

In vivo muscle function vs speed

II. Muscle function trotting up an incline

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Summary

Different locomotor tasks, such as moving up or down grades or changing speed, require that muscles adjust the amount of work they perform to raise or lower, accelerate or decelerate the animal's center of mass. During level trotting in the horse, the triceps had shortening strains of around 10.6% while the vastus shortened 8.1% during the stance phase. Because of the 250% increase in metabolic rate in horses trotting up a 10% incline which is, presumably, a result of the increased requirement for mechanical work, we hypothesized that muscle strain during trotting would be increased in both the triceps and the vastus over that observed when trotting on the level. Because times of contact are similar in level and incline trotting, we also hypothesized that strain rates of these muscles would be increased, accompanied by an increase in EMG activity. We examined the lateral head of the

triceps and the vastus lateralis while trotting up a 10% incline (5.7°) over a range of speeds. The triceps shortened by 18% compared with 10.6% shortening on the level, and the vastus shortened by 18.5% compared with 8.1% on the level. The increased shortening velocities that were observed in both muscles probably reduced the force that any given set of activated muscle fibers could produce. If this pattern held for other limb muscles that do work to elevate the horse's center of mass on an incline, then a greater volume of muscle would have to be recruited to generate an equivalent force for body support. This was reflected in significant increases in the EMG intensity (IEMG) of both muscles.

Key words: Locomotion, quadruped, sonomicrometry, muscle.

Introduction

One tenet of the comparative physiology of locomotion is that animals evolved to reduce the energetic costs of locomotion (Taylor, 1994): there is evidence that horses behaviorally choose speeds that are the most economical (Hoyt and Taylor, 1981; Wickler et al., 2000) and that when they change gaits, it affords metabolic savings (Wickler et al., 2002; Wickler et al., 2003). Another means of reducing metabolic costs during locomotion is to have the antigravity muscles contract with a minimum of strain, or under more nearly isometric conditions (Taylor, 1994); more force is produced in an isometric contraction than when a muscle shortens, so to produce a given force, less muscle is required if it is an isometric contraction and, thus, less energy is required. In distal leg muscles of turkeys (*Meleagris gallopavo*; Roberts et al., 1997) and wallabies (*Macropus eugenii*; Biewener et al., 1998), muscle strains during level locomotion are less than 6%. However, muscle function may not be dictated just by economics, but also by the requirements for mechanical work (Biewener, 1998). During level flight, the pigeon pectoralis

muscle undergoes total strain amplitudes of 32% (Biewener, 1998) and the rat biceps femoris shortens by 20% during trotting on the level (Gillis and Biewener, 2002). The varying locomotor behavior of animals under natural conditions, such as changes in grade or speed, also requires that muscles adjust the amount of work they perform to raise, accelerate or decelerate the animal's center of mass. Indeed, Roberts et al. (1997) found that the turkey gastrocnemius increased the amount of strain and work that it performed when running up an incline. The amount of strain in the rat biceps femoris increased from 20% to 24% when increasing the slope from 0 to 27% (Gillis and Biewener, 2002). Similarly, distal leg muscles in guinea fowl (Daley and Biewener, 2003) also exhibited increased strain during incline versus level locomotion, as well as strain modulation associated with stride-to-stride balance and stability.

In steady-state trotting at different speeds in the horse, an elbow extensor (the lateral head of the triceps) and a knee extensor (the vastus lateralis) provide a comparison of muscle

strains in paralleled-fibered muscles from limbs that have different roles in forward locomotion (Hoyt et al., 2005). In the horse, the triceps had shortening strains of around 10% while the vastus shortened 8% during the stance phase of level trotting (Hoyt et al., 2005). Although these strains are of similar magnitude, the patterns of length change are complex and reflect the different roles of the forelimb and the hindlimb: the former acts more as a stiff spring-like strut (McGuigan and Wilson, 2003) and the latter modulates power for propulsion (Dutto et al., 2004).

Because of the 250% increase in metabolic rate in horses trotting up a 10% incline (Wickler et al., 2000) which is, presumably, a result of the increased requirement for mechanical work, we hypothesized that muscle strain during trotting would be increased in both the triceps and the vastus over that observed when trotting on the level (Hoyt et al., 2005). Because the time of ground contact when going up a 10% incline is similar to that on the level (Hoyt et al., 2000), an increase in strain on the incline would produce increased strain rate. Owing to force-velocity effects, an increased shortening velocity means that active muscle fibers produce less force. So, although muscle forces on the incline need not be increased (Biewener et al., 2004; Roberts et al., 1997), an increase in shortening velocity suggests that a greater volume of muscle must be recruited to maintain the same force. Based on this reasoning, we hypothesized that the strain rate of both muscles would be elevated during incline trotting and that EMG (electromyographic) activity would be increased in both muscles as well. We examined the lateral head of the triceps and the vastus lateralis while trotting up a 10% incline (5.7°) over a range of speeds.

Materials and methods

Animals and training

Four physically conditioned Arabian horses *Equus caballus*, ranging in age from 4 to 7 years (mean \pm S.E.M.: 5.1 ± 0.7 years) with a body mass of 433 ± 13 kg, were used in this study and in the companion paper (Hoyt et al., 2005). More detail on the experimental protocols can be found in that paper. The Cal Poly Pomona Animal Care and Use Committee approved all procedures involving animals.

Surgical procedures

Surgery was done on standing, sedated horses (butorphanol tartrate, Fort Dodge Animal Health, Fort Dodge, IA; 0.1 mg kg^{-1} and detomidine hydrochloride, Pfizer Animal Health, Exton, PA; $20\text{--}40 \text{ }\mu\text{g kg}^{-1}$) and local anesthesia (lidocaine HCl, Pro Labs Ltd., St Joseph, MO, USA). The location of the lateral triceps (M. triceps brachii caput laterale) and vastus (M. vastus lateralis) was determined using palpable landmarks: anatomic locations were studied on several cadavers prior to surgery and anatomic validation of sonomicrometer crystal placement was done on three horses not part of this study that were euthanized for medical conditions not related to musculoskeletal dysfunction.

