

Intensity of activation and timing of deactivation modulate elastic energy storage and release in a pennate muscle and account for gait-specific initiation of limb protraction in the horse

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SUMMARY

The equine biceps brachii (biceps) initiates rapid limb protraction through a catapult mechanism. Elastic strain energy is slowly stored in an internal tendon and is then rapidly released to protract the forelimb. The muscle fibres are short, have little scope for length change and can therefore only shorten slowly compared with the speed at which the whole muscle must shorten, which makes them poor candidates for driving rapid limb protraction. We suggest that the muscle fibres in the biceps act to modulate the elastic energy output of the muscle–tendon unit (MTU) to meet the demands of locomotion under different conditions. We hypothesise that more elastic strain energy is stored and released from the biceps MTU during higher speed locomotion to accommodate the increase in energy required to protract the limb and that this can be achieved by varying the length change and activation conditions of the muscle. We examined the work performed by the biceps during trot and canter using an inverse dynamics analysis (IDA). We then used excised biceps muscles to determine how much work could be performed by the muscle in active and passive stretch–shorten cycles. A muscle model was developed to investigate the influence of changes in activation parameters on energy storage and energy return from the biceps MTU. Increased biceps MTU length change and increased work performed by the biceps MTU were found at canter compared with at trot. More work was performed by the *ex vivo* biceps MTU following activation of the muscle and by increasing muscle length change. However, the ratio of active to passive work diminished with increasing length change. The muscle model demonstrated that duration and timing of activation during stretch–shorten cycles could modulate the elastic energy storage and return from the biceps. We conclude that the equine biceps MTU acts as a tuneable spring and the contractile component functions to modulate the energy required for rapid forelimb protraction at different speeds.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/212/15/2454/DC1>

Key words: muscle, elasticity, locomotion, biomechanics, horse.

INTRODUCTION

Many larger animals including wallabies, humans and horses utilise elastic energy stored in tendons to minimise the energy required to do cyclical work (Biewener and Baudinette, 1995; Kubo et al., 2000; Alexander, 2002; Lichtwark and Wilson, 2006). The distal limb of the horse has been shown to function like a pogo stick, storing and returning energy in long, spring-like tendons throughout the gait cycle (Biewener, 1998; Wilson et al., 2001). The elastic energy stored in and recovered from tendons during cyclical locomotion can reduce the metabolic cost of locomotion (Cavagna et al., 1977; Alexander, 1988; Roberts et al., 1997). Tendons can also enable higher power output to be generated by the muscle–tendon unit (MTU) by enabling the contractile element (CE) to have a lower shortening velocity than the entire MTU (Roberts et al., 1997; Lichtwark and Wilson, 2006).

The biceps brachii (biceps) muscle in the proximal equine forelimb is a MTU with spring like properties. This muscle stores elastic energy in a thick internal tendon when the muscle is actively stretched during the stance phase. This energy is then released at the end of the stance phase when the leg buckles, allowing the tendon to recoil and catapult the limb forward into swing phase (Wilson et al., 2003). This increases the muscle's potential power output. The catapult mechanism is

important as it enables the horse to protract the limb more quickly than would be possible with muscle contraction alone. It has been estimated that the horse would need a muscle weighing 50 kg to produce enough power to protract the forelimb at observed speeds (Wilson et al., 2003). The biceps' spring-like mechanism provides the required power output from only 0.4 kg of tissue.

The biceps is not a simple tendon spring like some other apparently vestigial fibred horse muscles. It is composed of two short-fibred, multi-pennate, muscle heads, the lateral head and the medial head (Hermanson and Hurley, 1990). Muscle fibre lengths vary from 5 mm to 7.5 mm in the lateral head and 22 mm to 40 mm in the medial head and the muscle belly is approximately 400 mm long in a thoroughbred horse (Watson and Wilson, 2007). The two heads are separated longitudinally by a thick internal tendon which runs continuously from the muscle's origin on the supraglenoid tubercle of the scapula to the insertion on the medial radial tuberosity and acts as a parallel elastic element (Fig. 1). Long aponeuroses within the muscle heads represent a substantial additional series elastic element. The biceps is also connected by the lacertus fibrosus to the extensor carpii radialis. This link is presumed to act to help extend and stabilise the limb in late swing and stance.

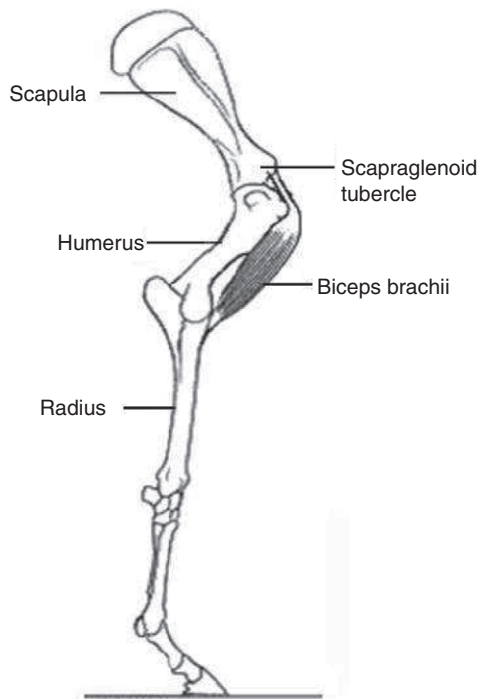


Fig. 1. The equine forelimb in lateral view showing the origin and insertion of the biceps brachii.

During the stance phase of locomotion the equine shoulder flexes and the elbow extends. This results in an increase in length of the biceps MTU and, if the muscle is active, enables elastic energy to be stored within the both the internal tendon and the series elastic elements (SEE; Fig. 2). This is supported by (1) experimental evidence that shows the moment at the shoulder increases during stance to oppose the ground reaction force (GRF) (Wilson et al., 2003), and (2) electromyographic (EMG) data that shows that both heads of the biceps are active throughout the stance phase of locomotion (Tokuriki et al., 1999). Short muscle fibres have little scope for length change and can therefore only shorten slowly compared with the speed at which the whole muscle must shorten, which makes them poor candidates for driving rapid limb protraction (Epstein and Herzog, 1998).

