

The effect of tendon stiffness on the development and degeneration of locomotion in the sport horse

Thesis by Pauline Robina Addis

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Abstract

Introduction

Injury to the flexor tendon is unfortunately a common cause of retirement of the sport horse, leading to concerns for equine welfare and economic loss. Maximising the quality of the tendon is only possible before tendon matrix synthesis ceases at around three years old. This cease is reflected in a change in tendon stiffness from an increase to a plateau. Throughout the rest of the horse's life, repeated loading cycles gradually weaken the tendon and lead to decrease in stiffness. Since the two flexor tendons support the joint of the distal limb, this change in stiffness is likely to be reflected in the joint angles, which could be used to determine both when tendon matrix synthesis has ceased and if degenerative changes are normal. A second area of investigation is into the development and deterioration of stride parameters such as length and frequency. Since these depend on both leg length and on the input of the central nervous system (CNS), normalising to leg length allows investigation of the influence of the CNS alone.

Method

The distal limbs of 57 horses from three months to 17.5 years old were videoed over one complete stride. Horses were divided into three age groups; young horses up to 35 months whose tendon stiffness and leg length was assumed to increase; adult horses 36 to 99 months old whose tendon stiffness and leg length was assumed to remain constant; and finally horses over 100 months old whose deteriorating tendons were assumed to show a decrease in stiffness but whose leg length would not change. To determine joint angles, the videos were processed with a new marker-free tracking system that was found to show comparable results to previously-published data. Since joint angles of the passive equine distal limb depend not only on tendon stiffness but also mass of the horse, angles were normalised to calculated mass to allow investigation of tendon stiffness alone.

Results

Maximum normalised angles decreased in the young horse, remained constant in the adult horse and increased towards old age; consistent with changes in tendon stiffness. Most stride parameters changed during growth, and all except velocity showed a change when normalised to leg length. Towards old age, two parameters showed a slight decrease but there was no change in the normalised parameters.

Conclusion

In terms of joint angle, a trend was seen that was consistent with previously-reported trends in tendon stiffness. An owner could track their horse's joint angles from birth to identify the characteristic change that accompanies the cease of matrix synthesis and therefore the limit of adaptive ability of the tendon. Tracking joint angles towards old age would also allow early identification of any abnormal change before the injury becomes severe enough to cause an overt change in the horse's gait.

The change seen in normalised stride parameters in the young horse indicates that these parameters are influenced by CNS maturation as well as a simple increase in leg length. Any abnormal change in stride parameters could indicate a lack of stability and the risk of a fall.

This thesis is dedicated to:

DOMINIQUE ADDIS

1940-2005

- *toujours chaude et étanche*

In memory also of Claude Simon-Duneau.

Riding is a partnership... together you can achieve a richness that alone neither can.

- Lucy Rees 1991

The ponies!

- Katharine Hull and Pamela Whitlock 1938

Horses are stupid, horses are mad.

- Benedict Addis, *circa* 1988



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Glossary of terms

Clinical	Observations of a subject or patient made using objective, evidence-based measures
Contralateral/ipsilateral	On the opposite side/same side of the body
Coronal plane	A plane that passes through the body medio-laterally, dividing it into anterior and posterior sections
Deep digital flexor tendon (DDFT)	One of the main elastic structures that supports the joints of the distal limb
Distal inter-phalangeal (DIP) joint	The most distal limb joint, contained within the hoof, and formed between the medial and distal phalanges
Distal	The section of a limb or segment furthest from the centre of the body
Gait/locomotion	Co-ordinated movement of the limbs that results in translation of the centre of mass over a distance
In vivo/in vitro	A type of study using a whole, live organism in its natural state; as opposed to using parts of an organisms that have been isolated from the whole body.
Invasive/non-invasive	A type of study that requires invasion into a body, such as taking blood samples/studies that do not require invasion
Kinematics	The study of how limb segments move in space, over time
Kinetics	The study of force
Longitudinal/cross-sectional	A study that takes place over a long period of time, usually investigating the same subjects
Metacarpo-phalangeal (MCP) joint	The joint between the third metacarpus and proximal phalanx and the primary distal limb joint
Proximal inter-phalangeal (PIP) joint	The joint formed between the proximal and medial phalanges, contained within the pastern
Proximal	The section of a limb or segment closest to the centre of the body
Sagittal plane	A plane that passes through the body cranio-caudally, dividing the body into left and right sections
Superficial digital flexor tendon (SDFT)	One of the primary elastic structures that spans and supports the MCP and PIP, commonly injured
Senescence	The period of life towards old age, when degenerative changes often take place
Suspensory ligament (SL)	One of the main elastic structures that support the distal limb

Stance phase	The part of the stride cycle when the hoof is on the ground
Stiffness/modulus of elasticity	A measure of the ability of a material to resist deformation
Strain	A measure of deformation: calculated as change in length divided by original length
Swing phase	The part of the stride cycle when the hoof is in flight
Tendon matrix	The fluid in which tendon fibres are suspended, consisting mainly of water and proteoglycans.
Toe on/off	The start of stance, when the front of the hoof first makes contact with the ground/the end
Training	Exercise aimed at improving a horse's gait and/or fitness

1. Introduction

The horse's speed, strength and stamina have been exploited by man for transport, warfare, agriculture and, during more recent times, in sporting disciplines. In strenuous sports such as racing and eventing, the distal limb tendons and ligaments are frequently injured, often with no clinical warning signs. The risk of injury can be decreased by optimising the quality of tendon during development, and also minimising loads placed on it during degeneration. During these periods of development and degeneration, tendon stiffness changes; first increasing until skeletal maturity and then decreasing towards old age. Since the flexor tendons wrap around the distal limb joints, these characteristic changes may be reflected in the horse's kinematics, possibly providing a useful and non-invasive method to investigate the mechanical properties of the tendon. This method would help to identify when the tendon is mature enough to begin training, and also to identify early-stage injury. This thesis studies the influence of tendon stiffness on equine locomotion to determine if these characteristic changes are detectable, with the ultimate aim of decreasing injury.

This chapter begins with the overall motivation for the research, describes the importance of tendon injury and investigates the influence of age on stride parameters. Next, it outlines the development of a new marker-free method of tracking the equine distal limb and how this method was applied to explore the link between tendon mechanical properties and kinematics through the use of a mass-spring model. This introduction chapter ends with an overview of the thesis and its scope.

1.1. Motivation: the importance of tendon injury

Injury to equine tendons and ligaments is common, especially in sports such as racing and eventing where these tissues undergo high strains. Tendon or suspensory ligament (SL) injury was found to account for 46% of all limb injury in British racehorses over a three-year period (Williams *et al.* 2001), similar to the 36% prevalence over two years reported by Pinchbeck *et al.* (Pinchbeck *et al.* 2004). More recently, Ely *et al.* (Ely *et al.* 2009) found the incidence of tendon or SL injury to be 16.1 per 1000 horse starts over two National Hunt seasons. As well as the high frequency of injury occurring at the racetrack, endemic tendinitis is also widespread; Avella *et al.* (Avella *et al.* 2009) reported a 24% prevalence of pathology in the superficial digital flexor tendon (SDFT)

determined by ultrasound examination during two National Hunt racing seasons. Eventing, another sport where tendon and SL undergo repeated high strains, also has a high prevalence of tendon and SL injury. Of the injuries that excluded Intermediate-level horses from long-term competition, 43% were to tendon or ligament (Singer *et al.* 2008). Since these studies only show reported injury occurrences, the actual figure for total tendon and ligament injury in the population of approximately one million (DEFRA 2004) horses in the UK is likely to be higher, as no database yet exists of injuries to non-competition horses.

Despite the frequency of tendon injury, there is currently no highly-effective treatment (Kjaer 2004; Rees *et al.* 2006) and management is mostly limited to prevention, although stem cell therapy is starting to show some encouraging results (Godwin *et al.* 2011; Smith 2008). Since the resulting scar tissue is stiffer than normal tendon, adjacent areas undergo greater strain (Smith *et al.* 2002), resulting in a high frequency of injury recurrence (Avella *et al.* 2009; Rees *et al.* 2006).

The principal consequence of injury is concern for the welfare of the horse, but injury also has two economic impacts. First, veterinary attention is an expensive outlay, and secondly, a horse that cannot compete due to injury will not earn money for its owner, especially in the case of tendinopathy which often requires long-term rehabilitation (Avella *et al.* 2009).

The 2004 Henley Report, produced by Department for Environment, Food and Rural Affairs (Defra) and the British Horse Industry Confederation (BHIC) estimated the value of the horse industry in Britain alone to be between £0.5 billion and £3.4 billion and to employ 50,000 people directly and 200,000 people indirectly (DEFRA 2004). More recently, the 2006 National Equestrian Survey published by the British Equestrian Trade Association (BETA) estimated the annual value of the industry at £4 billion. Part of this high value is due to the price of successful competition horses; the highest sum ever paid for a racehorse was \$16 million for The Green Monkey at the 2006 Fasig-Tipton sale in Calder, Florida¹. Understanding how to minimise tendon injury would help to maintain the economic value of this industry.

¹ Source: www.fasigtipton.com/about-us/history.asp.

Tendon injury occurs when loads exceed the mechanical limit of the tissue (Smith *et al.* 2002), either as a result of a single macro-trauma or due to cumulative micro-trauma that leads to fatigue failure (Buchanan and Marsh 2002; Dowling and Dart 2005; Kjaer 2004). This catastrophic stage is marked by an overt change in locomotion (Clayton *et al.* 2000a; Clayton *et al.* 2000b; Dow *et al.* 1991; Williams *et al.* 1999). Currently, injury is only identified once the damage is sufficient to alter locomotion; it would therefore be desirable to develop a method to detect subtle changes indicative of tendon degeneration *before* the horse exhibits clinical lameness (Weishaupt *et al.* 2004). The ability to detect this degeneration would allow trainers to reduce loads experienced by the tendon by, if necessary, decreasing exercise intensity and/or duration, before rupture occurs. Since each horse will exhibit an individual pattern of deterioration, there is a need for a subject-specific method to identify the point at which the tendon is at risk of injury.

1.2. Changes to tendon mechanical properties with age

Since the two flexor tendons and the suspensory ligament wrap around and support the joints of the distal limb, it is likely that joint angle will be indicative of the mechanical properties of these elastic structures (Butcher and Ashley-Ross 2002; McGuigan and Wilson 2003). The stiffness of the tendon has been found to change throughout the horse's life, from an initial low modulus of elasticity to a peak at around eight years old, followed by a slow decrease into senescence (Gillis *et al.* 1995) due to a weakening of the tendon. These changes are likely to be reflected in the kinematics, which owners could track longitudinally to determine the underlying mechanical properties of the tendon. Specifically, there are two stages that are important to identify. The first is at skeletal maturity between two and three years old, when tendon stiffness changes fairly markedly as matrix synthesis ceases (Smith *et al.* 2002) The second stage is the gradual process of degeneration from about ten years old onwards. Owners and trainers could therefore study the kinematics to determine when their horse's tendons are mature enough to enter training, and throughout the horse's competitive life to track degeneration of the tendon and subtle changes that may indicate sub-clinical tendon damage. This will now be discussed in greater detail.

1.2.1. Changes to tendon mechanical properties in the young horse

Since the storage and return of elastic strain energy in tendons and ligaments is a major factor contributing to the efficiency of equine locomotion, these tissues must be of optimal stiffness for the individual. From an initial low level, stiffness increases during development due to increasing production of tendon matrix by synthesis machinery; this change is described in detail in Section 2.5. A theory put forward by Smith *et al.* (2002) suggests that synthesis ceases once tendon stiffness is optimal. This cease occurs at skeletal maturity, between two and three years old, when the concentration of growth hormones that stimulate matrix production decreases (Champion *et al.* 2002; Fortier *et al.* 2005; Noble *et al.* 2007) to a level that can no longer support synthesis (Dowling and Dart 2005; Dowling *et al.* 2002; Smith *et al.* 2002). After this time, no more tendon matrix can be created and therefore any damage in the older horse is repaired with scar tissue. Since scar tissue is comparatively inelastic, adjacent tissue is subject to additional strain (Smith *et al.* 2002), leading to a high frequency of re-injury (Avella *et al.* 2009; Rees *et al.* 2006). Injured tendons are also hypothesised to be less effective at returning stored elastic strain energy, possibly contributing to hyperthermia-led degeneration (Dakin *et al.* 2011). Furthermore, high-speed exercise aimed at improving tendon quality can in fact cause degenerative change because instead of stimulating matrix production, the repeated high strains may cause micro-damage to the fibres (Smith *et al.* 2002). Thus it is essential to optimise tendon quality while it still has the capability to adapt to load and to minimise high, repeated strains after this age. It is therefore vital to identify the point at which tendon matrix production ends.

At present, it is only possible to determine whether matrix production has ceased either through post-mortem examination of the tendon or by analysis of the concentrations of growth hormones that stimulate matrix production - usually an invasive process performed by a veterinary surgeon. Ultrasonography of the tendon allows identification of pathological lesions and could therefore be useful in detection of early-stage injury. However, a study suggested that this technique was not able to predict later serious injury in the SDFT (Avella *et al.* 2009), and a recent study suggested that ultrasound underestimates the extent of long-term injury (Karlin *et al.* 2011). Other methods of analysis of soft tissue, for example magnetic resonance imaging, are expensive and impractical.

A simpler, non-invasive solution could be to investigate the kinematics of the horse; since the flexor tendons and suspensory ligament wrap around and support the distal limb joints, joint angles are likely to be influenced by the mechanical properties of these tissues, in particular their stiffness. Amongst other factors, tendon stiffness is closely linked to collagen quantity; so at skeletal maturity, when matrix production ceases, the trend of increasing tendon stiffness levels off to a plateau. This cease is likely to be reflected in a change in distal limb kinematics and indicates no further ability of the tendon to adapt to load. Debate currently exists over whether horses should commence training during development (Smith *et al.* 1999); investigation of the kinematics would allow individual, non-invasive identification of tendon maturity.

In the young horse, the longitudinal development of locomotion in untrained horses has been investigated by Back *et al.* (Back *et al.* 1994b; Back *et al.* 1995b) and Cano *et al.* (Cano *et al.* 2001) The maximum age of the horses studied by Back *et al.* was 26 months and since matrix production continues up until around 36 months, synthesis may have been ongoing on those horses, precluding investigation of the effect of synthesis cease. Although the study by Cano *et al.* extends the maximum age to 36 months, the minimum age was 12 months old and the period between data capture was 6 months, meaning that identifying the point of matrix production cease is difficult.

Other studies investigating the longitudinal development of kinematics included various other factors, making it difficult to separate the effects of age alone. For example, a study investigating longitudinal development of locomotion in Shetland ponies compared the effects of feeding and exercise regimes but did not present the data separately (Back *et al.* 2002). Another study examined the effect of training in horses from the age of around 3.5 to 4.5 years old (Cano *et al.* 2000); not only would training have masked the changes that resulted from tendon maturation, but also the study began after the assumed end of matrix synthesis. It is therefore necessary to study a population of horses from before matrix cease to well after it, to determine if the cease does result in a detectable change in locomotion. This would allow owners to study the kinematics of young horses to determine when to end exercise designed to improve tendon quality.

1.2.2. Changes to tendon mechanical properties in the older horse

During the horse's lifetime, the flexor tendons are subjected to repeated loading. This leads to a gradual degenerative change in tendon structure (Smith *et al.* 2002), in turn resulting in a weaker, more compliant tendon (Gillis *et al.* 1997; Parry *et al.* 1978a, b; Patterson-Kane *et al.* 1997a) as described in detail in Section 2.5. This weakening increases susceptibility to injury with age; the highest rate of tendon and ligament injury has been found to occur in horses over 12 years old and the lowest rate in horses less than five years old (Ely *et al.* 2009).