The fascia of the triceps and vastus was exposed by removing subcutaneous fat and, in the case of the triceps, incision through the omobrachialis muscle. One pair of 2 mm omni-directional, spherical, piezoelectric crystals (Tack crystals, Sonometrics Corporation, London, Ontario, Canada) was implanted 1 cm deep, 10–15 mm apart in a line parallel to muscle fiber orientation to measure changes in muscle fiber length. The crystals were anchored to muscle fascia using 0 silk suture and a tension relief loop.

Electromyography electrodes (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 EMG signal was amplified (1,000–10,000, depending on signal strength) and filtered (60 Hz notch and 100–1,000 Hz bandpass). A ground wire was implanted subcutaneously into the dorsal aspect of the horse's sacral region. Banamine® (flunixin meglumine, Schering-Plough Animal Health Corp., Union, NJ; $20\text{--}40 \text{ }\mu\text{g kg}^{-1}$) was administered post-surgery to reduce pain and act as an anti-inflammatory.

Data from the sonomicrometry crystals were obtained using Sonometrics System Software and output to the data acquisition software that also sampled EMG signals at 3704 Hz (LabVIEW®, National Instruments, Austin, TX, USA).

Data collection

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 hindlimb 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.

Each horse was run on a high-speed treadmill under two conditions: on the level and up a 10% incline. Incline data are the focus of this paper. Horses were run under each condition at speeds from $2.5\text{--}4.5 \text{ m s}^{-1}$ 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 conditions (nine speeds at 0% and 10% incline) were run in succession, with a 30 min. break after the first nine (results for 0%, level trotting, are given in Hoyt et al., 2005). Sonomicrometry crystals were removed at the end of the day and the surgical wounds sutured and dressed. No animal, either during the study or after removal of the crystals, experienced any lameness.

Kinematic data

Reflective markers (Peak Performance Technologies, Englewood, CO, USA) were glued to the skin on the lateral side of each limb, using standard palpable positions (Back et al., 1993). The horses were filmed at 125 Hz using a Model PCI Motion Scope® camera (Redlake Camera Corp., Morgan Hill, CA, USA) placed approximately 8.5 m away from the treadmill. A linear calibration was performed daily. Five consecutive strides were captured and digitized (Motus®, Peak Performance Technologies, Englewood, CO, USA) for

each horse at each speed and condition (level and incline). The angular data were smoothed using a cubic spline filter, normalized for time using a cubic spline fit, and five strides for each horse, speed and condition were averaged using the trial averaging feature of Motus. These data were used to determine mean joint angle of the knee and elbow at first hoof contact, mid-stance, maximum extension (elbow) and flexion (knee) and break-over, and analyzed for range of motion between these events. The angles reported are for the anterior aspect of the elbow joint and the posterior aspect of the knee.

Data processing

First hoof contact, break-over and second hoof contact were determined using the record from the accelerometer and the high-speed video, 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 timing of the EMG and sonomicrometry records, relative to stance phase, was based upon 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). All muscle fascicle lengths were normalized to their fractional length change (or strain) by dividing measured lengths L by the resting muscle length L_0 (L/L_0). The measurement of L_0 was recorded with the animal standing with its metacarpals and metatarsals perpendicular to the surface of the treadmill. In order to calculate total strain (muscle shortening) over the range of speeds, sonomicrometry records of individual strides were temporally normalized to 100% of time of contact (t_c) using a cubic spline interpolation. Changes in muscle lengths (ΔL) were measured at increments of 0.5% of t_c (201 increments per contact period). Because strain patterns were analyzed in conjunction with kinematics on the level (Hoyt et al., 2005), the average normalized muscle length data for results on the incline were divided into phases based upon the kinematics of the appropriate joint (joint kinematics were determined from five strides recorded simultaneously comprising 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 (quantified as $L s^{-1}$) 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 (IEMG) during stance.

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 muscle, and the four horses were used as the repeated measure. An additional ANOVA was run using speed and condition (level vs incline) to test for differences between conditions. For those conditions in which there was a significant interaction, differences between conditions were analyzed using a designed contrast analysis (Abacus Concepts Inc., Berkeley, CA).

Results

Characteristic patterns

A composite of a typical accelerometer record and EMG activity are given in Fig. 1. A composite of muscle strain for the triceps and vastus, average of all horses at all trotting speeds, is presented in Fig. 2. Representative muscle strains from the horses at 3.25 m s^{-1} , including kinematic data, are presented in Fig. 3. Because two horses displayed different vastus strains from the other two horses on the level (Hoyt et al., 2005) and on the incline, both of these patterns are presented in Fig. 3.

Strain and strain rate

In an effort to analyze better the concentric and eccentric contractions of the muscles during stance, muscle strain patterns were divided into phases based on the kinematics of the joint (Fig. 3) – an approach used by Hoyt et al. (2005).