The short fibres are, however, ideally placed and have a suitable activation pattern to modulate the amount of elastic energy stored in the biceps MTU. The biceps is activated during the stance phase of all gaits (which is when the muscle is stretching) and is deactivated at the end of stance (when the muscle is shortening) (Tokuriki et al., 1999). We propose that the timing and amplitude of activation can be adjusted to modulate force generated by the muscle contractile element and therefore the amount of energy stored in the SEE. Timing and duration of activation influences the power output of muscle (Curtin and Woledge, 1996) and this is influenced by the compliance of the elastic tissue (Lichtwark and Wilson, 2005). Increasing amplitude of activation increases the force-generating capacity of the muscle and may also result in more of the aponeurosis coming under load at lower muscle strains and hence increase stiffness (Hof, 1998). In addition, if the muscle deactivates during MTU shortening then some of the energy stored in the SEE can also be absorbed by the muscle rather than transferred to the limb. The modulation of elastic energy stored and returned by biceps is important for changes in everyday movement patterns; for example the sum of the potential and kinetic energy required for protraction

of the limb during the entire swing phase of trot is approximately only 30% of that required in gallop (Wilson et al., 2003).

Here we seek to understand how elastic energy for limb protraction might be modulated by the biceps contractile element by exploring three hypotheses.

(1) We hypothesised that more elastic strain energy is stored in and then released from the biceps during higher speed locomotion to accommodate the increase in energy required to protract the limb. To test this hypothesis we performed an inverse dynamics analysis (IDA) to determine the biceps MTU force-length relationship during both trot and canter and hence to examine how much positive work is performed by the muscle in each gait.

(2) We then hypothesised that the biceps is able to store and return more elastic energy at faster speeds through a combination of increased length change and increased stiffness though greater activation. To test the capacity of the biceps to store and return more elastic energy we performed *ex vivo* workloop experiments on excised live muscles under different length change and activation conditions to examine the relationship between activation, length change and energy stored/returned.

(3) Finally we hypothesised that changes in the timing, duration and level of stimulation will have a significant effect on the work absorbed by the MTU during active lengthening (much of which is stored in the SEE) and also on the amount of work performed by the MTU during active shortening [mainly by release from the previously stretched parallel elastic element (PEE) and SEE]. Direct measures on the biceps are very difficult to make, therefore to test this hypothesis, a Hill-type muscle model was developed to simulate the *ex vivo* results and investigate the influence of changes in activation parameters on energy storage and return *in vivo*.

MATERIALS AND METHODS

IDA: kinematic and kinetic analyses of trot and canter

Five Thoroughbred horses (height range 153–155 cm measured from the dorsal aspect of the spinous processes of thoracic vertebrae three to five to the ground, approximate body mass 500 kg) were trotted, and three horses were cantered, in hand, over a force plate (9287BA, Kistler, Switzerland) at self-selected speeds until a minimum of six right forelimb strikes had been recorded. Only three horses could canter sufficiently slowly to be led in hand. Hemispherical retro-reflective motion analysis markers, 40 mm in diameter, were placed over repeatable skeletal landmarks on the scapula, shoulder, elbow, radius, third metacarpal bone, metacarpophalangeal (MCP) joint and the distal interphalangeal joint of the right forelimb. Two motion analysis cameras (ProReflex, Qualisys, Sweden) were placed facing the force plate at 60° to one another and three-dimensional kinematic data were collected at 240 Hz.

The force plate was mounted on a purpose-built metal sub frame embedded in concrete, half way along a runway that was covered with rubber conveyor-belt matting. The force plate signal was amplified by integral eight channel charge amplifiers, filtered through a low pass filter (6 db per octave from 50 Hz) and logged using a 12 bit AD converter at 500 samples per second into a personal computer using software written in LabView (National Instruments, USA). A manual trigger was used to synchronise the motion analysis and force plate data.

For analysis, the forelimb was divided into five segments (scapula, humerus, radius, metacarpus and carpal bones, digit). Joint angles in the sagittal plane of the limb (measured on the cranial side of joints) were calculated from the kinematic data (supplementary material Figs S1 and S2). The shoulder angle was corrected for scapula marker displacement resulting from skin movement, using

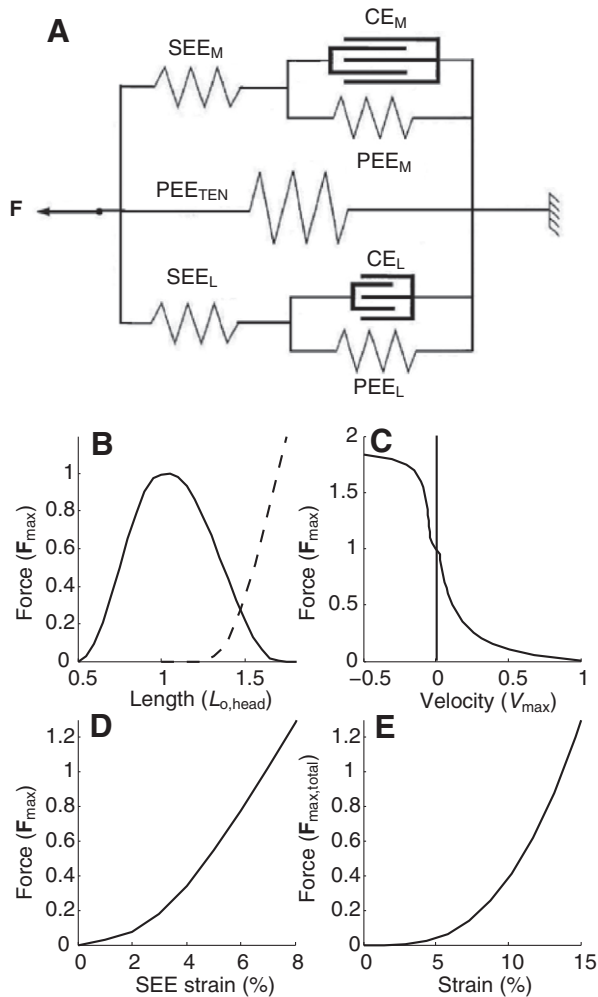


Fig. 2. Hill muscle model representation of the biceps brachii. (A) The muscle consists of two contractile components (a medial and lateral head, CE_M and CE_L, respectively) each with parallel elastic components (PEE_M and PEE_L), which are in series with aponeurosis (SEE_M and SEE_L). An internal tendon runs the length of the muscle and separates the two heads and therefore acts as a parallel elastic element (PEE_{TEN}). (B) Force-length relationship of each CE, where length (L_o) and F_{max} of each head is considered separately. (C) Force-velocity relationship of each CE, where positive velocity is shortening and F_{max} is considered separately for each head. (D) SEE force-strain relationship where F_{max} is considered separately for each head. (E) PEE_{TEN} force-strain relationship where force is considered relative to the maximum force of both heads.

the correction equation described in Appendix 1. The inertial properties of the segments were estimated from the data of Buchner (Buchner et al., 1997) on similarly sized horses. The angular velocities and accelerations of each segment were calculated from kinematic data. Kinematic information about the centre of mass and the positions of the proximal and distal ends of each segment enabled us to perform a two-dimensional IDA. We calculated the moments and forces acting at each joint (supplementary material Figs S3 and S4) across 120% of the stance phase. Stance was defined as the period during which the GRF was greater than 100 N.