Since degeneration is an individual process, it will vary between horses in terms of both rate and timing of onset. Therefore, there is no fixed age at which tendon weakness is critical. Not only is degeneration subject-specific, but it is also difficult to detect by traditional visual methods until after failure. These two factors will now be explored in greater detail.

Rate of degeneration is an individual process and depends on two main aspects. The first is the initial quality of the tendon and the second is the quantity and magnitude of loading cycles. A high quality tendon will be able to endure loading to a greater extent than a weaker one. The exact qualities which allow a tendon to withstand repeated loading are as yet undetermined; although ultimate tensile strength is important in resisting the single static load used in most testing conditions, a stronger tendon may not necessarily resist the repeated loading cycles experienced *in vivo* (Dowling and Dart 2005). Tendon strength is correlated to its cross-sectional area (CSA) and collagen content, and is measured as the stress at a given strain level or at rupture (Cherdchutham *et al.* 2001). Tendon quality, in terms of strength and stiffness, is greatly influenced by the type of exercise given in the young horse, because the synthesis machinery is still responsive to load at this stage. This was illustrated in a study that compared the mechanical properties of tendons from foals that had undergone difference exercise regimes (Cherdchutham *et al.* 2001), and found that the CSA and force required at rupture were significantly greater in foals left at pasture 24 hours a day compared to foals kept in a stable or subjected to forced exercise.

Secondly, exercise given throughout the horse's life is thought to influence tendon degeneration by accelerating the accumulation of micro-damage (Smith *et al.* 2002). This is supported by a study that found no areas of discolouration, indicative of

degenerative lesions, in wild horses that had not undergone superimposed exercise (Patterson-Kane *et al.* 1997a). The effect of exercise regime on tendon properties, during both development and deterioration, is fully explored in Section 2.6.9.

In summary, the rate of degeneration will depend on the individual horse due to differences in initial tendon quality, exercise regime and other factors such as genetics, blood type and accompanying chronic disease (Kjaer 2004). There is therefore no fixed age at which the integrity of the tendon becomes compromised. This is illustrated in a study that found a high inter-individual variability of cartilage oligomeric matrix protein (COMP), which is correlated to ultimate tensile stress (Smith *et al.* 1997).

A further challenge in identification of the point of critical tendon degeneration is that the gradual weakening does not necessarily result in clinical signs that are detectable by visual inspection. Although the process of degeneration begins as soon as the tendon matrix machinery ceases, the effects on tendon structure are not immediately apparent. For example, chondroid metaplasia - which is indicative of degeneration - has only been found in horses older than six years old (Crevier-Denoix *et al.* 1998).

Given the difficulty in assessing the point of critical tendon degeneration, it would be advantageous to devise a subject-specific detection method. However, as previously explained, existing methods of investigating the mechanical properties of the tendon are invasive, expensive and have practical limitations.

A possible solution could be to track the kinematics of the horse, since it is reasonable to assume that the kinematics are indicative of the mechanical properties of the tendon (Section 1.3.2). Therefore, owners could track the kinematics of their horses longitudinally for identification of any subtle change that could indicate a weakening of the tendon. This would allow the owner or trainer to decrease the level of intensity of exercise to reduce the loads placed on a weakened tendon with the aim of avoiding injury. Although there are several studies concerning the development of kinematics in the young horse (Back *et al.* 1994b; Back *et al.* 1995b; Back *et al.* 1999; Cano *et al.* 2000), there is currently little information on the change in locomotion towards old age. It would therefore be useful to have a database of kinematic data in older horses. These data could also be extrapolated to other species, for example to humans. In the UK, the human demographic is ageing; from 1983 to 2008 the number of people aged 65 and

over increased by 2.3%, while the number of people under 16 years old fell by 4.6% (Office of National Statistics, September 2009). The increasing number of elderly people creates a need for an active research field into the causes and effects of ageing. Since the horse has a shorter lifespan than humans, it is a convenient model for studying ageing and degeneration and results from the horse could be extrapolated into the human research field.

The population of horses in this thesis was cross-sectional. Although a longitudinal population would have been ideal, this was not possible due to time and financial constraints. This type of approach could allow abnormal changes in the adult horse to be revealed, as well as in those approaching old age.

Summary

It is necessary to develop a simple method whereby owners can identify characteristic changes in their horse's kinematics, both to determine when the tendon is mature enough for the young horse to begin training, and also to identify subtle degenerative changes that could precede tendon rupture in the older horse. This thesis aims to investigate the effect of changing tendon stiffness on kinematics throughout the horse's lifespan. Furthermore, a recently-developed model (Lawson *et al.* 2007) will be used to calculate the strains of the flexor tendons and SL from kinematic data for comparison to *in-vitro* data.

1.3. Mass-spring modelling

1.3.1. Overview of limb structure

This section introduces the concept of representing the equine distal limb as a passive mass-spring model. It starts with describing the underlying skeleton, then discusses the role of the tendons, and finally presents the assumptions and limitations of using this model. The structure of the equine distal forelimb is comprehensively covered in Chapter 2 and thus will only be briefly described here for the purpose of explaining the principles of the mass-spring model.

The skeleton of the distal forelimb is composed of the third metacarpus along with the vestigial second and fourth metacarpal bones, three phalanges and three sesamoid bones. There are three major distal limb joints: the metacarpo-phalangeal joint (MCP), where

the third metacarpus articulates with the proximal phalanx; the proximal interphalangeal joint (PIP) between the proximal and medial phalanges; and the distal interphalangeal joint (DIP) between the medial and distal phalanges (Figure 1.1). The skeleton serves as a framework for the distal limb and provides attachment points for the tendons and ligaments.

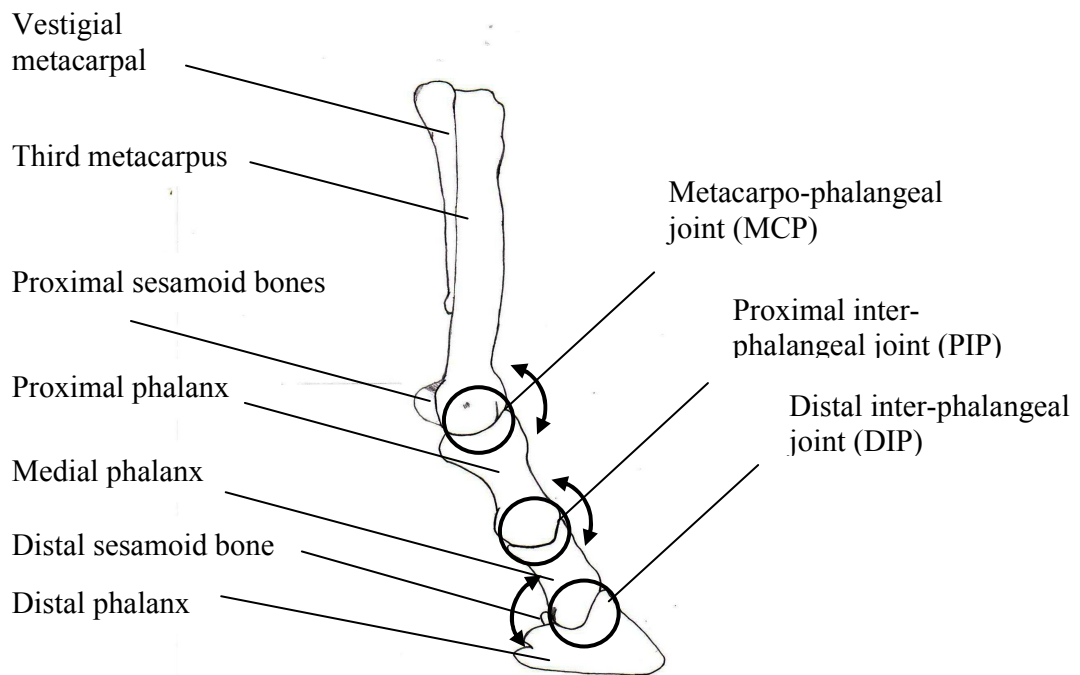


Figure 1.1 The skeleton of the equine distal limb. Arrows denote flexion of each joint

1.3.2. The role of the tendons

As a prey animal, the horse evolved an efficient means of locomotion to escape predation, by moving at high speed for a long period of time. An important contribution to efficient locomotion is the storage and return of energy in the flexor tendons and SL (Biewener 2006; Cavagna *et al.* 1977; Minetti *et al.* 1999). During the first half of stance phase, the weight of the horse passively flexes the MCP, PIP and DIP, stretching the elastic structures that span these joints. These stretched structures store energy in the form of elastic potential energy. Towards the end of stance, the elastic structures recoil and return the potential energy as kinetic energy, helping to propel the horse's centre of mass forwards and upwards and reducing the amount of work performed by the muscles. The visco-elastic nature of tendon means that although most of the energy is recovered,

a small percentage of the energy is lost as heat due to hysteresis between loading and unloading (Section 2.5).

This mechanism has been described as a mass-spring model (Biewener 2006; Blickhan 1989; Bullimore and Burn 2007; Cavagna *et al.* 1977), where the mass of the body is represented by a point mass, and the elastic structures are modelled as a massless spring. During a single stance phase, the spring contacts the ground and compresses under the horse's weight. The system rotates forwards and as the spring elongates towards the end of stance phase, the mass is forced upwards and forwards (Figure 1.2).

Although simple, this model does represent accurately various aspects of bouncing gaits, such as the simultaneous decrease of both the height and velocity of mass during the first half of stance and their simultaneous increase during the second half of stance. This model has been used extensively to determine various aspects of locomotion.

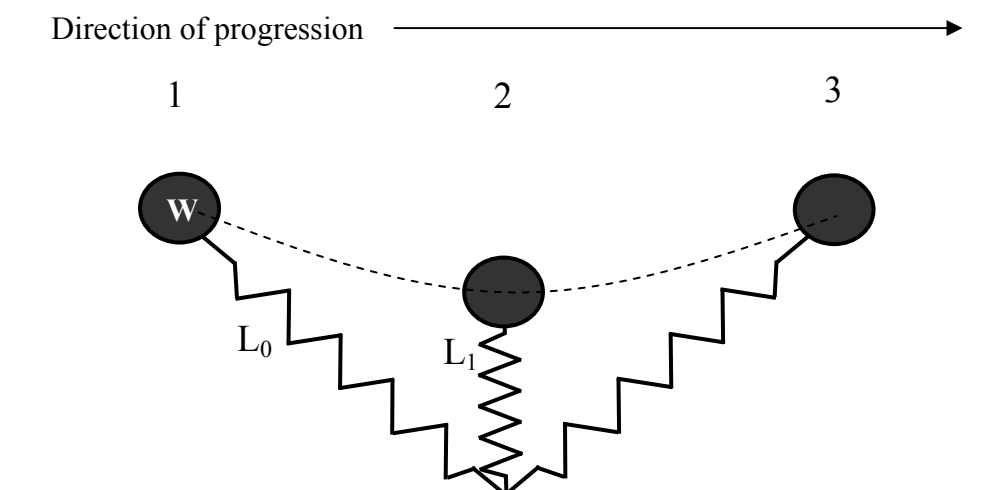


Figure 1.2 A mass-spring model of a limb at three stages of stance phase. W represents the weight of the body, L_0 is the initial length of the unloaded spring and L_1 is the length of the compressed spring. Δ_L , the change in spring length, is given by $L_0 - L_1$ and depends on W and the stiffness of the spring.

- 1: The limb contacts the ground and the mass compresses the spring,
- 2: The height of the mass decreases due to spring compression,
- 3: The spring elongates, propelling the centre of mass forwards and upwards.

The mass-spring model is passive in horses because the distal limb joints are compressed purely by the horse's weight, although it has been proposed that the limb can be tuned, to a limited extent, by the action of the muscles in the proximal limb

(McGuigan and Wilson 2003; Wilson *et al.* 2001). The passive nature of the system and compliance of the tendon reduce the need for energetically-expensive muscle length changes, improving locomotor efficiency (Wilson *et al.* 2001).

The spring component of the mass-spring model is primarily made up of the SDFT, the deep digital flexor tendon (DDFT) and the SL. The main function of these structures is to resist flexion of the distal limb joints by the weight of the horse during stance phase. The SDFT wraps around and supports the MCP and PIP, and the DDFT wraps around and supports the MCP, PIP and DIP. The SL divides into two branches, of which one fan-like branch inserts on the proximal sesamoid bones and helps to support the MCP (Figure 1.3), and the other continues forward and joins the common digital extensor tendon.

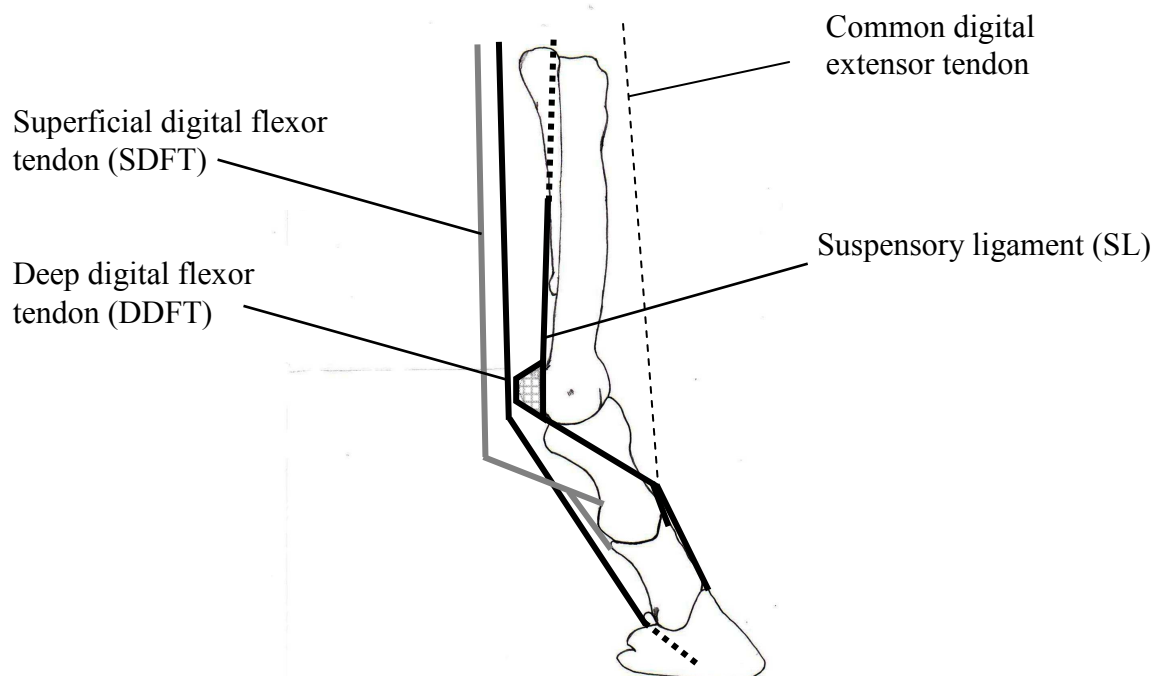


Figure 1.3 Lines of action of the SDFT, DDFT and SL.

1.3.3. Assumptions and limitations of mass-spring modelling

As with any model, there are various assumptions and limitations that have to be accounted for. The first assumption is that walk can be adequately represented by the mass-spring model, and the second is that joint angle changes are representative of tendon properties. The mass-spring model highlights the fact that compression of the distal limb is not only influenced by the stiffness of the tendon spring, but also by mass.

