Effects of surface grade on proximal hindlimb muscle strain and activation during rat locomotion

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Gillis, Gary B., and Andrew A. Biewener. Effects of surface grade on proximal hindlimb muscle strain and activation during rat locomotion. J Appl Physiol 93: 1731-1743, 2002. First published August 9, 2002; 10.1152/japplphysiol.00489. 2002.—Sonomicrometry and electromyography were used to determine how surface grade influences strain and activation patterns in the biceps femoris and vastus lateralis of the rat. Muscle activity is generally present during much of stance and is most intense on an incline, intermediate on the level, and lowest on a decline, where the biceps remains inactive except at high speeds. Biceps fascicles shorten during stance, with strains ranging from 0.07-0.30 depending on individual, gait, and grade. Shortening strains vary significantly among grades (P = 0.05) and average 0.21, 0.16, and 0.14 for incline, level, and decline walking, respectively; similar trends are present during trotting and galloping. Vastus fascicles are stretched while active over the first half of stance on all grades, and then typically shorten over the second half of stance. Late-stance shortening is highest during galloping, averaging 0.14, 0.10, and 0.02 in the leading limb on incline, level, and decline surfaces, respectively. Our results suggest that modulation of strain and activation in these proximal limb muscles is important for accommodating different surface grades.

incline; decline; vastus; biceps

MANY TERRESTRIAL ANIMALS live in environments that are topographically diverse and must often move up, down, and across slopes of varying degree. Grade-related changes in gravitational potential energy require that an animal expend more energy to move uphill at the same speed as on the level and dissipate energy to maintain this speed downhill. In accord with this, many physiological studies have shown that energetic costs, as measured by levels of oxygen consumption, increase during incline locomotion in diverse animal species (1, 9-11, 25, 30, 38-41) and decrease during decline locomotion (1, 6, 25, 30, 38, 41). Such shifts in locomotor energetics are largely due to changes in the recruitment and actions of various limb muscles. Whereas limb muscle recruitment typically increases on an incline (7, 8, 13, 20, 29, 31, 33), the reverse is true for decline locomotion relative to on the level (e.g., Ref. 18). In fact, Smith and Carlson-Kuhta (36) noted that several major hip extensor muscles in cats remain completely inactive during decline walking on grades as low as 10%.

In addition to these changes in muscle recruitment, alterations in limb posture and kinematics often accompany a change in surface grade (7, 17, 20, 37). For example, recent work on incline walking in cats demonstrated that, as grade increased from 25 to 100%, joint extension at the hip, knee, and ankle increased concomitant with electromyographic (EMG) intensity in extensors acting at those joints (7). In comparison, Smith et al. (37) showed that knee joint extension was reduced, whereas yield-related flexion at the ankle was increased on declined slopes of varying degree (also in cats). Such kinematic results suggest that limb muscles likely undergo relatively more active shortening on an incline but more stretching while active on a decline, which implies that shifts in muscle strain as well as recruitment are important for mediating changes in net muscle work and gravitational potential energy.

Although quantification of joint kinematics provides insight into the length-change patterns of muscle-tendon units spanning those joints, kinematic data cannot directly resolve the specific muscle and/or tendon strains that underlie a given joint excursion in vivo. Sonomicrometry permits direct measurements of limb muscle fascicle strain during dynamic behaviors, but few studies have employed this technique to quantify alterations in muscle strain regimes associated with shifts in locomotor surface grade. Recent work by Roberts et al. (32) and Gabaldon et al. (12) has shown that lateral gastrocnemius fascicles in running turkeys actively shorten on an incline, remain nearly isometric on the level, and lengthen while active on a decline. These data support the notion that shifts in distal limb muscle strain are important for mediating mechanical work output or absorption in response to changes in surface grade, but the role of more proximal limb muscles in this context remains unexplored.

To what extent might more proximal limb muscles also augment mechanical work output or dissipate energy in response to changes in surface grade? Hip and knee extensors generally have longer fibers than

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Table 1. Rats from which EMG activity and fascicle strain were recorded for the biceps femoris and vastus lateralis

	Biceps	Femoris	Vastus Lateralis			
No. Rat	EMG	Strain	EMG	Strain		
1	Х	Х	X	Х		
2	Х		Х	Х		
3	Х	Х	Х	Х		
4		Х	Х	Х		
5		Х				
6	Х	Х				
7		Х	Х	Х		

EMG, electromyogram; X, successful data collection and use in analysis.

more pinnate ankle extensors, which suggests the capacity to actively shorten or stretch over relatively large distances, thus facilitating substantial contributions to energy production or absorption. However, aside from the conceptual understanding that incline and decline locomotion tend to bias muscles toward concentric vs. eccentric contractions, respectively, we have little appreciation for how length-change patterns in proximal limb muscles actually change with grade.

