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Recruitment of faster motor units is associated with greater rates of fascicle strain and rapid changes in muscle force during locomotion

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1 **Abstract**

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Animals modulate the power output needed for different locomotor tasks through changes in muscle force production and fascicle strain rate. To generate sufficient force, appropriate motor unit recruitment must occur. Given that faster motor units contract with faster strain rates and have faster activation-deactivation rates, it is therefore likely that faster motor units are recruited for more rapid movements. The goals of this study were to 1) describe changes in motor unit recruitment patterns that occur with changes in locomotor dynamics and 2) test whether motor unit recruitment can be directly related to *in vivo* measures of muscle force and fascicle strain and strain rate, and thus mechanical work. Myoelectric, sonomicrometric, and muscle-tendon force data were collected from the lateral and medial gastrocnemius muscles of the goat hind limb during level and incline walking and trotting, and level galloping. Myoelectric signals were analyzed using wavelet and principal component analysis in order to quantify changes to the myoelectric frequency spectra across locomotor conditions. Fascicle strain and strain rate were calculated from the sonomicrometric data, and force rate was calculated from the tendon force data. The results of this study demonstrate that, under certain locomotor conditions such as level galloping and incline walking, where EMG activity were similar but had different frequency components, faster and slower motor units are recruited in patterns that were task-specific. The study also shows that the recruitment patterns of different motor unit types are related to *in vivo* fascicle strain rates in addition to myoelectric intensity and force. Together, these data provide evidence that changes in motor unit recruitment have an underlying mechanical basis, at least for certain locomotor tasks.

1 **Introduction**

2

3 Mammalian skeletal muscles are composed of multiple motor unit types, each of which
4 has defined physiological and mechanical properties. This enables these muscles to produce the
5 mechanical output required to perform a wide range of locomotor tasks with variable force and
6 velocity requirements. For example, fast fibres have strain rates and activation-deactivation rates
7 that are two to three times faster than those of slow fibers (He et al., 2000). It seems likely that
8 these differences influence the way motor units are recruited within individual muscles and
9 among synergistic muscles. Many studies have made indirect assessments of muscle force,
10 length, and shortening velocity (e.g. Hodson-Tole and Wakeling, 2008; Lichtwark et al., 2003;
11 Gillis et al., 2005). However, few studies have measured muscle force and strain together
12 directly during differing locomotor tasks due, in part, to methodological challenges.
13 Furthermore, the possible link between motor unit recruitment patterns and task-dependent
14 factors, such as muscle fascicle strain rate, has only been explored in a limited number of studies
15 (Hodson-Tole and Wakeling, 2009, Wakeling et al., 2006). Thus, whether the manner in which
16 recruitment patterns change with the force- or strain-related demands of different locomotor
17 tasks remains largely unknown.

18 Recent *in vivo* studies have established that muscles modulate their work output to meet
19 the changing mechanical demands associated with various locomotor tasks (Roberts et al. 1997,
20 Daley and Biewener, 2003; Gabaldon et al., 2004; McGuigan et al. 2009). For example, when an
21 animal runs uphill, the net output of the muscles must be increased to raise the body's center of
22 mass. Across a variety of species, in those muscles examined, muscle fascicle strain, strain rate
23 and force have been shown to vary substantially when animals and humans walk, hop, and run at
24 different speeds, or over different terrain (e.g. Roberts et al., 1997; Biewener, 1998; Biewener et
25 al., 1998; Daley and Biewener, 2003; Gillis and Biewener, 2002; Fukunaga et al., 2001;
26 Lichtwark et al., 2006, 2007; McGuigan et al., 2009). However, the manner in which work
27 output is modulated by recruiting different motor unit types remains largely unknown.

28 When there is a requirement for an increased level of muscle force output from a muscle,
29 motor units are typically recruited in an orderly fashion from slowest to fastest, originally
30 formulated as the size principle of motor unit recruitment by Henneman et al. (1965, 1974) and
31 others (Freund et al., 1975, Fedde et al., 1969). Different motor unit types innervate a range of

1 | [muscle fibres: type I – slow-twitch and type IIA-IIB fast twitch-fibres \(Burket et al., 1973\).](#)
2 | However, evidence for alternative, task-dependent recruitment patterns has emerged for muscles
3 | operating across a range of species (e.g. Gillespie et al., 1974; Grimby et al., 1981; Nardone et
4 | al., 1989; Sokoloff and Cope, 1996; Wakeling et al., 2006; Hodson-Tole and Wakeling, 2007,
5 | 2008, 2010). For example, there is evidence of preferential recruitment of faster motor unit types
6 | during rapid locomotor tasks. This evidence is based, in large part, on analyzing the time-
7 | varying frequency spectra of myoelectric signals (reviews: Hodson-Tole and Wakeling, 2009;
8 | Reaz et al., 2006). Different motor unit types have muscle fibres with different electrical
9 | membrane properties (Luff and Atwood, 1972), [and](#) there is an intrinsic speed dependence of the
10 | EMG frequency such that faster fibers generate higher-frequency signals (Wakeling et al., 2002;
11 | Hodson and Wakeling, 2008; Lee et al., 2011). Recently, Wakeling et al. (2006) demonstrated
12 | during cycling in humans that EMG signals shifted to higher frequencies [when humans cycle at](#)
13 | higher [pedaling frequencies and](#) muscle fascicle strain rates, [consistent with an earlier](#) cycling
14 | study [that](#) suggested preferential recruitment of the faster [quadriceps](#) muscle fibres [at](#) faster
15 | [contraction](#) speeds during locomotion (Citterio and Agostoni, 1984). Hodson-Tole and Wakeling
16 | (2008) [similarly](#) showed that myoelectric frequency content was associated with [changes in](#)
17 | [fascicle](#) strain rate in [rat hindlimb muscles](#) [over](#) a range of gait velocities and inclines.

18 | Other factors, such as the force magnitude and the force rise and relaxation rates of
19 | different fibres, may also influence motor unit recruitment. Since activation-deactivation rates
20 | vary for different motor unit types (Burke et al., 1973; Lee et al., 2011), it is logical that
21 | locomotor tasks that have [short](#) shortening-lengthening cycles, such as a cat paw-shake, require
22 | fast activation-deactivation rates and thus, recruitment of faster motor units (Smith et al., 1980).
23 | Roberts and Gabaldon (2008) observed [an increased](#) electromechanical delay for force relaxation
24 | in [turkey](#) ankle muscles during slow walking than during running. Because the delay was not
25 | correlated with the muscle's shortening velocity, they hypothesized that the shorter relaxation
26 | times during fast walking reflect the shorter relaxation times of faster muscle fibres. Hodson-
27 | Tole and Wakeling (2008) reported similar observations during incline walking where [rat](#) muscle
28 | strain rates were slower [during level walking](#) and were associated with the recruitment of slower
29 | motor units despite increased total EMG intensity. [Preferential recruitment of motor units types](#)
30 | [was further supported when comparing the EMG frequency content during level running and](#)
31 | [incline walking where the EMG intensity was similar, but the EMG frequency content was](#)

1 | significantly lower during incline walking. It is possible that when rapid force production and
2 | relaxation are required, recruitment of faster motor units — independent of slow units — is
3 | needed. If slower motor units are activated, then faster cycle rates may be hindered. During tasks
4 | where sustained force at low to moderate levels is needed, or where rapid force generation and
5 | relaxation are not required, orderly motor unit recruitment according to the size principle is more
6 | likely to be sufficient to meet the mechanical demands of the muscle.

7 | In this paper, we explore whether force rise and relaxation rates, along with fascicle strain
8 | rates, are associated with orderly recruitment patterns from slow to faster motor units of the
9 | lateral and medial gastrocnemius (LG and MG) muscles of goats during different *in vivo*
10 | locomotor tasks, or whether there are conditions in which faster units are preferentially recruited
11 | in a task-specific fashion. In goats and other mammals, the LG and MG muscles are composed
12 | of both slow and fast fibres. Goats were selected for this study because they allowed EMG,
13 | fascicle length and strain rate, as well as tendon force to be measured directly under *in vivo*
14 | conditions. The purposes of this study were to 1) describe changes in motor unit recruitment
15 | patterns due to changes in locomotor dynamics associated with speed, and grade; and 2) examine
16 | whether these motor unit recruitment patterns potentially tune or enhance the muscle's
17 | contractile properties for a specific motor task by characterizing the relationships between motor
18 | unit activity, fascicle strain rate, and force profiles. Given that the recruitment of faster motor
19 | units results in a higher frequency content of the EMG with the same or less intensity, we
20 | hypothesized that 1) faster gait speed leads to an increase in the high frequency component of the
21 | EMG signal and that 2) for similar EMG intensities, increased surface grade leads to an increase
22 | in the low frequency component of the EMG signal compared with fast level locomotion. We
23 | also hypothesized that 3) there is a significant association between myoelectric frequency
24 | content, force, and force rise and relaxation rates, as well as 4) there is a significant positive
25 | correlation between EMG frequency content and muscle fascicle shortening strain rate. These
26 | associations are anticipated because increases in muscle force likely depend on the recruitment
27 | of faster motor units within a muscle (in addition to slow units), with faster speeds (and higher
28 | fascicle strain rates) being linked to faster rates of force rise and relaxation.