This means that mass must be accounted for before the effect of tendon stiffness can be investigated. These assumptions and limitations are explored and justified in the following sections.

a. Representing walk as a mass-spring model

In walk, the low inertial forces in the limb are insufficient to compress the distal limb to a great degree, and thus minimal energy is stored and returned by the elastic structures (Biewener 1998). Walking gaits are in fact often represented by an inverse pendulum model (Biewener 2006; Cavagna *et al.* 1977), where the mass vaults up and over the supporting limb strut; this model is used to explain various stride parameters such as stride length in Chapter 3. However, a certain amount of limb compression does take place, evidenced by flexion in the MCP, PIP and DIP during stance (for example Chateau *et al.* 2006), causing a decrease in the maximum height of the centre of mass (Biewener 2006). For this reason, a mass-spring model was chosen to explore the link between tendon stiffness and locomotion.

b. Kinematics are representative of the tendon properties

Investigation of the tendon mechanical properties via the kinematics assumes these two properties are linked. This link can be justified because the SDFT, DDFT and SL wrap around and support the distal joints during stance, resisting compression of the distal limb (McGuigan and Wilson 2003). The mechanical properties of these structures are thus likely to affect the kinematics, such as a more elastic tendon allowing a greater range of motion. For example, Butcher and Ashley-Ross (2002) reported a faster rate of MCP dorsi-flexion in younger horses (presumed to have more compliant tendons) than older horses. Further evidence for the link is shown by the alteration of kinematics after induction of unilateral SDF tendinitis (Clayton *et al.* 2000a). It is therefore reasonable to assume that the kinematics are indicative of the underlying mechanical properties of the tendon.

c. Influence of mass on joint angle

Since the passive mass-spring model is influenced both by stiffness and mass, a greater mass would compress the joints to a greater extent for the same tendon stiffness; Back *et al.* (Back *et al.* 1995b) note that the larger range of motion (RoM) seen in the MCP in older horses could be due to increased mass. The influence of mass is especially important during development of the young horse, where mass gains are rapid

(Thompson 1995). Normalisation to mass eliminates its influence and allows determination of the separate effect of tendon stiffness on joint angle.

1.4. Development of a marker-free tracking system

Objective analysis of motion allows quantitative measurement of gait in a variety of applications. This is particularly important for tracking the subclinical changes in kinematics that may precede serious tendon injury. Traditional methods include visual assessment of the horse's gait, which has been reported to be subjective, inconsistent and may miss subtle changes; these drawbacks will now be explored. Subjective evaluation of lameness by experienced clinicians was not found to be significantly correlated to objective measurements using either a force plate (Merkens and Schamhardt 1988) or accelerometry (Weishaupt *et al.* 2001). Low intra-observer consistency has been reported, even in experienced clinicians (Arkell *et al.* 2006; Keegan *et al.* 2010). Observation of gait may not detect subtle changes; for example Dow *et al.* (Dow *et al.* 1991) reported that horses that had been judged as clinically sound subsequently showed force patterns indicative of injury. Similarly, Merkens and Schamhardt (Merkens and Schamhardt 1988) found that horses deemed clinically sound at walk actually had indices of lameness detectable by force plate analysis. Objectivity, consistency and accuracy are crucial for accurate assessment of lameness, especially throughout gradual changes. One objective measurement is the use of ultrasound, but whilst it can be used to detect lesions in the tendon, it has not been found to be predictive of tendon injury (Avella *et al.* 2009).

Currently, the most common method of objective motion capture is using specialised cameras to record the position of skin-based markers that reflect infra-red light (Mündermann *et al.* 2006). There are several drawbacks to this method, including skin displacement artefact where skin-mounted markers do not necessarily reflect the true movement of the underlying bone (Cappozzo *et al.* 1996), vibration of the markers introducing error when the raw signal data are differentiated to calculate velocity and acceleration data (Alonso *et al.* 2007), physical interference of markers with movement, especially in small subjects (van sint Jan *et al.* 1997), and marker self-occlusion (Mündermann *et al.* 2006). Finally, the application of markers is time-consuming - and in some situations impossible, such as during competition or when tracking a wild animal. A limitation of an infra-red based system is that excessive light can reduce the

contrast between the marker and the background, making it difficult for the position of the marker to be determined. These limitations are discussed fully in Sections 4.2.1 and 4.2.3.

To overcome some of these limitations, a marker-free system (MFS) was developed that automatically tracks the position of the limb from video-based data, thus eliminating the need for markers and controlled lighting. The MFS was developed specifically to track the stance phase kinematics of the equine distal limb, but can be adapted to suit a range of applications. The MFS can track kinematics in both the sagittal (MFSsa) and coronal (MFSCO) planes but this thesis will focus on the sagittal-plane kinematics since the majority of joint rotation, and therefore tendon elongation, occurs in this plane (Chateau *et al.* 2004; Clayton *et al.* 2007a; Clayton *et al.* 2007b)

The function of the MFSsa is detailed in Section 4.5, and will only be briefly described here. Firstly, the user selects the first frame of stance and manually registers the locations to track. In this instance, four locations were tracked: the top of the metacarpus, the geometric centre of rotation of the MCP, the middle of the coronet band and the middle of the hoof base (Figure 5.1). These points are tracked throughout stance phase using a combination of three constraints; first, all of the pixels of similar colour intensity to those found during initial manual registration are found. Next, those pixels located further than a given (and adjustable) distance from the previous frame are eliminated to effectively constrain the velocity and eliminate non-biological movement. Finally, to maintain the distal limb as a series of rigid segments, those results that returned a segment length greater than $\pm 3\%$ of the initial length were eliminated. If more than one solution remained, the average co-ordinates were returned. The MFSCO functions by tracking the lateral and medial boundaries of the limb through edge detection and then combining these coordinates to determine a virtual centroid in the middle of the limb. Trigonometry was applied to calculate the MCP and DIP joint angles throughout the stance phase. The results of validating both MFS against existing technology are described in Section 5.2.2.

In summary, a system was developed that does not require the attachment of surface markers, thus overcoming the limitations associated with this mode of motion capture. The system can be used outdoors, eliminating the need to remain inside a controlled

environment, possibly reducing cost and allowing a more natural gait. This also allows its use at a competition environment or to track wild animals.

1.5. Scope and overview of the thesis

The high frequency of musculoskeletal injury in the horse leads to concerns for equine welfare and economic loss. Understanding how gait varies throughout the horse's life under normal circumstances allows identification of abnormal development that could indicate future tendon failure. Tendon failure occurs due to rupture of fibres weakened by long-term cyclic loading. There may be subtle changes in kinematics that precede a catastrophic failure that cannot be identified by traditional visual techniques.

This thesis therefore aims to investigate the influence of tendon stiffness on the development and degeneration of locomotion by studying kinematic data from horses of foal age through to senescence.

Chapter 2 introduces the structure and function of the equine distal limb. The two main topics discussed are the role of the tendon in efficient locomotion and the change in tendon mechanical properties with age, both during development and degeneration.

Chapter 3 discusses changes in stride parameters with age. The development and degeneration of gait is examined with reference to the centralised control of locomotion and the pendulum-like nature of the limb.

Chapter 4 describes the current state of the art of motion analysis, including kinematic and kinetic capture, their uses and limitations.

Chapter 5 describes the data capture techniques used in this thesis and includes an overview of the novel marker-free tracking system used to record the kinematics, and the distal limb model used to calculate strain.

Chapter 6 presents the evidence for the link between changing tendon stiffness and joint angle with age, and covers the methods used, results obtained and conclusions drawn.

Chapter 7 concludes the thesis, summarises the implications of the previous chapters and discusses the limitations and suggests future directions for research in this field.

2. Structure and function of the equine distal limb

2.1. Introduction

The cursorial horse is well adapted for fast, efficient locomotion. Efficient locomotion allows the horse, as a prey animal, to move away from predators at speed for a prolonged period of time. Man has exploited this speed and endurance in the modern-day sport horse in disciplines such as racing and eventing. Features that allow efficient locomotion include an unguligrade stance (where only the extremities of the limb contact the ground) and the storage and return of elastic strain energy in the flexor tendons. The more upright stance aligns the axis of the limb more closely with the vertical ground reaction force, decreasing the turning moments experienced by the joints by reducing the lever arm needed to overcome them (Biewener 1989). This stance also loads the bone in compression, making it more able to resist force. During the first half of the stance phase, energy is stored in the flexor tendons and suspensory ligament (SL) and returned towards the end of stance phase, helping to propel the centre of mass forwards and reducing the amount of work the muscles have to perform *de novo* (Biewener 2006; Cavagna 1977; Minetti 1999), Section 1.3.2. The stiffness of the tendons and SL must be optimal for the individual's mass to maximise the energy recovered and thus the efficiency of the gait (Smith *et al.* 2002). The repeated stretch and recoil of the elastic structures during locomotion can weaken the tendons and SL, reducing their stiffness and predisposing them to injury, which is common during high-intensity exercise such as eventing and racing (Section 1.1). This degeneration may be exacerbated by imposed exercise regimes (Smith *et al.* 2002).

This chapter therefore describes the structure and function of the equine distal limb, discusses the relationship between the distal joints and the tendons and ligaments that support them, then describes the structural changes that occur in these elastic structures with age and the effect this has on their mechanical properties.

2.2. Structure of the equine distal limb

The skeletal structure of the equine distal limb includes the metacarpal bones, the three phalanges and the sesamoid bones (Figure 1.1). The associated tendons and ligaments are the SL, the superficial digital flexor tendon (SDFT) and its accessory ligament, the deep digital flexor tendon (DDFT) and its accessory ligament, and several short sesamoidean ligaments and collateral ligaments.

Of the metacarpal bones, the first and fifth are no longer present, and the second and fourth exist only in vestigial form. This evolutionary adaptation reduces the weight of the limb for greater efficiency of locomotion, leaving the third metacarpus as the predominant bone of the distal limb. The proximal articulations of this bone are with the first, second, third and fourth carpal bones, and the distal articulation is with the proximal phalanx to form the composite hinge of the MCP joint. The MCP joint primarily rotates in the sagittal plane (Clayton *et al.* 2007a), although a small degree of lateral rotation has been reported (Chateau *et al.* 2006).

There are three phalanges; the proximal, medial and distal, which together function as the supporting skeleton of the digit. The largest is the proximal phalanx, which articulates with the medial phalanx at the PIP joint. The PIP joint primarily undergoes sagittal-plane flexion and extension, although it has been observed to show limited medial rotation during the stance phase (Chateau *et al.* 2006). The medial phalanx articulates with the distal phalanx and distal sesamoid bone at the DIP joint, which again primarily shows flexion-extension, although Chateau *et al.* 2006 reported a certain degree of medial rotation followed by lateral rotation.

The superficial digital flexor muscle originates at the medial epicondyle of the humerus and continues to the SDFT just proximal to the carpus. The SDFT also attaches via an accessory ligament (AL-SDFT) on the radius proximal to the antebrachio-carpal joint. After bifurcating just distal to the MCP joint, the SDFT primarily inserts at the proximal collateral tubercles of the medial phalanx, and a few fibres insert at the distal collateral tubercles of the proximal phalanx; the tendon thus spans the PIP joint.

The deep digital flexor muscle originates at three heads; the humeral, ulnar and radial. These attach respectively to the medial epicondyle of the humerus, medial olecranon and the caudal surface of the centre of the radius. The DDFT begins proximal to the

antebrachiocondylar joint but remains partially muscular until the level of the joint, where the tendons from all three heads fuse to form the DDFT. It is joined by its accessory ligament distal to the carpus, and inserts on the palmar surface of the distal phalanx.

The SL has two origins; the distal row of the carpal bones and the proximal end of the metacarpal bones, predominantly the third metacarpus. At around the level of the mid metacarpus, the SL divides into two fan-like branches, each of which inserts onto each of the proximal sesamoid bones. Each branch then crosses the axis of the proximal phalanx and blends with the dorsal digital extensor tendon (DDET) just proximal to the PIP joint. The tension on the SL is continued into the digit by the oblique, cruciate, short and straight distal sesamoidean ligaments. All four originate at the proximal sesamoid bones and palmar ligament, but whereas the first three insert on the proximal phalanx, the last inserts on the middle phalanx.

The main role of the SL, SDFT and DDFT is to support the distal limb joints, which is now described in detail.

2.3. Sagittal plane action of the equine distal limb

The function of the equine distal limb is twofold. First, it acts as a strut to support its share of the horse's weight during the stance phase, and secondly the tendons and ligaments store and return energy, increasing the efficiency of locomotion (Section 1.3.2). These two functions are intimately linked because when the weight of the horse flexes the distal limb joints, the elastic components that span these joints are stretched, storing elastic strain energy.

The importance of the flexor tendons in supporting the distal joints is illustrated by the effect of injury on kinematics. For example, unilateral tendonitis in the SDFT led to a significant decrease in MCP and DIP joint maximum angle compared to the unaffected contralateral limb (Clayton *et al.* 2000a). Injury has been found to decrease the stiffness of the tendon (Dakin *et al.* 2011), which would indicate an increase in maximum angle in these two joints. However, to minimise pain horses adapt their gait to minimise load on the affected leg (Clayton 2000a; Weishaupt 2006, 2004). Therefore, the joints of the passive distal limb are likely to show a decrease in maximum angle, consistent with

previous findings. Owners should consider filming the contralateral limb of a horse with suspected injury to monitor possible redistribution of load through kinematic tracking.

The importance of the DDFT in DIP joint support has been demonstrated by a lack of stability in the DIP joint after AL-DDFT desmotomy (Denoix 1994). Pathology in the SL has been found to limit PIP joint flexion (Denoix 1994). Similarly, Azevedo *et al.* (2009) found that knee flexion was significantly smaller in human athletes who presented with Achilles tendinopathy compared to a control group of unaffected athletes.

2.3.1. Stance phase action of the MCP joint

After the hoof contacts the ground, the MCP joint flexes; after a slight extension, it flexes again to a peak at mid stance. It then extends until just before toe off, where an inflection is seen (Back *et al.* 1995c; Clayton *et al.* 2007a; Hodson *et al.* 2000). The range of motion in flexion has been found to be around 28° and in extension about 25° (Chateau *et al.* 2004), giving a total range of motion of around 53°. Although the MCP principally rotates in the sagittal plane, it has been shown to show limited rotation in the coronal and transverse planes (Chateau *et al.* 2004).

The MCP joint is primarily supported throughout the stance phase by the SDFT and DDFT (Butcher and Ashley-Ross 2002; Denoix 1994; McGuigan and Wilson 2003; Parry *et al.* 1978b), which are attached to bone via their accessory ligaments. The other components that support the MCP joint are the SL, the palmar ligament, the proximal sesamoid bones and the four sesamoidean ligaments; these latter components make up the suspensory apparatus (Denoix 1994) and help to prevent over-extension.

During the first part of stance, MCP flexion loads these elastic structures, causing them to lengthen and store elastic strain energy. Towards the end of stance, the recoil of the structures, together with active contraction of the flexor muscle bodies, extend and elevate the MCP (Denoix 1994).

2.3.2. Stance phase action of the PIP joint

During initial ground contact, the PIP joint extends slightly, followed by flexion until about 13% of stance phase, where it reaches peak flexion. Throughout the rest of stance

phase, the PIP extends until just before toe off (Chateau *et al.* 2004; Clayton *et al.* 2007b). The range of motion in extension has been found to be minimal, and in flexion about 10° (Chateau *et al.* 2004). Again, rotation is primarily craniocaudal although limited mediolateral and axial rotation also occurs (Chateau *et al.* 2004; Clayton *et al.* 2007b).

PIP extension in stance is limited by the SDFT due to its insertion on the palmar aspect of the joint, and also by the action of the oblique and straight sesamoidean ligaments and the extensor branches of the SL. Due to its insertion on the dorsal aspect of the PIP joint, the DDET also prevents PIP flexion. In contrast, extension of the PIP is limited by the action of the DDFT, which acts as an antagonist to the SDFT. Towards the end of stance and during the propulsive phase, the PIP joint is passively extended by tension in the SDFT.