IDA: biceps brachii MTU force and length calculations during trot and canter

The biceps moment arm at the shoulder (0.102 ± 0.009 m; mean \pm s.d.) was measured from radiographs of standing horses (Watson

and Wilson, 2007) as this joint does not have a large angular range during gait (supplementary material Fig. S2). The biceps moment arm at the elbow varies as the elbow joint extends throughout a stride (trot 0.049–0.054 m; canter 0.051–0.055 m) and was therefore approximated at different elbow angles using a musculoskeletal modelling package (SIMM, MusculoGraphics Inc., Santa Rosa, CA, USA) (Weller, 2006; Watson and Wilson, 2007). Biceps IDA force was then calculated by dividing the shoulder moment by the shoulder moment-arm. Seventy percent of the shoulder moment was attributed to biceps. The remaining 30% was attributed to the forelimb protractors supraspinatus and brachiocephalicus. This was apportioned from the relative isometric force generating capacity of biceps (10,600–21,400 N), supraspinatus (3700–6000 N) (Watson and Wilson, 2007) and brachiocephalicus (1860 N) (Payne et al., 2005). The brachialis muscle, a small flexor of the elbow joint, exhibits very little EMG activity during locomotion (Tokuriki et al., 1999) and so its contribution to elbow flexion was ignored in this analysis.

A different percentage of the shoulder moment attributed to biceps would have altered the absolute value of the calculated IDA stiffness and thus energy stored. It is possible that this percentage contribution may vary depending on the gait condition; however, this could not be calculated in this study because of the redundancy in the system, and is therefore a limitation of this approach. However, given that the biceps will be required to contribute the majority of the large forces applied in all gaits, then the variation due to actions of other muscles is considered to be small.

Biceps MTU length change at the elbow and shoulder was calculated by multiplying the instantaneous elbow and shoulder angle changes by the respective moment arms at the instantaneous angle (fixed for the shoulder which was assumed to have a fixed moment arm). Elbow and shoulder angle changes were measured relative to reference angles of 130° and 250°, which represented length at zero force of the biceps MTU. These values were chosen as this was the length at which the calculated biceps force began to increase from zero during the stance phase (approximately 20% of the stance phase for both trot and canter). Integration of the biceps force *versus* MTU length relationship (known as a workloop) gave a measure of the work absorbed by the biceps MTU (negative work – lengthening) and the amount of work performed by the MTU (positive work – shortening) during the stance and initial swing period. The positive and negative work values were summed to give the net work across the cycle.

Ex vivo biceps MTU force-length properties

The force and length conditions calculated from the IDA were reproduced *ex vivo* in order to determine the force-length properties of biceps with and without activation. The force-length relationship was obtained by mechanically stretching MTUs while recording force. In order to determine the role of the muscle fibres, the MTU was initially stretched and shortened while passive and was then immediately activated and mechanically stretched and allowed to shorten again.

Five biceps MTUs (length 190–269 mm) were removed immediately *post mortem* from five ponies (approximate body mass 200 kg) euthanased for reasons unrelated to this study. The neck of the scapula, proximal radius and ulna were sectioned and the MTU mounted in a loading jig by a narrow chain and shackle around the origin and insertion. The muscle bellies were wrapped in plastic food wrap to prevent evaporative cooling. A linear variable differential transformer (LVDT) was used to measure MTU length change while a 10 kN load cell (both from Instron, Norwood, MA,

USA) simultaneously measured the force. The data were logged into a PC.

Once mounted, each MTU was cyclically stretched with a triangular waveform (constant lengthening and shortening velocity) at 1 Hz with different amplitudes of length change (12 mm, 22 mm and 26 mm) using a servo-hydraulic tension ram (Instron). Length changes of 10–14 mm would be expected *in vivo* from ponies with biceps MTU lengths 190–269 mm, assuming 5% strain, based on data from horses with biceps MTUs approximately 400 mm long (Wilson et al., 2003; Watson and Wilson, 2007). Five MTUs were lengthened by 12 mm and 22 mm and three of these were also lengthened by 26 mm. We adjusted the initial length of the muscles so that when muscles were stimulated, they were at the bottom of the ascending limb of the force–length relationship and generating only a small active force (<200 N). The muscles were cyclically loaded twice for each length change condition; once when not stimulated and once when stimulated (the order randomised to eliminate bias) with no interruption between cycles. During the active cycles, both muscle heads were activated for the whole stretch shorten cycle (1 s) using a muscle stimulator (Grass SD9, Quincy, USA) and wire electrodes inserted into both ends of the muscle belly (pulse duration 2 ms, 50 Hz, 150 V). Voltage settings were chosen to give the maximum contraction possible throughout the entire muscle and the frequency was set sufficiently high to ensure fused tetanus. During the active cycles the 1 Hz cycle speed resulted in a 0.5 s period of active muscle stretch. This is equivalent to stance phase duration in a walking horse (Hodson et al., 1999). As mentioned above, the biceps is active for the entire stance phase *in vivo* (Tokuriki et al., 1999). All experiments were carried out less than 30 min *post mortem*, which has previously been shown to be reasonable for retaining muscle function (Wilson et al., 2001).

Each muscle was subjected to one passive and one active stretch at each length change cycle (immediately following each other), with approximately a 1-min rest period before increasing the length change. The shortest length change was always carried out first to prevent any damage from over extension. The final set of data is from only three muscles and is the least reliable because the length change of 26 mm (MTU strain of ~11%) exceeded that expected *in vivo* for muscles of this size. In addition to this, the muscles at this point had already been subjected to four previous stretches and may have been damaged and/or fatigued.

Integration of the *ex vivo* biceps force *versus* MTU length relationship gave a measure of the work of the biceps MTU, which was further divided into the work absorbed by the biceps MTU during lengthening and the amount of work performed by the MTU during shortening. The positive and negative work values were also summed to give a measure of the net work across the cycle.