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1 **Methods**

2

3 | _____Six African pygmy goats (*Capra hircus* L; 3 males, 3 females; mean \pm standard
4 | deviation; age: 21.0 ± 15.5 months, **body mass**: 25.85 ± 6.20 kg) were tested at Harvard
5 | University's Concord Field Station. EMG, fascicle strain, and tendon force data were recorded *in*
6 | *vivo* during a variety of locomotor tasks, as detailed below. The experimental protocol was
7 | conducted over a three-day period, which included surgical implantation of transducers, *in vivo*
8 | testing, and *in situ* testing (Lee et al., 2011). The results of the *in situ* experiments have been
9 | previously reported (Lee et al., 2011). All surgical and experimental procedures followed
10 | IACUC approval.

11

12 *Surgical implantation of transducers*

13

14 | Initially, animals were sedated with a mixed injection of ketamine and xylazine (8mg/kg
15 | body mass and 0.05 mg/kg body mass, respectively) into the jugular vein. The animals were
16 | then intubated and maintained on a closed system anesthesia machine (Matrix, Orchard Park,
17 | NY, USA) at 0.5 – 1.0 % isoflurane.

18 | Offset twist-hook bipolar silver-wire electrodes (0.1 mm, California Fine Wire Inc.,
19 | Grover Beach, USA), with tips bared of 0.5 mm of insulation and an offset of 2 mm, were
20 | implanted about 3 mm deep into proximal, mid-belly, and distal regions of the lateral and medial
21 | gastrocnemius muscles (Lee et al., 2011).

22 | Sonomicrometry crystals (2 mm, Sonometrics Inc., London, Ontario, Canada) were
23 | implanted into the mid-belly region of the lateral and medial gastrocnemius muscles in either
24 | paired or triad configurations (Lee et al., 2011). For the paired configuration, the crystals were
25 | inserted parallel to the fascicles (proximo-superficial to distal-deep $\sim 25^\circ$ pennation angle),
26 | which allowed length changes of the fascicles to be estimated. For the triad configuration, an
27 | additional crystal was implanted directly below the superficial crystal to measure muscle depth,
28 | which also allowed changes in pennation angle to be estimated (these results will be reported
29 | elsewhere). The signal output was amplified (Triton 120.2; Triton Technology, San Diego, CA,
30 | USA) and monitored on an oscilloscope (2245A; Tektronix, Beaverton, OR, USA). The
31 | alignment of the crystals was optimized to maximize the signal-to-noise ratio.

1 A lateral incision proximal to the ankle joint was made to expose the underlying tendons.
2 The common tendon sheath was then exposed to separate the gastrocnemius tendon from the
3 superficial digital flexor tendon. Gas-sterilized “E”-shaped stainless-steel buckle transducers,
4 equipped with a metal foil strain gauge (type FLA-1, Tokyo Sokki Kenkyujo) bonded to the
5 central arm (Biewener & Baudinette, 1995), were attached to the Achilles tendon. On several
6 goats, a longer 2 cm incision was made along the distal portion of the Achilles tendon and two
7 separate tendon buckles were attached to the lateral and medial portions of the Achilles tendon,
8 enabling separate recordings of LG and MG force (Lee et al., 2011).

9 Lead wires from all transducers were passed through a subcutaneous tunnel to a
10 connector that was sutured to the skin proximal to the hip. Animals received post-operative
11 analgesia (buprenorphine 0.1 to 0.5 mg/kg, subcutaneously) every 12 hrs during the following 40
12 hr recovery and *in vivo* recording periods.

13

14 *In vivo testing*

15

16 Goats were allowed 20-24 hrs for recovery following surgery. The animals had been
17 previously trained to walk, trot, and gallop on a large, motorized treadmill (belt, 2.50 m long and
18 0.75 m wide) on the level and 15 ° incline. The gait speeds ranged from 1.1 – 1.4 ms⁻¹, 1.7 – 2.8
19 ms⁻¹, and 3.3 – 4.9 ms⁻¹ across all goats. For each goat, the same speed was kept for the level and
20 incline conditions. Recordings were therefore collected at typical walking and running speeds
21 such that three trials of at least 15-20 strides each were collected for each condition. Sufficient
22 rest was given to the goats between trials to ensure that they could complete the experiments.
23 Myoelectric signals were amplified (gain of 100-1000) and recorded with minimal filtering
24 (bandpass 30-3000 Hz, notch filtered at 60 Hz, P511 amplifier, Grass, West Warwick, USA).
25 Tendon buckle signals were connected to a bridge amplifier (Vishay 2120, Micro-Measurements,
26 Raleigh, NC). All signals were recorded at 5000 Hz using a 16-channel acquisition device (NI-
27 6259, National Instruments, Austin, TX).

28 Tendon buckle transducers were calibrated the following day, after the *in situ*
29 experiments were completed and the animals euthanized (sodium pentobarbital, 150 mg kg⁻¹).
30 For these calibrations, a cut was made across the distal aponeurosis of the LG and MG to free a
31 portion of the Achilles tendon with the buckles intact. The cut end was clamped and frozen with

1 liquid nitrogen. A series of cyclical loads were imposed using a force transducer (model 9203,
2 Kistler, Amherst, MA), and the relationship between buckle voltage and applied force was
3 determined.

4

5 *Data Analysis*

6

7 Stride times were determined from the tendon force profiles of each trial. The beginning
8 and end of each stride were defined as 60 ms prior to when the force began to increase (Fig. 1).
9 This ensured that our analysis captured the entire muscle activation profile.

10

11 *Analysis of force data*

12

13 Tendon forces were filtered using a low-pass third-order Butterworth filter with a cut-off
14 frequency of 115 Hz and notch filter at 60 Hz. [The cut-off frequency was chosen to](#)
15 [accommodate testing the models against *in situ* experiments where we had 40 Hz tetanic](#)
16 [stimulations \(Wakeling et al., 2012\).](#) Force rise and relaxation rates were calculated as the first
17 derivative of tendon force with respect to time. Data from each condition were partitioned into
18 20 equal time windows, and mean force and force rates were calculated for each time window
19 (Fig. 1).

20

21 *Analysis of sonomicrometric data*

22

23 Raw sonomicrometry signals were converted to a distance measurement between the
24 crystals based on the speed of sound through skeletal muscles, adjusted by a +0.82 mm
25 correction due to differences between the speed of sound through the epoxy coating of the
26 crystals and the speed of sound through muscle (Daley and Biewener, 2003). Sonomicrometry
27 signals were first filtered using a low-pass, second-order Butterworth filter with a cut-off
28 frequency of 30 Hz. [Filters with different parameters were tested, but these parameters were best](#)
29 [in smoothing out noise, while still capturing time-varying features of the signal.](#) A custom-
30 written program was used to identify and remove “extraneous” peaks, after which the signals
31 were fit with a 4th order polynomial using a 200-point (40 ms) size window. [Extraneous peaks](#)

1 | [would occasionally occur in the signal when one or two values deviated greatly from the signal.](#)

2 Fascicle lengths were converted to strain by subtracting the resting length (distance measured
3 between the crystals during quiet stance) then dividing by the resting length (Gillis et al., 2005).
4 Strain rate was calculated as the first derivative of strain with respect to time. Data from each
5 condition were partitioned into 20 equal time windows and mean strain and strain rate were
6 calculated for each time window (Fig. 1).

8 *Analysis of EMG data*

9
10 EMG signals from the LG and MG were analyzed using wavelet analysis, a time-
11 frequency decomposition technique, similar to previously published methods (von Tscharner,
12 2000; Wakeling, 2002; Wakeling and Syme, 2002; Lee et al., 2011). A filter bank of 24 wavelets
13 $0 \leq k \leq 23$ was used to decompose the myoelectric signals into intensities as a function of time
14 and frequency. To exclude low frequency noise, the first four wavelet domains were excluded
15 from further analysis (≤ 70 Hz) such that the data were analyzed for wavelets 3 to 23, Hodson-
16 Tole and Wakeling, 2007). Thus, the frequency band of 70–1857 Hz is presented in this analysis.
17 This ensures that the signals of the slow motor units (central frequencies of ~150 Hz, Lee et al.,
18 2011) are included in the analysis and are consistent with the cut-off frequency used in other
19 fine-wire myoelectric studies (Daley and Biewener, 2003; Gabaldon et al., 2004; Gillis and
20 Biewener, 2001 & 2002). As with the force data, myoelectric signals were partitioned into 20
21 equal-time windows and the mean intensity for each wavelet for each time window was
22 calculated. Total intensity of the signal was calculated as the sum of the intensities determined
23 using wavelets $3 \leq k \leq 23$.

