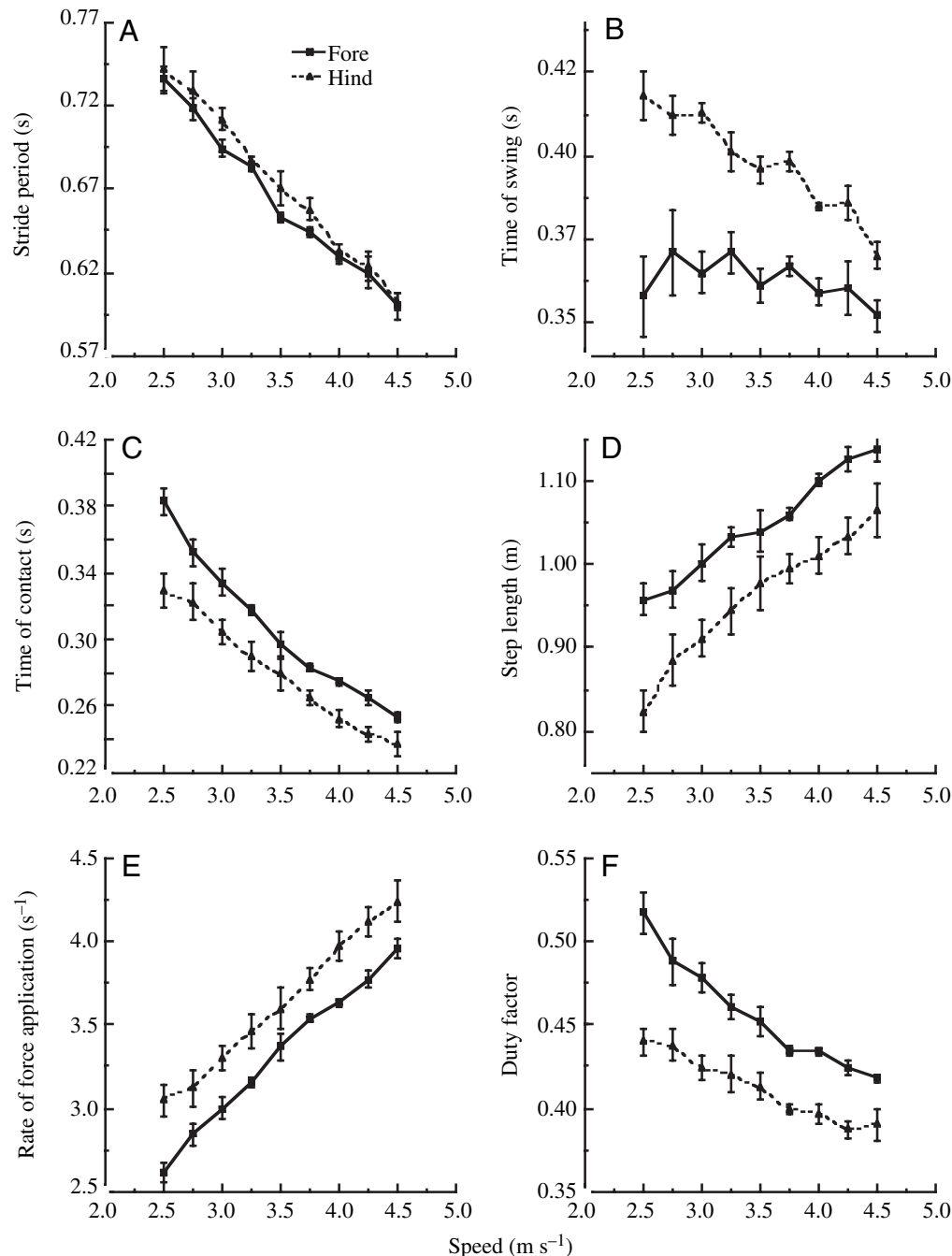


Fig. 5. Stride parameters in the forelimbs (filled squares) and hindlimbs (filled triangles) of horses trotting on the level. (A) Stride period decreased with speed but was the same in both limbs. There were differences between the limbs for all of the other parameters (B–F). (B) Swing time is shorter for the forelimb and does not change with speed but it decreases with speed in the hindlimb. (C) Time of contact is longer for the forelimb and decreases with speed in both limbs. As a consequence of the longer time of contact in the forelimb (C), step length (D) is longer in the forelimb, the rate of force application (E) is lower, and duty factor (F) is larger. Notice that duty factor is greater than 0.5 in the forelimb at a speed of 2.5 m s^{-1} .

shortening are very similar (-9.7% predicted and -11.3% observed).

The differences are more pronounced in the vastus (Table 4). During the two concentric phases in the vastus (phases 3 and

5), the amount of strain observed via sonomicrometry is about 3.8 times the amount predicted from kinematics (-1.9% predicted and -7.2% observed). However, during phase 4 (the expected eccentric phase near mid-stance) the muscle was predicted to lengthen by 3.2% but it actually shortened by 3.7% . In both muscles the largest difference between predicted and observed values occurred during phase 1 (triceps: -6.4% vs $+3.0$; vastus: $+2.9\%$ vs -9.4% ; predicted and observed, respectively).