In this study we use sonomicrometry and electromyography to measure patterns of muscle strain and activation in the biceps femoris (a biarticular muscle that acts in hip extension and knee flexion) and vastus lateralis (a major uniarticular knee extensor) of rats during uphill and downhill locomotion by having the rats move on a treadmill over a range of speeds and gaits. Our specific goals are threefold. First, we wish to test whether proximal muscles acting at the hip and knee exhibit different strain patterns depending on the surface grade. More specifically, we sought to determine whether these muscles shorten more on an incline than on the level and shorten less, or perhaps stretch, on a decline. Second, we address whether deactivation of the biceps femoris during stance, as observed in cats (36, 37), is characteristic of decline locomotion in rats as well. If both rats and cats exhibit this pattern of deactivation, this may represent a relatively widespread neuromotor response to downhill grades among mammalian quadrupeds. Finally, by examining a range of speeds, we can explore the interaction between gait and grade on limb muscle actions during locomotion. For example, are differences observed among grades during walking also observed during running, or are the effects of grade more prominent at a particular gait or range of speeds?

MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats weighing 225-305 g (mean = 257 g) were obtained from Charles River Laboratories. Rats were housed in pairs in cages and maintained on a diet of IsoPro 3000. The room in which rats were held was kept at 21°C, and a 12:12-h light-dark cycle was established. Individuals were initially trained to walk, trot, and gallop on a small level treadmill with a 60×20 -cm working section. Once individuals could maintain speed for 1 min at each gait, rats were then trained to move over a range of speeds with the treadmill either inclined or declined 15° (27% grade). Many animals were resistant to declined treadmill locomotion at high speeds, and thus the sample size of rats that performed trotting and galloping gaits downhill is relatively low. Animals ranged between 8 and 15 mo of age at the time of experiments, and all experimental procedures were approved by the University Committee for the Use and Care of Animals at Harvard University.

Implantation procedures. To record patterns of muscle electrical activity and length change, fine-wire bipolar electrodes and piezoelectric sonomicrometry crystals were implanted unilaterally into the cranial or anterior aspect of the biceps femoris and central region of the vastus lateralis. The biceps is the largest muscle in the rat's hindlimb (3). Although it is biarticular, fascicles within the most anterior region of the biceps act primarily in hip extension and have little, if any, effect in flexing the knee. The vastus is the largest muscle of the quadriceps complex (3) and acts as a major extensor of the knee. Thus the two muscles of interest act as major extensors of the proximal hindlimb.

In preparation for electrode and crystal implantation, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (35 mg/kg body mass). After anesthetization, the hindlimb and skull of the rat were shaved and scrubbed with a Povidone-iodine solution (EZ Prep, Becton Dickinson) for disinfection. A small skin incision was made over and parallel to the femur to expose the hindlimb muscles for implantation. A second incision was made through the skin over the skull, and a subcutaneous passage was created between the two incisions. To minimize wire exposure, all electrode and crystal wires were pulled through the incision at the skull, subcutaneously, to the limb incision.

Table 2. Average speeds of locomotor trials at each gait and grade for every individual

Rat No.	Slow Walk		Fast Walk		Trot		Gallop (1st Limb)		Gallop (2nd Limb)						
	L	Ι	D	L	I*	D	L	Ι	\mathbf{D}^*	L*	I*	D*	L	Ι	D*
1	30	26	31	40	51	50	68	60		100			103	84	84
2	17	20	22	41	45	40	66			78			65	65	
3	28	25	27	42		45	69	60		106		93	88	92	93
4	28	28	29	36		57	58	51				88	79	75	
5	26	28	19	44		46	62	56		115				112	
6	26	24	26	38	40	46	70	57		78	74		66		
7	27	24	30	39		50	72	55	64	89		96	92	83	
Mean	26	25	26	40	45	48	66	56	64	94	74	92	82	85	88

Values are given in cm/s. Animals rarely trotted during decline trials (D) and rarely used their experimental limb as the 1st limb down when galloping on an incline (I). L, level trial. *Data from these trials were not used in statistical comparisons among grades.

On the dorsal surface of the skull, a 10×15 -mm area was cleared of all tissue, and a small hole was drilled into its center by using a dental drill. An epoxy block was secured onto the dorsum of the skull by using a small stainless-steel machine screw and dental cement. Before surgery, electrode and crystal wires were soldered into female miniature connectors (Microconnectors, GF-6), which in turn were glued to the sides of the epoxy block. Once the block was fixed on the skull, skin from the scalp was sutured around its base and sealed with silicone adhesive (Dow Corning).

At the limb incision, two small pockets were created within the muscle tissue of the biceps and vastus by using the tips of fine watchmaker forceps. In each muscle, pockets were aligned along the trajectory of the muscle fibers ~ 10 mm apart. Sonomicrometry crystals (1-mm + 38-gauge lead wires, Sonometrics) were placed within the pockets, which were then closed with 6-0 silk suture. Offset twist-hook bipolar silver-wire electrodes (22) with tips bared of insulation were implanted immediately adjacent to and between crystal pairs in each muscle by using a 21-gauge hypodermic needle. Because of the potential for motor unit compartmentalization within these muscles, there was the possibility that EMG recordings did not reflect the pattern of activation throughout the muscle as a whole. Nevertheless, because of the proximity of the electrode implant to the crystal implants, it is likely that fascicles in which length changes were measured are also the fascicles in which electrical activity was measured.

Crystal and electrode wires were sutured with 6-0 silk through several superficial fibers onto the surface of the muscle to help prevent any dislodging during experiments. Once implantations were complete, the skin incision was sutured closed by using 4-0 silk. On completion of surgery, rats were allowed 24–48 h to recover before locomotor trials and recordings were started.