24 We used principal component analysis (PCA) to identify the major features of the
25 intensity spectra (Wakeling, 2004; Hodson-Tole 2008; Lee et al., 2011). We randomly selected
26 30 strides per gait condition for statistical analysis. The spectra were compiled into a $p \times N$
27 matrix **A**, where $p = 20$ wavelets and $N = 36,000$ (6 goats x 2 muscles x 5 gait conditions x 30
28 strides x 20 partitioned time windows). The principal components of the intensity spectra, PCs,
29 defined in terms of eigenvector-eigenvalue pairs, were calculated from the covariance matrix **B**
30 of the data matrix **A** without prior subtraction of the mean (Wakeling and Rozitis, 2004). This
31 | ensured that the whole signal, and not just its variance, was described. The principal component

1 weightings of each PC were given by the eigenvectors ξ of covariance matrix **B** and the amount
2 of the signal explained by each PC was determined from the eigenvalues. The PC loading scores
3 were calculated from $\xi'A$, the product of the transpose of the weighting matrix and matrix **A**. We
4 calculated the angle, θ , as the angle formed between the vector of the first and second principal
5 component, PCI-PCII, loading scores and PCII loading score axis (Wakeling and Rozitis, 2004).
6 This measure describes the contribution of high and low frequency content in the signal. A small
7 θ has a positive contribution of the PCII loading scores and indicates relatively high frequency
8 content. Mean PCI and PCII loading scores were calculated for each of the 20 time windows of
9 the stride, thus allowing changes in their contributions to be quantified throughout each stride for
10 each gait condition.

11

12 *Statistical analysis*

13

14 Differences in myoelectric intensity, muscle force, force rise and relaxation rates, muscle
15 fascicle strain and strain rate between muscles, activity (walk, trot, and gallop), and grade
16 (incline and level) were determined using general linear model ANOVA. Tukey *post hoc* tests
17 were conducted if significant differences were identified to locate differences within each factor.
18 A general linear model ANCOVA was conducted to identify significant associations between θ
19 and intensity, θ and muscle fascicle strain rate, and θ and force rise and decline rates, with strain,
20 strain rate, force, force rate, and myoelectric intensity as covariates. [Myoelectric intensity was
21 included as a covariate so that associations between \$\theta\$ and strain rate could be detected that were
22 independent from the effect of myoelectric intensity.](#) Muscle fascicle strain was [also](#) included as
23 a covariate so that changes in strain would not confound the results (Wakeling et al., 2006), as
24 strain can influence myoelectric frequency content (Doud and Walsh, 1995). Greater strains can
25 be associated with decreased frequency content, so if muscle fascicle strain and θ were
26 significantly positively correlated, no further analysis was conducted. [The analyses were
27 conducted for time periods corresponding to concentric contraction, when the strain rate was
28 negative. Separate analyses were done for force-rise and force-relaxation periods so that the
29 association between angle \$\theta\$ and force rise and relaxation rates could be calculated since these
30 rates were positive and negative, respectively.](#)

31

Results

General changes in EMG intensity and frequency content in relation to muscle contractile patterns across differing locomotor tasks

Across all gait conditions, the LG and MG showed a single shortening cycle during limb support with typically one burst of muscle activity and period of active force production per stride (e.g., Fig. 1). In general, total EMG intensity, force, and force rise *and* relaxation rates, *as well as fascicle* strain and strain rate, all tended to increase as speed increased from walking to trotting to galloping. These measures were even higher when animals walked and trotted uphill (Tables 2 to 5, Figs. 2 *and* 3).

From the PCA, the first two principal components explained about 76% of the myoelectric signal (Fig. 4). The first PC had positive weightings for all frequencies and had a shape that was similar to the mean intensity spectrum ($r^2 = 0.98$). The second PC contained positive and negative weightings with a transition occurring at 387 Hz. In general, θ tended to decrease as speed increased from level walking to trotting to galloping, indicating an increase in the high frequency component of the EMG signal with locomotor speed. By contrast, θ tended to increase as surface grade increased (Tables 2 to 5, Fig. 4), indicating *an* increase in the low frequency component of the EMG signal with surface grade

Association between myoelectric frequency content and myoelectric intensity and force

A significant negative association was observed between θ and myoelectric intensity (all $p < 0.001$) (Table 1), as well as between θ and force (all $p < 0.001$) for both LG and MG force rise and relaxation rates. *Thus, as* myoelectric intensity increased, the high frequency component of the EMG signal increased and force increased.

Association between myoelectric frequency content and force rate

1 A significant negative association was observed between θ and force rate during LG
2 relaxation ($p = 0.031$) (Table 1), but not during LG force rise, MG force rise, or MG force
3 relaxation. Thus, except for LG relaxation, these data do not support the hypothesis that
4 myoelectric frequency content is associated with rates of force rise and relaxation.

5

6 *Association between myoelectric frequency content and fascicle strain and fascicle strain rate*

7 A significant negative association was observed between θ and fascicle strain for LG
8 force relaxation ($p < 0.001$), but not for LG force rise, MG force rise, or MG force relaxation
9 (Table 1). Consequently, except during LG relaxation, our interpretations of recruitment are not
10 confounded by fascicle strain, as changes in the myoelectric frequency content were not affected
11 by strain.

12 For the LG, a significant positive association was observed between θ and shortening
13 strain rate, during both force rise and force relaxation ($p < 0.001$, Table 1). Since negative strain
14 rates represent shortening, a positive association between θ and strain rate indicates that higher
15 frequency signals are associated with faster shortening strain rates. This supports our hypothesis
16 that the frequency content of the EMG signal increases with faster shorting strain rates. For the
17 MG, the association between θ and strain rate was not statistically significant ($p = 0.198$ during
18 rise and $p = 0.256$ during relaxation).

19

20 **Discussion**

21

22 Our study was designed to detect associations between myoelectric frequency and
23 myoelectric intensity, force rise and relaxation rates, fascicle strain, and fascicle strain rate, all
24 measured *in vivo* during different locomotor tasks. Myoelectric frequency properties were
25 characterized by the variable θ , which emerged from the PCA, with higher θ corresponding to
26 lower frequency content (and lower θ corresponding to higher frequency content). In general,
27 the results for goat LG and MG muscles revealed a positive association between θ and
28 shortening strain rate and a negative association between θ and myoelectric intensity,
29 demonstrating that faster motor units associated with higher myoelectric frequency content are
30 correlated with increased fascicle shortening strain rates and increased myoelectric intensity.
31 Although this was the case for level locomotion, shifts in recruitment (increased θ) during incline

1 | gait were less clear and failed to correlate with reduced fascicle strain rates. Increases in
2 | myoelectric intensity, force, force rate, fascicle strain, and fascicle strain rate were also generally
3 | observed as exercise demand increased from level walking to trotting to galloping, and from
4 | level walking and trotting to incline walking and trotting.

5 |
6 | General changes in total EMG intensity, muscle force and rate, and fascicle strain and rate
7 | across different locomotor tasks
8 |

9 | Several of our findings are consistent with previous *in vivo* experiments. For example,
10 | total EMG intensity increased with locomotor speed and surface grade (Tables 2 to 5, Figs. 2 and
11 | 3 to 4), consistent with previous studies of goats (McGuigan et al., 2009), rats (Gillis and
12 | Biewener, 2002), turkeys (Gabaldon et al., 2004), and horses (Wicker et al., 2005). This was
13 | linked to increases in LG and MG force as goats increased speed from level walking to trotting
14 | to galloping, and when they moved from the level to the inclined surface (Tables 2 to 5, Figs. 2
15 | and 3). These results are again consistent with data from goats reported by McGuigan et al.
16 | (2009) and with data from guinea fowl reported by Daley and Biewener (2003). Our measures of
17 | increased fascicle shortening strain and strain rate during incline walking and trotting (Tables 2
18 | to 5, Figs. 2 and 3) are also consistent with previous studies. For example, Roberts et al. (2007)
19 | reported increased shortening strain and strain rates when turkeys switched from level running to
20 | incline running, concluding that muscle fascicle strain was less affected by speed than by slope.
21 | Using ultrasound to track human MG fascicles, Lichtwark and Wilson (2006) also reported
22 | increased fascicle strain during walking and running as surface grade was increased. These
23 | previous studies have shown that the work output of individual muscles may be modulated by
24 | changes in force or fascicle shortening, or both. The timing of muscle force relative to fascicle
25 | strain also contributes to changes in work output (Daley and Biewener, 2003; Gabaldon et al.,
26 | 2004). Consequently, muscle activation and motor unit recruitment likely play important roles in
27 | modulating muscle work across different locomotor tasks (McGuigan et al., 2009). Our current
28 | study extends previous research and by demonstrating that the net work performed by muscles
29 | may also be modulated by recruiting different motor unit types.
30 |
31 |