Reliability of sonomicrometry data

The variance ratio was used to assess repeatability of the temporal muscle waveform data obtained with sonomicrometry for full strides. It is the ratio of the average variance at regular points along the waveforms for consecutive strides to the total variance of all points in these strides. This ratio is zero if there is perfect repeatability of the strides since each point is exactly the same for each stride, i.e., the variance at each point is zero. Likewise, the ratio is one if the waveforms are completely random (no repeatability) since the average for variances at each point should approach the variance of all points. Normalized vastus muscle length data from two consecutive years in two different horses are presented in Fig. 6. These two figures represent the range of variance ratios obtained in this analysis. The reliability (Table 5), which is the variance ratio for ten consecutive strides in one trial averaged 0.029 (range 0.009 – 0.08). Constancy (Table 6), which is the variance ratio over the 2 years, averaged 0.149 (0.027 – 0.250). An ANOVA indicated that reliability and constancy did not vary significantly between speeds or years.

Table 3. *Triceps comparison of observed strain at 3.5 m s⁻¹ with strain predicted from kinematics*

Phase of activity	Joint range of motion (degrees)	Predicted strain (% of fascicle length)	Observed strain (% of fascicle length)	<i>P</i>
1	14.7±1.9	-6.4±0.8	3.0±1.1	0.007
2	-2.3±1.0	2.0±0.4	-0.7±0.4	0.023
3	22.3±3.2	-9.7±1.4	-11.3±2.1	0.616

Values are mean ± S.E.M.; *N*=4.

P value is for paired *t*-test of predicted and observed.

Table 4. *Vastus comparison of observed strain at 3.5 m s⁻¹ with strain predicted from kinematics*

Phase of activity	Joint range of motion (degrees)	Predicted strain (% of fascicle length)	Observed strain (% of fascicle length)	<i>P</i>
1	-10.4±1.4	2.9±0.4	-9.4±1.8	0.008
3	3.3±0.6	-0.9±0.2	-4.5±1.6	0.086
4	-11.6±1.4	3.2±0.4	-3.7±1.9	0.030
5	3.5±0.7	-1.0±0.2	-2.7±1.5	0.282
3+4+5		1.3±0.2	-11.0±2.0	
3+5		-1.9±0.2	-7.2±1.8	

Values are mean ± S.E.M.; *N*=4.

Discussion

This study was undertaken to test several hypotheses regarding the function of extensor muscles that act at two serially homologous proximal joints, but which exhibit different kinematic patterns. These hypotheses were: (1) the triceps shortens because the elbow extends during most of stance; (2) the vastus will exhibit two cycles of eccentric activity followed by concentric activity; (3) the amount of strain in both muscles increases with speed because step length increases with speed; (4) strain rate in both muscles increases with speed because the duration of stance phase decreases with speed; (5) the increase in strain rate will necessitate an increase in the volume of active muscle as indicated by an increase in the integrated EMG. We also compared the strain patterns observed in the vastus in two consecutive years to evaluate the reproducibility of strain patterns in individuals.

While the triceps did shorten during stance, perhaps the most interesting result of this study was the fact that, in both extensor muscles, periods of joint flexion were not associated with eccentric activity. The strain pattern of the triceps, a parallel-fibered extensor of the elbow, can be considered to be composed of three phases on the basis of elbow kinematics (Fig. 2). The second of these phases is an eccentric phase during which the elbow flexes by 2.3°. The triceps would be expected to lengthen by 1%, but it actually shortened 0.7% (values that are significantly different). The vastus, a unipennate extensor of the knee exhibits a complex strain pattern (Fig. 2) including a phase of flexion (phase 4) of 11.5° during which the muscle might be expected to lengthen by 3.2% but actually shortened by 3.7%. In both muscles, these phases of activity occurred when the muscle was

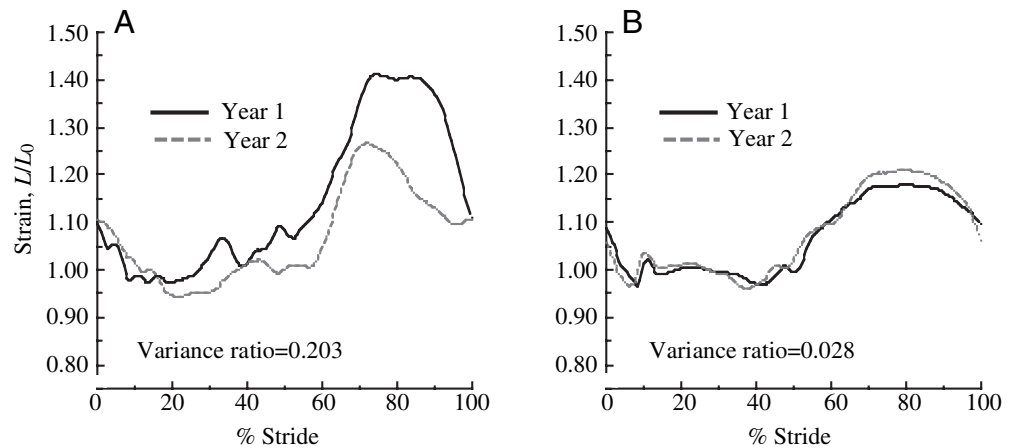
unambiguously active, starting at about 20% of stance and ending just prior to the end of EMG activity (Tables 1 and 2).