Locomotor trials and data collection. The miniature female connectors fastened to the epoxy block on the rat's skull were connected via male counterparts (Microconnectors, GM-6) to lightweight shielded cables (Cooner Wire) that were attached to the EMG and sonomicrometry amplifiers. EMG signals were amplified (\times 1,000) and filtered (100- to 3,000-Hz band pass; 60-Hz notch) by using Grass P-511 preamplifiers. Sonomicrometry signals were amplified by using a Triton sonomicrometer (model 120-1001). Raw voltage data were digitized at 5,000 Hz with a 12-bit analog-to-digital converter (Digidata 1200B; Axon Instruments) and recorded onto computer with Digidata software (Axoscope). Table 1 shows the individuals for which successful EMG and strain data were collected for the biceps and vastus.

Locomotor trials were performed on the same treadmill used for training, and all trials were recorded from a lateral perspective by using a digital high-speed video system (Redlake Motionscope; 125 frames/s). Once placed on the treadmill, rats were exercised at a given speed on the level until at least three to six strides were obtained at a steady speed; most trials recorded many more strides than this. Animals were then allowed to rest for 1-2 min before repeating another trial at a similar speed. Trials were then replicated with the treadmill inclined and declined at 15°, with rest intervals of 1-2 min between trials. The order of grade introduced was random. This procedure was repeated over a broad range of speeds until trials spanned gaits from walking to galloping. Because precise treadmill speeds could not be determined until after trials were completed, comparable speeds at each grade could only be approximated. As a result, data were organized by gait rather than speed for each individual. All trials were placed into one of five categories:



Fig. 1. Graphs of step-cycle duration (A), stance-phase duration (B), swing-phase duration (C), and duty cycle (D) as a function of speed for incline, level, and decline locomotion (black, gray, and white symbols, respectively). Different symbols represent different individuals (n = 7 rats). Note the comparable decrease in step-cycle and stance-phase duration, whereas swing-phase durations remain relatively constant or decrease only slightly.

slow walking (17-32 cm/s), fast walking (33-59 cm/s), trotting (52-83 cm/s), galloping first limb down (56-122 cm/s), and galloping second limb down (60-113 cm/s). The latter two categories distinguish between trials in which the experimental hindlimb was used as the first or second hindlimb down during a stride (i.e., "trailing" vs. "leading" limb). Table



2 shows the breakdown of average speeds for each gait and grade combination from each individual.

An attempt was made to obtain 1-3 locomotor trials for each gait/grade combination. However, as Table 2 reflects, there were some combinations that were quite rare: trotting downhill, first-limb gallop uphill, second-limb gallop downhill. For each trial, visual frame-by-frame examination of video files was used to determine the timing of the experimental limb's foot-down and foot-up times for each stride. These times allowed determination of three statistical variables that were used to assess basic step-cycle parameters from each stride: stance duration, swing duration, and total cycle duration (stance + swing). With the use of these data, duty cycle was also calculated (stance duration/total cycle duration).

Axoscope files were used to quantify fundamental parameters of the EMG bursts and fascicle strain regimes. Four statistical variables were used to assess EMG activity in the biceps and vastus: EMG onset and offset time (calculated relative to the timing of stance), duration, and intensity. Onset and offset times were determined visually by using magnified traces of the EMG signals. EMG intensity was calculated from the raw EMG data by averaging the spike amplitude of each rectified EMG signal. Intensities were scaled for every individual relative to the maximum value observed during level locomotion for each muscle (always observed during galloping). Thus an EMG intensity of 0.4 would reflect an average spike amplitude that was 40% of the maximum value observed during high-speed level galloping in that individual.

Fascicle strains were estimated based on the changes in distance between sonomicrometry crystal pairs implanted into the muscles of interest. The signal-to-noise ratio of the sonomicrometry measurements averaged ~40 across all animals and was estimated by comparing the baseline width of the signal at rest to the average amplitude of the signal during walking. More complete descriptions of the sonomicrometry technique are found in Olson and Marsh (28) and Biewener et al. (4), and more detail on our specific methods can be found in Gillis and Biewener (14). Briefly, any change in distance between crystals was assumed to represent a proportional length change over the entire fascicle. Fascicle strains were calculated as fractional length changes relative to a resting length. Rest length was determined while the animal was anesthetized. The animal was laid on its side (opposite the experimental limb), and the limb was then manually positioned so that the femur and tibia were oriented as during quiet stance. In this position, the distance between crystals in each muscle was measured, and these distances were used as resting lengths. Although these rest lengths likely represented a value that was slightly distinct from what would have been measured in an awake standing animal, making such measurements was impractical as animals tended to be quite mobile when awake and hooked up to the recording cables. Moreover, because rest lengths mea-

Fig. 2. Effects of surface grade on step-cycle duration (A), stancephase duration (B), swing-phase duration (C), and duty cycle (D) during slow walking. Black bars, incline locomotion; gray bars, level locomotion; white bars, decline locomotion. Data are from the same 7 rats as Fig. 1 at each grade. Speeds averaged 25 cm/s on the incline, 26 cm/s on the level, and 26 cm/s on the decline. Repeated-measures ANOVAs demonstrate a significant effect of grade on step-cycle, stance-phase, and swing-phase duration (P < 0.05). In all cases, durations are longer on an incline than on a decline. No significant effect of grade on duty cycle is detected (P > 0.05). Error bars denote standard errors.

sured in vivo are often not linked to the force-length properties of the muscle under study, their main role is to serve as a standard metric against which fascicle length changes can be scaled, which our anesthetized measurements permit. Biceps strain patterns were characterized by a single statistical variable: stance-related shortening. Two variables were used to characterize vastus strain patterns: the initial lengthening strain observed during stance and the subsequent shortening strain (also during stance). Only stancerelated strains were analyzed because these were the periods during which EMG activity was present and the muscles were likely generating active force.