1
2
3 *Differences in the EMG frequency spectra across gait conditions and between muscles*
4

5 Our results support previous work in which locomotor tasks associated with increasing
6 speed (walking to trotting to galloping) elicit decreasing θ values (Hodson-Tole and Wakeling,
7 2009), indicative of higher myoelectric frequency components at higher locomotor speeds
8 (Tables 2 to 5, Fig. 4). Our results also agree with previous work showing a significant increase
9 in low frequency myoelectric signal component (increasing θ) from level to incline locomotion
10 (Hodson-Tole and Wakeling, 2009) (Tables 2 to 5, Fig. 4). Higher myoelectric frequencies occur
11 when there is an increase in the activation level and proportion of faster fibres recruited,
12 however, this could occur with either recruitment in an orderly pattern of recruitment from slow-
13 to-fast fibres, or with preferential recruitment of faster fibres (Wakeling, 2009). Therefore, the
14 observation of a higher myoelectric frequency alone is insufficient to detect differences in
15 recruitment patterns between gaits. In order to make such a distinction, situations are needed that
16 have similar levels of myoelectric intensity (corresponding to similar activation levels), but
17 differing myoelectric frequencies (indicating a shift in recruitment).

18 Our experiment was designed to test for task-specific recruitment by comparing
19 recruitment at high speed (reduced θ , indicative of increased fast motor unit recruitment) versus
20 slower speed inclined gait (increased θ , indicative of increased slow motor unit recruitment) as
21 two conditions with similar overall EMG intensities (and force requirements). However, the
22 comparison between gaits revealed that in general the gaits with greater muscle activation
23 (myoelectric intensity) also resulted in higher myoelectric frequency, and so comparisons
24 between gaits are unable to resolve differences in recruitment patterns (Table 2-5) due to the
25 manner in which myoelectric intensity covaried with frequency. For this reason it was necessary
26 to conduct an analysis of covariance (Table 1, and discussed in the following two sections), in
27 which the intensity effect was accounted for as a covariate, and in which we could test the
28 associations of myoelectric frequency with fascicle strain rates and forces. The EMG intensities
29 for galloping were comparable to those measured during incline walking and/or trotting and in
30 these specific instances we observed significant associations between θ and gait condition
31 consistent with increased slow unit recruitment for slower speed incline gait relative to faster unit

1 recruitment at a gallop (Tables 2-5). However, these shifts in motor unit recruitment were
2 inconsistent with measured LG and MG fascicle strain rates (Tables 2-5) with the incline walk
3 and trot resulting in greater fascicle strain rates than those during level walk and trot due to the
4 need for increased muscle shortening and work output while moving up an incline. Nevertheless,
5 shifts in motor recruitment were observed between incline walking and level galloping where
6 strain rates were similar.

7 Although similar shifts in myoelectric frequency content between gait conditions were
8 found for the LG and MG, EMG recordings from the LG yielded smaller values of θ for all gait
9 conditions (Fig. 4), indicating that LG myoelectric signals contained a higher frequency content
10 than MG signals for any given condition. This finding is consistent with previously reported
11 patterns of *in situ* motor unit recruitment from these muscles (Lee et al., 2011), and is likely
12 caused by the higher proportion of fast fibres within the goat LG (which is supported by
13 unpublished immunohistochemistry observations, Carr, Miara, Lee, Wakeling and Biewener).
14 This finding also likely explains the larger total EMG intensity and faster fascicle strain rate of
15 the LG compared with the MG during level galloping, when faster shortening is required. In
16 addition, this difference in θ and the supporting evidence that MG contains a higher proportion
17 of slow fibres may explain why myoelectric intensity was higher for MG during incline walking
18 and trotting, for which recruitment of slower motor units was dominant. McGuigan et al. (2009)
19 also reported higher myoelectric intensity in the MG during incline walking and trotting versus
20 higher intensity in the LG during level walking and trotting in goats.

21 Principal component analysis of motor unit recruitment temporal profiles allowed us to
22 test our proposed hypotheses regarding motor unit recruitment across different locomotor tasks.
23 PCI loading scores correlated closely to the total myoelectric intensity ($r^2=0.98$) and so can be
24 used as a measure of muscle excitation. Negative and positive PCII loading scores indicate
25 greater low and high frequency components, respectively, corresponding to activation of faster
26 and slower motor units, respectively (Fig. 4). The angle θ between the vector of the PCI-PCII
27 loading score and PCII loading score axis (Fig. 4) can therefore be used to indicate the relative
28 frequency component of the myoelectric signal which corresponds to the different motor unit
29 type recruitment (Wakeling and Rozitis, 2004, Lee et al., 2011). Our *in situ* results from a
30 previous study of the same goats, where we stimulated the tibial nerve to illicit single twitches
31 recruiting either slow motor units or fast motor units or both, validates this method of EMG

1 analysis to signify frequency content and the corresponding motor unit recruitment (Lee et al.,
2 2011). A higher θ value is interpreted as having a relatively greater low frequency signal content
3 associated with slower motor unit recruitment. Conversely, a smaller θ value is interpreted as
4 having a relatively higher frequency signal content associated with faster motor unit recruitment.
5 Preferential recruitment, where faster motor units are recruited in the absence of or decreased
6 slow motor unit activation, can only be distinguished from orderly recruitment if the [level of](#)
7 [muscle excitation and thus](#) PCI loading score is the same, but there is a different θ . Hence, PCI
8 (or total intensity) was included as a covariate in the ANCOVA, allowing θ to be characterized
9 independent of intensity.

10 When evaluating myoelectric signals, it is important to consider fatigue, which can affect
11 the frequency content. Past studies have most commonly investigated the effect of fatigue until
12 exhaustion (e.g. Naeije and Zorn, 1982). However, this was not done in our study. Instead, we
13 adopted an experimental design that involved five short recording intervals of approximately 20
14 seconds each for trot and gallop to avoid fatiguing the animal. In addition, the order of
15 presenting incline versus level and speed of locomotion was varied among animals to minimize
16 any bias that fatigue may have had on these factors. Therefore, we believe our results are not
17 affected by fatigue.

18 One challenge in determining associations between myoelectric frequency content and
19 strain rate relative to force rate is examining appropriate locomotor tasks. In this study, the
20 incline locomotor tasks elicited an increase in θ (slower frequency content), but also a
21 simultaneous increase in strain rates. An activity like jumping is also not an ideal locomotor task
22 because the total myoelectric intensity increases, in addition to strain rate and force. Thus, it is
23 difficult to uncouple force, strain rate, and myoelectric intensity during most locomotor tasks.
24 Identifying a locomotor task that requires rapid, but small muscle forces would be ideal, and this
25 challenge has been acknowledged in previous studies ([Smith et al., 1980](#); Hodson-Tole and
26 Wakeling, 2008, Wakeling et al., 2006).

27

28 *Association between myoelectric frequency and force rise and relaxation rates*

29

30 For single twitch contractions, both the contraction time, the time from an action
31 potential to peak force (Burke et al., 1973), and the activation-deactivation rates (Lee et al.,

1 2011) differ between slow and fast motor units, and these properties affect the mechanical output
2 of a muscle. Roberts and Gabaldon (2008) examined the relaxation electromechanical delay of
3 the turkey LG (from the end [of](#) EMG to the end of force and found that this delay varied
4 inversely with locomotor speed). However, they found no association with fascicle velocity, and
5 therefore suggested that changes in the muscle fibre recruitment pattern might explain the
6 association between the observed electromechanical relaxation delay and locomotor speed.
7 Preferential recruitment of faster motor units — with reduced recruitment of slower motor units
8 — could explain why slow activation-deactivation rates and slow strain rates may not dominate
9 at all speeds. Muscles need to generate force and relax at rates that are appropriate for the speed
10 of the movement to be most effective. Since faster motor units have faster activation and
11 relaxation rates (Burke et al., 1973), it seems likely that the rate of muscle force development or
12 relaxation could be an influential factor related to motor unit recruitment. To our knowledge, our
13 study is the first to test this hypothesis. However, our results for the goat LG and MG provide
14 little evidence to support this hypothesis. However, we observed a negative association between
15 θ and LG force relaxation rate, suggesting that lower frequency content is associated with fast
16 relaxation rates for this one muscle during relaxation, in contrast to what we predicted.