For neither muscle does it seem likely that the difference between observed and predicted strain during the eccentric phase can be completely explained by strain in an external tendon. First, the triceps has no external tendon to account for this difference. Secondly, the amount of calculated strain in the tendon across the knee seems unreasonable. For example, during the initial phase of activity and knee flexion (phase 1), when the muscle was predicted to lengthen by 2.9% it actually shortened by 9.4%. The total difference (12.3%) amounts to about 2.5 cm in the vastus, which has an average fascicle length of about 20 cm. The total length of the middle patellar tendon is about 12 cm, so if all of this difference were absorbed by the muscle's tendon, the strain would be about 20% – far greater than any tendon can sustain without breaking (Shadwick, 1990). Some of the difference between predicted and observed strain may be due to the fact that measurements were made on different animals (tendon lengths, muscle moments and fascicle lengths were calculated from cadavers). An additional factor that contributes to some uncertainty in the present prediction of strain from kinematics and morphometrics is the fact that there is a range of fascicle lengths in both muscles. However, some confidence in these calculations is supported by the observation that in both muscles there were phases of concentric activity (triceps phase 3; vastus phases 3 and 5) when the muscle shortened by values that are not significantly different than predicted from kinematics (Tables 3 and 4).

If the external tendon cannot account for all of the difference between length changes of the fascicles and the length change

Fig. 6. Comparisons, in two horses trotting, of the patterns of vastus fascicle strain over the whole stride period at 3.0 m s^{-1} in two different years. Implants were placed in approximately the same area of the vastus muscle in each animal. Each strain pattern represents the mean of ten consecutive strides. The variance ratio is a quantitative measure of the similarity of two waveforms. It can range from a value of zero (identical) to 1.0 (no similarity). These two horses represent the range of constancy (variance ratios in two different years) obtained in this study:

(A) variance ratio=0.203 and (B) variance ratio=0.028. Constancy is the term used to describe the similarity between two different experiments and reliability the similarity between successive strides from the same experiment. Constancy and reliability are reported in Tables 5 and 6 for three horses and four speeds. These two horses exhibit the two different patterns of vastus strain shown in Fig. 2. Focusing on the stance phase (up to ~40% of stride) it can be seen that the two patterns are reasonably similar in the two different years.



of the muscle–tendon unit, then some of the difference must be due to strain in the aponeuroses within the muscle. There is strong evidence that aponeuroses are an important component of a muscle's series elasticity (Fukashiro et al., 1995; Huijing and Ettema, 1988; Maganaris et al., 2001; Monti et al., 2003; Scott and Loeb, 1995) but there is conflicting evidence as to whether there are differences in the amount of strain in tendons and aponeuroses and whether there is heterogeneity of strain within the aponeuroses (Finni et al., 2003; Maganaris, 2002; Maganaris and Paul, 2000b; Muramatsu et al., 2001). Maximum reported strain values for tendons are usually around 5–7% and values for aponeuroses range from 2% to 14.3% (Muramatsu et al., 2001). However, in some cases the tendon is reported to be more compliant than the aponeurosis (Magnusson et al., 2003). While there have been no direct studies of series elasticity in the equine vastus lateralis, there is substantial strain in the elastic elements of the human vastus lateralis (Bojsen-Moller et al., 2003; Fukunaga et al., 2002; Ichinose et al., 2000; Muraoka et al., 2001) and at least one study (Finni and Komi, 2002) supports the idea that, in muscles in general, the aponeurosis may be more compliant than the tendon (Kawakami and Lieber, 2000). In the absence of data on the compliance of the series elasticity of the equine vastus lateralis, we cannot unambiguously account for the differences in observed and predicted strain.

While both muscles probably store some strain energy in series elastic elements such as the aponeurosis, it is striking that this energy does not appear to be recovered through subsequent extension of the joints. The end of vastus activity is estimated to be at about 75% of stance and the timing of maximum flexion changes from 63% of stance at 2.5 m s^{-1} to 72% of stance at 4.5 m s^{-1} , indicating that the joint extends for only a short period of time at the end of muscle activity when energy stored in stretched elastic structures might be recovered. During this time the knee extended only slightly (an average of 4°), suggesting that some elastic energy may have

been recovered from the stretched patellar tendon and the muscle's aponeurosis.