Development of a muscle model to reproduce the *ex vivo* experimental results

A muscle model was created to determine the influence of muscle activation on the biceps MTU energy storage and to reproduce the *ex vivo* experimental results. The biceps muscle was modelled as a Hill-type muscle model (Zajac, 1989) (Fig. 2) with separate short and long fibred contractile elements representing the lateral and medial heads and created in Simulink (Matlab, Mathworks, MA USA).

The model consists of two contractile elements [CE_M (medial) and CE_L (lateral)] which have different lengths and standard force–length and force–velocity relationships relative to the length of the CE (Fig. 2). The CE (fibre) length and MTU length were given anatomically similar values to the pony biceps, determined by dissection following the *ex vivo* experiment. The

CE_M fibre length was set to 30 mm, the CE_L fibre length was set to 10 mm and the MTU length was set to 215 mm. The series elastic element (SEE) slack length for both heads was calculated as the length of the MTU minus the corresponding CE length. Rome (Rome et al., 1990) reported a weighted average maximum shortening velocity of approximately 1.68 lengths s^{-1} in mixed fibre type muscle of the horse (soleus) at 15°C. Scaling this to 37.5°C using a Q_{10} of 2 (Bennett, 1984), we estimated a maximum shortening velocity of 8 fascicle lengths s^{-1} . A maximum isometric force (F_{max}) of 1200 N and 1000 N of the lateral and medial heads was calculated from the average physiological cross-sectional area (PCSA) of the two heads assuming a maximum isometric stress of 0.3 MPa. An exponentially rising and falling relationship between stimulation and active state (the proportion of bound cross-bridges relative to the maximum in activated sarcomeres) was used with a time constant of 0.3 s (Zajac, 1989). This accounted for the time it takes for the active state to develop and fall during and after stimulation.

The muscle model was subjected to the same length change over time as in the *ex vivo* experiments. The model MTU was stimulated at the start of lengthening and stimulated for 0.9 s during the first cycle (90% of the *ex vivo* cycle) and was inactive during the second cycle to obtain a force–length relationship for the entire MTU during both active and passive lengthening.

In early simulations it was apparent that activating the muscle to 100% levels (i.e. all muscle fibres fully active), produced larger forces than were measured in the *ex vivo* experiments. It is unlikely that 100% of the muscle fibres in our dissected preparation would be able to activate (as a result of death of fibres and also limitations in the stimulation procedures), therefore we scaled the maximum activation level to 70% to achieve similar forces to the *ex vivo* experiments. The elastic properties (stress–strain) of the PEEs and SEEs were also varied until a good match between the model and the *ex vivo* experimental results was achieved (see Fig. 2).

Adaptation of the muscle model to represent biceps IDA force and length during trot and canter

When the muscle model could predict the *ex vivo* experimental data for ponies, it was then adapted to represent the larger biceps of the Thoroughbreds used in the IDA. The SEE slack length was changed from 215 to 400 mm, while muscle fibre lengths remained unchanged from the previous model, as the fibre lengths of larger horses are very similar to that of the ponies measured here (Watson and Wilson, 2007). A maximum contraction velocity (V_{max}) of 8 lengths s^{-1} was again assumed and an F_{max} of 10,000 N and 3500 N for the lateral and medial heads was used to account for the increase in PCSA of the Thoroughbred biceps heads (Watson and Wilson, 2007). The muscle model was then subject to the length change calculated in biceps MTU during the stance phase and early swing phase of trot in the IDA. The duration of the muscle model stimulation (as a percentage of trot stance time), maximum activation level (proportion of muscle recruited) and timing of stimulation (relative to the start of the stance phase) were then altered to determine their influence on the amount of energy stored and returned from the biceps MTU. When the duration of stimulation was altered the intensity was set to 70% of maximum activation (i.e. 70% of fibres activated) and the MTU was stimulated 0.025 s into the stance phase (~10% stance phase). When the maximum activation level was altered, the duration of stimulation was set to 40% of stance time and the MTU was stimulated 0.025 s into the stance phase. Finally, when the timing of the onset and duration of stimulation was varied,

the maximum activation level was set to 70% and the duration of stimulation was set to 40% of the stance time.

Integration of the simulated biceps force *versus* MTU length relationship gave a measure of the negative and positive work over the cycle, as explained previously. We also integrated the CE force *versus* CE length relationship for both heads and summed these to gain insight into the net contribution of the CE to the total work. The CE work was again divided into positive and negative work phases.

Statistical analysis

The calculated negative work (lengthening) and positive work (shortening) of the biceps in both trot and canter were compared with a Student's *t*-test assuming a two-tailed distribution and unequal variance in both groups, where *P*-values less than 0.05 were considered as significant. A paired Student's *t*-test was used in each condition to compare the negative and positive work of the biceps. The same analysis was performed for all the *ex vivo* muscle data.

RESULTS

Biceps IDA at trot and canter

Biceps force during trot and canter was estimated by IDA and the measured change in shoulder and elbow joint angles were used to calculate biceps MTU length change. The biceps force–length curves for trot and canter (Fig. 3A) show that the MTU had a higher maximum force and stretched further in canter compared with trot. There was significantly greater negative work ($P=0.02$) and positive

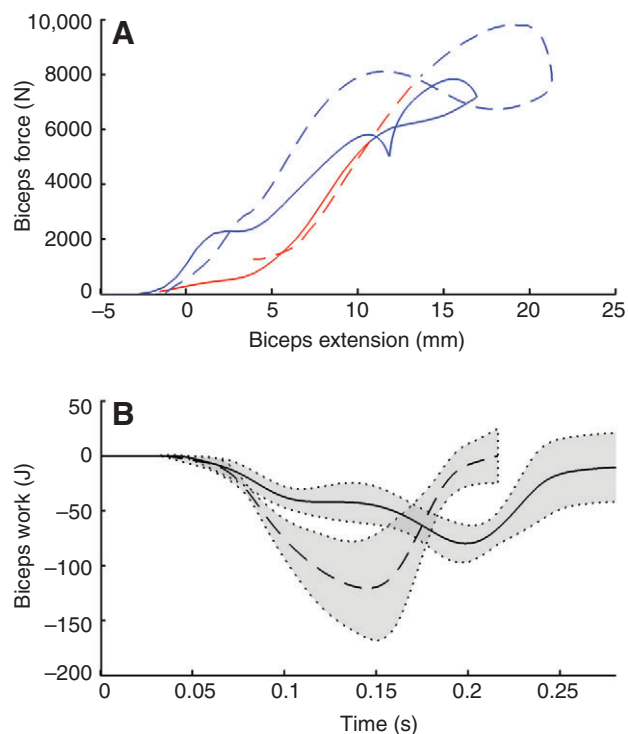


Fig. 3. (A) Average calculated force–extension relationship for biceps brachii at trot (solid line) and canter (dashed line) during 120% of stance phase (blue indicates 1–100% stance, red indicates 101–120% stance). Extension was measured relative to the length changes from the average shoulder and elbow joint angle at 20% of stance for trot and gallop, where biceps force production begins. (B) Average work of the biceps brachii during the 120% of stance period during trot (solid line) and canter (dashed line). Negative work corresponds to work done on the muscle whereas positive work corresponds to work done by the muscle. Shaded area indicates ± 1 standard deviation.