Triceps

Phase 1 was a period of elbow extension, and the end of this phase increased with speed from 20 to 30% of stance. This was an anomalous phase when the joint extended but the muscle lengthened 5.2% (Table 1). Phase 2 was a period of elbow flexion that lasted for 10% of the stance phase at all speeds, and during which the triceps shortened by 1.4%. In the third

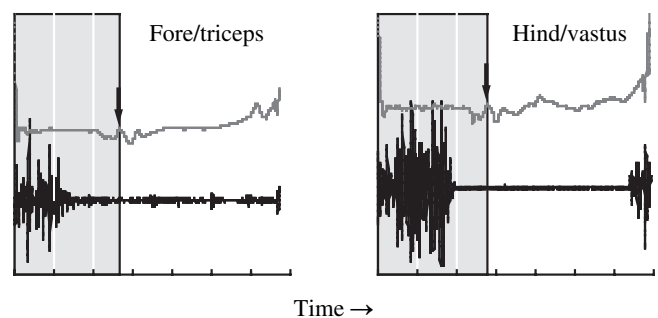


Fig. 1. Typical recordings for the entire stride for a forelimb and hindlimb during trotting up an incline. The top graphs include tracings of accelerometer records that were used to identify stance phase (gray shaded area with hoof lift-off denoted by vertical arrow). EMG activity patterns are shown below the accelerometer records to demonstrate when the muscle was active.

phase, the elbow extended while the triceps shortened concentrically by 18%. There was no change with speed in the

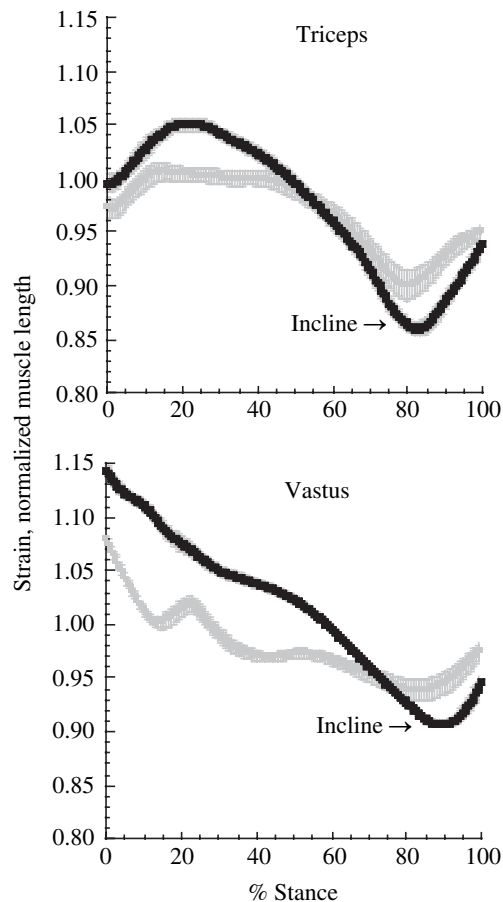


Fig. 2. Normalized stance phase muscle lengths (means \pm 1 S.E.M.) of the triceps and vastus for all horses at all speeds ($N=4$). The grey curve represents the mean of the same horses for locomotion over all speeds at 0% slope (from Hoyt et al., 2005). On the incline, both muscles shortened more than on the level.

amount of strain during phase 3 (Fig. 4). Because phase three lasted for 50% of the entire stance period and included mid-stance, when ground reaction forces reach a maximum, it seems probable that most of the concentric work required to trot uphill was done during this phase. For this reason, and because there was no effect of slope on the strain observed during phases 1 and 2, only the data for phase 3 are plotted in Fig. 4 and considered in subsequent discussions.

Shortening strain rate (Fig. 4) of the triceps increased with speed ($P=0.005$) and was greater on the incline than on the level ($P=0.050$). At the lowest speed the shortening rate during incline trotting was approximately three times greater than on the level but it was only 1.2 times greater at the highest speed (4.5 m s^{-1}).

Vastus

There were two distinct patterns of muscle strain observed in each of two horses during level locomotion (Hoyt et al., 2005) but these differences were attenuated on an incline. Phase 1 (Fig. 3) was a period of knee flexion and muscle shortening that lasted for the first 14% of stance (Table 1), a period that did not change with speed but was shorter than during level trotting. The vastus shortened by 6.4%, an amount similar to that on the level. Phase 2 was only observed in two horses, and lasted for only a brief period of stance (about 3%). During level trotting, this phase was characterized by substantive lengthening in two horses. However, when these horses trotted up an incline this lengthening was attenuated, amounting to 0.6%. The magnitude of this change was unaffected by speed. Phase 3 on the incline was characterized by a brief period of knee extension (starting at 17% of the stance period and ending at 31%) and vastus shortening of 3.6% (Fig. 5). Shortening strain was not different with speed ($P=0.49$). No difference in phase 3 shortening strain was observed between level and incline. Phase 4 was a period of knee flexion that lasted from 31% of stance until about 60%, during which the vastus shortened by