These data are consistent with the hypothesized triceps strain pattern but not the hypothesized vastus strain pattern. The total strain in the triceps (Table 1) during phases 2 and 3 (-10.6%) is very similar to that observed in the vastus (Table 2) during phases 3 through 5 (-8.1%), despite the fact that the two joints have very different actions: the elbow extending and the knee flexing during limb support. The fact that the vastus shortens even though the knee flexes during most of stance is a good example of the importance of series elasticity as a mechanical buffer (i.e. protecting against potentially damaging rapid stretch of muscle fibers to longer lengths) during potentially eccentric activity (Fukunaga et al., 2001; Griffiths, 1991; Reeves and Narici, 2003).

The results of this study can also be used to test Taylor's 'economical force hypothesis' (Taylor, 1994) that the muscles responsible for supporting the body during the stance phase of the stride function largely in an isometric fashion. Although both muscles have complex strain patterns, our results generally support Taylor's hypothesis. The net amount of strain observed in the horse triceps (-10.6%) and vastus (-8.1%) is only slightly more than the ~6% shortening strain reported for more distal, pennate muscles in other species. The lateral gastrocnemius of wild turkeys (Roberts et al., 1997) shortened by $6.6 \pm 1.9\%$ of resting length. In tammar wallabies (Biewener et al., 1998), the plantaris shortened less than 2%, and the lateral gastrocnemius less than 6%.

The strain in the horse vastus (-8.1%) is somewhat less than the amount of strain reported in extensors of other species. In trotting dogs, the amount of shortening in the vastus lateralis (Carrier et al., 1998) ranged from 8.7% to 20.1%, whereas the semimembranosus shortened by 4.5% to 6.6%. The biceps femoris (a hip extensor) shortened by an average of 27% during trotting in rats (Gillis and Biewener, 2001), whereas the vastus lateralis lengthened by 8% to 16% while electrically

Table 5. *Constancy of sonomicrometry measured at four speeds over 2 years in three horses*

Horse	Speed (m s ⁻¹)				
	2.5	3.0	3.5	4.0	All
Charlie	0.209	0.181	0.223	0.250	0.215
Reign	0.176	0.203	0.226	0.187	0.198
Royalty	0.046	0.028	0.032	0.027	0.033
All	0.143	0.137	0.160	0.155	0.149

Constancy is the variance ratio calculated over 10 strides/trial during two trials.
Overall s.d. = 0.023 (based on RM-ANOVA MSE).

active during stance. It is remarkable that, although the kinematics of the knee are relatively similar in the rat, dog and horse, the strain pattern in the rat vastus differs substantially. In all three species, the knee flexed during at least the first half of stance while the muscle was electrically active. In the rat, the vastus lengthened continuously by about 10% during this period of time.

Strain vs speed

The results of this study support, in part, our third hypothesis that muscle strain should increase with speed. Although concentric (shortening) strain in the triceps did not change with speed, there was a significant increase during both concentric phases in the vastus. For the triceps this was not surprising in view of the fact that the range of motion of the elbow, from contact to maximum extension, did not change with speed. However, the range of motion of the knee, from contact to maximum flexion, did increase with speed (by 5°), and as a knee extensor, the vastus might have been expected to shorten less at higher speeds. In the tammar wallaby (Biewener et al., 1998) fascicle strain within the plantaris and gastrocnemius muscles did not increase with hopping speed between 2.5 and 6.0 m s⁻¹. In the report on the turkey (Roberts, 1997) it is not clear whether the increased muscle work with speed (fig. 3 in Roberts, 1997) was due to increased strain, increased force development, or both. None of the other *in vivo* studies of muscle function (Carrier et al., 1998; Gillis and Biewener, 2001; Gregersen et al., 1998) examined the effect of speed within a single gait.

Strain rate

Our results support the fourth hypothesis that strain rate would increase with speed. In both muscles, strain rate increased with speed during the concentric phases: triceps phase 3 (Fig. 3) and vastus phases 3 and 5 (Fig. 3). An increased muscle strain rate with speed might result from the following three conditions: the same amount of strain occurring in less time, more strain occurring in the same time, or a combination of more strain and less time. In the triceps the amount of strain did not change with speed but the absolute duration of phase 3 did decrease. In the vastus there was increased strain with speed during both phases. The

Table 6. *Reliability of sonomicrometry measured at four speeds in two different years in three horses*

Horse	Year	Speed (m s ⁻¹)				
		2.5	3.0	3.5	4.0	All
Charlie	1	0.053	0.023	0.055	0.033	0.041
	2	0.084	0.022	0.042	0.018	0.042
Reign	1	0.014	0.023	0.028	0.020	0.021
	2	0.054	0.081	0.021	0.018	0.043
Royalty	1	0.020	0.009	0.009	0.010	0.012
	2	0.026	0.015	0.011	0.008	0.015
All		0.042	0.028	0.029	0.018	0.029

Reliability is the variance ratio calculated over 10 strides during one trial.
Overall s.d. = 0.023 (based on RM-ANOVA MSE).