2.3.3. Stance phase action of the DIP joint

After initial ground contact, the DIP joint undergoes a high rate of flexion, which continues at a slower rate until its peak of 16° at about 20% of stance (Chateau *et al.* 2004). The joint then shows extension until its peak of 42° at toe off (Chateau *et al.* 2004; Clayton *et al.* 2007b; Hodson *et al.* 2000). As with the MCP and PIP, rotation occurs mostly in the sagittal plane (Chateau *et al.* 2004).

DIP joint flexion due to the weight of the horse during stance phase is limited by the DDFT, SL and the DDET. During the final part of stance and throughout the propulsive phase, tension in the DDFT and active contraction of the deep digital muscle belly extend the joint.

2.3.4. Section summary

In summary, the role of the distal limb is to support the weight of the horse and to store and return strain energy in the elastic structures that span the distal joints. The link between tendon mechanical properties and kinematics can also be demonstrated by the compromised movement of an individual suffering from tendinopathy.

2.4. Tendon structure

Tendon has a hierarchical structure, from molecular polypeptide chains up to the tendon itself (Figure 2.1; Kannus 2000, Patterson-Kane and Firth 2008). This layered structure not only improves structural strength but also ensures that minor damage is localised to one region (Kjaer 2004).

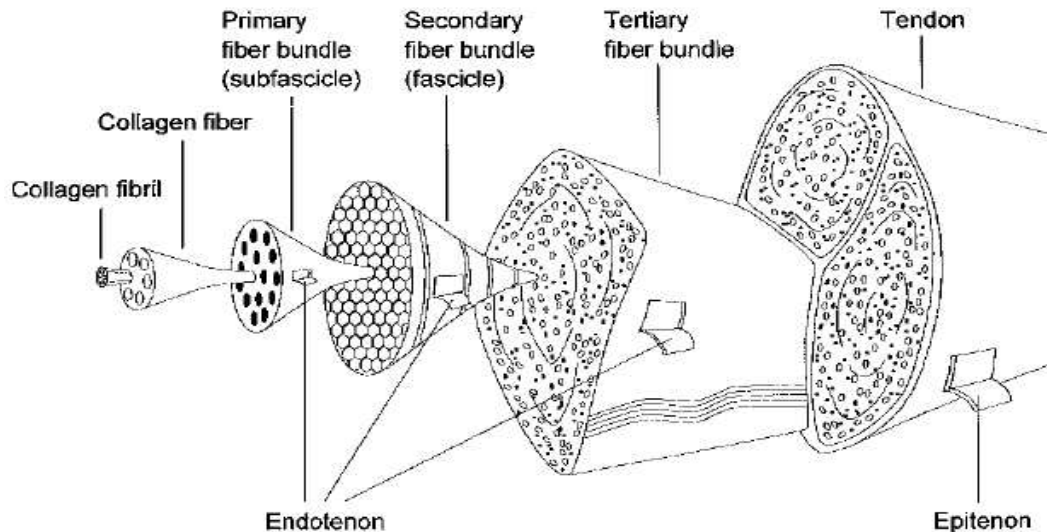


Figure 2.1 Tendon has a hierarchical structure. From Kannus (2000)

A triple helix of polypeptide chains forms the collagen molecule, otherwise known as tropocollagen, and via several progressive levels of structure, these molecules form collagen fibrils. These fibrils are bound together by cross-links that reduce shear forces and increase the tensile strength of the tendon (Patterson-Kane *et al.* 1997b). The collagen fibrils follow a zig-zag waveform that gives the tendon fascicle a banded appearance (Crevier-Denoix *et al.* 1998). This wave structure has been termed the crimp, or degree of undulation. Crimp angle is measured between the axis of the overall tendon and the tangent fibril (Figure 2.2).

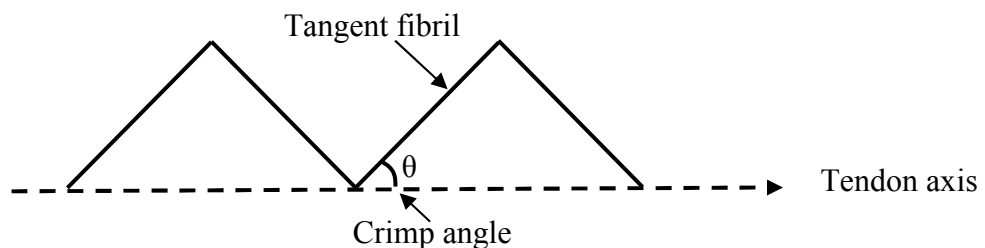


Figure 2.2 Diagrammatic view of tendon crimp angle.

Fibrils make up collagen fibres, which are bound together by endotenon. These fibres in turn make up primary and secondary bundles, known as subfascicles and fascicles, respectively. The tendon itself consists of tertiary bundles, and the whole structure is encased in epitenon.

Collagen fibrils, which are the smallest mechanical unit, are principally made up of type I collagen, with small amounts of type II and III also present (Birch *et al.* 1999a; Kjaer 2004). Together with elastin fibres, the fibrils lie within a matrix of water, proteoglycans and other small molecules that are thought to play a role in spacing and lubrication (Kjaer 2004). The matrix and collagen molecules are produced by tenocytes that are located between fibril bundles. Although tendon is not highly vascularised, there is a network of gap junctions that may allow transport of nutrients and which also allow the tendon to respond to mechanical loading (Stanley *et al.* 2007). Most of the structures that make up tendon show a change with age; these changes are explored in Section 2.6.

2.5. Tendon functional behaviour under load

When load is first applied to tendon, the crimp straightens out (Dowling and Dart 2005), which is represented by the characteristic non-linear “toe” region of a stress-strain graph (Figure 2.3). The end of this region, where crimp angle is minimal, is called the toe limit strain. Once the crimp has been fully straightened, the stretching of the collagen fibrils themselves corresponds to the linear part of the graph. The gradient of the linear region represents the stiffness of the tendon, and the area under the graph represents the amount of energy stored. Under even greater load, the fibrils start to break and eventually the tendon ruptures.

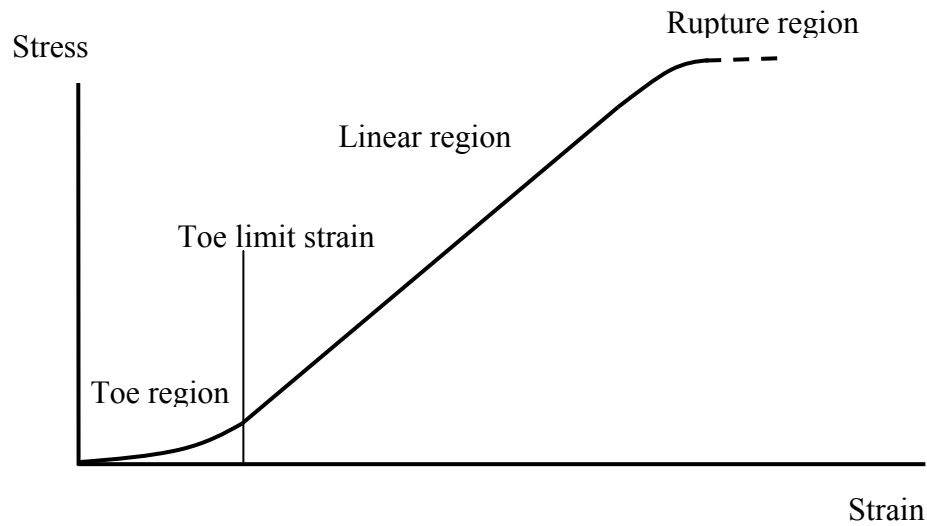


Figure 2.3 The tendon stress-strain curve shows a characteristic shape under load

Under repeated loading cycles, tendon demonstrates two important characteristics. The first is hysteresis, where energy is lost during the stretch and recoil process and is represented by the characteristic loop shape of the loading curve (Shadwick 1990) (Figure 2.4). This energy is usually dissipated as heat, which may contribute to hyperthermic degeneration of the tendon (Section 2.6.8, (Birch et al. 1997; Dakin *et al.* 2011; Farris et al. 2011; Wilson and Goodship 1994).

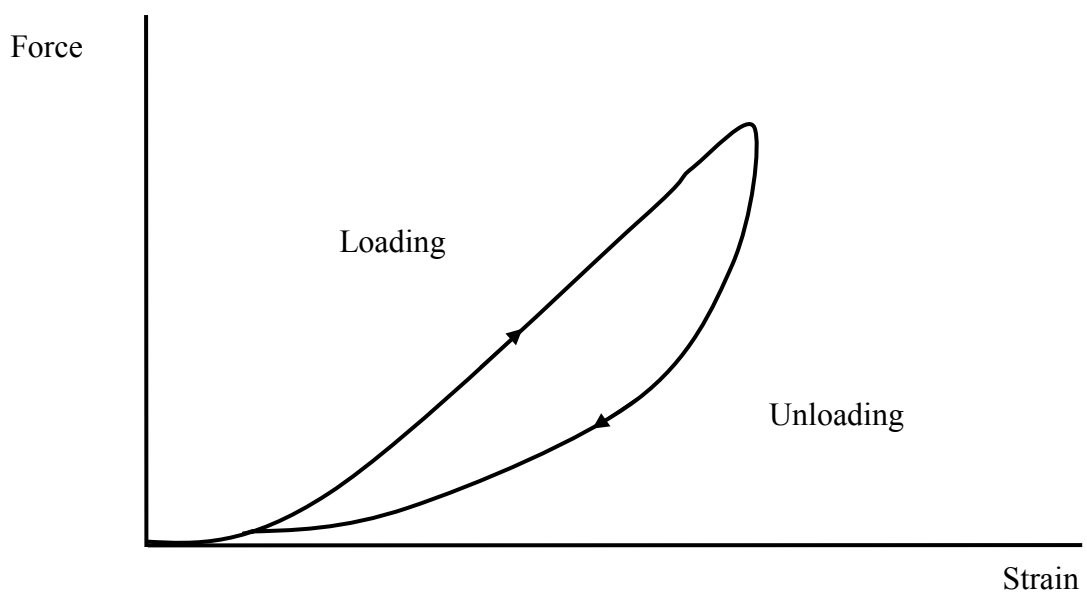


Figure 2.4 Tendon demonstrates hysteresis under load

The second feature is known as pre-conditioning, where the curve shifts to the right with each loading cycle until it reaches a steady state (Figure 2.5).

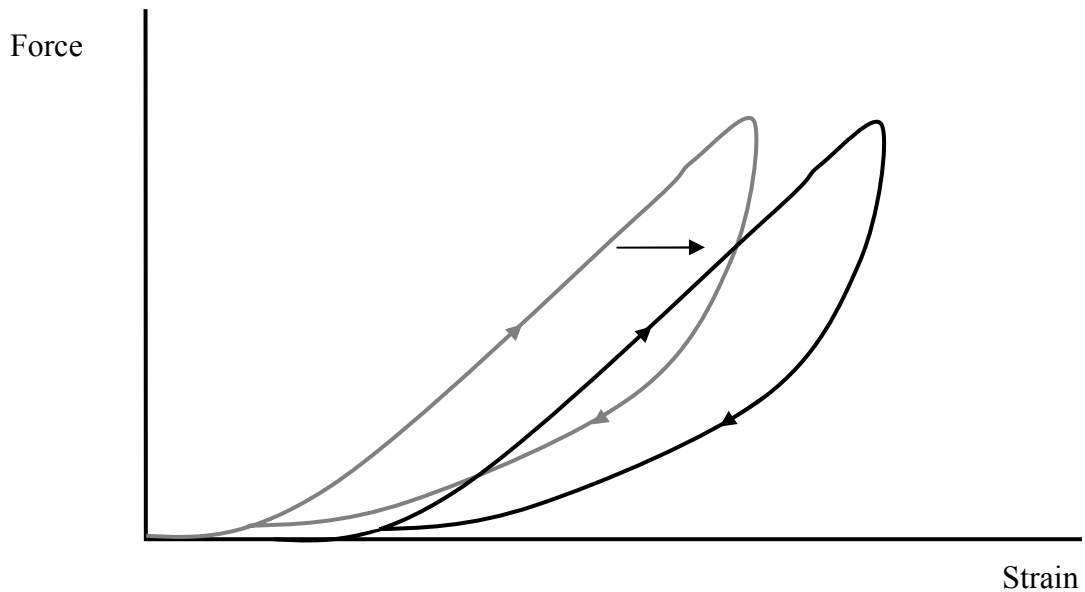


Figure 2.5 Pre-conditioning of the tendon results in a shift of the curve to the right

This shift may be due to a re-arrangement of fibrils or water being forced out of the matrix (Dowling and Dart 2005). These same authors report that pre-conditioning is brief and is unlikely to alter maximum load, although maximum strain in the muscle-tendon unit has been found to increase with number of conditioning stretches or loading cycles performed (Hawkins *et al.* 2009; Morse *et al.* 2008).

2.6. Changes in tendon composition and biomechanical properties with age

2.6.1. Introduction

From birth, the structure of the equine tendon undergoes changes in response to growth hormones and increasing load as a result of the growing body. Towards old age, the repeated loading cycles start to weaken the tendon and cause degeneration. Lifelong changes of the tendon structure and morphology, and the subsequent effect on the biomechanical properties of the SDFT and DDFT, will be discussed in the following sections.

As discussed in Section 2.4, tendon consists of several levels of units that lie parallel to the length of the tendon, starting with collagen fibrils connected by covalent cross-links.

2.6.2. Changes to cross links with age

There are two main types of covalent cross-link; those derived from an enzymatically-mediated process, and via the non-enzymatic process of glycation (Last *et al.* 1990). The nature of the enzymatically-derived cross-links has been found to change with age. At birth, they are mostly reducible and divalent in form, and these are gradually replaced with the mature, non-reducible, trivalent form (Avery and Bailey 2005; Bailey *et al.* 1998; Moriguchi and Fujimoto 1978). In horses, the concentration of immature cross-links has been found to be negligible by two years old, whereas the level of mature cross-links were found to increase rapidly from around one year old, then show a slower increase up to three years old (Patterson-Kane *et al.* 1997b). Gillis *et al.* (Gillis *et al.* 1997), who extended this work to horses up to 23 years old, reported that mature cross-link levels increased up to the age of 15 years before showing a gradual decline into senescence. Since concentrations of mature cross-links have been found to be positively correlated with tendon stiffness (Gillis *et al.* 1997; Parry *et al.* 1978a), the likely mechanical effect of the change in cross-links type is that the tendon will show a rapid increase in stiffness during growth, then a slow decrease in stiffness towards old age. The concentration of non-enzymatic cross-links increases after maturity (Cherdchutham *et al.* 1999; Reiser 1994) and could play a part in the general degeneration associated with ageing (Last *et al.* 1990), and therefore probably contributes to the weakening of the tendon.

2.6.3. Changes to fibrils with age

The cross-links hold together the collagen fibrils, whose structure shows an age dependency. The overall trend is an increase in the mass-average diameter (MAD) of the fibrils from before birth until skeletal maturity between two and three years old (Firth 2006; Patterson-Kane *et al.* 1997a; Patterson-Kane *et al.* 1997b). A similar increase in collagen fibril MAD has been reported in rabbit Achilles tendon (Cetta *et al.* 1982), chicken and cow flexor tendon (Scott 1984) and in the skin of various mammals and birds (Flint *et al.* 1984).

Larger fibrils have been suggested to have a higher density of intrafibrillar covalent cross-links (Parry *et al.* 1978a; Patterson-Kane *et al.* 1997b), giving the fibril greater tensile strength. However, smaller fibrils allow a greater number of interfibrillar

connections, inhibiting creep (Parry *et al.* 1978a) and decreasing elasticity. This means that a bimodal distribution is necessary. This bimodal distribution only appears at maturity, indicating that whilst tendons are strong from an early age, stiffness increases gradually during growth.