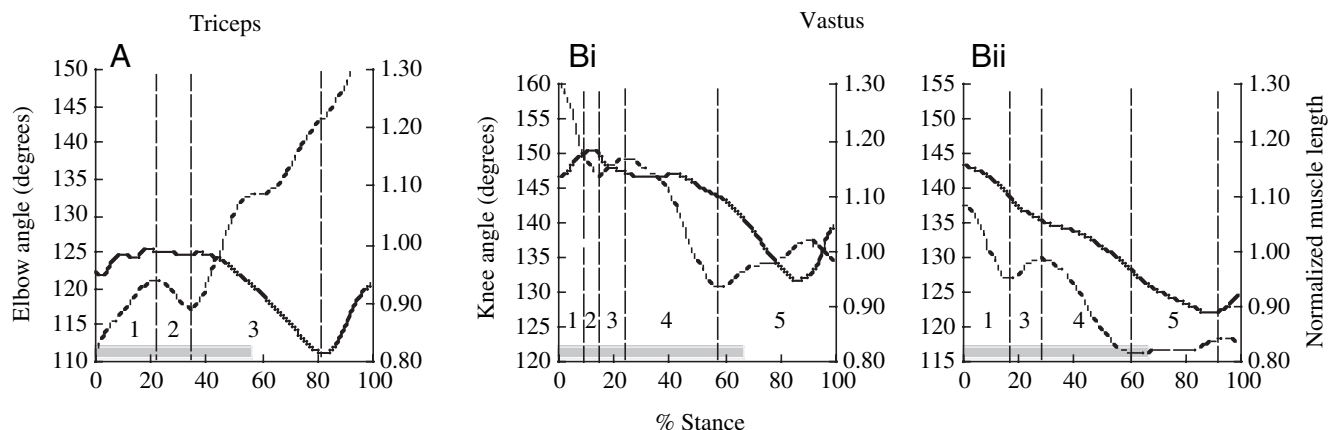


Fig. 3. Joint angles (broken lines) and normalized muscle lengths (solid lines, where 1.0 = length at rest), at 3.25 m s^{-1} for the triceps (A) and for the two patterns of muscle strain observed in the vastus (B; referred to in Hoyt et al., 2005). Phases (denoted by vertical broken lines and numbers), based on kinematics, were the same as used in the analysis of the data obtained during level locomotion. Mean duration of EMG activity is indicated by shaded grey bars.

Table 1. Strain during the phases identified by the kinematics of the joint

<i>Triceps</i> Phase	Average strain (S.E.M.)	Effect of speed (<i>P</i>)	Difference from level (<i>P</i>)
1	5.2% (0.7)	Increased (0.028)	Not different (0.129)
2	-1.4% (0.2)	Decreased (0.012)	Not different (0.194)
3	-18% (0.7)	Not different (0.408)	Increased (0.026)
% Stance			
1	25	Increased (0.0001)	Not different (0.787)
2	34	Increased (0.0001)	Not different (0.188)
3	83	Increased (0.038)	Not different (0.595)
<i>Vastus</i> Phase	Average strain (S.E.M.)	Effect of speed (<i>P</i>)	Difference from level (<i>P</i>)
1	-6.4% (0.7)	Not different (0.900)	Not different (0.209)
2	0.6% (0.2)	Not different (0.448)	Not different (0.210)
3	-3.6% (0.4)	Not different (0.525)	Not different (0.525)
4	-5.4% (0.5)	Not different (0.376)	Not different (0.062)
5	-9.7% (0.7)	Not different (0.135)	Decreased (0.003)
% Stance			
1	14	Not different (0.557)	Decreased (0.046)
2	17	Increased (0.005)	Decreased (0.004)
3	31	Increased (0.001)	Decreased (0.011)
4	59	Increased (0.001)	Not different (0.101)
5	88	Not different (0.810)	Increased (0.004)

Strain, percent of normalized muscle length; negative strain values indicate shortening.

Percent stance indicates the time when the phase ended.

Values are averages of the triceps and vastus for all four horses at all nine speeds on the incline.

5.4%, a value statistically not different from the 2% shortening observed in the muscle during this phase of level locomotion. Phase 4 muscle shortening did not differ with speed. Phase 5 lasted from 59% of stance until approximately 90% of stance and was characterized by a period of knee extension and vastus shortening. The vastus shortened 9.7% compared to 3.1% during level locomotion but did not change with speed. Thus total shortening strain during phases 3, 4, and 5 was greater (18.5% vs 8.1%) on the incline. There was no effect of speed ($P=0.194$) when phases 3-5 were combined and analyzed.

For phase 3 and 5, shortening rates (Fig. 6) increased with speed ($P=0.023$, $P<0.001$, respectively) but not for phase 4 ($P=0.103$). Shortening rates were higher on the incline than on the level for phases 4 ($P=0.040$) and 5 ($P=0.022$), but not for phase 3 ($P=0.729$). Thus the average shortening rate during phases 3-5 was 1.7 times as fast on the incline (0.958 L s^{-1}) as on the level (0.568).

EMG

Triceps

EMG activity (Fig. 7) of the triceps initiated prior to foot contact (termed here as 'activation phase advance') and

increased slightly with trotting speed ($P=0.005$), so that at 4.5 m s^{-1} triceps EMG activity preceded foot contact by 8.5 ms. This differed from level trotting, where triceps activation was phase advanced by an average of 36 ms relative to foot contact (Hoyt et al., 2005) and did not change with speed ($P=0.746$). The percentage of time during the stance that the triceps EMG was active decreased with speed ($P<0.001$) and was 19.4% longer on the incline than on the level ($P=0.001$). Even though triceps EMG duration decreased with speed, its integrated activity (IEMG) increased ($P<0.001$) with speed and averaged 80% higher on the incline compared with the level ($P=0.003$).

Vastus

At low trotting speeds on the incline, the vastus became active at hoof contact, and similar to the triceps, became more phase advanced relative to limb contact as speed increased ($P<0.001$). The percentage of time that vastus EMG was active during stance also decreased with speed ($P=0.006$) but the muscle was active 12% longer on the incline compared with the level ($P=0.001$). Similar to triceps, vastus IEMG increased with speed ($P=0.024$) and was 113% greater on the incline than on the level ($P=0.003$).