absolute duration of phase 3 did not change with speed but it decreased for phase 5. Thus, considering the three concentric phases in these two muscles, the increased strain rate resulted from all three combinations of the two variables. The fascicle-shortening rate observed during phase 3 in the triceps (0.8 lengths s⁻¹) was much less than the long head of triceps in the dog (1.4 to 3.6 lengths s⁻¹; Gregersen et al., 1998). The horse vastus strain rates during phases 3 and 5 (0.6 to 0.8 lengths s⁻¹) were also slower than those reported for the dog vastus (1.26–2.62 lengths s⁻¹; Carrier et al., 1998), but similar to those of the dog semimembranosus (0.66–1.07 lengths s⁻¹; Gregersen et al., 1998). Because the strain rates reported in previous sonometric studies were obtained from longer, sustained periods of concentric activity, rather than from shorter, kinematically defined, components of stance, the values may not be directly comparable. Gillis and Biewener (2001) reported that during trotting the strain rate of the rat biceps femoris increased with speed, averaging about 2 fascicle lengths s⁻¹ (at a speed of 0.64 m s⁻¹; see fig. 8A in Gillis and Biewener, 2001). In the rat, the vastus lengthened during stance and the rate of lengthening increased with speed when walking and trotting data are considered, averaging about 1.5–2.0 lengths s⁻¹ (at a speed of 0.64 m s⁻¹; see fig. 8B in Gillis and Biewener, 2001). The ankle extensors of cats have also been reported to exhibit increased strain rates as a function of speed (Gregor et al., 1988; Prilutsky et al., 1996a; Whiting et al., 1984).

EMG – phase advance, duration and IEMG

EMG activity of antigravity muscles generally ends part way through stance. In the present study of the horse, EMG activity ended at 40% of stance in the triceps and at 59% of stance in the vastus. In the rat (see fig. 3 in Gillis and Biewener, 2001) EMG activity during trotting ended at about 51% of stance in the biceps femoris and at about 63% of stance in the vastus. In the tammar wallaby, EMG activity continued for 67% and 71% of the period of force development (plantaris and lateral

gastrocnemius, respectively) and tended to decrease with speed (Biewener and Baudinette, 1995).

In the triceps, EMG activity commenced an average of 0.036 s before initial hoof contact (phase advance) and this did not change with speed. In the vastus, EMG phase advance changed with speed from about zero seconds at low speeds to 0.068 s at the highest trotting speed studied, averaging 0.042 s. In the rat (Gillis and Biewener, 2001) there was a small EMG phase advance in the biceps femoris but not the vastus lateralis, which did not change significantly with gait, suggesting that it did not change with speed. A phase advance of about 0.050 s was also observed in the dog semimembranosus during trotting (Gregersen et al., 1998) as well as in the tammar wallaby plantaris and gastrocnemius (mean: 0.042 s; Biewener and Baudinette, 1995).

Muscle recruitment with speed

As predicted by hypothesis five, IEMG increased with speed in both muscles (Fig. 4C) indicating that a greater volume of muscle was actively recruited at higher speeds. Gillis and Biewener (2001) reported that EMG intensity (determined from relative average spike amplitude of the rectified EMG signal) increased with speed in both the biceps and the vastus of the rat. Because of the force–velocity relationship, an increase in the volume of active muscle would be necessary if an increase in strain rate required an increase in the number of fibers needed to produce the same force. The increased IEMG might be explained by the speed-related increase in strain rate of both muscles. However, an additional factor that should require an increase in forelimb muscle force is the need to exert greater force on the ground at faster trotting speeds. The peak vertical ground reaction force acting on the equine forelimb increases about 23% (Dutto et al., 2004b) and peak elbow moment about 60% (D. F. Hoyt, S. J. Wickler, H. M. Clayton, E. A. Cogger and D. J. Dutto, unpublished data) as speed increases from 2.5 to 4.5 m s⁻¹. While the ground reaction force under the hindlimb was not found to increase over the same range of trotting speeds (Dutto et al., 2004b), peak knee moment near the middle of stance increased 18% (D. J. Dutto, D. F. Hoyt, H. M. Clayton, E. A. Cogger and S. J. Wickler, unpublished data). Thus, the observed increase in IEMG of both muscles appears to be consistent with increased force development, reflecting an increased volume of active muscle resulting from either a force–velocity effect of increased strain rate or because of increased muscle force requirements at faster speed, or both.

Stride parameters

Duty factor differed between limbs and was longer in the forelimb than in the hindlimb. Interestingly, at 2.5 m s⁻¹ (a speed that is more than 0.5 m s⁻¹ above the walk-trot transition speed) duty factor averaged 0.517 in the forelimb but 0.445 in the hindlimb, indicating that there is an aerial phase between the hindlimbs but not the forelimbs. It is well documented that bipeds run with duty factors greater than 0.5 (Gatesy, 1999; Gatesy and Biewener, 1991), but it is surprising to find a

quadruped trotting without an aerial phase, and it is even more surprising that there is an aerial phase between the hindlimbs but not the forelimbs. Two separate studies from our laboratory (Catterfeld et al., 2002; Dutto et al., 2004a) have shown the same phenomenon during over-ground locomotion.