2.6.4. Changes to fibril crimp with age

At birth, tendon has been found to have a high crimp angle, which decreases rapidly during growth until it reaches a plateau at skeletal maturity (Crevier-Denoix *et al.* 1998; Patterson-Kane *et al.* 1997a; Patterson-Kane *et al.* 1997b; Wilmink *et al.* 1992). Repeated loading of the fibrils, as well as an overall increase in loading due to greater body weight, gradually straightens the fibrils (Buckwalter *et al.* 1993; Patterson-Kane *et al.* 1997a; Patterson-Kane *et al.* 1997b) and hence decreases crimp angle. Beyond skeletal maturity and into senescence, crimp angle decreases further; one study found that the fibrils in 23 year-old horses were completely straight (Crevier-Denoix *et al.* 1998).

It has been proposed that the crimp acts as a reserve of length to help to protect the tendon when load is applied suddenly (Crevier-Denoix *et al.* 1998; Patterson-Kane *et al.* 1997a). As described in Section 2.5, a tendon stress-strain curve follows a distinct pattern, where the characteristic “toe” region represents the straightening of the crimp. The effect of the age-induced decrease in crimp angle is to decrease the toe limit strain, because the crimp has to be stretched less far before it is straight (Figure 2.3).

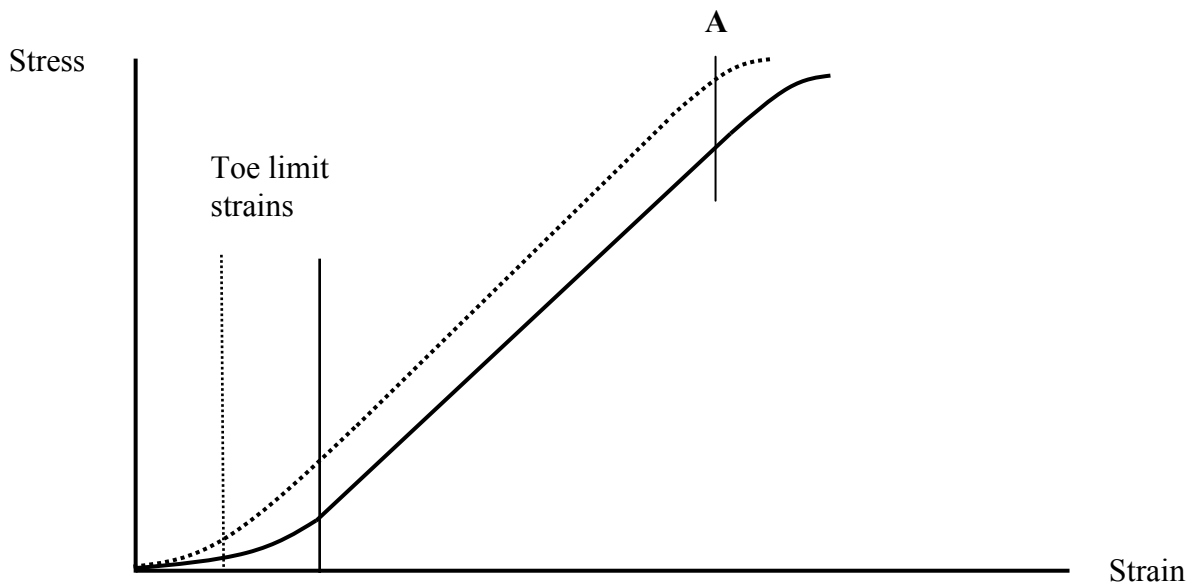


Figure 2.6 The crimped nature of the tendon fibrils leads to the characteristic toe region of a stress-strain graph. The effect of the decrease in crimp angle is to decrease the toe limit strain. Indicates a small crimp angle, — a high crimp angle. Point A represents the point of rupture of tendons with a small crimp angle. Adapted from Patterson-Kane *et al.* 1997a.

Therefore, for a given stress, an adult tendon with a small crimp angle shows a smaller degree of strain than a foal tendon with a larger crimp angle (Patterson-Kane *et al.* 1997a). The overall effect of decrease in crimp angle during growth is therefore an increase in tendon stiffness.

Towards old age, a minimal crimp angle predisposes the tendon to injury since at a given strain the stress experienced by the older tendon will be greater. Also, at the point of rupture of the tendon with a lower crimp angle (Figure 2.3, point A) the tendon with a higher crimp angle is still within the linear region (Patterson-Kane *et al.* 1997a). Thus, as well as altering the stiffness, the decrease in crimp angle also increases the risk of tendon rupture towards old age.

2.6.5. Changes to fascicles with age

The collagen fibrils are packed in bundles known as fascicles, which show a general decrease in cross-sectional area (CSA) with age (Crevier-Denoix *et al.* 1998; Gillis *et al.* 1997). As fascicle CSA has been found to be negatively correlated with tendon elastic modulus (Gillis *et al.* 1997; Riemersma and Schamhardt 1985), this suggests that

tendon stiffness is low in the very young horse and increases with age. The increase in stiffness with decreasing CSA has been suggested to be due to the replacement of interfascicular connective spaces with collagen fibres (Crevier-Denoix *et al.* 1998; Gillis *et al.* 1997), which are positively correlated with stiffness (Dowling 2005; Gillis 1997; Parry 1978a; Riemersma 1985). Therefore, the overall effect is to increase tendon elastic modulus with age.

2.6.6. Changes in concentration of COMP with age

It has been suggested that the collagen fibrils are aligned during growth by a large protein found in the tendon matrix known as cartilage oligomeric matrix protein (COMP) (Buckwalter *et al.* 1993; Smith *et al.* 2002; Sodersten *et al.* 2005). The function of COMP is probably to resist load, since it is distributed mainly in tissues that serve this function.

The concentration of COMP has been reported to increase until skeletal maturity, followed by a decrease in the metacarpal region and a plateau in the MCP region (Cherdchutham *et al.* 1999; Smith *et al.* 1997). The results from the study by Smith *et al.* 1997, however, showed a high degree of variability, especially for the latter region, and the inclusion of only one “old” horse (20 years old) perhaps gives these results false significance. A later review article by the same authors (Smith *et al.* 2002) shows similar findings: COMP concentrations in the metacarpal region increased sharply to a peak at around two years old, fell to a plateau at around five years old, and then remained constant to about 25 years old. In the metacarpo-phalangeal region, concentration rose more slowly to peak around four years old, and then remained constant. Although these results are from unpublished work from another author, they are consistent with a later study (Sodersten *et al.* 2005) which found the highest concentration of COMP in three year-old horses. Further evidence for the age-related change in COMP concentration comes from Buckwalter *et al.* (1993), whose review of soft tissue aging included the observation that collagen fibrils and bundles become less organised with age, which is a possible result of a decrease in COMP.

COMP is produced by the tenocytes in response to greater load (Smith *et al.* 2002) from increasing body weight, and to stimulation by growth hormones, particularly insulin-like growth factor-1 (IGF-1) and transforming growth factor β (TGF- β) (Dowling *et al.*

2002; Smith *et al.* 2002). Concentrations of growth hormones have been found to decline from an initial high level at birth to a plateau at around three years old (Champion *et al.* 2002; Hess and Roser 2001; Malinowski *et al.* 1996; Noble *et al.* 2007); Hess *et al.* (Hess and Roser 2001) found a significantly higher concentration in horses less than two years old than in any adult group. In contrast, a study by Fortier *et al.* (Fortier *et al.* 2005) that focussed on horses up to two years old found two peaks of IGF-1 concentration; in addition to the neonatal peak, another was reported at around 15 months old. No other authors reported this double peak, possible because Fortier *et al.* presented IGF-1 concentration as a function of days of age, rather than as function of years of age as employed by the other authors. Taken together, these results suggest that COMP, whose production is stimulated by growth hormones, also shows a high concentration in the neonate, decreasing to a plateau at skeletal maturity.

In addition to age, an effect of gender has been found, with intact males (stallions) having a higher concentration of IGF-1 per kg of body mass than females (mares) or castrated males (geldings) (Champion *et al.* 2002; Noble *et al.* 2007). In contrast, Fortier *et al.* (Fortier *et al.* 2005) discovered no gender effect on IGF-1 concentrations, but concluded that this may have been due to the young age of the horses studied. With a higher concentration of IGF-1 present, it is possible that stallions produce a higher level of COMP than mares or geldings.

Since COMP concentration is positively correlated with tendon stiffness (Smith *et al.* 2002), the overall effect is likely to be a sharp increase in stiffness until around three years old, and a decrease into senescence. One study that contradicts these findings was carried out by Dowling *et al.* (Dowling *et al.* 2002); they did not find any significant effect of recombinant Equine Growth Hormone (rEGH), assumed to stimulate production of COMP, on mechanical properties including stiffness. However, this may be because the age of the horses in the study was six to nine years old, and at this age the tendon has limited ability to respond to hormonal stimulation (Smith *et al.* 2002).

2.6.7. Matrix production cease

As efficiency of locomotion is achieved through the storage and release of elastic strain energy in the spring-like SDFT (Cavagna 1977; Minetti 1999; Biewener 2006), the tendon must be at optimal stiffness for the mass of the individual (Smith *et al.* 2002).

These authors state that once tendon stiffness is optimal, matrix synthesis may cease. This cease is likely to occur at skeletal maturity, when decreasing concentrations of growth hormone no longer stimulate matrix production. This is a possible explanation for results indicating that tendon has limited capacity to adapt to change after skeletal maturity, and implies that tendon begins the process of degeneration after this time, although pathological changes may not become immediately apparent. It is also consistent with the finding that collagen content increases from birth to maturity then shows a slow decline into senescence (Parry *et al.* 1978a, b). As collagen content is positively correlated with tendon stiffness (Gillis *et al.* 1997; Parry *et al.* 1978a), it suggests that the tendon becomes stiffer throughout growth, then becomes less stiff towards old age.

2.6.8. Theories of tendon degeneration

Tendon degeneration is a complex process and is the result of several interacting factors. In general, degeneration leads to a decrease in function from the cellular level to the organ level (Buckwalter *et al.* 1993). Although the exact mechanism of tendon degeneration is not properly understood, several theories have been put forward including passive mechanical degeneration, active degradation through the action of enzymes, vascular reduction, neural overstimulation, hyperthermia, relative hypoxia, and finally a fall in circulating growth hormone concentration. These will now be discussed in further detail.

Passive mechanical degeneration

The passive mechanical theory states that during the lifespan of the individual, repetitive loading leads to microdamage of the collagen fibres or even breakdown of larger fibrils into smaller ones (Patterson-Kane *et al.* 1997c; Rees *et al.* 2006; Smith *et al.* 1999; Smith *et al.* 2002). The fibres eventually fail, especially if the load is extreme or prolonged, and the tendon ruptures. Since load depends on the type and intensity of exercise, the precise contribution of imposed exercise to the age-induced degenerative process is difficult to determine, but has been shown to accelerate the process. For example, no discolouration was found in feral horses up to 15 years old (Patterson-Kane *et al.* 1997a), which indicates that degenerative lesions were not present in horses where no exercise had been imposed. This is consistent with *in vivo* findings from several other authors (Buckwalter *et al.* 1993; Patterson-Kane and Firth 2008). One *in vitro*

study found that cyclical loading of tendon explants significantly reduced ultimate tensile stress compared to mechanically-unstimulated controls (Dudhia *et al.* 2007). Smith *et al.* (Smith *et al.* 2002) add that the superimposition of exercise on aging accelerates the degeneration of tissue, and rupture occurs when the tissue is too weak to withstand the loads placed on it.

As noted by Smith *et al.*, the importance of a direct injury in disrupting the tendon matrix should not be underestimated, especially because the resulting disorganised scar tissue affects the function of the tendon (Patterson-Kane and Firth 2008; Smith 2008).

Into senescence, the gradual decrease in MAD (Birch *et al.* 2008; Gillis *et al.* 1995; Patterson-Kane *et al.* 1997a) has been suggested to be a result of larger-diameter fibres breaking down into smaller ones (Birch *et al.* 2008; Parry *et al.* 1978a). The biomechanical significance of this is that smaller-diameter fibrils have been suggested to be more elastic than ones of larger diameter (Birch *et al.* 2008; Parry *et al.* 1978a), implying that the tendon becomes more elastic into senescence.

Active enzyme breakdown

A second possible mechanism of tendon breakdown is through an active process involving matrix metalloproteinases (MMPs), enzymes that are responsible for the degradation of the matrix. The concentration of MMPs increases throughout the horse's life (Birch 2007), and since they are associated with a loss of matrix integrity (Riley *et al.* 2002), it is likely that matrix degradation increases over time. This was demonstrated by Dudhia *et al.* (Dudhia *et al.* 2007) who found minimal loss of ultimate tensile strength in tendon explants cyclically loaded with an MMP inhibitor, implicating MMP activity in the decrease of tendon integrity.

Decrease in vasculature

A third theory is that the metabolic processes of the tendon require a vascular supply, and therefore a reduction in vasculature may lead to degeneration (Kjaer 2004; Rees *et al.* 2006). However, as noted by Kannus (Kannus 2000) metabolic activity in tendon shows a decrease with age, to the extent that aerobic glycolysis was not detected at all in the fibroblasts of older individuals (Buckwalter *et al.* 1993). Dowling *et al.* (2002) also state that mature equine tendon has been found to have a very low rate of metabolic

activity. Whether this decrease in metabolic activity is due to or independent from the decrease in vasculature is therefore unclear.

Chronic nervous overstimulation

Rees *et al.* (2006) describe a fourth theory, which they point out is a collection of observations that need further evidence before their significance can be determined. The observations include the close link between tendon innervation, mast cells and the raised concentration of a possible inflammatory factor in certain types of tendinopathy. The possible inference is that chronic overstimulation of the nerves due to tendon overloading may cause degranulation of the mast cells. This theory requires further work before conclusions should be drawn from it.

Hyperthermia

A fifth mechanism of tendon degeneration was put forward by Wilson and Goodship (1994), who argue that hyperthermia of the tendon could cause its degeneration. During exercise, some of the energy stored during tendon loading is lost as heat energy (Section 2.5). The current theory is that this heat causes hyperthermia within the tendon, which could result in the disruption of collagen fibrils as well as damaging the fibroblasts. A more recent study (Gevorkian *et al.* 2009) reported a decrease in elastic modulus in rat tail tendon when the temperature reached 45°, similar to the peak values recorded by Wilson and Goodship in equine flexor tendon (mean core temperature 43.3°C). Gevorkian *et al.* (Gevorkian *et al.* 2009) interpret their findings as instability of the collagen fibrils but conclude that the changes in structure were temporary because the fibril was unchanged after ten trials of the heating process. However, as the equine tendon undergoes many orders of magnitude more loading cycles (and therefore heating cycles) during its lifetime, it is possible that hyperthermia-induced accumulated microdamage could lead to tendon degeneration. This is consistent with findings by Birch *et al.* (1997), who did not find any significant cell death after 10 minutes of heating the tendon to 45°, but found that cell survival was less than 10% after 180 minutes. A recent study into human Achilles tendon, that used a mathematical model predicted values of up to 44°, concluded that heat may play a role in tendon degeneration (Farris *et al.* 2011).

Relative hypoxia

Smith *et al.* (2002) state that hypoxia of the tissue could be a cause of degeneration, but results from other studies are ambiguous. One study (Sivakumar *et al.* 2008) found a significantly higher level of hypoxia in human patients with rheumatoid arthritis than those without, but another study (Rempel and Abrahamsson 2001) did not find a significant decrease in cell proliferation, synthesis of new proteoglycans or collagen synthesis in hypoxic patients. Further work is needed to determine the effect of hypoxia on tendon tissue.