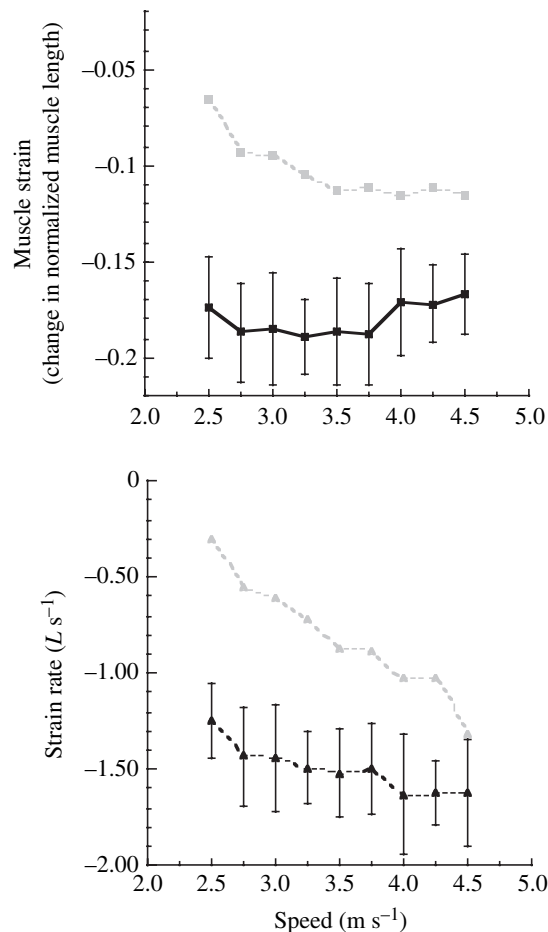


Fig. 4. Total strain (change in normalized muscle length) and strain rate ($L s^{-1}$) of the triceps (during phase 3) as a function of trotting speed on a 10% incline (black symbols, mean \pm 1 S.E.M.). Total strain on the level is denoted by gray symbols (Hoyt et al., 2005). Strain and strain rate during incline locomotion did not change with speed but were greater than during level locomotion.

Stride parameters

Fig. 8 gives details of the stride parameters. The horses' stride period decreased with speed ($P < 0.001$) and averaged 5% longer on the incline than on the level ($P = 0.048$). The horses' swing period in the forelimb did not change with speed ($P = 0.499$) but decreased in the hindlimb ($P < 0.001$) and was 11% shorter in the forelimb than the hind when averaged over all speeds ($P = 0.001$). When compared to level trotting, there was no difference in swing time for the forelimb ($P = 0.149$) or the hindlimb on the incline ($P = 0.075$). The time of ground contact, t_c , decreased with speed in both limbs ($P < 0.001$) and was 11% longer for the forelimb than the hindlimb ($P = 0.048$), matching the 11% decline in forelimb swing time. When compared to level trotting, t_c during incline trotting was 5% longer in the forelimb ($P < 0.001$) but not different for the hindlimb ($P = 0.139$). Step length (the distance the body moves during limb contact), increased with speed for both limbs ($P < 0.001$) and was 11% longer for the forelimb ($P = 0.052$), associated with the longer contact time of the forelimb. When

compared to level locomotion, step length was 5% longer on the incline for the forelimb ($P = 0.001$) but did not differ for the hindlimb ($P = 0.207$). Duty factor, the proportion of time the foot is in contact with the ground during the entire stride, decreased with speed ($P < 0.001$) and was 12% greater for the forelimb than the hindlimb ($P = 0.014$). When compared to level locomotion, no difference in duty factor was observed for either limb (forelimb, $P = 0.897$; hindlimb, $P = 0.727$).

Discussion

Our first hypothesis was that when animals move on an incline, shortening strain in the extensors of the elbow (triceps) and the knee (vastus) would be increased compared with level locomotion. This was supported by the results obtained for both muscles (cf. Hoyt et al., 2005): the triceps shortened by 18% compared with 10.6% shortening on the level, and the vastus shortened by 18.5% compared with 8.1% on the level (Figs 2–6).

Because shortening strains in both muscles were increased on an incline compared to on the level, and limb contact times remained unchanged (Fig. 8), it makes sense that there was an increase in the muscles' shortening strain rates on the incline for both the triceps (Fig. 4) and vastus (Fig. 6). Therefore, because of the force–velocity relationship of skeletal muscle, one would expect to find that more motor units within each muscle must be activated to produce the same force. This would seem to explain the observation that both muscles' EMG activity increased in magnitude and duration (Fig. 7) on an incline. However, the explanation may be more complex than this if muscle forces are changed on an incline.

In general, forces in distal leg muscles have not been found to increase during incline locomotion in running turkeys and hopping wallabies (Biewener et al., 2004; Roberts et al., 1997). Whereas no change in the medial gastrocnemius force was observed in turkeys running on a 10% incline (Roberts et al., 1997), lateral gastrocnemius force decreased by 8% and plantaris force increased by 9% in tammar wallabies hopping on a 10% incline (Biewener et al., 2004). However, in a smaller avian biped, lateral gastrocnemius forces were observed to increase by 38% and digital flexor (DF-4) by 12% when guinea fowl ran on a 16% incline (Daley and Biewener, 2003). The increase in muscle force for this species may reflect the steeper incline and/or its smaller size and differences in muscle–tendon architecture.

There have been few direct measurements of muscle forces in quadrupeds locomoting on the level and incline but the available data suggest that muscle forces may be different under these two conditions. In cats walking up a 30–60° incline, forces in the tendon of the medial gastrocnemius are higher than on the level but those in the soleus are not changed (Kaya et al., 2003). In the horse, unless there are changes in limb mechanical advantage, muscle forces may not be the same on the incline as on the level because peak ground reaction force (GRF) changes on an incline: forelimb peak GRF is lower on an incline than on the level and hindlimb peak GRF