Statistical analyses. Basic statistics (i.e., mean and standard error) were calculated for each of the variables mentioned above to quantitatively assess aspects of the step cycle and patterns of strain and EMG activity in the biceps and vastus on incline and decline grades. Our recent work (14) on these proximal muscles during level locomotion allows for a direct quantitative assessment of the influence of surface grade on muscle recruitment and strain. To determine the effects of surface grade on these variables, repeated-measures ANOVAs were computed by using the mean values from each individual. Because slow walking was the only gait for which trials were obtained from all individuals on level, incline, and decline grades, this was the only gait for which repeated-measures ANOVAs were performed incorporating all three grades. Most other gaits had enough data from two of the grades to facilitate quantitative comparison. In these cases, *t*-tests were used to compare between grades, and they allowed for determination of whether trends observed in slow walking were also present at faster speeds and gaits. *t*-Tests



were only used if data from at least the same four individuals were available at each grade. Thus comparisons were made between level and decline fast walking, level and incline trotting, and level and incline galloping (second limb). We used regression to analyze the relationships between speed and EMG intensity and relative duration. Analysis of covariance (ANCOVA) was then used, when appropriate, to compare these relationships among grades. A *P* value of <0.05 was used for all statistical tests to determine significance.

RESULTS

Step cycle. Step-cycle duration decreased in a curvilinear fashion with locomotor speed on level, incline, and decline surfaces (Fig. 1A). At all grades, these decreases are mirrored by similar reductions in stancephase duration (Fig. 1B). Swing-phase duration decreases minimally but significantly (P < 0.001; Fig. 1C). As a result, the proportion of the step cycle occupied by the stance phase (i.e., the duty cycle) exhibits a shallow curvilinear decrease with increasing speed regardless of grade (Fig. 1D).

Plots of step-cycle, stance-phase, and swing-phase durations as a function of speed generally show substantial overlap among grades (Fig. 1). However, more detailed analysis indicates that surface grade can influence the temporal nature of the step cycle, at least during relatively slow locomotor speeds (Fig. 2A). For example, comparison of level, uphill, and downhill

> Fig. 3. Schematic diagrams indicating the average timing of electromyographic (EMG) activity in the biceps and vastus during the step cycle for various gaits and grades. Within each gait, the stance and swing phases are distinguished by dark and light gray shading, respectively. Biceps data are shown in the top half of each diagram (above solid line), and vastus data are shown in the bottom half (below solid line). Average periods of EMG activity from level, incline, and decline locomotion are shown using gray, black and white bars, respectively. Numbers within each bar reflect the average speed of trials (in cm/s), followed by the number of rats (in parentheses) used to derive the EMG timing data. Data are only shown if at least 3 rats exhibited measurable EMG signals for that gait and grade. Note that EMG data from downhill trials for the biceps are often lacking due to little or no EMG activity in this muscle during downhill locomotion and not because of faulty recordings. Similarly, few animals exhibited downhill trotting or used the experimental limb as the trailing limb during uphill galloping. Error bars denote standard errors.

J Appl Physiol • VOL 93 • NOVEMBER 2002 • www.jap.org

walking at comparable walking speeds reveals that the duration of the step cycle differs significantly among surface grades (P = 0.02; n = 7). Specifically, step-cycle durations are significantly shorter on a decline than on an incline (P = 0.03), and decline durations are also significantly shorter than on the level (P = 0.03); however, step-cycle durations are not significantly different between level and incline walking (P = 0.11). These effects on step-cycle duration are related to gradedependent effects on both stance duration (P = 0.04; n = 7) and swing duration (P = 0.01; n = 7), which are always longer, on average, on an incline than on a decline (Fig. 2, B and C). Despite these differences in the absolute timing of the step cycle, duty cycle is not significantly affected by grade during slow walking (P > 0.05; n = 7) and averages 0.71 on both incline and decline surfaces (Fig. 2D). Because temporal aspects of the step cycle depend strongly on the speed of locomotion, quantitative comparisons of faster gaits are compromised by larger differences in average speeds across individuals.

Muscle activity patterns. EMG activity in both the biceps and vastus typically begins near the time the foot makes ground contact during each stride and ends in the second half of the stance phase, regardless of gait or grade (Fig. 3). Biceps activity, on average, begins slightly before the stance phase starts (Fig. 3). The major burst of vastus activity can begin slightly before or after the foot makes ground contact, but on

average it starts after the onset of biceps activity (Fig. 3). Although a small burst of electrical activity is often present in the vastus late in the swing phase, this burst does not appear to be affected much by speed or grade. Absolute EMG burst durations in both muscles decrease with speed in a manner similar to the speed-dependent decrease in stance-phase duration (Fig. 4, A and C). As a result, on a given surface grade, EMG duration remains a nearly constant fraction of stance-phase duration, regardless of speed or gait (Fig. 4, B and D).