($P=0.02$) work performed by the biceps MTU during canter compared with trot. Integration of the force–length curves gave a peak negative work of approximately 82 J and 127 J at trot and canter respectively (Fig. 3B, Table 1), and the total positive work was 71 and 121 J. Peak negative work occurred at approximately 90% of stance in both trot and canter, after which, a rapid decline in force and length results in work performed by the biceps MTU, which is used for limb protraction. The net CE work was negative in both trot and canter (–10 and –0.4 J, respectively), indicating an energy absorbing role. However, the difference between the negative and positive work values was not found to be significant in both trot ($P=0.25$) and canter ($P=0.97$).

Ex vivo biceps force–length relationship

The purpose of the *ex vivo* experiments were to determine the influence of activation and length change on the energy storing capacity of the biceps. The average force–length relationship under three different length change conditions for both passive and active contractions are shown in Fig. 4. In all three length change conditions the active curve was stiffer than the passive curve and more negative and positive work was done as length change increased (Table 1). In all three conditions more work was done on the MTU during lengthening than was returned during the shortening phase, with a total CE energy absorption of 5, 16 and 23 J in the 12, 22 and 26 mm length change conditions, respectively. There was also a difference between negative and positive work during the passive conditions for length changes of 22 mm and 26 mm ($P=0.02$ and $P=0.03$), which indicates energy loss due to hysteresis. Despite an increase in work done on the muscle, the total positive work was greatest when the muscle was active compared with the passive condition; however, the proportion of energy returned in the passive condition compared with the active condition increased from 32, 56 and 86% with increasing length changes.

Muscle model *versus ex vivo* experimental results

The biceps muscle model was subjected to the same length changes as in the *ex vivo* experiments. Force predictions close to those observed experimentally were achieved in all length change conditions when the maximum activation level was scaled to 70% of maximum (Fig. 4, thin lines). As with the experimental results, the model shows an increase in total energy returned with increases in peak MTU length and in activation.

Influence of activation parameters during simulated gait conditions

The biceps model was scaled up to represent the anatomy of the Thoroughbred horses and subject to the same length change trajectory as that calculated in the IDA. The activation parameters were then varied to examine the influence this has on the energy stored and returned during the cyclical movement.

First the duration of the model stimulation (as a percentage of trot stance time) was varied with a maximum activation level of 70%. Stimulation began at 0.025 s and the stimulation durations used were 0.023 s, 0.059 s, 0.118 s and 0.177 s (which corresponds to 10, 25, 50 and 75% of the stance phase). The activation level (or proportion of bound cross-bridges relative to the possible number of bound cross-bridges) corresponding to each stimulation pattern is shown in Fig. 5A and the resulting force–length relationships are shown in Fig. 5B. With increasing stimulation duration, the model predicts an increase in both negative and positive work (Table 2), however, net MTU energy absorption was greatest during the shorter stimulation durations.

Table 1. Negative, positive and net muscle–tendon unit work during trot and canter and during *ex vivo* experiments with different length changes and both active and passive conditions

	Negative work (J)	Positive work (J)	Net work (J)	
IDA				
Trot	-81.9 (4.2)	71.4 (8.6)	-10.5 (8.6)	$P=0.25$
Canter	-127.2 (15.1)	126.8 (8.8)	-0.4 (8.8)	$P=0.97$
	* $P=0.02$	$P=0.02$		
Ex vivo				
12 mm – passive	-1.67 (0.14)	1.72 (0.17)	–	$P=0.68$
12 mm – active	-7.12 (1.32)	4.94 (0.91)	-2.18 (0.91)	* $P<0.01$
	* $P=0.01$	* $P=0.02$		
22 mm – passive	-10.51 (0.84)	8.22 (0.41)	–	* $P=0.03$
22 mm – active	-32.65 (1.22)	15.99 (0.88)	-16.66 (0.88)	* $P<0.01$
	* $P<0.01$	* $P<0.01$		
26 mm – passive	-24.62 (1.33)	19.91 (0.58)	–	$P=0.02$
26 mm – active	-46.80 (4.25)	22.95 (0.59)	-23.85 (0.59)	$P=0.03$
	* $P=0.03$	* $P=0.02$		

Values are means \pm s.e.m.

Work during trot and canter calculated from inverse dynamic analysis (IDA).

*Significant differences between conditions (either trot/canter or active/passive) for absolute positive and negative work are indicated as well as differences between absolute positive and negative work for each condition.

We then tested how the maximum activation level influences energy stored and returned by the MTU. We stimulated the muscle model for 40% of the stance phase (0.118 s) starting 0.025 into the stance phase while varying the maximum activation level (or proportion of muscle fibres stimulated) to values of 10, 25, 50 and 100%. The greater the maximum activation level the more energy

was both absorbed and performed/released by the biceps, while CE energy absorption also decreased with increasing maximum activation level (Fig. 5B, Table 2). All active conditions produce more positive work than the equivalent passive conditions (Fig. 5A–C, thick grey line; Table 2).

Finally we tested the influence of changing the timing of stimulation relative to the stance phase while maintaining a fixed maximum activation level (70%) and a constant duration of stimulation (40% of stance phase). We used starting times for stimulation of 0, 0.03, 0.04 and 0.06 s relative to the start of the stance phase. Although the amount of negative work decreased with increasing time of the start of activation, the amount of positive work was greatest when activated at 0.03 and 0.04 s after the start of stance. As the timing of stimulation relative to the stance phase increased, the net CE increased to be only -2 J in the 0.04 s condition (nearly all of the work done on the MTU is returned during shortening) and 7.2 J in the 0.06 s condition (where the muscle fibres do more work than that done on the MTU).