Decreased growth hormone concentration

A final theory is that the reduced concentration of growth hormones after skeletal maturity may no longer be sufficient to stimulate tendon synthesis. Cellularity, collagen synthesis and rate of tendon metabolism have all been found to be stimulated by growth hormones *in vitro* (Dahlgren *et al.* 2002; Murphy and Nixon 1997; Smith *et al.* 2002). As the concentration of these growth hormones has been found to decrease with age (Champion *et al.* 2002; Fortier *et al.* 2005; Hess and Roser 2001; Malinowski *et al.* 1996; Noble *et al.* 2007; Smith *et al.* 2002), cellularity, rate of collagen synthesis and metabolism are also likely to decrease towards senescence. A decrease in cellularity with age has been reported (Gillis *et al.* 1995), to the extent that Crevier-Denoix *et al.* (1998) found zones of total acellularity in the two 23 year-old horses studied. As well as a general decrease in cellularity with age, a significant change in cell type has also been found, from the more metabolically-active type II cells in foals to the less active type I cells found in the adult horse (Dowling *et al.* 2002). Rate of collagen synthesis has been found to decrease with age after skeletal maturity (Parry *et al.* 1978a, b). The positive correlation of collagen concentration with elastic modulus (Gillis *et al.* 1997; Parry *et al.* 1978a) means that stiffness is likely to decrease with age. Metabolic activity also decreases towards old age (Buckwalter *et al.* 1993), which has been suggested to contribute to the decreased ability of mature tendon to respond to load and injury (Gillis *et al.* 1995).

Summary

It is likely that tendon degeneration with age is a result of several factors, which may well interact. The overall effect of degeneration is to weaken the tendon, reducing its stiffness and predisposing it to injury.

2.6.9. Influence of exercise

Exercise has been found to be influential during both maturation and degeneration of the tendon. In the growing horse, evidence suggests that exercise regime affects both the composition (Cherdchutham *et al.* 1999) and the mechanical properties (Cherdchutham *et al.* 2001) of the tendon. It has been suggested that there is a narrow margin between too little and too much exercise for all tissues of the body (Moffat *et al.* 2008; van Weeren *et al.* 2008); too little exercise may not be sufficient to stimulate change, and too much exercise may lead to injury, especially in immature tissue. However, the optimum type and amount of exercise in the growing horse has yet to be determined. The degeneration process can be minimised by reducing excessive loading of the weakened tissue, but the point at which loading becomes excessive depends on the individual tendon and is therefore difficult to establish. Selecting an appropriate exercise regime for the horse during the two critical time periods of development and degeneration could help to minimise the risk of tendon injury and prolong the horse's useful working life. The influence of exercise on the structural and mechanical properties of the tendons will now be explored.

Structural response of tendon to exercise

Load influences the production of tendon matrix at all levels from gene expression up to protein synthesis. The mechanism has been proposed to be via mechanical stimulation of the tenocytes (Kjaer 2004) or due to a change in hormone receptor activity (Bailey *et al.* 1998). Since the production of tendon matrix ceases at around three years old (Section 2.5.7), it is important to give appropriate exercise before this point, whilst the mechanism can still adapt to loading (Smith *et al.* 2002). Towards old age, structural changes to the tendon matrix are mostly degenerative, due to repeated loading cycles and minimal synthesis of new matrix. The influence of exercise on the different levels of tendon structure will now be discussed.

Exercise appears to promote the turnover of type I collagen, although the effect on collagen production depends on the type of exercise (Kjaer 2004). The collagen content of SDFT was found to be significantly higher after box rest (Cherdchutham *et al.* 1999), although this could be due to a decrease in concentration of the other components. However, the collagen content in the SL was found to increase after 18 months high-intensity exercise (Birch *et al.* 2008), similar to results found in chicken Achilles tendon when compared to caged birds (Romero Nakagaki *et al.* 2010). A review concluded that

although exercise has little effect on collagen production in flexor tendons, it appear to promote production in extensor tendons (Buchanan and Marsh 2002). These authors state that the apparent increase of collagen percentage in extensor tendons may in fact be due to a decrease in the non-collagenous components. Since the non-collagenous components are already at a low concentration in flexor tendons, exercise appear to cause minimal further change, as seen in pigs (Woo et al. 1981). These results suggest that high-intensity exercise increase the rate of collagen turnover, decreasing the degree of maturity of the collagen.

Exercise did not appear to affect the concentration of cross-links, although the concentration is generally higher in the high load-bearing flexor tendons compared to the low-loaded extensors (Kjaer 2004). Foals on low-intensity pasture exercise had a higher concentration of cross-links than on box-rest or high-intensity exercise, but this difference was not significant (Cherdchutham *et al.* 1999). Another study reported no difference in concentration between high and low levels of exercise intensity (Birch *et al.* 2008). However, chicks that underwent physical exercise were found to have a lower concentration compared to non-trained birds.

Studies into the effect of exercise on crimp angle are limited, but indicate that training leads to a decrease in angle. A study of feral, untrained, horses found no change in angle at the periphery of the SDFT (Patterson-Kane *et al.* 1997a), but did find a change in the core. In contrast, a decrease in angle has been found in both regions in trained horses (Patterson-Kane *et al.* 1997b). The authors concluded from these results that enforced exercise induces a considerable, long-term, change to SDFT structure.

In terms of CSA, a pasture-based regime from one week old resulted in a significantly larger SDFT CSA than for a box-rest or exercised group (Cherdchutham *et al.* 2001). This is in contrast to a study by Birch *et al.* (1999b) who reported no difference in CSA of the SDFT either between low- and high-intensity exercise regimes or short- and long-term training period. They did, however report a significant increase in the DDFT across both of these variables. This indicates that although the SDFT is not susceptible to further adaptation with imposed exercise, the DDFT may be altered.

The MAD of DDFT fibrils has been found not to be influenced by exercise (Patterson-Kane *et al.* 1998). However, the MAD of SDFT fibrils was significantly smaller in an

exercised group compared to untrained horses (Patterson-Kane *et al.* 1997c) or a low-intensity exercise regime (Birch *et al.* 2008). Since the CSA tends to increase with exercise, this could indicate that exercise leads to an increase in the number of fibrils (Kjaer 2004). This suggests that exercise could lead to the break-down of larger molecules, especially since there has found to be a prevalence of small-diameter fibrils in horses that have undergone long-term high-intensity exercise (Smith *et al.* 1999).

Cellularity has been found to be significantly higher in young horses left at pasture than horses either placed on box rest or given forced exercise (Cherdchutham *et al.* 1999). This indicates that pasture exercise is the best regime in terms of promoting tenocyte numbers.

The concentration of COMP has also found to be affected by exercise, being highest in the pastured group (Cherdchutham *et al.* 1999). It was also higher in a weight-bearing limb compared to a non-weight bearing limb (Smith *et al.* 1997). These results suggest that exercise promotes the production of COMP.

Even within the same individual, differences in structure and mechanical properties exist between the digital flexor and extensor tendons, which reflect the different demands placed on them. The flexor tendons require high elasticity to withstand sudden high loads whereas the extensors are subject to low-level repeated stress (Parry *et al.* 1978b). Collagen concentration has been found to be higher in the flexor tendons than in the extensor tendons (Parry *et al.* 1978b). The increase in pig extensor CSA after a 12-week training programme (Woo 1982) was not seen in the flexor tendons (Woo 1981). Both strength and stiffness of flexor tendons has been found to be twice that of the extensors, and age has been suggested to have a greater effect on the flexors than on the extensors (Woo *et al.* 1981; Woo *et al.* 1980). These differences in structure and mechanical properties reflect the different demands placed on the tendons during exercise.

The importance of long-term exercise was illustrated by several studies where short-term regimes failed to elicit a change. For example, Birch *et al.* found that a 5-month exercise regime did not influence DDFT CSA, whereas an 18-month regime did lead to a significant increase (Birch *et al.* 1999b). A similar result was found in pigs, where a 3-month exercise regime did not affect CSA but a 12-month regime did lead to an

increase (Woo *et al.* 1981). This finding indicates that the intensity of exercise and the ratio between stress and recovery time are important in how the matrix adapts to exercise.

These structural adaptations result in altered mechanical properties of the tendon in terms of its stiffness and strength; these will now be discussed.

Influence of exercise on mechanical properties

Tendon stiffness is generally greater in individuals given low-intensity exercise. For example, young foals left at pasture had a greater stiffness than groups given either box-rest or forced exercise (Cherdchutham *et al.* 2001). Stiffness of the SDFT was found to be higher after low-intensity training compared to a high-intensity regime, but this difference was not significant (Birch *et al.* 2008). Similar results have also been found in the Achilles tendon of chickens left to roam freely, representing low-intensity exercise (Romero Nakagaki *et al.* 2010), and in other species such as rats, turkeys and humans that have undergone long-term distance running exercise (Kjaer 2004). Immobilisation appears to result in the opposite effect: the Achilles tendon of immobilised rabbits has been found to be less stiff than in controls (Matsumoto *et al.* 2003), a similar finding to a study investigating the effects of unloading on humans (Reeves 2006). The increase in stiffness leads to a decreased extensibility and therefore chance of damage (Buchanan and Marsh 2002) but may decrease efficiency (Birch *et al.* 2008). Towards senescence, resistance training in humans resulted in an increase in stiffness, suggesting loss of stiffness in older adults can be partially offset by this type of training (Kjaer 2004; Reeves 2006). This maintenance of stiffness helps to increase force transmission (Kjaer 2004; Reeves 2006).

In terms of tendon strength, the general consensus is that low-intensity exercise improves strength in a range of species compared to restriction of exercise (Cherdchutham *et al.* 2001; Matsumoto *et al.* 2003; Romero Nakagaki *et al.* 2010; Woo *et al.* 1981; Woo *et al.* 1980). There was, however, no further effect on strength of equine SDFT when a high-intensity exercise regime was applied (Birch *et al.* 2008).

Summary

Exercise given during both development and degeneration of the tendon affects its structural and mechanical properties. During growth, low-intensity exercise seems to

stimulate optimum development of the tendon and minimises degeneration towards old age. Exercise given at too low an intensity provides insufficient stimulation, whereas too high an intensity may not allow sufficient recovery time for physiological adaptation. The exact intensity and duration of exercise for optimum tendon quality has yet to be found.

Towards old age, resistance training appeared to counteract the decrease in stiffness. Complete immobilisation or high-intensity exercise leads to an increase in degenerative changes.

2.6.10. Summary of changes to tendon properties with age and exercise

Tendon shows phases of growth, maturation and degeneration. During growth, the tendon becomes stiffer due to a hormone-mediated increase in the ratio of mature to immature cross-links, a change in MAD distribution, a decrease in fascicle diameter and crimp angle, and an increase in COMP concentration. Once the tendon in an individual horse is at optimal stiffness for efficient locomotion, matrix synthesis ceases at skeletal maturity, leading to a plateau in tendon stiffness until the onset of degenerative changes at around eight years old. Beyond this age, a decrease in circulating growth hormones leads to a decrease in the synthesis of new tendon matrix, and therefore the tendon has a much reduced capacity to respond to stimulus. The lack of any new material, along with the breakdown of the fibrils and a straightening of the crimp, leads to a weaker tendon with a lower elastic modulus. As these degenerative changes are exercise-linked, the age at which they appear is affected by the duration and intensity of exercise.

The overall trend in tendon stiffness throughout the horse's life is a sharp increase during maturation, followed by a slower decrease towards senescence, and has also been found in rat tail tendon (Bailey *et al.* 1998; Vogel 1980) and rabbit medial collateral ligament (Woo *et al.* 1990). The trend was neatly illustrated by Gillis *et al.* (1995) (Figure 2.6).

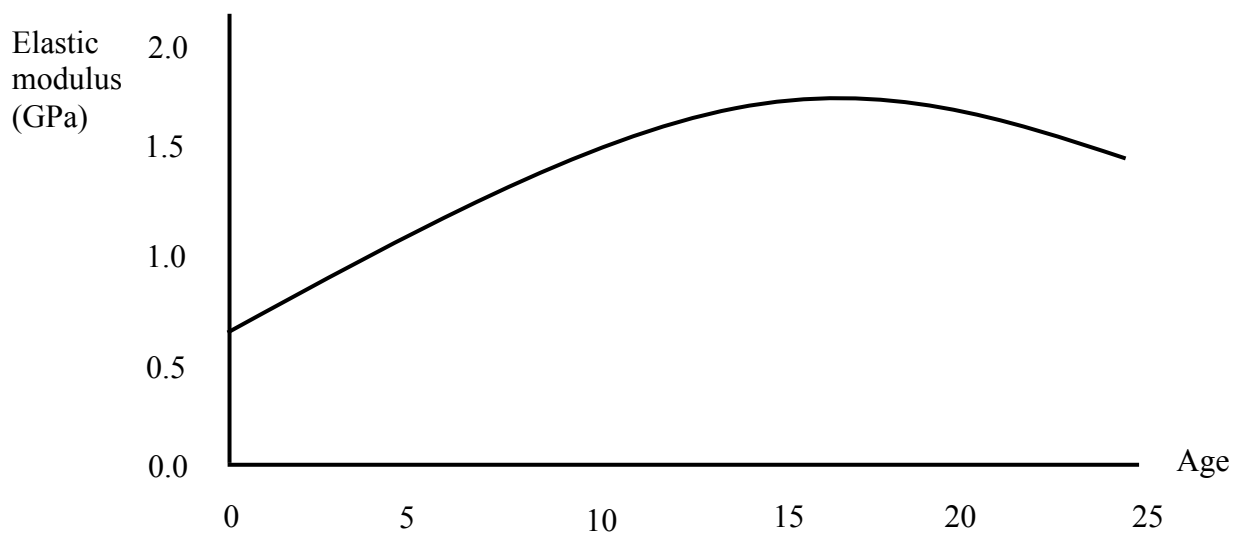


Figure 2.7 Tendon stiffness increases with age until maturity, reaches a plateau then decreases into senescence. From Gillis *et al.* (Gillis *et al.* 1995).

2.7. Chapter summary

The equine distal limb skeleton is composed of one main metacarpal bone and two vestigial metacarpal bones, the three phalanges and two sets of sesamoid bones. These articulate at the metacarpo-phalangeal, proximal inter-phalangeal and distal inter-phalangeal joints. The elastic structures that wrap around and support these joints include the superficial and deep digital flexor tendons and their accessory ligaments, the suspensory ligament, distal sesamoidean ligaments and the collateral ligaments. These elastic structures contribute to efficient locomotion through storage and return of elastic strain energy during the stance phase, so optimum stiffness of the tendons is vital to achieve efficient locomotion. During the course of the horse's life, age affects both the structure and biomechanical properties of the tendon, with exercise given during both development and degeneration being influential. The close link between the tendon mechanical properties and kinematics could mean that the change in tendon stiffness affects joint angle during the horse's lifetime. Therefore, the change in joint angle could provide useful information about the underlying properties of the tendon, especially degenerative changes that occur before clinical injury is seen.

3. The influence of age on development of stride variables

3.1. Introduction

Understanding age-related changes in gait is important to determine when the horse is mature enough to begin training, and also to identify and interpret pathological development. Maturation of stride parameters is influenced by the development of both the central nervous system (CNS) and the musculoskeletal system, particularly leg growth (Sutherland 1997). Research carried out in growing children isolated the effect of CNS maturation by normalising stride parameters to leg length (Vaughan *et al.* 2003).

Development of locomotion in the young horse and pony has been extensively researched by Back *et al.* (Back *et al.* 1994b; Back *et al.* 2002; Back *et al.* 1995b; Back *et al.* 1999) and Cano *et al.* (Cano *et al.* 2000; Cano *et al.* 2001) up to the age of 36 months. However, these studies did not normalise all results to leg length and therefore separating the effect of CNS maturation from leg growth is difficult. Additionally, little work has yet been carried out to determine how stride parameters change in the ageing horse, during deterioration of both the musculoskeletal system and the CNS.

The aim of this chapter was therefore to investigate the development of walk parameters during growth, separating the effects of physical growth from maturation of the CNS; and to investigate how these change parameters towards old age. A further purpose of analysing stride parameters is to validate the joint angles obtained as the main data of this thesis (Chapter 6).