In the biceps, EMG duration as a fraction of stance is greater, for any given speed, on an incline (mean =(0.85) than on the level (mean = (0.76)) (P = (0.006), ANCOVA; Fig. 4B). This difference in duration is due more to a shift in the relative timing of EMG offset than of EMG onset (Fig. 3). During decline locomotion, little or no EMG activity is present in the biceps except at high locomotor speeds (Figs. 5A and 6). Biceps EMG intensity increases significantly with speed on both level and incline surfaces. However, for decline locomotion, the slope of the line relating speed and EMG intensity is not significantly different from zero (Fig. 5A). For any speed, biceps EMG intensity tends to be greater on an incline than on the level (P = 0.0001,ANCOVA) and is much reduced, on average, on a decline (Fig. 5A).

In the vastus, EMG duration as a fraction of stance is comparable across grades (Fig. 4D) and slightly

Fig. 4. Effects of locomotor speed and surface grade on absolute EMG burst durations and durations as a fraction of stance-phase duration for the biceps (A and B; n = 4 rats) and vastus (C and D; n = 5 rats). Different symbols represent different rats (black = incline, gray = level). Best-fit linearregression lines are shown in B and D for all incline and level locomotion trials. Data for decline locomotion are not included because EMG activity was rarely present across the entire range of speeds for an individual. For all regression lines shown, the slope is not significantly different from zero (see graphs for statistics). Thus, although absolute EMG burst duration decreases with speed, bursts occupy a consistent fraction of the stance-phase duration at all speeds. Analysis of covariance (ANCOVA) results show that this fraction is greater in the biceps on an incline than on the level (P = 0.006); however, the fraction of the stance phase occupied by vastus EMG activity is similar across these 2 grades (P = 0.34).



J Appl Physiol • VOL 93 • NOVEMBER 2002 • www.jap.org



Fig. 5. Effects of speed and grade on the relative intensity of EMG signals from the biceps (A; n = 4 rats) and vastus (B; n = 5 rats). Relative intensity values were obtained for each individual by dividing the absolute intensity of every EMG signal by the maximum intensity observed during level locomotion for that individual. Different symbols represent different individuals (black = incline, gray = level, white = decline). Best-fit linear-regression lines are shown for each grade (dashed line corresponds to decline data). Except for decline biceps data, all regression lines have a positive slope significantly different from zero that demonstrate increasing intensities with increasing speed (see graphs for statistics). ANCOVA results demonstrate that EMG intensities are significantly greater, on average, on an incline than on the level in the biceps (P =(0.0001) and vastus (P < 0.0001) and that vastus intensities are significantly greater on the level than on a decline (P < 0.0001). A lack of homogeneity among slopes precluded the use of ANCOVA for comparing biceps data from decline locomotion with other grades.

lower, on average, than in the biceps (mean = 0.70, 0.67, and 0.70 for incline, level, and decline locomotion, respectively, when averaged across all speeds and individuals). In animals for which successful EMG recordings were obtained for both the vastus and biceps muscles, vastus activity typically began and ended slightly after biceps activity, regardless of grade (Fig.

3). Vastus EMG intensity increases significantly with speed on all three grades (Fig. 5*B*). At a given speed, vastus intensity is greater on an incline than on the level (P < 0.0001, ANCOVA) and is greater on the level than on a decline (P < 0.001, ANCOVA).

Muscle-strain patterns. In both muscles, fascicles undergo consistent and cyclic patterns of length change during each stride. In the biceps, fascicles generally shorten during stance and lengthen during swing, but both gait and grade influence the magnitude and/or trajectory of this length-change pattern (Fig. 6). Biceps fascicles generally begin to shorten just before the foot makes ground contact. After a brief bout of rapid shortening, fascicles often undergo a short period in which they remain relatively isometric, creating a small "shoulder" in the biceps strain trace early in stance (Fig. 6). This shoulder is typically prominent on level and decline grades but is diminished on an incline. After this shoulder, biceps fascicles shorten more considerably throughout the rest of stance. The biceps then begins to lengthen at the stance-swing transition and is stretched passively through all of the swing phase (Fig. 6).

Total biceps shortening strains during stance increase with speed from a walk to a trot, and then decrease on transition to a gallop, regardless of grade (Fig. 7). In addition, for a given gait, shortening strains are generally highest on an incline, intermediate on the level, and lowest on a decline (Fig. 7). This effect of grade is highly significant during slow walking (P <0.001; n = 6). Specific comparisons show that strains on an incline (mean = 0.21) are significantly higher than strains on the level (mean = 0.16; P = 0.007) and on a decline (mean = 0.14; P = 0.003). During fast walking, strains are significantly lower on a decline (mean = 0.15) than on the level (mean = 0.18, P <0.01; n = 6), and during trotting strains are significantly greater on an incline (mean = 0.24) than on the level (mean = 0.20, P < 0.01; n = 6). By averaging across all gaits, it was found that biceps shortening strains are 35% greater than those on the level (standard deviation among average values for each gait = 7%).