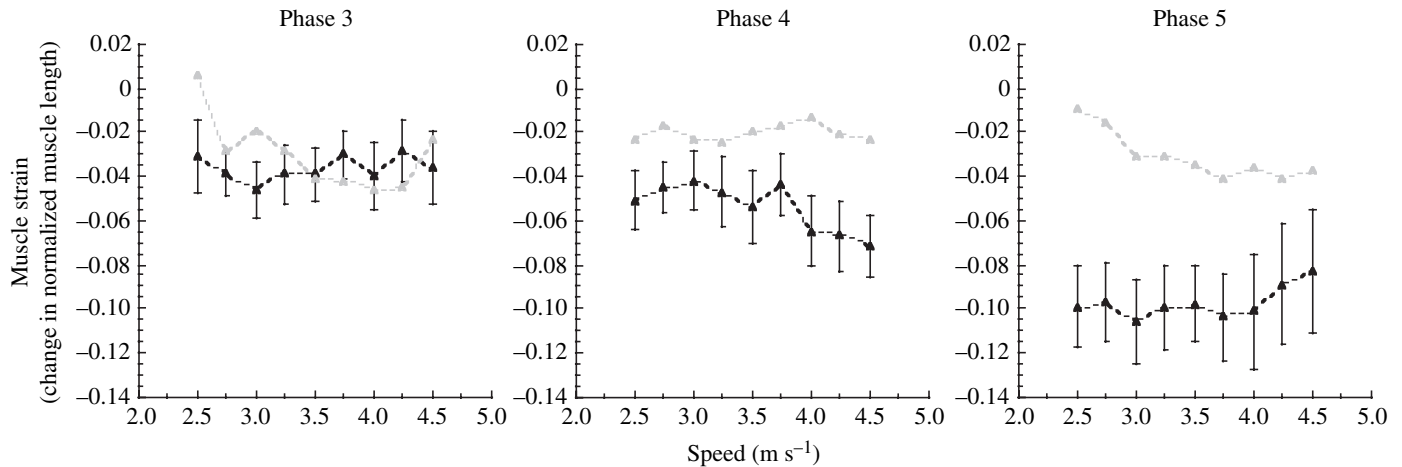


Fig. 5. The average strain (change in normalized muscle lengths; means \pm 1 S.E.M.) of the vastus for the three phases of stance identified in Fig. 3 (phases 3–5). There was no overall effect of trotting speed. The muscle contracted concentrically during all three phases and the total concentric strain during the three phases was greater on an incline (black symbols) than on the level (gray symbols).

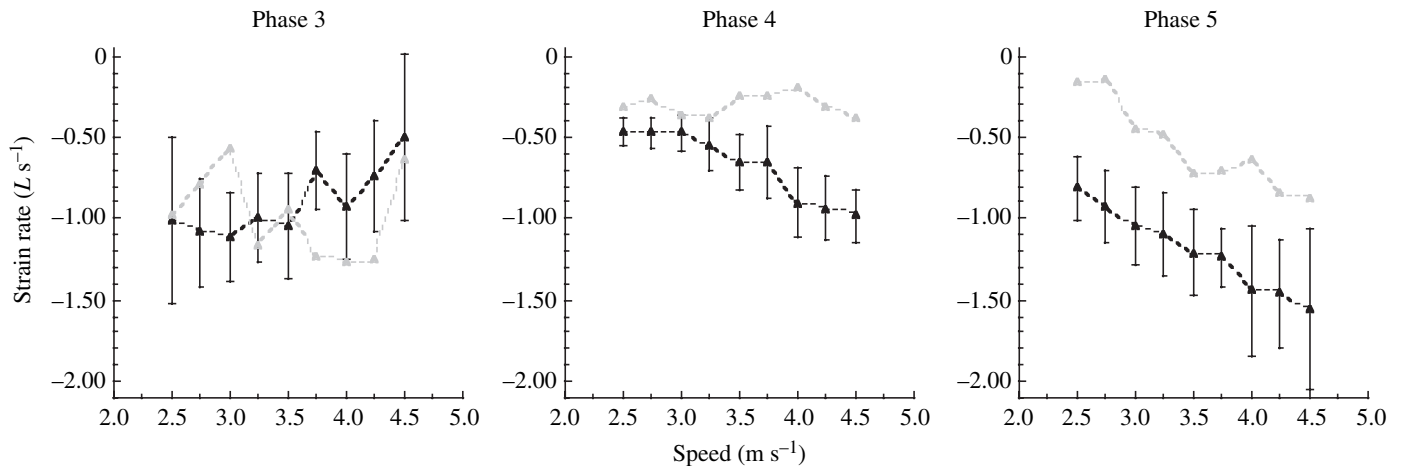


Fig. 6. Shortening rates ($L s^{-1}$; means \pm 1 S.E.M.) of the vastus for phases 3–5. For phases 4 and 5 the shortening rates increased with speed and were greater on the incline (black symbols) than on the level (gray symbols).

is elevated at higher trotting speeds on an incline (Dutto et al., 2004). Therefore, until muscle forces can be empirically determined in horses, it is not clear whether the increased EMG activity on an incline is due to increased strain rate alone or also reflects an increase in muscle force development.

Changes in timing of EMG activity (Fig. 7) are similar to those observed in other species. In the horse triceps, the muscle was activated 20 ms after contact at low speeds, and 10 ms prior to contact at 4.5 m s⁻¹. This activation was later than observed on the level (Hoyt et al., 2005). In the vastus, the EMG started at contact at lower speeds and 60 ms prior to contact at 4.5 m s⁻¹ – again, later than on the level. This delay in the timing of onset of EMGs in the vastus on an incline is also apparent in the trotting rat (Gillis and Biewener, 2002) and the trotting horse (Robert et al., 2000).

The increase in integrated EMG (IEMG) with speed and with incline has also been observed in: (a) another knee extensor of the horse, the tensor fasciae latae, and a hip

extensor, the gluteus medius (Robert et al., 2000); (b) the vastus and biceps femoris of the rat (Gillis and Biewener, 2002), and (c) some hindlimb muscles (but not all) of the cat (Roy et al., 1991). The 80% increase of IEMG does not seem to reflect the 250% increase in metabolic rate that occurs with a 10% incline (Eaton et al., 1995; Wickler et al., 2000). However, this is not surprising, as we only measured the length changes and activity of one muscle in each limb, and we do not know the force or power output of these muscles or the joints at which they act (principally the elbow and knee) compared with other joints of the limbs. Furthermore, the electrodes are small, superficial, parallel to the surface fibers and, because the volume of the muscle is large relative to the region sampled by the electrodes, such recordings are sensitive to compartmentalization of recruitment patterns within the muscle (English, 1984; Scholle et al., 2001).