3.2. Mechanical concepts of walk

Horses in this thesis were studied in walk due to difficulty in maintaining control of the younger horses at faster gaits. The four parameters that this chapter investigates are step length, frequency, velocity and stance percentage. Step length is a function of leg length and the range of motion (RoM) through which the leg moves during swing phase (Section 3.2.1). Frequency can be explained in terms of an inverted pendulum, the concept of which is explained in Section 3.2.1, and velocity is a function of step frequency and length. Stance percentage, the proportion of the gait cycle when the limb

is in contact with the ground, has been used a measurement of stability in humans (Judge *et al.* 1996b; Sutherland 1997). However, since the quadrupedal horse has different methods of maintaining balance from the human, results cannot be fully extrapolated. Differences between quadrupedal and bipedal stability are discussed further in Section 3.2.2.

3.2.1. Step length as a function of leg length and range of motion

Step length is a function of leg length and the RoM through which it moves during the swing phase (Figure 3.1). Therefore, the increase in limb length during growth will affect step length.

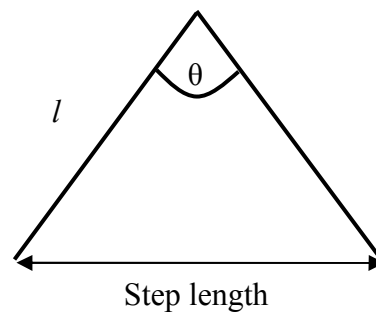


Figure 3.1 Step length is a product of limb length l and the angle θ through which it moves

3.2.2. Step frequency: the inverse pendulum

During the stance phase in walk, the path travelled by the supporting limb can be compared to an inverted pendulum (Biewener 2006; Cavagna *et al.* 1977) as it swings from the protracted position to the retracted position. The centre of mass describes an arc, rising up and over the supporting limb before falling forwards (Figure 3.2).

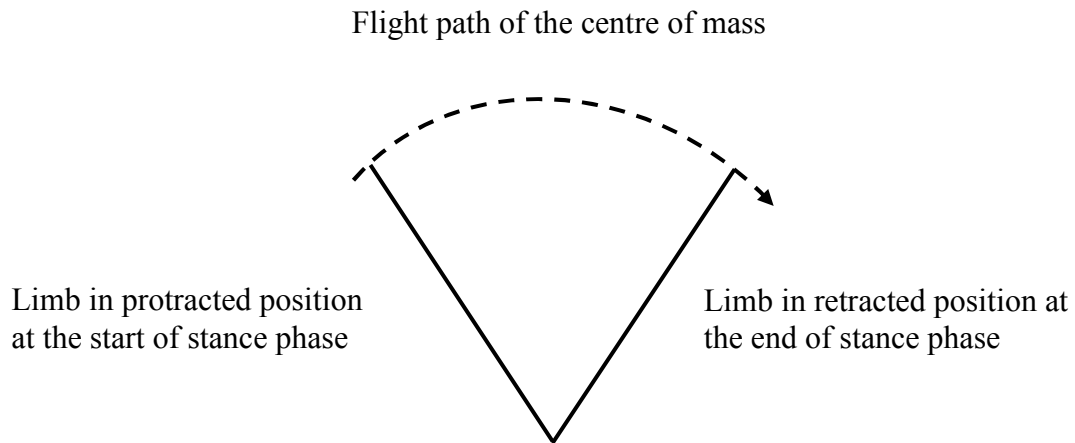


Figure 3.2 In walk, the supporting limb acts as an inverted pendulum. The limb is shown as a solid line and the path of the centre of mass is shown as a dotted line. Adapted from (Cavagna *et al.* 1977)

Despite its simplicity, this model can be used to explain some of the characteristics of walk, such as the energy-saving mechanism via the exchange of kinetic and potential energy (Biewener 2006; Cavagna *et al.* 1977; Minetti *et al.* 1999). More relevant to this thesis is its use in explaining stride characteristics such as frequency.

The time taken for a pendulum to complete one swing (T) is approximately described by Eqn. 1, where L is pendulum length and g is acceleration due to gravity (9.81 ms^{-2}).

$$T = 2\pi \sqrt{L/g} \tag{Eqn. 1}$$

This equation is, however, only accurate for displacements of up to around 20° . Since the pro-/retraction angle in horses is around 44° (Back *et al.* 1994b), the inaccuracy introduced when calculating T in horses was determined² using a more complex series, of which the first three terms are:

$$T = 2\pi \sqrt{L/g} [1 + 1/16 \theta_0^2 + 11/3072 \theta_0^4 \dots]$$

Using this method, the inaccuracy was found to be less than 4% and therefore a simple pendulum model was deemed to be acceptable for the current study.

² Calculation performed using <http://hyperphysics.phy-astr.gsu.edu/hbase/pendl.html#c2>

Frequency, as the inverse of eqn. 1, is therefore approximated by:

$$F = \frac{1}{2\pi \sqrt{L/g}}$$

Eqn. 2

Since the other elements are constants, step frequency is highly dependent on the length of the leg pendulum, where a longer length results in a lower frequency. Therefore, frequency will be influenced by the increase in leg length during growth.

3.2.3. Quadrupedal stability

Since the majority of studies into the development and deterioration of gait have been carried out in humans, most of the evidence presented in Section 3.3 has been obtained from humans. However, it is recognised that the bipedal human has a different method of maintaining balance than the quadrupedal horse, and therefore data that concern stability (such as stance percentage) cannot be directly extrapolated.

Static equilibrium only occurs in the walking human when both feet are in contact with the ground, because the centre of mass (CoM) falls within the base of support formed by the two feet (Judge *et al.* 1996b). However, the quadrupedal walk includes periods where three (or more) limbs are in contact with the ground. During these times, the horse can achieve constant static equilibrium because the CoM falls within the triangle formed by the three limbs that are in contact with the ground (Biewener 2003) pg 54-55, Figure 3.3.

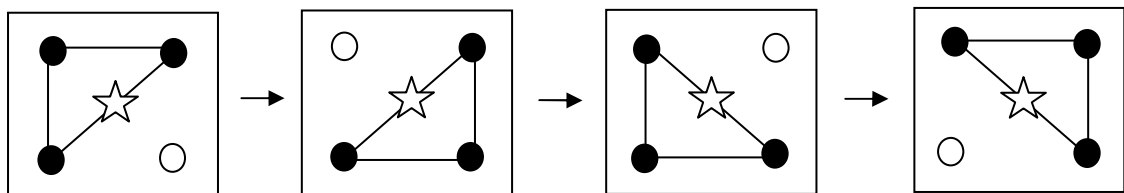


Figure 3.3 Constant static equilibrium can be achieved in the quadruped since the centre of mass (represented by the star) falls within the triangular base of support. Filled circles represent hooves in contact with ground, white circles represent in hooves in flight. Adapted from Biewener 2003.

Achieving constant static equilibrium gives the quadruped greater stability than the biped and therefore variables such as stance percentage cannot be directly compared.

3.3. The effect of maturation and deterioration on stride parameters

Gait production

Gait is a product of several interacting components, mainly the neurological and musculoskeletal systems. The main neurological component is a group of interconnected neurones called central pattern generators (CPGs), which are responsible for rhythmical, intrinsic processes such as breathing and chewing as well as locomotion (Fénelon *et al.* 1998; Kiehn *et al.* 2008; Mackay-Lyons 2002; Marder and Bucher 2001; Marder and Calabrese 1996). In his seminal paper, Wilson (1961) reported that flight patterns in deafferented locusts were slowed but still rhythmic. Since there was no link to external stimuli, he concluded that the motor pattern originated within the motor ganglia. This paper was the first to provide evidence that opposed the long-held theory that rhythmic behaviour was purely reflex-based (Edwards 2006). The purpose of the CPGs is to free the supraspinal centres to control other processes and allow co-ordinated timing of efferent nerve impulses (Biewener 2003) pg 254. The CPGs send efferent nervous impulses to the actuator muscles, which then contract to bring about locomotion via the musculoskeletal system.

The influence of the development and degeneration of the CNS and musculoskeletal system on gait will now be explored, although a full investigation of the neurological effects of ageing on gait is beyond the scope of this thesis.

3.3.1. Overall changes to gait during maturation

Development of the CPGs begins *in utero* to ensure that essential, rhythmical movements such as breathing are fully functional at birth (Fénelon 1998). In humans, kicking movements are seen in the lower limb before the onset of walking, suggesting that the CPGs are functional before they are necessary (Sutherland 1997). Early locomotor function is particularly important in the horse which, as a prey animal, must be able to co-ordinate its limbs shortly after birth to escape potential predation.

After birth, CPG development to the adult form can be via one of several routes, including a gradual maturation or a switch from controlling one process to controlling another (Fénelon *et al.* 1998). However, the final process for all maturation routes is the fine tuning achieved through external modulatory input towards the end of development (Fénelon *et al.* 1998; Marder and Rehm 2005).

During development, the CPGs mature and muscle strength increases along with medio-lateral stability (Vaughan *et al.* 2003). The adult gait pattern emerges and, as neural networks are tuned, the variability of muscle activation patterns decreases. The overall effect is a change in gait characteristics, and disorders such as cerebral palsy can lead to delays in development (Vaughan *et al.* 2003).

The primary musculoskeletal factor in gait development is limb growth, so gait cannot be said to be mature until skeletal growth is complete. In the horse, although wither height reaches its maximum at about 1.5 - 2 years old (Anderson and McIlwraith 2004; Lovšin *et al.* 2001; Thompson 1995), a review of radius growth found that the growth plate did not close until around 36 months old (Fretz *et al.* 1984). This means that limb growth is likely to influence gait until skeletal growth finishes at around 36 months old.

Since the growing human experiences both CNS maturation and leg growth, (Beck *et al.* 1981; Ganley and Powers 2005; McGibbon and Krebs 2004; Sutherland 1997; Vaughan *et al.* 2003) parameters can be normalised to leg length to investigate the effect of CNS maturation alone. Evidence suggests that child values for stride length and frequency, once scaled to leg length, are no different from adult values (Ganley and Powers 2005). The methods employed to calculate normalised values are described in the following sections.

3.3.2. Overall changes to gait towards old age

In humans, deterioration of the CNS and musculoskeletal system results in a decline of both static and dynamic balance (Judge *et al.* 1996b), and means that gait is once again adapted to prevent falls. Neuromuscular changes take place that compensate for physical impairments (McGibbon 2003), and result in a reorganisation of torque and power in limb joints (DeVita and Hortobagyi 2000). These changes serve to improve stability towards old age (Judge *et al.* 1996b; Menz *et al.* 2003; Winter *et al.* 1990) and

result in alterations to stride parameters such as stance percentage and speed (Judge *et al.* 1996b; McGibbon and Krebs 2004).

Evidence suggests neuromuscular changes have a greater influence on deterioration of gait than conscious alterations (McGibbon 2003; McGibbon and Krebs 2004), suggesting that changes towards old age are a reflection of the deterioration of the CNS and musculoskeletal system, rather than a deliberate attempt to improve stability.

Ageing itself has been reported to have a different effect on gait than impairment (for example arthritis) or reduced function (for example decreased muscle contractility) in humans (McGibbon and Krebs 2004). This means that the degree of impairment or reduced function in subjects must be assessed. All horses in this study underwent routine clinical examination and those filmed at evaluation events were also examined by a vet on the day of assessment. Their gait was therefore presumed to be free from impairment and influenced only by their age. Although a full clinical assessment would have confirmed this, the cost was prohibitive.

Changes to individual gait parameters with age

The following sections review the current literature on the effect of maturation and ageing on stride parameters. Since literature on the ageing horse is limited, evidence from the human field is also examined. As step length may not be the same between contralateral limbs, parameters are measured in terms of a step (consecutive contact of contralateral feet or hooves), as distinct from a stride (consecutive contact of the same foot or hoof).

3.3.3. Step length

Step length is a product of limb length and the RoM at the most proximal limb joint (Section 3.2.1). In humans, the RoM at the hip has been found to change minimally throughout development, with a negligible increase reported between two and seven years old (Sutherland 1997) and no significant change between seven years old and adults (Ganley and Powers 2005). Similarly, a minimal change in shoulder and hip RoM has also been reported in horses between four and 26 months old (Back *et al.* 1995b) and between 12 and 36 months old (Cano *et al.* 2001). Therefore, the major determinant of step length is limb length, and since this increases in length during development, the

increase in step length is expected in both horses (Back *et al.* 1994b; Back *et al.* 2002; Cano *et al.* 2001) and humans (Ganley and Powers 2005; Sutherland *et al.* 1980; Vaughan *et al.* 2003).

The close relationship between limb length and step length is a useful test of neuromuscular maturation, since a low correlation may indicate delayed maturation (Sutherland *et al.* 1980). Failure to increase step length with age could indicate an inability to flex the hip or extend the knee, or a lack of stability in the supporting limb.

This direct linear relationship between leg length and step length in children between one and seven years old appears to indicate that growth alone determines the increase in step length. This would mean that step factor (step length normalised to leg length) shows no change with age. However, step factor has been found to increase up until four years old. This indicates that both growth and CPG maturation influence development between one and four years old, and growth alone beyond this age (Sutherland 1997).

In the ageing human, musculoskeletal impairment such as reduced knee extension (DeVita and Hortobagyi 2000) decreases the RoM through which the leg moves and therefore can limit step length. The need to maintain gait stability (Winter *et al.* 1990), also results in an increase in variability (Judge *et al.* 1996a; Judge *et al.* 1996b).

The hypothesis was that horses would show an increase in step length with age up until the cease of skeletal growth at around 36 months, and would then show a decrease towards old age. Step factor was hypothesised to increase in the young horse, due to maturation of the CPGs and plateau at around 36 months. Since stride length is likely to decrease towards old age and the mature CPGs are not likely to influence step factor, this parameter was hypothesised to decrease towards old age.

3.3.4. Step frequency

As described in Section 3.2.2, a longer pendulum has a longer period and therefore a lower frequency. Therefore, the increasing leg length of the growing individual results in a decrease in stride frequency. A decrease in stride frequency has been previously

reported in the growing horse (Back *et al.* 1994b; Back *et al.* 2002; Cano *et al.* 2001) and human (Ganley and Powers 2005; Sutherland 1997).

To eliminate the effect of leg growth and assess the influence of CPG maturation alone, Vaughan *et al.* (2003) calculated dimensionless step frequency ϕ as follows:

$$\phi = SF / \sqrt{g/LL}$$

Eqn. 3

where SF is step frequency, g is the acceleration due to gravity (9.81m^{-2}) and LL is leg length. In growing children, Vaughan *et al.* found that dimensionless frequency increased with age, indicating an effect of CNS maturation over and above that of increasing leg length.

In the ageing human, step frequency has been found to increase when walking at the same speed as younger subjects (DeVita and Hortobagyi 2000; Menz *et al.* 2003). This may be to maintain stability (Judge *et al.* 1996b; Menz *et al.* 2003) as a consequence of the reduction in joint power with age (Winter *et al.* 1990). Since the horse can achieve constant static equilibrium in walk, maintaining stability is less of a concern; and since leg length does not change, frequency towards old age in the horse is unlikely to change.

The hypothesis was that both step frequency and dimensionless step frequency would decrease in the growing horse and then plateau.

3.3.5. Stance percentage

At the same speed, the duration of both swing phase and stance phase is determined by the length of the leg pendulum (Eqn. 1), where the only variable component is leg length. Therefore, the change in duration of both phases with age should theoretically be identical and stance percentage (stance duration as a percentage of total stride duration) should remain constant. A normal stance time indicates stability in the human and allows adequate time for flight of the contralateral limb (Sutherland 1997).