In all conditions the positive CE work only accounted for a small proportion of the MTU positive work, which suggests that the elastic elements were contributing to the majority of the work. The CE performed significant amounts of negative work (absorbing mechanical energy) and the proportion of this to the total negative work varied between 10% and 70% depending on the conditions. The least amount of negative work was achieved when the stimulation timing started between 0.04 and 0.06 s after the start of the stretch–shorten cycle.

DISCUSSION

Overview

The aims of this research were to understand how elastic strain energy may be modulated by the contractile component of the biceps brachii in order to control the speed of limb protraction. To achieve this we used three approaches. Firstly, IDA was used to examine the force–length profile of the biceps at trot and canter, where more energy is required to protract the limb in canter. The results of this experiment found that more work was done on the biceps during the stance phase of canter, primarily by extending the muscle further, which corresponded with a greater amount of positive work

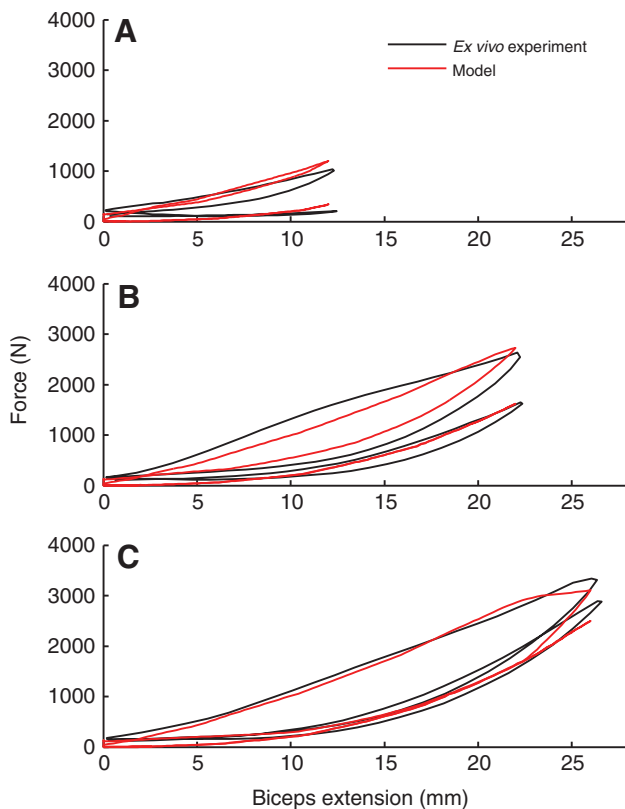


Fig. 4. Biceps brachii force versus extension during *ex vivo* experiments on excised pony muscles (solid black lines) and using the muscle model depicted in Fig. 2 (red lines) at three different lengths: (A) 12 mm, (B) 22 mm, (C) 26 mm.

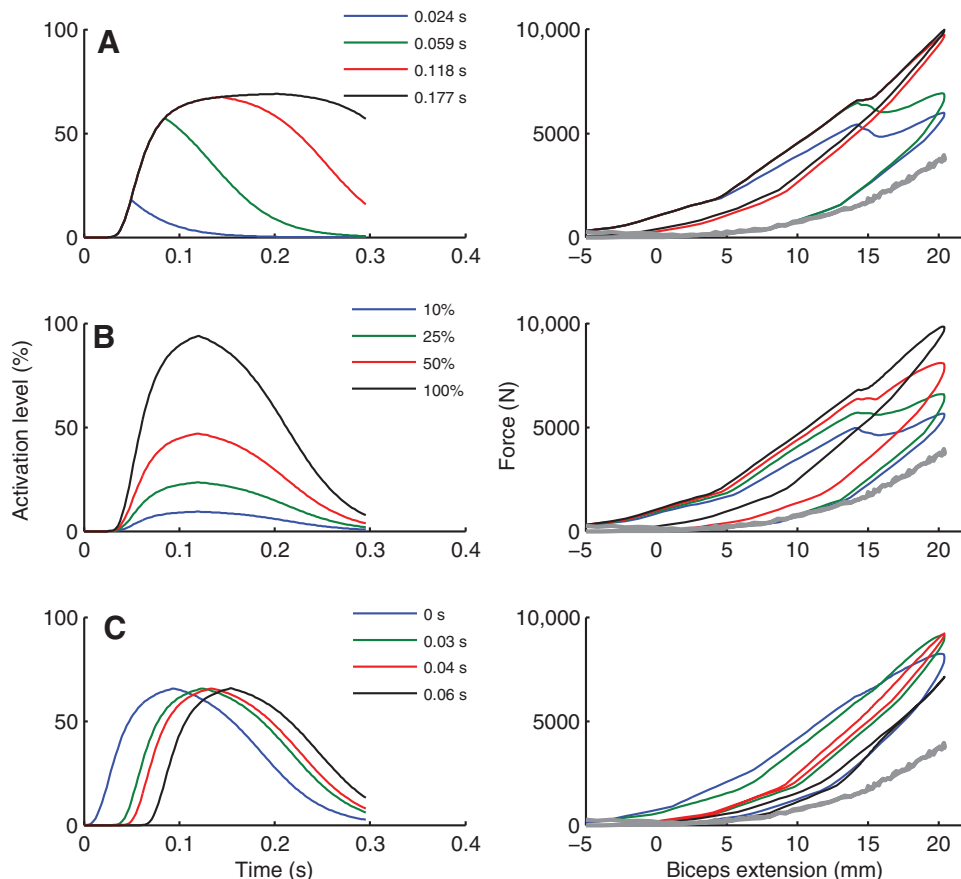


Fig. 5. Influence of changing the activation conditions during length changes measured from 120% of the stance phase of trot. Left panels indicate how the muscle model is activated with respect to time (i.e. how the proportion of total bound cross-bridges changes, compared with maximum number) and right panels show the corresponding biceps muscle–tendon unit (MTU) force–extension relationship calculated from the model. A passive MTU force–extension plot (no stimulation) is shown as the thick grey line in all activation conditions (A–C). (A) Influence of changing the duration of activation (maximum activation=70%, timing of stimulation=0.025 s). (B) Influence of changing the maximum activation level (proportion of muscle fibres that are stimulated; duration of stimulation=0.118 s, timing of stimulation=0.025 s). (C) Influence of changing the timing of activation relative to the start of the stance phase (maximum activation=70%, duration of stimulation=0.118 s).

performed by the MTU during late stance and into the swing phase. Secondly, we performed passive and active stretch–shorten cycles on excised pony biceps muscles with different amplitudes of length change. The results indicate that a greater amount of positive work can be achieved with activation compared with no activation for all length change trajectories. The results also indicate that more positive work is produced with greater length changes. However, passive forces contribute a greater proportion of the total force during very long length changes. Finally, we used a model (validated against the *ex vivo* data) to examine how changing the activation parameters might change the amount of work done on the MTU (negative work) and done by the MTU (positive work). The results of this analysis showed that by changing the duration, magnitude and timing of stimulation it is possible to both absorb more energy with the biceps MTU and also generate more energy with the biceps MTU. The majority of the positive work produced by the muscle is generated by the elastic elements. Although the CEs only contributed a small amount of the positive work, the net work that they produced, because of activation timing, was important in modulating the amount of positive work generated by the MTU.