Repeatability of sonomicrometry data

Our results show that sonomicrometry yields highly consistent data when comparing a series of consecutive strides in the same animal and when comparing separate studies of the same animals in different years. The reliability (Table 5), which is the variance ratio for ten consecutive strides in one trial averaged 0.029 and constancy (Table 6), which is the variance ratio over two (or more) trials, averaged 0.149. ANOVA indicated that reliability and constancy did not vary significantly between years or speeds. This is the first published evaluation of the constancy of sonomicrometry data and indicates, even when data are collected from different implants in approximately the same part of a muscle of the same animal, the patterns are quite similar.

Conclusion

In this study we tested several hypotheses regarding the function of the lateral triceps and vastus lateralis, two relatively long-fibered proximal limb extensor muscles. Our data indicated that the triceps, a muscle with no external tendon, shortened and helped cause the elbow to extend during most of stance. The vastus, however, shortened in spite of the fact that the knee flexed during stance, suggesting substantial stretch of its series elasticity. The difference in the length change of the fascicles and the muscle-tendon unit exceeded what could be accounted for by tendon strain alone, suggesting there must be a large amount of strain in the aponeurosis through which the muscle transmits its force to the patellar tendon. The amount of strain in the triceps did not change with speed, but in the vastus strain increased during both of the concentric phases of muscle activity. The strain rate and the integrated EMG in both muscles increased with speed.

We gratefully acknowledge the assistance of Sylvia Magaña and Robert McGuire in data collection and reduction. Horses were trained by Charnelcie Lewis and Deborah Mead with the help of Shannon Garcia. We would also like to acknowledge Holly M. Greene, MS, Equine Research Technician, for providing technical support. This study was funded by N.I.H. grant S06 GM053933 to D.F.H. and S.J.W.

References

- Alexander, R. M., Maloiy, G. M. O., Hunter, B., Jayes, A. S. and Nturibi, J. (1979). Mechanical stresses in fast locomotion of buffalo (*Syncerus caffer*) and elephant (*Loxodonta africana*). *J. Zool.* **189**, 135-144.
- Back, W., Barneveld, A., van Weeren, P. R. and van den Bogert, A. J. (1993). Kinematic gait analysis in equine carpal lameness. *Acta Anat.* **146**, 86-89.
- Back, W., Schamhardt, H. C., Savelberg, H. H. C. M., van den Bogert, A. J., Bruin, G., Hartman, W. and Barneveld, A. (1995a). How the horse