Increased muscle fascicle strain on an incline was observed in the turkey lateral gastrocnemius (Roberts et al., 1997), in

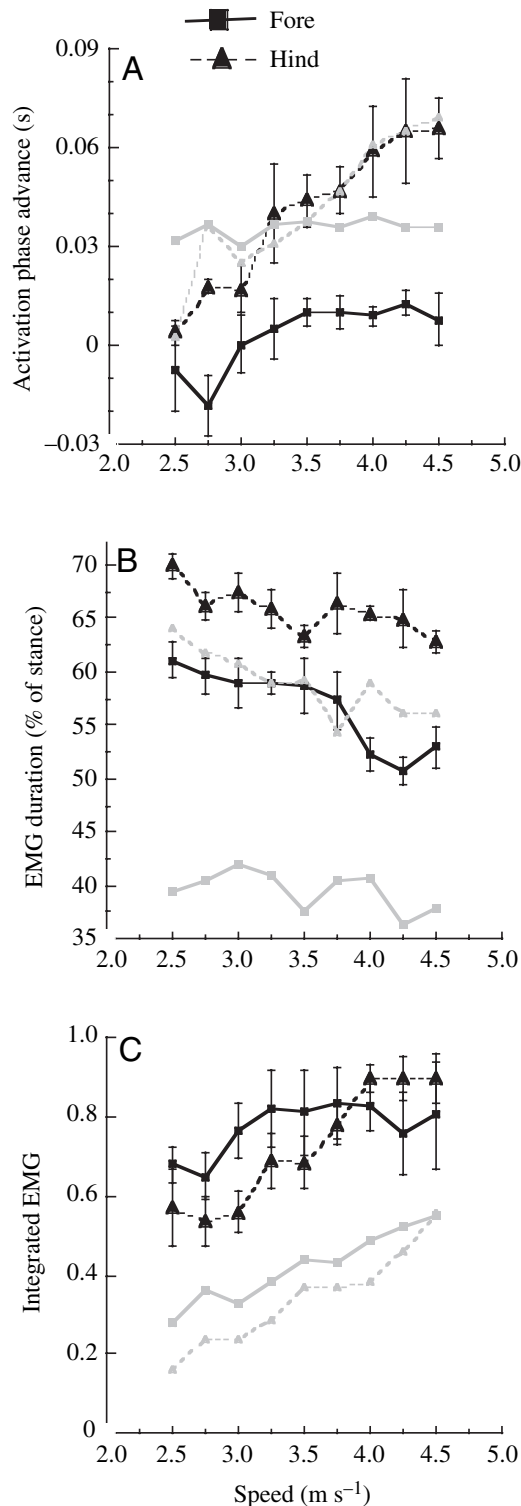


Fig. 7. EMG patterns (means \pm 1 S.E.M.) for the triceps and vastus as a function of speed on the incline (black) *versus* the level (gray; Hoyt et al., 2005). (A) On an incline at low speeds, EMG activity of both muscles started near the time of foot contact, but as speed increased the muscle was activated earlier. (B) As speed increased on the incline, EMGs of both muscles were active for a smaller percentage of stance, but for a larger fraction than at the same speed on the level. (C) Integrated EMG activities of both muscles increased with speed and were greater on the incline.

guinea fowl lateral gastrocnemius (45% increase) and digital flexor (Daley and Biewener, 2003), but, in tammar wallabies, neither the lateral gastrocnemius nor the plantaris increased their strain (Biewener et al., 2004). In rats moving up an incline (Gillis and Biewener, 2002), strain increased in the biceps femoris, but not in the vastus (at least during trotting). In contrast to the horse vastus, which shortens, the rat vastus undergoes substantial lengthening even when trotting uphill. This suggests that the horse vastus contributes positive work during incline trotting, but the rat vastus absorbs energy, requiring that other muscles (including the biceps) increase their shortening to raise the animal's mass. These differences are probably due to locomotion limb design and pattern (digitigrade *vs* unguligrade), or other anatomical features of muscle origins and insertions. Such differences are certainly underscored by the different strain patterns observed in the vastus lateralis of rats (Gillis and Biewener, 2001) and dogs (Carrier et al., 1998) – despite the similarities in the kinematics of their knees during trotting.

The scaling of metabolic rate during level locomotion over a wide range of size in mammals and birds is correlated with the inverse of time of contact (Kram and Taylor, 1990). It has been hypothesized that $1/t_c$ reflects the rate of force development by the antigravity muscles. In the present study of incline trotting in the horse, we observed a decrease in t_c for both fore- and hindlimb as speed increased, consistent with the increased metabolic rate at higher trotting speeds (Wickler et al., 2000). However, t_c was not different between incline and level conditions at a given trotting speed, even though the horses' metabolic rate was 2.5 times greater at all trotting speeds (Wickler et al., 2000). Much of the observed increase in metabolic cost on the incline, therefore, must reflect increased muscle recruitment associated with the increased muscle shortening strain and the resulting loss of force produced by active fibers.

As expected, the time of contact decreased relatively more than swing time as speed increased, resulting in a decrease in duty factor. Typically (Alexander et al., 1979; Biewener, 2003; Dutto et al., 2004), a decrease in duty factor results in an increase in peak ground reaction force (GRF). On the level, this generalization was true for the forelimb as speed increased, but not for the hindlimb, where forces were independent of speed (Dutto et al., 2004). While duty factor of both limbs was the same on the incline as on the level, peak GRF on an incline was not the same as on the level (Dutto et al., 2004).