17 [However, increased force rise and relaxation rates may simply be a reflection of the](#)
18 [increased force due to an increase in the number of motor units recruited and not due to](#)
19 [recruitment of different motor unit types. Thus, we tested force rise and relaxation rates](#)
20 [calculated from forces normalized to peak forces, but also did not find a significant association](#)
21 [between the force rise and relaxation rates and angle \$\theta\$.](#)

23 *Association between myoelectric frequency and strain rate*

24
25 A major finding of our study was a shift from lower to higher EMG frequencies during
26 faster muscle strain rates during LG shortening [during locomotion on a level surface](#). Using
27 wavelets and PCA, and accounting for EMG intensity, we determined that this shift was not of
28 the mean frequency; rather, this shift was due to a decrease in low frequency components and an
29 increase in high frequency components of the spectra. A significant positive association between
30 fascicle strain rate and θ was found for LG shortening during [force rise and relaxation periods](#).
31 As LG shortening rates increased for faster gaits (Figs. 2 [and](#) 3), θ decreased indicating that there

1 were greater high frequency components. However, because this association was not observed
2 in the MG muscle during shortening, we conducted further analysis to assess the association
3 between θ and strain rate. [Muscle fascicle strain was included as a covariate so that changes in](#)
4 [strain would not confound the results \(Wakeling et al., 2006\), as strain can influence myoelectric](#)
5 [frequency content \(Doud and Walsh, 1995\). Greater strains can be associated with decreased](#)
6 [frequency content, ANCOVA was only conducted if muscle fascicle strain and \$\theta\$ were](#)
7 [significantly negatively correlated. Given that \$\theta\$ decreased while strain increased from level to](#)
8 [incline walking and trotting, and the possibility of strain being a confounding factor, we repeated](#)
9 [the PCA and ANCOVA for only the level gait conditions.](#) This yielded a positive association
10 between θ and strain rate during MG force development, similar to the results obtained for LG,
11 suggesting that preferential recruitment of faster motor units may allow for faster MG strain rates
12 as needed, when gait velocity increases [on a level surface](#). Our results are supported by similar
13 findings in the MG, soleus, and plantaris muscles in rats (Hodson-Tole and Wakeling, 2008) and
14 in the MG muscles in humans (Wakeling et al., 2006) where increases in gait velocity (rats) and
15 cycling frequency (humans) and increased strain rates were associated with shifts to high
16 frequencies in the EMG signal. One advantage of preferentially recruiting faster motor units
17 during rapid movements is that maximum mechanical power and maximum mechanical
18 efficiency occur at higher strain rates in faster motor units (He et al., 2000). A positive
19 association between recruitment of faster motor units and fascicle shortening rate may therefore
20 be driven by the necessity of generating high mechanical power at high efficiency. Preferential
21 recruitment of faster motor units at faster gait speeds would also favor rates of muscle shortening
22 better suited to necessary increases in limb segment velocity.

23

24 *Conclusions*

25

26 Our results for the goat LG and MG support previous observations in other animals and
27 man that different motor units may be recruited in a pattern that is task-specific during
28 locomotion. In particular, our analyses of myoelectric patterns related to *in vivo* muscle fascicle
29 strain and force showed that there are times where preferential recruitment of faster fibres is
30 related to faster shortening strain rates of the muscle fascicles. The shift to higher [myoelectric](#)
31 frequencies is due to a decrease in low frequency components and an increase in high frequency

1 | components of the spectra, providing evidence that [a greater number of](#) fast motor units are
2 | recruited while fewer slow motor units are recruited. These results support other evidence
3 | suggesting motor units form task groups that are selectively recruited for rapid tasks (Hodson-
4 | Tole and Wakeling, 2008, Wakeling, 2004; Wakeling et al., 2001; Loeb, 1985) [and](#) has
5 | implications for muscle modeling where independent slow and fast contractile elements can be
6 | incorporated into novel, more complex muscle models (Wakeling et al., 2012). To our
7 | knowledge, this is only the third report that has identified and quantified [the influence of](#)
8 | myoelectric intensity, strain rate, and force [on](#) motor unit recruitment [and investigated if there is](#)
9 | [a mechanical basis for preferential recruitment of different motor unit types](#). Further work is
10 | therefore needed to determine if an association between preferential motor unit recruitment and
11 | strain rate exists across species and whether other biomechanical factors drive motor unit
12 | recruitment strategies.

13

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17

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20

References

Biewener, A.A. (1998). Muscle-tendon stresses and elastic energy storage during locomotion in the horse. *Comp. Biochem. Physiol. B* **120**, 73-87.

Biewener, A. A. and Baudinette, R. (1995). In vivo muscle force and elastic energy storage during steady-speed hopping of tammar wallabies (*Macropus eugenii*). *J. Exp. Biol.* **198**, 1829-41.

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.

Biewener, A.A., McGowan, C., Card, G.M., and Baudinette, R.V. (2004). Dynamics of leg muscle function in tammar wallabies (*M. eugeni*) during level versus incline hopping. *J. Exp. Biol.* **207**, 211-223.

Buchtal, F., Guld, C. and Rosenfalk, P. (1955). Innervation zone and propagation velocity in human muscles. *Acta Physiol. Scand.* **35**, 174-190.

Burke, R.E., Levine, D.N., Tsairis, P., and Zajac, F.E. (1973). Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *J. Physiol.* **234**, 723-748.

Citterio, G and Agostoni, E. (1984). Selective activation of quadriceps muscle fibers according to bicycling rate. *Journal of Applied Physiology*, **57**, 371-379.

Daley, M.A. and Biewener, A.A. (2003). Muscle force-length dynamics during level versus incline locomotion: a comparison of *in vivo* performance of two guinea fowl ankle extensors. *J. Exp. Biol.* **206**, 2941-2958.

Doud, J.R. and Walsh, J.M. (1995). Muscle fatigue and muscle length interaction: effect on the EMG frequency components. *Electromyography Clinical Neurophysiology.* **35**, 331-339.

Fedde, M.R., DeWet, P.D., and Kitchell, R.L. (1969). Motor unit recruitment pattern and tonic activity in respiratory muscles of *Gallus domesticus*. *J. Neurophysiol.* **35**, 331-339.

Freund H.J., Büdingen, H.J., and Dietz V. (1975). Activity of single motor units from human forearm muscles during voluntary isometric contractions. *J Neurophysiology* **38(4)**, 933-46.

Fukunaga, T., Ichinose, Y., Ito, M., Kawakami, Y. and Fukashiro, S. (1997). Determination of fascicle length and pennation in a contracting human muscle *in vivo*. *J. Appl. Physiol.*, **82**, 3540358.

Gabalton, A.M., Nelson, F.E. and Roberts, T.J. (2004). Mechanical function of two ankle extensors in wild turkeys: shifts form energy production to energy absorption during incline versus decline running. *J. Exp. Biol.* **207**, 2277-2288.

Gerdle, B., Karlsson, S., Crenshaw, A.G., Elert, J. and Friden, J. (2000). The influences of muscle fibre proportions and areas upon EMG during maximal dynamic knee extensions. *Eur. J. Appl. Physiol.* **81**, 2-10.

Gillespie, C.A., Simpson, D.R., and Edgerton, V.R. (1974). Motor unit recruitment as reflected by muscle fibre glycogen loss in a prosimian (bushbaby) after running and jumping. *Journal of Neurology, Neurosurgery & Psychiatry* **37**, 817-824.

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 a hip and knee extensor of the rat (*Rattus norvegicus*). *J. Exp. Biol.* **204**, 2717-2731.

Gillis, G.B. and Biewener, A.A. (2002). Effects of surface grade on proximal hindlimb muscle strain and activation during rat locomotion. *J. Appl. Physiol.*, **93**, 1731-1743.

Grimby L, Hannerz J, and Hedman B. (1981). The fatigue and voluntary discharge properties of single motor units in man. *J Physiol.* **316(1)**, 545-554.

He, Z.H, Bottinelli R., Pellegrino, MA, Ferenczi, MA, and Reggiani, C. (2000). ATP consumption and efficiency of human single muscle fibres with different myosin isoform composition. *Biophys. J.* **79**, 945-961.

Henneman, E., Somjen, G. and Carpenter, D.O. (1965). Excitability and inhibibility of motoneurons of different sizes. *J. Neurophysiol.* **28**, 599-620.

Henneman, E., Somjen, G. and Carpenter, D.O. (1965b). Functional significance of cell size in spinal motoneurons. *J. Neurophysiol.* **28**, 560-580.

Henneman E and Olson CB. (1965c). Relations Between Structure and Function in the Design of Skeletal Muscles. *J Neurophysiology* **28**, 581-98.

Henneman, E., Clamann, H.P., Gillies, J.D. and Skinner, R.D. (1974). Rank order of motoneurons within a pool: law of combination. *J. Neurophysiol.* **37**, 1338-1349.

Higham, T., Biewener, A.A. and Wakeling, J.M. (2008). Functional diversification within and between muscle synergists during locomotion. *Biology Letters* **4**, 41-44.