The strain regime of the vastus is markedly different from that observed in the biceps (Fig. 8). Vastus fascicles are typically stretched over the first half of the stance phase (Figs. 8 and 9). The amount of stretching incurred during this period varies among individuals but generally ranges between 8 and 15% of resting length, regardless of grade. After this stretch, fascicles exhibit one of three patterns through mid to late stance, depending on gait and grade: continued lengthening (generally observed on a decline), a brief period of nearly isometric behavior (most typical during walking), and a variable amount of shortening (generally greatest on an incline and at higher speeds). This "poststretch" or "late-stance" shortening strain is most prominent in the second limb down during galloping (Figs. 8 and 9), and it is significantly greater on an incline than on the level (P = 0.01;n = 5), averaging 0.14 and 0.10, respectively. During the transition from stance to swing, the vastus again begins



Fig. 6. In vivo recordings of biceps strain and activation from the same rat during walking (top) and galloping (bottom) (all recordings from second limb down) for level, incline, and decline surfaces (left, middle, and right, respectively). Dark gray shading represents the stance phase, and light gray shading represents the swing phase. Note that the biceps largely shortens during stance, regardless of gait or grade, and that for both gaits stance-related shortening strains are greatest on an incline. In addition, traces from decline trials demonstrate minimal EMG activity, even at relatively fast speeds. All panels have the same scales for strain and EMG amplitude. Points of maximum strain, rather than the timing of stance onset, were used to define the intervals shown in each panel. Because maximum length was typically reached late in swing, the leftmost portion of each panel typically encompasses the very end of the swing phase (the small light gray area). L, muscle length; L_o , optimal muscle length.

to stretch as the knee is flexed and the foot is lifted off the ground (Figs. 8 and 9). Vastus fascicles continue to lengthen over the first third of the swing phase as the knee continues to flex and then shorten rapidly and over a large distance (15-20% of rest length) as the knee extends until the foot makes ground contact (Figs. 8 and 9).

During decline locomotion, almost no late-stance shortening is ever observed in vastus fascicles. For example, during walking, vastus fascicles are stretched throughout the entire stance phase and exhibit only a shift in the relative velocity of stretch rather than a period of isometric or shortening behavior (Figs. 8 and 9). Even in the second limb down during decline galloping, fascicles often remain nearly isometric after their initial stretch, and, if they do shorten, strains are often no more than 2-3%(Figs. 8 and 9).

DISCUSSION

We undertook this study to determine the response of two proximal hindlimb muscles to changes in surface grade. The vastus lateralis is an uniarticular extensor of the knee; the biceps femoris is a biarticular muscle

with an extensor moment at the hip and a flexor moment at the knee. Our results demonstrate that, at all speeds and gaits, both recruitment intensity and fascicle strain change markedly with grade in these muscles. Muscle activation levels are greater, for a given speed, on an incline than on the level and lower on a decline. In fact, the biceps femoris remains inactive during decline locomotion, except at relatively high speeds. Biceps fascicles shorten during stance on all grades, but for any gait they shorten more on an incline than on the level and more on the level than on a decline. The vastus lateralis is generally stretched 8–15% over the first half of stance, regardless of grade. After this initial stretch, vastus fascicles undergo a variable amount of shortening during the second half of stance on level and incline surfaces. This shortening is higher, on average, on an incline than on the level and increases with speed, reaching a maximum in the second limb down during galloping. In contrast, on a decline, vastus fascicles are generally stretched throughout stance, except during galloping, where a brief period of nearly isometric behavior follows the



Fig. 7. Histograms of average biceps shortening during stance at different gaits and grades for one individual. Gray, black, and white bars represent level, incline, and decline trials, respectively. Although variation exists among individuals with respect to shortening strain values, the grade-related changes exhibited here are generally representative of other animals. The only exception is the difference between incline and level galloping (lead limb), which is substantially greater in the animal depicted here than in the other rats (mean = 20% difference). Regardless of gait, however, shortening strains are typically highest on an incline, intermediate on the level, and lowest on a decline. Error bars represent standard errors.

initial stretch. Taken together, these results suggest that proximal hindlimb muscles are likely as important for mediating levels of mechanical work output and absorption during grade locomotion as the distal ankle extensors that have been studied previously (e.g., Refs. 12, 15, 31).

Surface grade and the step cycle. Moderate slopes can influence temporal aspects of the step cycle during locomotion. For example, rats in this study used longer stride periods (i.e., lower stride frequencies) when walking up a 27% grade than when walking on the level at comparable speeds. Grade-related increases in stride duration have also been observed in rats moving up a 30% grade over a range of speeds (33), in cats walking up a 36% grade [see Fig. 1 in Pierotti et al. (29)], and in horses trotting up a 6-10% grade (16, 35). Although fewer studies have examined the effects of a decline grade on stride parameters, both rats (this study) and cats (37) appear to adopt relatively higher stride frequencies (i.e., shorter stride periods) and shorter stride lengths during decline walking than on the level. In fact, the study by Smith et al. (37) suggests that the steeper the slope, the more stride period and stride length decrease during decline walking.