Biceps energy during trot versus gallop

Calculation of the work performed by the MTU during canter compared with trot confirmed the capacity of biceps to provide the additional energy required for limb protraction at higher speeds. The amount of work performed by the biceps MTU during trot, 71 J, was quite a bit larger than that calculated to be required to protract the limb; 33 J (Wilson et al., 2003). Even during a canter, there was more energy produced by the MTU (127 J) than was required for protraction at gallop (120 J). This suggests that the biceps is the primary muscle responsible for limb protraction. It is

probable that a good proportion of this energy was later absorbed by muscles such as the triceps brachii and the leg extensors as the leg is prepared for contact. Although it might seem energetically inefficient to absorb energy produced by the biceps, we calculate that the total cost is relatively small and may be outweighed by the requirement for adequate power for hoof clearance and positioning the limb for controlled ground contact. Furthermore, the alternative of having a larger muscle would be even more costly because of the extra weight and activation costs.

To achieve an increase in positive work, it seems that the biceps was actively extended further in canter compared with trot. To achieve this, more negative work must be performed on the muscle. This was achieved through an increase in both GRF and limb sweep angle with increased speed which contributed to increasing the elbow and shoulder moments. This effectively allowed more work to be done on the active MTU and hence more energy to be stored.

There are several limitations to this approach. Firstly, the inverse dynamics calculations used inertial properties of the equine forelimb taken from Buchner et al. (Buchner et al., 1997). These inertial properties are based on Dutch Warmblood horses that had a slightly greater body mass and slightly heavier limbs than the Thoroughbred horses used here (~40 kg). Use of Buchner's data may therefore have resulted in a small overestimation of the inertial properties. In addition, the use of estimated moment arms along with noise in our motion analysis signal are likely to affect both the force and length measures calculated for the biceps. However, our results are based on comparisons of mechanics between the trot and canter conditions as opposed to the absolute calculated values and the differences are large, so we have confidence in our conclusions.

Table 2. Influence of changing the stimulation or activation conditions on positive and negative work performed by the biceps brachii muscle–tendon unit and by the contractile element during length changes measured across 120% of the stance phase of trot

	Negative work (J)	Positive work (J)	Net work (J)	Negative CE work (J)	Positive CE work (J)
(A) Duration of stimulation (s)					
0.024 (10% stance)	-70.23	30.59	-39.64	-48.97	0.20
0.059 (25% stance)	-81.88	33.71	-48.17	-59.72	0.48
0.118 (50% stance)	-93.06	47.14	-45.91	-30.35	1.31
0.177 (75% stance)	-102.73	73.11	-29.62	-26.02	2.41
(B) Maximum activation level (%)					
10	-77.81	33.70	-44.11	-43.78	0.21
25	-89.69	35.12	-54.57	-52.49	0.26
50	-99.41	75.87	-23.54	-51.15	0.59
100	-99.67	81.63	-18.04	-36.27	1.09
(C) Timing of stimulation (s)					
0 (0% stance)	-89.56	45.76	-43.80	-48.55	0.22
0.03 (10% stance)	-86.52	63.45	-23.06	-29.50	1.22
0.04 (17% stance)	-70.11	67.60	-2.51	-12.03	3.95
0.06 (27% stance)	-43.34	50.36	7.02	-5.18	7.57
(D) Passive (no activation)	-25.95	25.42	-	-	-

(A) Influence of changing the duration of activation (maximum activation=70%, timing of stimulation=0.025 s). (B) Influence of changing the maximum activation level (proportion of muscle fibres that are stimulated; duration of stimulation=0.118 s, timing of stimulation=0.025 s). (C) Influence of changing the timing of activation relative to the start of the stance phase (maximum activation=70%, duration of stimulation=0.118 s). (D) Influence of passive condition i.e. no stimulation of muscle during length change.

Biceps energy during active and passive stretch-shorten cycles

The *ex vivo* experiments enabled us to test the hypothesis that muscle activation coupled with increased MTU length change would result in increased elastic energy storage in biceps. Indeed, we found that both activation and increasing length change led to an increase in both the work done on the MTU and the work done by the MTU. It is not surprising that more force was generated during the active stretch than during the passive stretch, particularly given the force–velocity characteristics of muscle. However, it was interesting to note that the proportion of work performed by the MTU in the passive compared with active conditions increased with increasing length change; effectively more load was going through the PEE. This suggests that the muscle fascicles were stretched beyond optimal length and hence could not produce much active force and that PEE was becoming more important in resisting muscle elongation. It is probable that stretches of this magnitude are rarely imposed during normal locomotion; however, this experiment demonstrates how the active capacity of the muscle diminishes with very long stretches. The much smaller forces recorded in the *ex vivo* experiments compared with the IDA are primarily due to the large differences in PCSA between the ponies and the Thoroughbreds, and hence the difference in force producing capacity.

It is important to note that during the *ex vivo* experiments the biceps were activated for 1 s, which was the entire duration of the stretch shorten cycle. *In vivo*, the muscle is active during the lengthening cycle and perhaps the start of the shortening cycle (Tokuriki et al., 1999). In addition, the cycle frequency used (1 Hz) is somewhat longer than that of the trotting or cantering horse (they are more consistent with those of a walking horse). Therefore, we cannot make direct comparison between the results of the *ex vivo* experiment and real life energy stored and returned in the elastic tissues of the biceps. These experiments do, however, demonstrate the ability of the muscle to do more positive work after actively stretching (absorbing work). The modelling part of our study highlights how the interaction between the CE and the elastic elements can further modulate energy produced by the muscle for limb protraction under different conditions.