Hodson-Tole, E.F. and Wakeling, J.M. (2007). Variations in motor unit recruitment patterns occur within and between muscles in the running rat (*Rattus norvegicus*). *Journal Experimental Biology.* **210**, 2333-45.

Hodson-Tole, E.F. and Wakeling, J.M. (2008). Motor unit recruitment patterns 2: the influence of myoelectric intensity and muscle fascicle strain rate. *Journal Experimental Biology* **211**, 1893-902.

Hodson-Tole, E.F. and Wakeling, J.M. (2010). The influence of strain and activation on the locomotor function of rat ankle extensor muscles. *Journal Experimental Biology* **213**, 318-30.

Ker, R.F., and Bennett, M.B., Bibby, S.R., Kester, R.C., and Alexander, R.M. (1987). The spring in the arch of the human foot. *Nature* **325**, 147-149.

Kupa, E.J., Roy, S.H., Kandarian, S.C. and de Luca, C.J. (1995). Effects of muscle fibre type and size on EMG median frequency and conduction velocity. *J. Appl. Physiol.* **79**, 23-32.

Lee, S.S.M., de Boef Miara, M., Arnold, A.S., Biewener, A.A. and Wakeling J.M. (2011). EMG analysis for determining the timing and level of activation in different motor units. *J. Electromyography and Kinesiology* **21**, 557-565.

Lichtwark, G.A. and Wilson, A.M. (2006). Interaction between the human gastrocnemius muscle and the Achilles tendon during incline, level, and decline locomotion. *J. Exp. Biol.* **209**, 4379-4388.

Lichtwark, G.A., Bougoulas, K., and Wilson, A.M. (2007). Muscle fascicle and series elastic element length changes along the length of the human gastrocnemius during walking and running. *J. Biomechanics.* **40**, 157-164.

Lindstrom, L.H. and Magnusson, R.I. (1977). Interpretation of myoelectric power spectra: a model and its applications. *Proceedings of the IEEE*, **65(5)**, 653-662.

Luff, A.R. and Atwood, H.L. (1972). Membrane properties and contraction of single muscle fiber in the mouse. *Am. J. Physiol.* **222**, 1435-1440.

McGowan, C.P., Baudinette, R.V. and Biewener, A.A. (2007). Modulation of proximal muscle function during level versus incline hopping in tammar wallabies (*Macropus eugenii*). *J. Exp. Biol.* **210**, 1255-1265.

McGuigan, M.P., Yoo, E., Lee, D.V., and Biewener, A.A. (2009). Dynamics of goat distal hind-limb muscle-tendon function in response to locomotor grade. *J. Exp. Biol.* **212**, 2092-2104.

Minetti, A.E., Ardigo, L.P. and Saibene, F. (1994). Mechanical determinants of the minimum energy cost of gradient running in humans. *J. Exp. Biol.*, **195**, 211-225.

Naeije, M. and Zorn, H. (1982). Relation between EMG power spectrum shifts and muscle fibre action potential conduction velocity changes during local muscular fatigue in man. *European Journal of Applied Physiology*, **50**, 23-33.

Nardone, A., Romano, C., and Schieppati, M. (1989). Selective recruitment of high-threshold human motor units during voluntary isotonic lengthening of active muscles. *J Physiol.* **409**, 451-471.

Reaz, M.B.I., Hussain, M.S., and Mohd-Yasin F. (2006). Techniques of EMG signal analysis: detection, processing, classification, and applications. *Biol Proceedings Online*, **8**, 11-35.

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**, 1113-1115.

Roberts, T.J., Higginson, B.K., Nelson, F.E., and Gabaldon, A.M. (2007). Muscle strain is modulated more with running slope than speed in wild turkey knee and hip extensors. *Journal of Experimental Biology*, **210**, 2510-2517.

Roberts, T.J. and Gabaldon, A. (2008). Interpreting muscle function from EMG-lessons learned from direct measurements of muscle force. *Integrative and Comparative Biology*, **48**, 312-320.

Rome, L.C., Funke, R.P., Alexander, R.M., Lutz, G., Aldridge, H., Scott, F., and Freadman, M. (1988). Why animals have different muscle fibre types. *Letters to Nature* **335**, 824-827.

Sadoyama, T., Masuda, T., Miyata, H. and Katsuta, S. (1988). Fibre conduction velocity and fibre composition in human vastus lateralis. *Eur. J. Appl. Physiol. Occup. Physiol.* **57**, 767-771.

Smith, J.L., Betts, B., Edgerton, V.R. and Zernicke, R.F. (1980). Rapid ankle extension during paw shakes: selective recruitment of fast ankle extensors. *J. Neurophysiology* **43**, 612-620.

Sokoloff, A.J. and Cope, T.C. (1996). Recruitment of triceps surae motor units in the decerebrate cat. II. Heterogeneity among soleus motor units. *J Neurophysiol.* **75**, 2005-2016.

Von Tscherner, V. (2000). Intensity analysis in time-frequency space of surface myoelectric signals by wavelets of specified resolution. *Journal Electromyography and Kinesiology* **10**, 433-445.

Wakeling, J.M., Kaya, M., Temple, G.K., Johnston, and W., Herzog, W. (2002). Determining patterns of motor recruitment during locomotion. *J Exp Biol.* **205**, 359-369.

Wakeling, J.M. (2004). Motor units are recruited in a task dependent fashion during locomotion. *J. Exp. Biol.* **207**, 3883-3890.

Wakeling, J.M. and Rozitis, A. I. (2004). Spectral properties of myoelectric signals from different motor units distinguished during ramped contractions of the leg extensors. *J. Exp. Biol.* **207**, 2519-2528.

Wakeling, J.M., Uehil, K, Rozitis, A.I. (2006). Muscle fibre recruitment can responds to the mechanics of the muscle contraction. *J. R. Soc. Interface* **3**, 533-544.

Wakeling, J.M., Lee, S.S.M., de Boef Miara, M., Arnold, A.S., and Biewener, A.A. (2012). A Muscle's force depends on the recruitment patterns of its fibers. *Annals of Biomedical Engineering* DOI 10.1007/s10439-012-0531-6.

Table 1. The effects of mechanical and excitation variables on angle θ for the force rise and force relaxation phases. Arrows denote the direction of association: \uparrow positive and \downarrow negative when the covariates are significant ($p < 0.05$ from the ANCOVA).

	LG		MG	
	Force Rise	Force Relaxation	Force Rise	Force Relaxation
Total intensity	\downarrow	\downarrow	\downarrow	\downarrow
Force	\downarrow	\downarrow	\downarrow	\downarrow
Force rate		\downarrow		
Strain		\downarrow		
Strain rate	\uparrow	\uparrow		

Table 2. Mean (standard error of the mean) for mechanical and excitation variables during LG force rise during concentric contractions. Values are averages across the measurement period. Asterisks in the columns “level walking, trotting, and galloping” indicate significant differences between gaits (compared to level trotting) on the level surface in *post hoc* comparisons (* p<0.05, **, p<0.01, *** p<0.001). Addition signs in the columns “incline walking and trotting” indicate significant differences between level and incline surface for each gait (+ p<0.05, ++ p<0.01, +++ p<0.001).

	Level walking	Level trotting	Level galloping	Incline walking	Incline trotting
Strain	0.130** (0.006)	0.151 (0.007)	0.157 (0.008)	0.166+++ (0.008)	0.212+++ (0.012)
Strain rate (s ⁻¹)	-1.212*** (0.059)	-1.537 (0.061)	-1.755*** (0.080)	-1.567+++ (0.065)	-2.486+++ (0.161)
Force (N)	19.42 (1.77)	15.23 (1.49)	20.95** (2.24)	36.11+ (2.63)	51.70+++ (4.10)
Force rate (N s ⁻¹)	368.67*** (34.56)	536.31 (47.66)	881.03*** (98.66)	533.46++ (47.00)	1274.88+++ (119.18)
Total intensity (mV ²)	1.042*** (0.075)	2.386 (0.154)	2.891*** (0.294)	2.144+++ (0.144)	6.058+++ (0.447)
Angle θ (rad)	1.512 (0.014)	1.509 (0.015)	1.407* (0.016)	1.504 (0.014)	1.527++ (0.016)

Table 3. Mean and standard error of the mean for mechanical and excitation variables during LG force relax during concentric contractions. Values are averages across the measurement period. Asterisks in the columns “level walking, trotting, and galloping” indicate significant differences between gaits (compared to level trotting) on the level surface in *post hoc* comparisons (* p<0.05, ** p<0.01, *** p<0.001). Addition signs in the columns “incline walking and trotting” indicate significant differences between level and incline surface for each gait (+ p<0.05, ++ p<0.01, +++ p<0.001).