- moves: 1. Significance of graphical representations of equine forelimb kinematics. *Equine Vet. J.* **27**, 31-38.
- Back, W., Schamhardt, H. C., Savelberg, H. H. C. M., van den Bogert, A. J., Bruin, G., Hartman, W. and Barneveld, A.** (1995b). How the horse moves: 2. Significance of graphical representations of equine hind limb kinematics. *Equine Vet. J.* **27**, 39-45.
- Biewener, A. A. and Baudinette, R. V.** (1995). In vivo muscle force and elastic energy storage during steady-speed hopping of tammar wallabies, (*Macropus eugenii*). *J. Exp. Biol.* **198**, 1829-1841.
- Biewener, A. A., Konieczynski, D. D. and Baudinette, R. V.** (1998). In vivo muscle force-length behavior during steady-speed hopping in tammar wallabies. *J. Exp. Biol.* **201**, 1681-1694.
- Bojsen-Moller, F., Hansen, P., Aagaard, P., Kjaer, M. and Magnusson, S. P.** (2003). Measuring mechanical properties of the vastus lateralis tendon-aponeurosis complex in vivo by ultrasound imaging. *Scand. J. Med. Sci. Sports* **13**, 259-265.
- Carrier, D. R.** (1996). Function of the intercostal muscles in trotting dogs: ventilation or locomotion? *J. Exp. Biol.* **199**, 1455-1465.
- Carrier, D. R., Gregersen, C. S. and Silverton, N. A.** (1998). Dynamic gearing in running dogs. *J. Exp. Biol.* **201**, 3185-3195.
- Catterfield, G. E., Dutto, D. J., Wickler, S. J. and Hoyt, D. F.** (2002). Deviant forces in trotting horses. *Int. Comp. Biol.* **42**, 1207.
- Dutto, D. J., Hoyt, D. F., Clayton, H., Cogger, E. A. and Wickler, S. J.** (2004a). Moments and power generated by the horse (*Equus caballus*) hind limb during jumping. *J. Exp. Biol.* **207**, 667-674.
- Dutto, D. J., Hoyt, D. F., Cogger, E. A. and Wickler, S. J.** (2004b). Ground reaction forces in horses trotting up an incline and on the level over a range of speeds. *J. Exp. Biol.* **207**, 3507-3514.
- Ettema, G. J. C.** (1996). Mechanical efficiency and efficiency of storage and release of series elastic energy in skeletal muscle during stretch-shorten cycles. *J. Exp. Biol.* **199**, 1983-1997.
- Ettema, G. J. C.** (2001). Muscle efficiency: the controversial role of elasticity and mechanical energy conversion in stretch-shortening cycles. *Eur. J. Appl. Physiol.* **85**, 457-465.
- Farley, C. T., Glasheen, J. and McMahon, T. A.** (1993). Running springs speed and animal size. *J. Exp. Biol.* **185**, 71-86.
- Finni, T., Hodgson, J. A., Lai, A. M., Edgerton, V. R. and Sinha, S.** (2003). Nonuniform strain of human soleus aponeurosis-tendon complex during submaximal voluntary contractions in vivo. *J. Appl. Physiol.* **95**, 829-837.
- Finni, T. and Komi, P. V.** (2002). Two methods for estimating tendinous tissue elongation during human movement. *J. Appl. Biomech.* **18**, 180-188.
- Fukashiro, S., Itoh, M., Ichinose, Y., Kawakami, Y. and Fukunaga, T.** (1995). Ultrasonography gives directly but noninvasively elastic characteristic of human tendon in vivo. *Eur. J. Appl. Physiol.* **71**, 555-557.
- Fukunaga, T., Kawakami, Y., Kubo, K. and Kanehisa, H.** (2002). Muscle and tendon interaction during human movements. *Exerc. Sport Sci. Rev.* **30**, 106-110.
- Fukunaga, T., Kubo, K., Kawakami, Y., Fukashiro, S., Kanehisa, H. and Maganaris, C. N.** (2001). In vivo behavior of human muscle tendon during walking. *Proc. R. Soc. Lond. B* **268**, 229-233.
- Gans, C. and DeVree, F.** (1987). Functional bases of fiber length and angulation in muscle. *J. Morphol.* **192**, 63-85.
- Gatesy, S. M.** (1999). Guinea-fowl hind-limb function. I Cineradiographic analysis and speed effects. *J. Morphol.* **240**, 115-125.
- Gatesy, S. M. and Biewener, A. A.** (1991). Bipedal locomotion: effects of speed, size, and limb posture in birds and humans. *J. Zool.* **224**, 127-147.
- Gillis, G. B. and Biewener, A. A.** (2001). Hindlimb muscle function in relation to speed and gait: *in vivo* patterns of strain and activation in hip and knee extensor of the rat (*Rattus norvegicus*). *J. Exp. Biol.* **204**, 2717-2731.
- Gregersen, C. S., Silverton, N. A. and Carrier, D. R.** (1998). External work and potential for elastic storage at the limb joints of running dogs. *J. Exp. Biol.* **201**, 3197-3210.
- Gregor, R. J., Roy, R. R., Whiting, W. C., Hodgson, J. A. and Edgerton, V. R.** (1988). Mechanical output of the cat soleus during treadmill locomotion: in vivo vs in situ characteristics. *J. Biomech.* **21**, 721-732.
- Griffiths, R. I.** (1991). Shortening of muscle fibers during stretch of the active cat medial gastrocnemius muscle the role of tendon compliance. *J. Physiol. Lond.* **436**, 219-236.
- Hershler, C. and Milner, M.** (1978). An optimality criterion for processing electromyographic (EMG) signals relating to human locomotion. *IEEE Trans. Biomed. Engin.* **25**, 413-420.
- Hof, A. L., Geelen, B. A. and van den Berg, J. W.** (1983). Calf muscle moment, work and efficiency in level walking; role of series elasticity. *J. Biomechanics* **16**, 523-537.
- Hoyt, D. F., Molinari, M., Wickler, S. J. and Cogger, E. A.** (2002). Effect of trotting speed, load and incline on hind limb stance-phase kinematics. *Equine Vet. J. Suppl.* **34**, 330-336.
- Hoyt, D. F., Wickler, S. J. and Cogger, E. A.** (2000). Time of contact and step length: The effect of limb length, running speed, load carrying and incline. *J. Exp. Biol.* **203**, 221-227.
- Huijing, P. A. and Ettema, G. J. C.** (1988). Length-force characteristics of aponeurosis in passive muscle and during isometric and slow dynamic contractions of rat gastrocnemius-muscle. *Acta Morphol. Neerlando-Scand.* **26**, 51-62.
- Ichinose, Y., Kawakami, Y., Ito, M., Kanehisa, H. and Fukunaga, T.** (2000). In vivo estimation of contraction velocity of human vastus lateralis muscle during 'isokinetic' action. *J. Appl. Physiol.* **88**, 851-856.
- Ito, L., Kawakami, Y., Ichinose, Y., Fukashiro, S. and Fukunaga, T.** (1998). Nonisometric behavior of fascicles during isometric contractions of a human muscle. *J. Appl. Physiol.* **85**, 1230-1235.
- Kawakami, Y. and Lieber, R. L.** (2000). Interaction between series compliance and sarcomere kinetics determines internal sarcomere shortening during fixed-end contraction. *J. Biomech.* **33**, 1249-1255.
- Kram, R. and Taylor, C. R.** (1990). Energetics of running: a new perspective. *Nature* **346**, 265-267.
- Maganaris, C. N.** (2002). Tensile properties of in vivo human tendinous tissue. *J. Biomech.* **35**, 1019-1027.
- Maganaris, C. N., Kawakami, Y. and Fukunaga, T.** (2001). Changes in aponeurotic dimensions upon muscle shortening: in vivo observations in man. *J. Anat.* **199**, 449-456.
- Maganaris, C. N. and Paul, J. P.** (2000a). In vivo human tendinous tissue stretch upon maximum muscle force generation. *J. Biomech.* **33**, 1453-1459.
- Maganaris, C. N. and Paul, J. P.** (2000b). Load-elongation characteristics of in vivo human tendon and aponeurosis. *J. Exp. Biol.* **203**, 751-756.
- Magnusson, S. P., Hansen, P., Aagaard, P., Brond, J., Dyhre-Poulsen, P., Bojsen-Moller, J. and Kjaer, M.** (2003). Differential strain patterns of the human gastrocnemius aponeurosis and free tendon, in vivo. *Acta Physiol. Scand.* **177**, 185-195.
- Marsh, R. L., Ellerby, D. J., Carr, J. A., Havalee, T. H. and Buchanan, C. I.** (2004). Partitioning the energetics of walking and running: Swinging the limbs is expensive. *Science* **303**, 80-83.
- Monti, R. J., Roy, R. R., Zhong, H. and Edgerton, V. R.** (2003). Mechanical properties of rat soleus aponeurosis and tendon during variable recruitment in situ. *J. Exp. Biol.* **206**, 3437-3445.
- Muramatsu, T., Muraoka, T., Kawakami, Y. and Fukunaga, T.** (2002). Superficial aponeurosis of human gastrocnemius is elongated during contraction - implications for modeling muscle-tendon unit. *J. Biomech.* **35**, 217-223.
- Muramatsu, T., Muraoka, T., Takeshita, D., Kawakami, Y., Hirano, Y. and Fukunaga, T.** (2001). Mechanical properties of tendon and aponeurosis of human gastrocnemius muscle in vivo. *J. Appl. Physiol.* **90**, 1671-1678.
- Muraoka, T., Kawakami, Y., Tachi, M. and Fukunaga, T.** (2001). Muscle fiber and tendon length changes in the human vastus lateralis during slow pedaling. *J. Appl. Physiol.* **91**, 2035-2040.
- Prilutsky, B. I., Herzog, W. and Allinger, T. L.** (1996a). Mechanical power and work of cat soleus, gastrocnemius and plantaris muscles during locomotion - possible functional-significance of muscle design and force patterns. *J. Exp. Biol.* **199**, 801-814.
- Prilutsky, B. I., Herzog, W., Leonard, T. R. and Allinger, T. L.** (1996b). Role of the muscle belly and tendon of soleus, gastrocnemius, and plantaris in mechanical energy-absorption and generation during cat locomotion. *J. Biomech.* **29**, 417-434.
- Reeves, N. D. and Narici, M. V.** (2003). Behavior of human muscle fascicles during shortening and lengthening contractions in vivo. *J. Appl. Physiol.* **95**, 1090-1096.
- Roberts, T. J.** (2002). The integrated function of muscles and tendons during locomotion. *Comp. Biochem. Physiol.* **133A**, 1087-1099.
- Roberts, T. J., Marsh, R. L., Weyand, P. G. and Taylor, C. R.** (1997). Muscular force in running turkeys: The economy of minimizing work. *Science* **275**, 1113-1114.
- Scott, S. H. and Loeb, G. E.** (1995). Mechanical properties of aponeurosis and tendon of the cat soleus muscle during whole-muscle isometric contractions. *J. Morphol.* **224**, 73-86.
- Shadwick, R. E.** (1990). Elastic energy storage in tendons: mechanical differences related to function and age. *J. Appl. Physiol.* **68**, 1033-1040.