This study originated from an interest in integrating whole animal energetics and muscle function. While the observations of increased muscle strain are consistent with the increased energetic demands for uphill locomotion, we only measured one muscle in each limb. A better linking of muscle contractile patterns to their significance for muscle work, joint dynamics and whole limb movement will require analysis of more muscles of varying architecture, as well as measurements of muscle forces.

Conclusion

The mechanical work required to elevate the center of mass

of a quadruped during locomotion up an incline requires an increase in shortening strain of the antigravity muscles of both the fore- and hindlimbs. Because fore- and hindlimb contact times do not differ between level and incline trotting at the same speed, limb muscles that contribute increased work to

move up an incline must contract with an increased strain rate. We confirmed these changes for two representative antigravity muscles of the horse: triceps (lateral head) and vastus lateralis. The increased shortening velocities of these two muscles probably reduce the force that any given set of activated

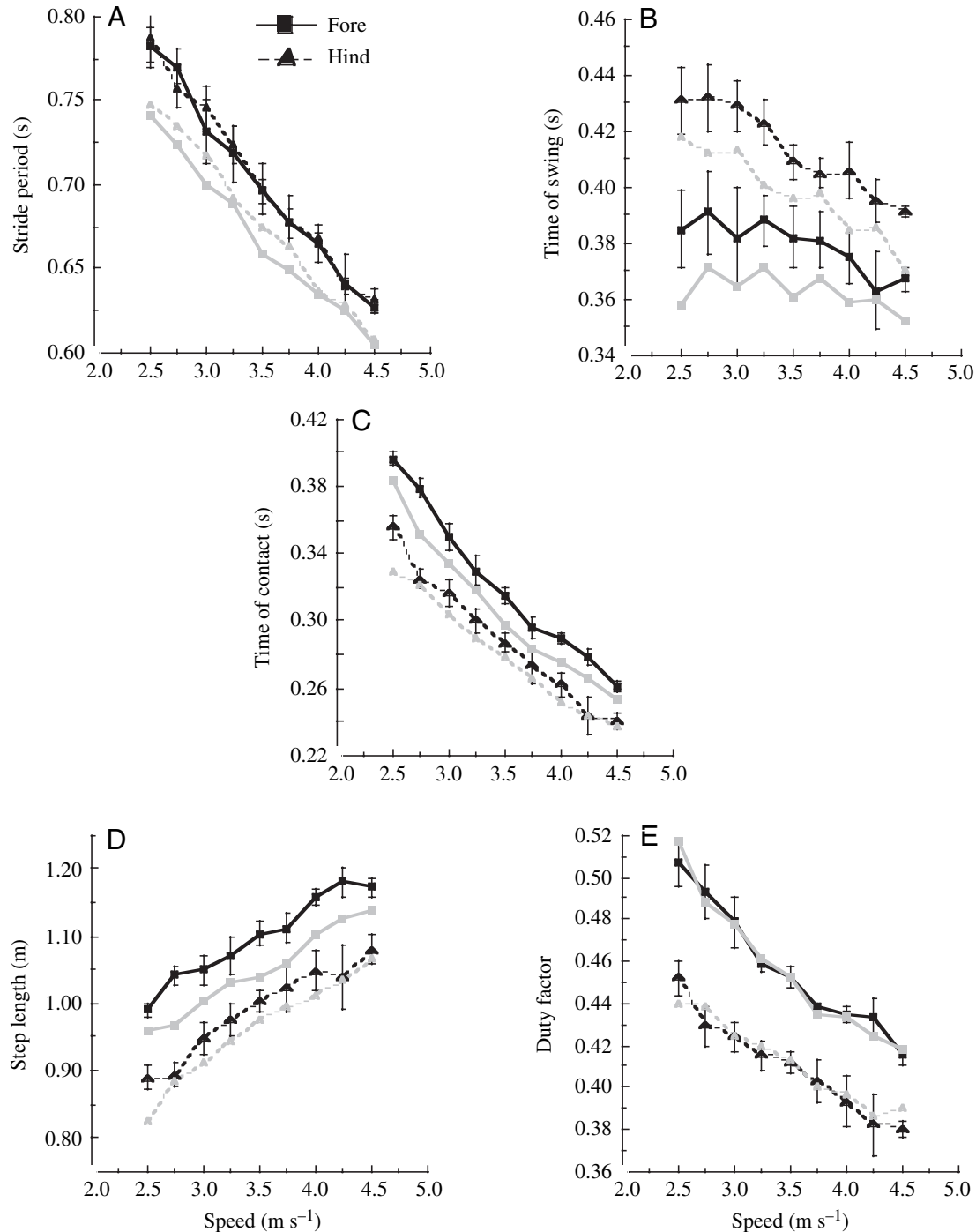


Fig. 8. Stride parameters (means \pm 1 S.E.M.) for both limbs measured on the incline (black) versus on the level (gray; Hoyt et al., 2005). (A) Stride period decreased with speed and was longer on the incline than on the level, and was not different between the limbs. There were differences between the limbs for all the other parameters listed. (B) Swing time on the incline was not different from that on level for the forelimb, but was shorter for the hindlimb. (C) Time of contact on the incline was longer in the forelimb but not different in the hind limb. (D) Step length on the incline was longer for the forelimb but not different for the hindlimb. (E) The duty factor on the incline was not different from that on the level.

muscle fiber can produce. If this pattern holds for other limb muscles that do work to elevate the horse's center of mass on an incline, then a greater volume of muscle must be recruited to generate an equivalent force for body support. This was reflected in significant increases in the EMG intensity (IEMG) of both muscles. With increasing speed, time of contact (and duty factor) decreases, compounding the need for additional motor recruitment.

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