However, data regarding such grade-dependent temporal shifts are conflicting, even within the same species. Inclined grades had no effect on stride frequency in various studies of horses exercising at different speeds (9, 18, 31) or in cats walking freely (7) or running on a treadmill (29). Despite such disparity among compiled grade data for various animals to date, it seems fair to draw several tentative conclusions with respect to the effects of grade on temporal aspects of the step cycle. First, when an effect is present, it is manifested by an increase in stride duration on an incline (e.g., Refs. 16, 33, 35) and a decrease in duration on a decline (e.g., Ref. 37). Second, the magnitude of this effect may change with speed or gait; for example, several studies demonstrate an effect of grade during slow walking but not at faster locomotor speeds (e.g., Ref. 29). Third, the nature of the effect of grade may differ among species. Without more controlled studies using similar methods and large sample sizes, the impact of moderate surface grades on the temporal nature of the step cycle during quadruped locomotion will remain somewhat obscure.

Surface grade, gait, and speed. Although we did not set out to examine the interactions among grade, gait, and speed explicitly, examination of Table 2 provides some insight into these issues. First, animals were typically resistant to moving at high speeds on a decline (e.g., only 4 of 7 animals exhibited high-speed locomotion on a decline, whereas all 7 moved at high speeds on incline and level surfaces). We doubt such results are indicative of any natural disinclination toward downhill galloping in the wild but instead likely reflect the unnatural circumstance of running downhill fast on an enclosed treadmill. Second, animals rarely used trotting on a decline (e.g., only 1 of 7 animals exhibited downhill trotting). Most rats that were willing to gallop downhill were unwilling to exhibit a trotting gait on a decline at the speeds typically used for trotting on level and incline surfaces. Instead, these animals extended use of their fast walking gait at these speeds. This could reflect either a natural disinclination toward decline trotting or a response to using an enclosed treadmill. The fact that rats were willing to gallop on a decline but were nevertheless unwilling to trot at the speeds examined suggests that the reasons underlying the lack of one gait vs. the other may be different. Additional experiments are required to tease apart these issues.

Surface grade and muscle recruitment. It is the activation and contraction of limb muscles that provide the forces and energy required to support and propel terrestrial animals during locomotion. Although there have been relatively few studies of the effects of surface grade on muscle activity patterns during animal locomotion, a number of results from this study are consistent with those observed among various vertebrate species moving up and down inclines. In general, extensor muscles become activated shortly before or at the time the foot makes ground contact, and activity persists over 60-80% of the stance phase. Although the effects of surface grade on the timing of muscle activity are relatively minor, the intensity of this activity is altered substantially. Relative to level locomotion, incline locomotion at similar speeds generally elicits an increase in EMG intensity in hindlimb extensors (7, 8, 19, 31, 32), except those composed predominantly of slow-twitch fibers (29, 33). In contrast, decline locomotion generally elicits a reduction in EMG intensity in various limb extensors (37). Such alterations are typically interpreted as reflecting shifts in the level of



Fig. 8. In vivo recordings of vastus strain and activation from the same rat during walking (*top*) and galloping (*bottom*) (all recordings from second limb down) for level, incline, and decline surfaces (*left, middle*, and *right*, respectively). Dark gray shading represents the stance phase, and light gray shading represents the swing phase. Note that during stance the vastus always undergoes an initial period of stretch, regardless of gait or grade. After this relatively rapid stretch, fascicles exhibit a period of shortening, continued stretching, or relatively isometric behavior, depending on gait and grade. Late in stance, the muscle is again stretched rapidly as the limb transitions into swing. During the initial portion of the swing phase, fascicles continue to stretch before shortening rapidly and over a long distance until the foot again makes ground contact. The vastus typically exhibits EMG activity during the first two-thirds of stance, when the predominant strain is stretching. However, in the second limb down during galloping (*bottom*), the vastus typically undergoes more substantial shortening strains in the latter half of stance, and EMG activity is often present during much of this shortening. All panels have the same scales for strain and EMG amplitude.

activation or volume of muscle recruited to mediate the mechanical output required on different grades.

A rather unexpected result of decline slope walking in cats was reported by Smith and colleagues in the mid 1990s (36, 37). Major muscles that act to extend the hip (e.g., anterior biceps femoris and anterior semimembranosus) remained inactive during the stance phase on downhill grades as shallow as 10%. Whereas previous experiments had revealed an "immutable" synergy among limb extensors at the hip, knee, and ankle during various locomotor tasks (5), this synergy was broken during decline locomotion. Our data for downhill locomotion confirm the absence of EMG activity in the anterior region of the biceps femoris in rats as well, even at speeds faster than slow walking. In fact, even during slow galloping, EMG activity was occasionally absent on a downslope. Thus, among mammalian quadrupeds, inactivation of major muscles that act in extension at the hip joint may be a widespread neuromotor response to decline grades, thereby allowing hip extension to be largely passive (i.e., gravitational potential energy of the body can used to extend the hip when a rear foot is in contact with the ground), whereas active hip flexors may actually absorb mechanical energy during stance (37).