1190 D. F. Hoyt and others

- Taylor, C. R.** (1994). Relating mechanics and energetics during exercise. *Advances Vet. Sci. Comp. Med.* **38A**, 181-214.
- Tokuriki, M. and Aoki, O.** (1995). Electromyographic activity of the hindlimb muscles during the walk, trot and canter. *Equine Vet. J. Suppl.* **18**, 152-155.
- van Weeren, P. R., van den Bogert, A. J. and Barneveld, A.** (1992). Correction models for skin displacement in equine kinematic gait analysis. *Equine Vet. Sci.* **12**, 178-192.
- Walmsley, B., Hodgson, J. A. and Burke, R. E.** (1978). Forces produced by medial gastrocnemius and soleus muscles during locomotion in freely moving cats. *J. Neurophysiol.* **41**, 1203-1213.
- Whiting, W. C., Gregor, R. J., Roy, R. R. and Edgerton, V. R.** (1984). A technique for estimating mechanical work of individual muscles in the cat during treadmill locomotion. *J. Biomech.* **17**, 685-694.
- Wickler, S. J., Hoyt, D. F., Biewener, A. A., Cogger, E. A. and De La Paz, K. L.** (2005). In vivo muscle function vs speed. II. Muscle function trotting up an incline. *J. Exp. Biol.* **208**, 1191-1200.