Biceps energy under different activation conditions

The muscle model enabled alteration of activation parameters in ways that would have been impossible experimentally given the time restrictions associated with experiments of this nature on live muscle. There is good agreement between the *ex vivo* experimental force–length relationship and the model force–length relationship, particularly at the longer length changes (Fig. 4). It was necessary to reduce the maximum activation level of the model (which might be thought of as proportion of activated fibres compared with the total number), however, this is probably justified based on the fact that a proportion of the fibres are probably not be activated given our stimulation procedure and some exposure to air (which may cause cell death; and indeed in life).

After scaling the model to represent the anatomy of the Thoroughbreds tested with the IDA, we were able to examine the influence of changing the duration, amplitude and timing of biceps muscle activation in the model (Fig. 5). Although longer durations of stimulation were found to produce the required work performed by the MTU, a greater proportion of work had to be done on the MTU to achieve this than was calculated in the IDA (102 J vs 81 J). Changing the amplitude of the activation level had a similar effect to changing duration. The closest match between the model and the IDA results was found by changing the timing of activation (relative to the lengthening phase). In Fig. 5C, the duration of activation was set to 40% of the stance time and the maximum activation level was set to 70%. By adjusting this timing to 0.04 s it was possible to elicit minimal net work from the muscle fibres, yet achieve similar positive work performed by the MTU as calculated in the IDA (67.6 J compared with 71.4 J). Therefore, by adjusting these parameters it is possible to effectively adjust energy utilised by the biceps for limb protraction and to achieve this with minimal fibre work.

The model also demonstrated that the majority of the shortening work was performed by the elastic tissues rather than the CEs of either head. In nearly all cases considered here, the positive CE work was very small compared with the positive MTU work (<10% in all cases). Given that the fibres of the medial head are only 10 mm, and the length changes that occur during trot and canter exceed 20 mm, most of the length change has to occur in the elastic tissues

to prevent these fibres from overextending. Therefore we can safely conclude that the majority of the work done on the muscle is stored in elastic tissues and elastic recoil of these tissues contributes to most of the energy required for limb protraction. One role of the CE is therefore to generate sufficient force so that the elastic tissues can stretch and store the elastic strain energy.

It should be noted that modelling this muscle using a multi-element Hill type muscle model is likely to be an oversimplification of how the muscle operates. With such a complex structure, it is difficult to assess whether homogenous strains would occur across each head and in the two heads and the interaction of the two heads is likely to be critical, which includes when the two are activated relative to each other (Higham et al., 2008; Nelson and Roberts, 2008). The model also does not take account of effects such as history dependence of force production (Herzog and Leonard, 2000) or myofascial force transmission (Maas et al., 2003), which might alter the results slightly. However, the final conclusions are unlikely to change a great deal given that the elastic tissues performed much of the positive work of the MTU during shortening. One interesting finding was that in our modelling, the net CE work across the cycle did not always match the net MTU work. This is because in many cases the muscle is still active at the end of the cycle (hence energy is still stored in elastic tissues) and also because the muscle is not returned to its length at the start of the cycle (a full cycle is not assessed). Small integration errors in the model also contribute to these differences.

The muscle model does not currently consider activation of the biceps muscle before the start of the stance phase. It is possible that, *in vivo*, the biceps becomes active shortly before the stance phase, which would result in a faster rate of force increase during the stance phase and thus greater energy storage and return. The nervous system has the ability to change the number and distribution of muscle fibres activated through a contraction (and hence maximum activation level), which was not attempted in the modelling presented here. Results from Hof (Hof, 1998) suggest that the stiffness of the SEE increases with increasing activation level, because of sequential activation of CE plus SEEs acting in parallel. This phenomenon was not modelled here because of the limited knowledge of this mechanism, however, it would provide further scope for modulating the energy stored and returned from elastic structures. Further permutations of muscle activation parameters (such as the proportion of activated fibres) would provide interesting future work, and measurements of biceps activation timing, duration and amplitude and muscle fibre length changes during locomotion would provide further insight into the biceps muscle physiology. However, the biceps is situated on the medial aspect of the limb so *in vivo* assessment of biceps activation and fibre length change is limited to challenging invasive fine wire electromyography (EMG) and sonomicrometry techniques rather than ultrasound and surface electrode EMG.

Conclusion

These experiments highlight the potential for the contractile element of biceps to modulate the energy storage capacity of the MTU. The results indicate that this occurs using a combination of MTU length change, muscle activation level, activation duration and an optimal start time of activation to facilitate precise control of the equine forelimb enabling multi-speed locomotion over varying terrains.

APPENDIX 1

Algorithms to correct for skin movement error over the scapula spine

Six thoroughbred-type horses (height range 158–173 cm) were recorded using high-speed video (JVC, GR-DVL9700,

200 frames s⁻¹) on a treadmill (high speed Sato, Uppsala, Sweden) at trot and canter at 4 m s⁻¹ and 8.2 m s⁻¹, respectively. Oil was applied to the shoulder region to make the scapula spine more easily visible and a marker was placed at the top of the scapula spine when the horse was standing square. The bony scapula spine could thus be seen moving beneath the scapula marker when the horse moved. The aim was to calculate the angle between the shoulder marker and scapula spine, about the shoulder joint in order to estimate the shoulder angle error due to skin displacement. The video data were digitised and angles were calculated using Silicon Coach software (Silicon Coach Pro, Version 6.1.2.0, Dunedin, New Zealand). Six complete strides were recorded from each horse at each gait and data were interpolated to 100 points. The mean angle of all strides from all horses at each gait was plotted against percentage stride and a sixth order polynomial regression line was fitted to the trace. This gave the shoulder angle error at all times throughout a stride and was subsequently subtracted from all shoulder angles calculated in these experiments. The polynomial equations describing the shoulder angle error (deg.) in both trot and canter are shown below, where *x* is the percentage of the stride. It is necessary to include all decimal places in order to calculate accurate values.

Trot shoulder angle error =

$$4.59303727646729 \times 10 - 10.x^6 - 1.32207167288009 \times 10 - 07.x^5 + 0.0000139935283518455.x^4 - 0.000657197452042729.x^3 + 0.0124306076744823.x^2 - 0.0324638832142215.x + 2.10202373582735, \quad (A1)$$

Canter shoulder angle error =

$$-9.92192724286989 \times 10 - 11.x^6 + 3.32758481196076 \times 10 - 08.x^5 - 4.49549532039671 \times 10 - 06.x^4 + 0.00030609336817928.x^3 - 0.0104594125297431.x^2 + 0.141358592008581.x + 2.8874932038934. \quad (A2)$$

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