	Level walking	Level trotting	Level galloping	Incline walking	Incline trotting
Strain	0.065* (0.003)	0.078 (0.005)	0.105*** (0.006)	0.091+++ (0.005)	0.118+++ (0.008)
Strain rate (s ⁻¹)	-0.669*** (0.031)	-1.138 (0.061)	-1.416*** (0.068)	-0.914+++ (0.044)	-1.439+++ (0.067)
Force (N)	23.81* (1.43)	20.94 (1.72)	29.06*** (2.86)	39.73+++ (2.06)	62.21+++ (3.79)
Force rate (Ns ⁻¹)	-264.14*** (15.88)	-618.34 (42.06)	-962.09*** (79.65)	-408.09++ (22.16)	-1050.67+++ (65.96)
Total intensity (mV ²)	0.513 (0.046)	0.770 (0.084)	2.621*** (0.296)	1.455+++ (0.149)	3.255+++ (0.469)
Angle θ (rad)	1.330*** (0.012)	1.270 (0.014)	1.291 (0.015)	1.347 (0.013)	1.314++ (0.014)

Table 4. Mean and standard error of the mean for mechanical and excitation variables during MG force rise during concentric contractions. Values are averages across the measurement period. Asterisks in the columns “level walking, trotting, and galloping” indicate significant differences between gaits (compared to level trotting) on the level surface in *post hoc* comparisons (* p<0.05, **, p<0.01, *** p<0.001). Addition signs in the columns “incline walking and trotting” indicate significant differences between level and incline surface for each gait (+ p<0.05, ++ p<0.01, +++ p<0.001).

	Level walking	Level trotting	Level galloping	Incline walking	Incline trotting
Strain	0.155** (0.007)	0.175 (0.007)	0.172 (0.008)	0.243+++ (0.010)	0.255+++ (0.010)
Strain rate (s ⁻¹)	-1.35* (0.079)	-1.58 (0.07)	-1.89*** (0.09)	-2.04++ (0.219)	-2.05+++ (0.116)
Force (N)	17.91 (1.71)	18.96 (1.73)	25.08 (2.45)	41.25+++ (3.79)	51.62+++ (4.52)
Force rate (N s ⁻¹)	471.17*** (30.42)	769.27 (58.80)	1062.93*** (99.08)	864.16+++ (81.97)	1436.76+++ (149.16)
Total intensity (mV ²)	0.888*** (0.070)	2.052 (0.153)	2.925*** (0.205)	3.085+++ (0.242)	7.741+++ (0.667)
Angle θ (rad)	1.490*** (0.012)	1.423 (0.011)	1.467 (0.011)	1.452+++ (0.012)	1.494+++ (0.014)

Table 5. Mean and standard error of the mean for mechanical and excitation variables during MG force relaxation during concentric contractions. Values are averages across the measurement period. Asterisks in the columns “level walking, trotting, and galloping” indicate significant differences between gaits (compared to level trotting) on the level surface in *post hoc* comparisons (* p<0.05, **, p<0.01, *** p<0.001). Addition signs in the columns “incline walking and trotting” indicate significant differences between level and incline surface for each gait (+ p<0.05, ++ p<0.01, +++ p<0.001).

	Level walking	Level trotting	Level galloping	Incline walking	Incline trotting
Strain	0.129 (0.006)	0.120 (0.007)	0.140*** (0.008)	0.211+++ (0.007)	0.221+++ (0.008)
Strain rate (s ⁻¹)	-0.510*** (0.062)	-0.722 (0.049)	-1.055*** (0.051)	-0.905++ (0.083)	-1.451+++ (0.142)
Force (N)	23.62 (1.95)	29.64 (2.97)	40.01** (4.16)	51.00+++ (3.52)	64.76+++ (4.75)
Force rate (N s ⁻¹)	-415.37*** (23.97)	-997.90 (77.66)	-1429.71*** (111.94)	-511.19++ (29.94)	-1274.25+++ (72.61)
Total intensity (mV ²)	0.466*** (0.047)	0.647 (0.078)	0.964*** (0.128)	1.920+++ (0.142)	2.356+++ (0.217)
Angle θ (rad)	1.427*** (0.011)	1.370 (0.012)	1.326*** (0.013)	1.414+++ (0.011)	1.379++ (0.012)

Figure Legends

1

2 Figure 1. Representative lateral gastrocnemius force, fascicle length, and myoelectric signals
3 from fine-wire EMG of a goat during different locomotor tasks. Shaded bars indicate the stance
4 phase.

5

6 Figure 2. Mean (thick lines) \pm S.E.M. (thin lines) of lateral gastrocnemius a) fascicle strain, b)
7 fascicle strain rate, c) force, d) force rate, and e) normalized intensity for different locomotor
8 tasks over a stride (level walking, trotting, and galloping, incline walking and trotting).

9

10 Figure 3. Mean (thick lines) \pm S.E.M. (thin lines) of medial gastrocnemius a) fascicle strain, b)
11 fascicle strain rate, c) force, d) force rate, and e) normalized intensity for different locomotor
12 tasks over a stride (level walking, trotting, and galloping, incline walking and trotting).

13

14 Figure 4. Principal component (PC) analysis of the myoelectric signals. a) The first two principal
15 components defined from the myoelectric spectra of LG and MG. The percentage values reflect
16 the percentage of the signal explained by the PC. b) Principal component I and II loading scores
17 for medial gastrocnemius (solid) and lateral gastrocnemius (dotted) during different locomotor
18 tasks (diamond: level walking, triangle: level trotting, star: level galloping, circle: incline
19 walking, square: incline trotting).

Figure 1.

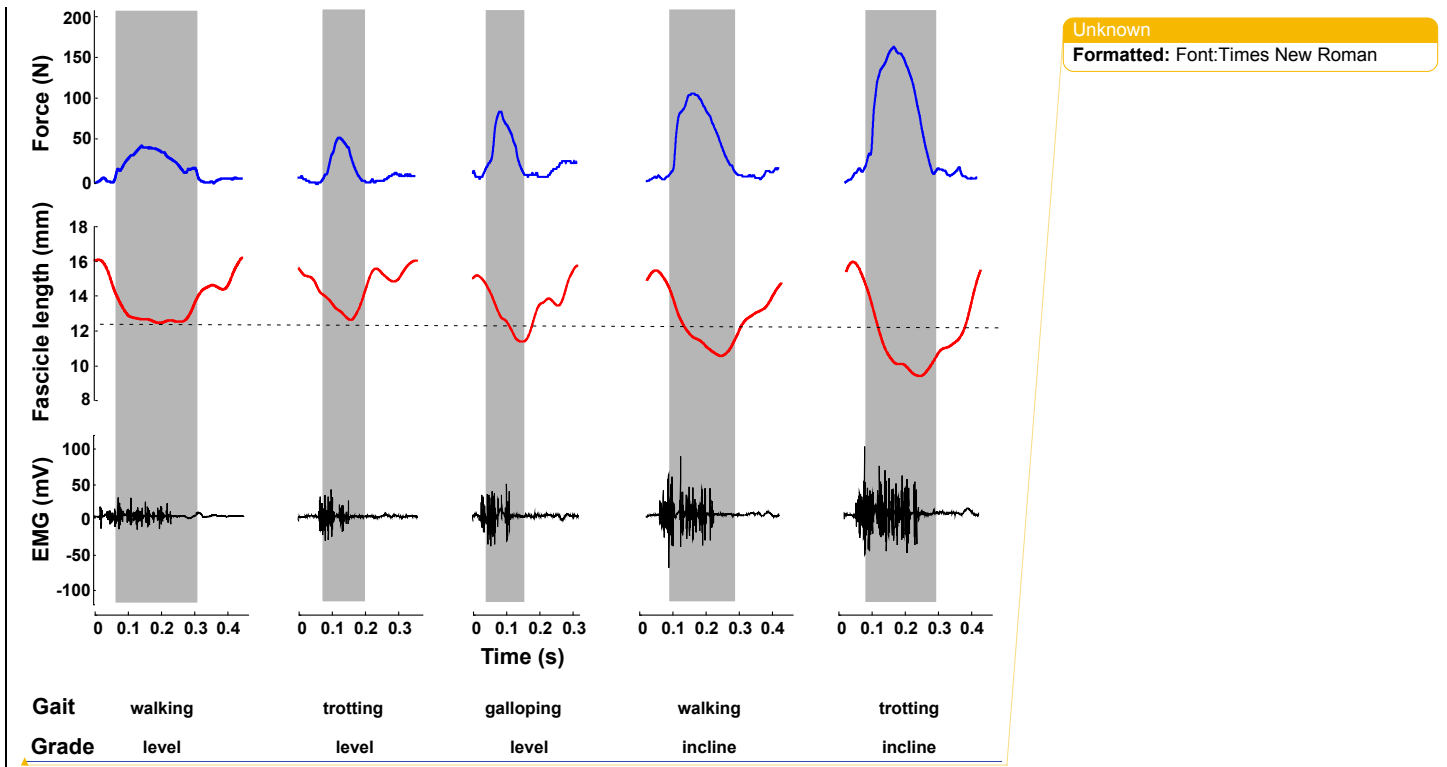


Figure 1. Representative lateral gastrocnemius force, fascicle length, and myoelectric signals from fine-wire EMG of a goat during different locomotor tasks. Shaded bars indicate the stance phase. The dotted line indicates fascicle length during standing.