Surface grade and muscle strain. It is well known that certain limb muscles exhibit biochemical and histological responses to exercise on different grades. More specifically, antigravity muscles typically experience substantial physical damage in untrained animals after bouts of downhill locomotion but less damage on the level (2). Such changes are assumed to be linked to grade-dependent differences in the mechanical actions of the underlying muscles. Limb muscles are presumed to undergo mainly concentric (i.e., shortening) contractions during uphill locomotion that result in relatively minimal damage. In contrast, downhill locomotion is known to bias limb muscles toward "eccentric" contractions, in which muscle fibers are stretched while actively generating force. Active muscle stretching dissipates energy as animals move downhill but also may result in rapid- and high-force devel-



Fig. 9. Schematic vastus strain profiles for different gaits and grades. Vastus strain trajectories typically comprise 4 phases during each stride: 1) an initial stretch in the first half of stance; 2) a subsequent period in which fascicles exhibit variable degrees of shortening, lengthening, or remain nearly isometric; 3) another stretch during the transition from stance to swing; 4) a final period of substantial shortening over the second half of swing. Panels show the average timing and fascicle length excursions during each of these phases for different gaits and grades (n = 5 rats). Error bars are not shown in the figure because they obscure the presentation. Solid black lines reflect incline trials, gray lines represent level trials, and dashed lines represent decline trials. Within each gait, the stance and swing phases are distinguished by dark and light grav shading, respectively. Average speeds (in cm/s) and sample sizes (in parentheses) are shown at *right*. Animals rarely trotted downhill or used the experimental limb as the first limb down when galloping uphill; hence, strain trajectories for these gaits and grades are not shown. Note that the phase after the initial stretch in stance is what varies most across gaits and grades. Fascicles generally exhibit more shortening during this period as speed increases and as the grade inclines. In contrast, decline trials show little to no shortening over the same period.

opment and injury, particularly to muscles composed mainly of slow fibers. For example, the vastus intermedius in rats, which is largely composed of slow oxidative fibers, incurs more damage after downhill locomotion than the vastus lateralis and medialis, which both have smaller proportions of slow fibers (2). Short-term training can mitigate the extent of muscle damage (34), perhaps by leading to an increase in the number of sarcomeres in series within myofibrils (23, 24) or by degeneration and/or regeneration of fibers susceptible to injury (2, 34).

Despite recognizing major differences in the response of certain limb muscles to locomotion on different grades, the actual strain profiles experienced by such muscles have remained largely unknown. Strain data from the vastus lateralis of rats demonstrate a large degree of stretching over the first half of stance at all speeds (14) and grades (present study). EMG activity is generally coincident with this stretching, which suggests that eccentric contractions characterize vastus lateralis behavior regardless of grade. If one assumes that vastus intermedius strains are grossly similar to those of the vastus lateralis, this suggests that any differential morphological response of the muscle to training on different grades is not simply due to the presence of eccentric contractions on a decline and absence of such contractions on an incline. Instead, grade-dependent differences in vastus strain appear to be more subtle.

Most in vivo studies of eccentric muscle contraction in rats exercise animals on declined slopes comparable to those used in this study (i.e., $\sim 15^{\circ}$) at 14–16 m/min or 23-27 cm/s (2, 23, 24, 34), which is categorized herein as slow walking. At this grade and speed, vastus fascicles are typically stretched throughout all of stance and can experience continuous lengthening strains of nearly $25\overline{\%}$, which has been shown to elicit substantial damage in situ in different mammalian muscle models (21, 27). Moreover, given the relatively higher limb-cycle frequencies used on a decline grade at this speed, stretching rates early in stance are between 5 and 50% greater than on an incline. Hence, differences in initial stretch velocity and/or the lack of any discrete fiber shortening are what differentiate the vastus strain regime between decline and incline grades at these speeds. The absence of fiber shortening and rapid rate of early stretch are likely the mechanical factors that induce the biochemical and morphological changes observed in vastus intermedius fibers after downhill locomotion (e.g., Refs. 2, 23, 24, 34).

Incline locomotion requires that the limb musculature as a whole produces larger amounts of mechanical work than on the level. Muscles produce mechanical work by actively shortening, and the greater the force produced and distance shortened, the higher the work output. Previous work on in vivo limb muscle force production and strain have typically focused on muscles acting at the ankle joint. Results from this work suggest that ankle extensors exert higher forces (15) and/or undergo greater shortening strains (32) in response to an inclined grade. Although we lack data on force production and thus cannot directly measure work output of these muscles on any grade, our strain and EMG data suggest that more proximal muscles also augment work output on inclined grades. During walking, trotting, and galloping, total biceps shortening and EMG intensity average 30-35% more on an incline than on the level. If one ignores force-velocity issues and makes a variety of other simplifying assumptions (e.g., EMG intensity is proportional to the volume of muscle recruited, all biceps shortening is active), these increases in shortening distance and EMG activity would suggest that the biceps likely increases its mechanical work output substantially in response to a 15° incline. Knee extensors might also augment work output during incline locomotion as the shortening observed late in stance on an incline is typically greater than that observed on the level in all gaits. For example, in the second limb down during galloping, vastus fascicles undergo 40% more latestance shortening on an incline than on the level and show comparable increases in EMG intensity as well. Given the simplifying assumptions mentioned above, these changes likely lead to a major increase in work output by this muscle during late stance on uphill grades.

Thus modulation of both strain and activation likely leads to substantial shifts in mechanical work production and absorption in major hip and knee extensor muscles of the rat during locomotion on different grades. Previous work on ankle extensors in different vertebrate species suggests that more distal pinnate muscles, with relatively long tendons, shift their function in a similar manner in response to changes in surface grade. Hence, despite substantial differences in architecture, major muscles throughout the hindlimb are likely important for amplifying and/or absorbing mechanical energy during locomotion on slopes of different degree.

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