Figure 2.

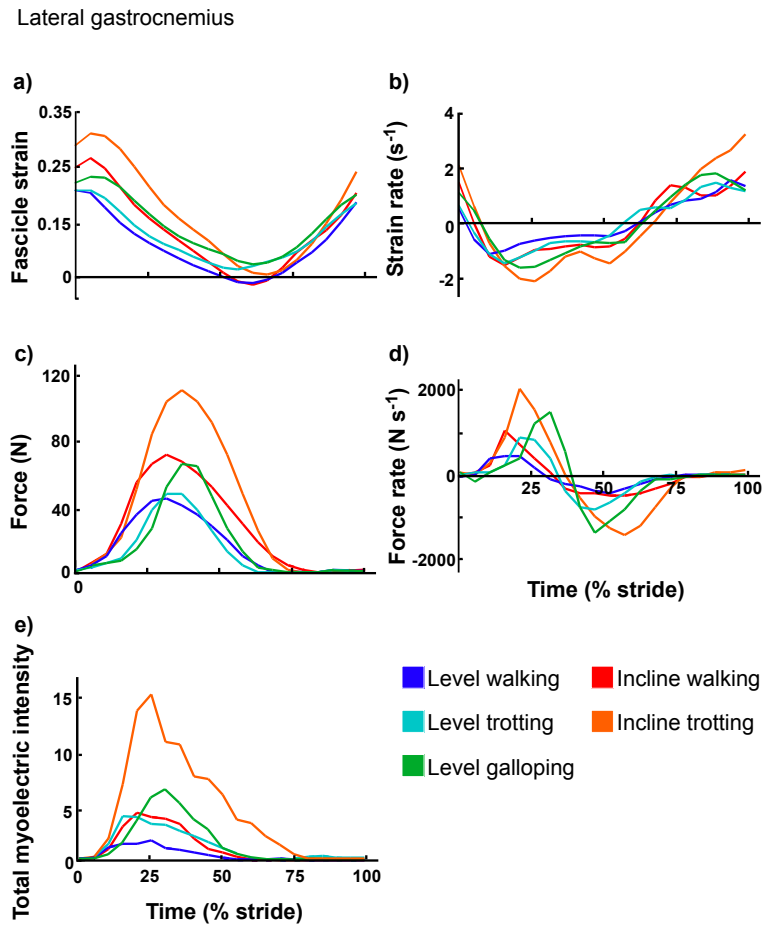


Figure 2. Mean values of lateral gastrocnemius a) fascicle strain, b) fascicle strain rate, c) force, d) force rate, and e) normalized intensity for different locomotor tasks over a stride (level walking, trotting, and galloping, incline walking and trotting).

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Figure 3.

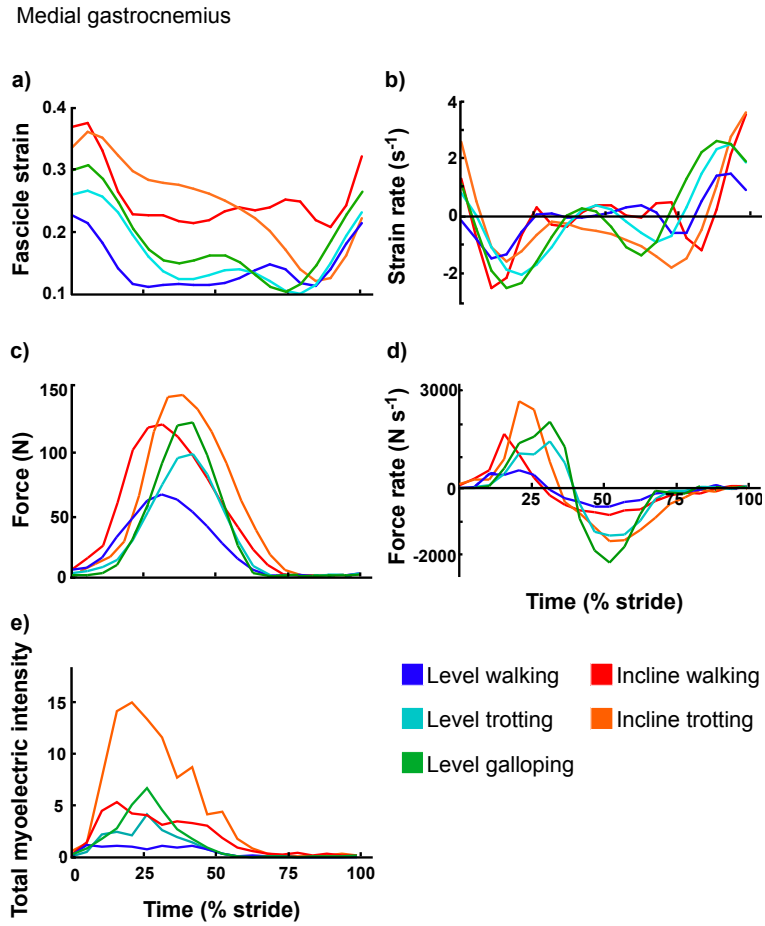


Figure 3. Mean values of medial gastrocnemius a) fascicle strain, b) fascicle strain rate, c) force, d) force rate, e) normalized intensity for different locomotor tasks over a stride (level walking, trotting, and galloping, incline walking and trotting),

Figure 4.

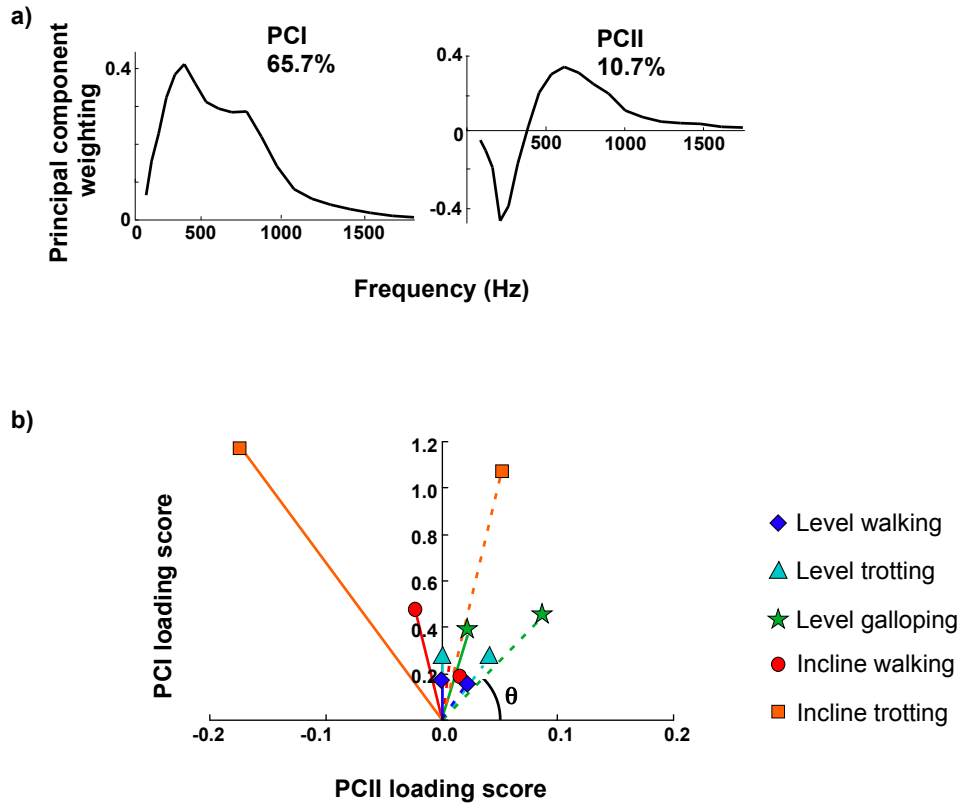


Figure 4. Principal component (PC) analysis of the myoelectric signals. a) The first two principal components defined from the myoelectric spectra of LG and MG. The percentage values reflect the percentage of the signal explained by the PC. b) Principal component I and II loading scores for medial gastrocnemius (solid) and lateral gastrocnemius (dotted) during different locomotor tasks (diamond: level walking, triangle: level trotting, star: level galloping, circle: incline walking, square: incline trotting).