An increase in stance percentage has been found in horses (Back *et al.* 1994b; Cano *et al.* 2001), which seems to be in contrast to one of the aims of training across all

disciplines - to reduce stance duration (Section 3.3.6), (Back *et al.* 1994a; Holmström *et al.* 1994; Morales *et al.* 1998). A possible explanation for this is offered in Section 3.5.4. To investigate the possible effects of CNS maturation and training, the influence of leg length was eliminated by normalising stance percentage to leg length.

Towards old age, one of the goals of human gait is to maintain stability and prevent falling (Judge *et al.* 1996b). Bipedal humans are stable when the centre of mass (CoM) falls within the base of support - the area formed by the feet that are in contact with the ground. Therefore, they are less stable during periods of single stance, where only one foot is in contact with the ground, than in double stance, where both feet are in contact with the ground. Periods of double support (Judge *et al.* 1996b) and stance percentage (DeVita and Hortobagyi 2000) have both been found to be greater in the elderly human than in the younger adult. However, the quadrupedal horse is in static equilibrium in walk and is therefore more stable than the human. Therefore, ageing is unlikely to affect stance percentage.

It was hypothesised that stance percentage would not change, but that normalised stance percentage would decrease in the growing horse to a plateau at skeletal maturity, and remain constant until old age.

3.3.6. Velocity

Velocity is a product of step length and frequency, which are both influenced by leg length. Although a longer leg length results in a greater step length (Section 3.2.1), it results in a smaller frequency (Section 3.2.2), and therefore the overall effect is that velocity will not change significantly with age. A reduction in walking speed in humans may be indicative of delayed or pathological development (Sutherland 1997) and therefore is a useful indicator of maturation level.

Dimensionless velocity, β , is calculated using step factor, λ , and dimensionless step frequency, φ (Vaughan *et al.* 2003):

$$\beta = \lambda \times \varphi.$$

Eqn. 4

Slow gait is typical of the elderly human (Judge *et al.* 1996b) and is due to the deterioration of cognitive ability (Priest *et al.* 2008) and various organ systems (Studentski 2009). For example, taking muscle as a Hill-type model where force and velocity are inversely related (Hill 1938), an ageing, weaker muscle must contract more slowly to maintain the force of contraction. Therefore, the overall effect may be to reduce the speed of walking. Reduced speed is primarily a product of shorter step length (Winter *et al.* 1990), which may be due to the need to increase balance (Judge *et al.* 1996b).

In the horse, it was hypothesised that velocity would remain constant in the growing horse and decrease towards old age. Dimensionless velocity was hypothesised to show no change with age.

3.3.7. Variability

One of the characteristics of immature gait is greater inter- and intra-subject variability (Sutherland 1997) than older children (Lasko-McCarthy *et al.* 1990) and teenagers (Gorton *et al.* 1997). Similarly, the variability of various gait parameters have been found to decrease in the growing horse (Back *et al.* 1994b). The decrease in variability with age may indicate gait stability in terms of consistent step patterns, co-ordination (Hollman *et al.* 2007) and maturity of neuromuscular control (Hausdorff *et al.* 1999).

Towards old age, human gait variability increases (Hollman *et al.* 2007; Judge *et al.* 1996b; Kang and Dingwell 2008; Priest *et al.* 2008) possibly due to a decrease in stability (in terms of controlling displacements of the CoM) (Priest *et al.* 2008) and physiological function (Kang and Dingwell 2008), aggravated by deterioration of the CNS (Hollman *et al.* 2007). Several studies found that performing a second task such as counting aloud whilst walking increased gait variability in older adults only (Beauchet *et al.* 2003; Priest *et al.* 2008). The disruption of gait regularity by the second task suggests that higher cortical regions become more involved in the neuromuscular control of gait with age (Beauchet *et al.* 2003), and therefore could indicate deterioration of the CPGs. Increased variability in older human adults has been found to be independent of speed (Kang and Dingwell 2008), suggesting the increase is due to reduced physiological function.

For each parameter, the coefficient of variation (CV) was obtained as a measure of gait stability and maturity in the younger horses, or deterioration in the older subjects. Variability of each parameter was expected to decrease in the growing horse and then increase towards old age.

3.3.8. The influence of training

As well as being influenced by physical growth and CNS maturation, a horse's stride parameters may also be affected by training. Most horses will undergo training to improve their movement and hence chance of competition success, although the specific parameters that an owner will try to improve will depend on the discipline. In the racehorse, where outright speed is most important, a trainer will focus on increasing variables such as horse's stride length and frequency (Drevemo *et al.* 1980; Ferrari *et al.* 2009; Rogers *et al.* 2005) to enable it to gallop as fast as possible. At the other end of the spectrum is the dressage horse, where parameters such as swing phase percentage and diagonal advanced placement have been correlated with superior movement (Holmström *et al.* 1994; Morales *et al.* 1998) and are therefore the focus of training. Possibly the one parameter where training has a consistent effect is stance percentage. In racehorses a decrease allows a faster gait (Corley and Goodship 1994; Drevemo *et al.* 1980; Rogers *et al.* 2005); and in dressage horses a decrease has been correlated with superior gait (Holmström *et al.* 1994; Morales *et al.* 1998).

The variety of disciplines in which a horse can take part means that the literature regarding training must be interpreted with reference to a particular discipline. Since the population in the present study was used for general purpose riding, with no racehorses or top-level dressage horses included, the influence of training on stride parameters is discussed with respect to the amateur-level horse undergoing training in jumping and dressage.

Stride length has been found to decrease with training in horses used for general-purpose work (Cano *et al.* 2000). This finding is in contrast to work that has found a longer stride length to be correlated with superior performance (Holmström *et al.* 1994), and may be explained by the slower velocity of horses after training in the former study ($3.8 \pm 0.3 \text{ ms}^{-1}$ compared to $4.8 \pm 0.08 \text{ ms}^{-1}$).

Stride frequency has been shown to increase compared to a pastured group after training for both 70 days (Back *et al.* 1995a) and 10 months (Cano *et al.* 2001). These results are in contrast to the ideal result of training, since superior movement has been correlated with a lower stride frequency (Holmström *et al.* 1994; Morales *et al.* 1998). However, the horses in the study by Holmström *et al.* were substantially older (10 years old; age was not given in the study by Morales *et al.*) than the young horses used in the studies by Back *et al.* (four to 26 months old) and Cano *et al.* (45 to 55 months old), and therefore perhaps the effect of training takes some years to become apparent.

Stance percentage has been found to decrease after training only in the hindlimb (Back *et al.* 1995a; Cano *et al.* 2000). This is consistent with a paper that found a significant difference in stance phase duration between horses judged as good and poor movers only in the hindlimb (Holmström *et al.* 1994).

Velocity has not been found to be influenced by training in the general-purpose horse (Cano *et al.* 2000). This was possibly because training was found to have opposing effects on the two variables that affect velocity - stride length and frequency - and therefore there was no overall effect.

3.4. Method

3.4.1. Study population and protocol

The study population, fully described in Section 6.2.1, included mares, geldings and stallions from three to 212 months old subject to approximately similar exercise routines. The data capture protocol described in Section 6.2.2 was applied to capture the following stride variables: step length, step frequency, percentage of stance phase and velocity. Horses were walked in hand perpendicular to a single camera at a distance of approximately four metres (Figure 3.4). Three trials were recorded for each horse, with at least one complete stride per trial captured. Horses were led close to a calibration object of known size to calculate lengths. The length of the metacarpus (MC), representing limb length, was calculated to allow normalisation.

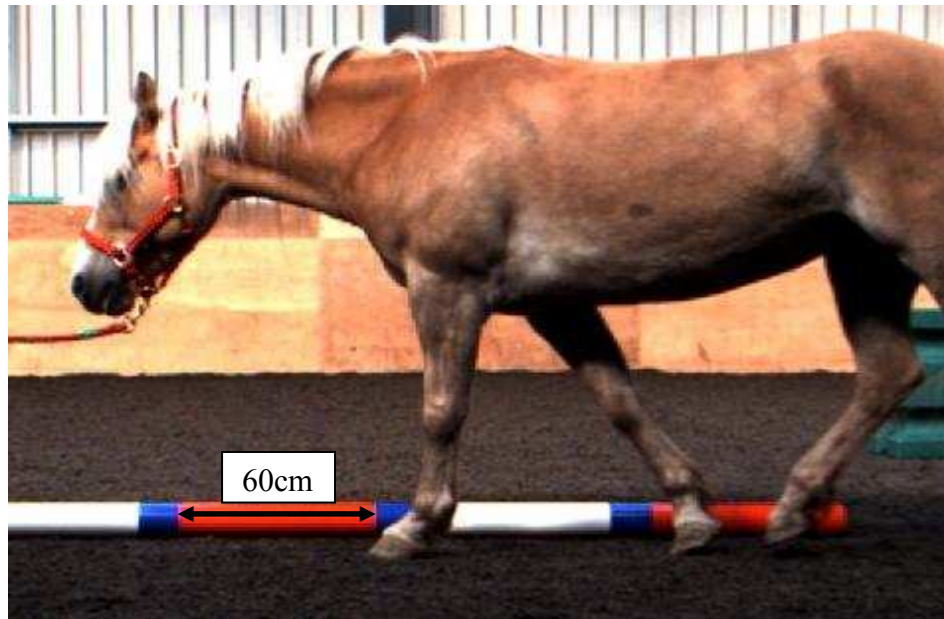


Figure 3.4 A still showing the camera's eye-view of a subject. The striped pole was used to calibrate distances.

A programme was custom-written in Matlab (The Mathworks, Inc., USA) that allowed the user to locate a repeatable point on the subject's hooves and top of the withers during one complete stride, to calculate distances and velocities. The dimensions of the calibration object were also digitised, and used to convert distances calculated in pixels into real-world units.

To begin, the user selected the first frame of stance for the near-side limb. In this frame, the user manually digitised the location of the front of the hoof (Figure 3.5a and 3.6a) and the top of the withers (Figure 3.7a). Next, the user identified the first frame of the contralateral limb stance phase and digitised the location of the contralateral hoof (Figure 3.5b). At the start of the subsequent near-side stance phase, the user located the position of the near-side hoof (Figure 3.6b) and the top of the withers (Figure 3.7b). Finally, the user noted the frame where toe-off of the near-side hoof occurred, defined as when the toe was no longer visibly in contact with the ground. Since some data capture was performed at evaluation events where it was not possible to control the way in which horses were led, stride parameters could not be obtained for all horses.

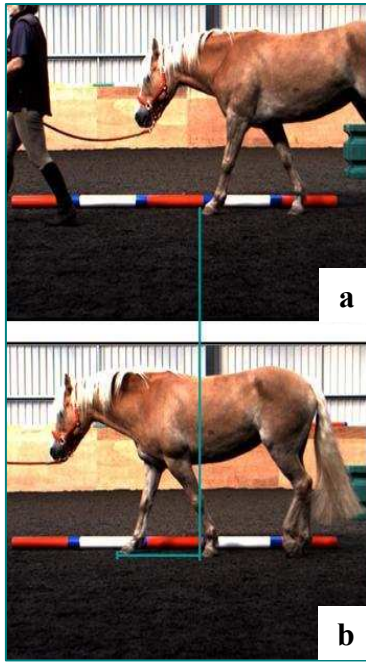


Figure 3.5 Locations of the front contralateral hooves were digitised in their respective first frames of stance phase, to determine step length.

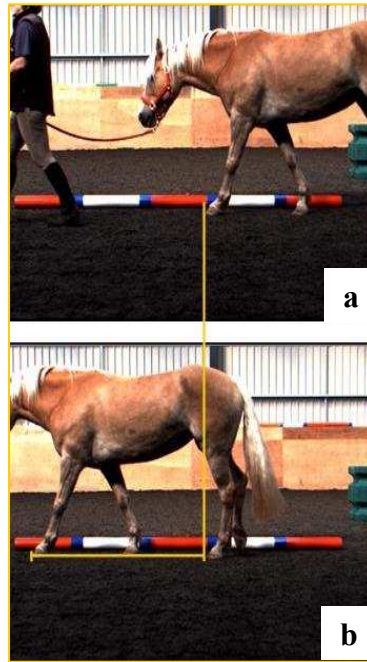


Figure 3.6 Total stride duration was calculated, from which the percentage of stance could be determined

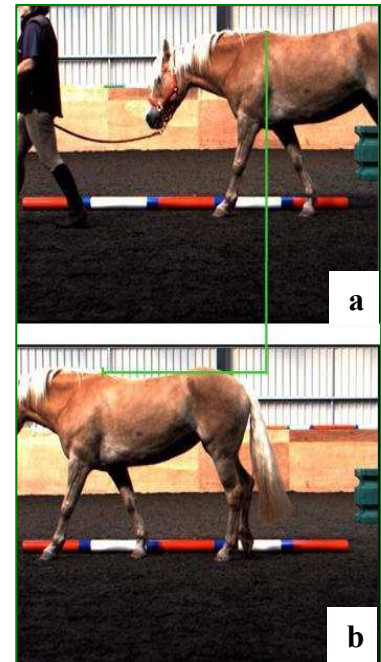


Figure 3.7 The location of the top of the withers was digitised in the first frame of consecutive stance phases to allow calculation of velocity.

Step length was determined by measuring the horizontal displacement of contralateral hooves over one complete step in pixels (Figure 3.5), and then converting this to real-world units using a calibration object of known size. MC length was calculated in the same way. Stride duration was calculated as the time taken between toe-on of the same hoof for successive strides (Figure 3.6). Stance duration was calculated as the time between the start of stance and toe-off for the near-side hoof, and stance percentage was calculated as the proportion of stance phase over the total stride. Step frequency, measured as steps per second, was calculated as the inverse of step duration (Vaughan *et al.* 2003). Velocity was calculated by first converting the horizontal displacement of the withers in pixels to real-world units (Figure 3.7), and then dividing this by the same elapsed. To test the repeatability of the manual digitisation method, the same point on the same frame was manually digitised ten times, and the standard deviation (SD) of the horizontal and vertical co-ordinates calculated.

Results for young horses in the current study were compared to fundamental work carried out by Back *et al.* (Back *et al.* 1994b; Back *et al.* 1995b) and Cano *et al.* (Cano

et al. 2001) into the development of the young horse. Since these authors investigated horses in trot, the trends of development were compared rather than the raw data. No data currently exist on the deterioration of gait in the older horse.

Studies by Back *et al.* report only stride duration, so the values for stride frequency, length and factor were calculated as follows. Frequency was taken as the inverse of stride duration; stride length was obtained by dividing the given velocity by the calculated frequency; and stride factor was calculated as stride length divided by MC length. This length was only given for the 4- and 26-month old horses, so data for the 10- and 18-month old horses were obtained from a study by Thompson (Thompson 1995). Since both studies investigated Sport Horses, the influence of breed was assumed to be minimal.

3.4.2. Statistical methods

The population was divided into three age groups (young, adult and old) based on the following criteria. As the horse's limb acts like a pendulum, most time and distance characteristics will depend on leg length (Sutherland 1997), and therefore it was expected that there would be a strong influence of age in the growing horse until skeletal maturity at 36 months. It was then hypothesised that stride parameters would remain constant in the adult horse. Finally, the effect of mechanical degeneration on gait was assessed by studying the influence of age in older horses. In contrast to bone, tendon undergoes minimal turnover and therefore shows little response to repeated loading cycles (Chapter 2). It is therefore representative of the state of degeneration of the musculoskeletal system. Since the average age of onset of degenerative change in tendon is 100 months old (Section 2.5) this age was selected as the upper age limit of adult horses.

On the basis of these two age boundaries, horses were divided into three age groups:

- Group 1: 16 immature horses from three to 35 months old, whose leg length is assumed to be increasing.
- Group 2: 20 adult horses from 36 to 99 months old, which are assumed to have finished growing.
- Group 3: 21 mature horses from 100 to 212 months old, which are likely to be undergoing degenerative changes.

The influence of age within each group was determined by calculating the Pearson's correlation coefficient for each parameter using Minitab (Minitab Ltd., UK), with significance set at 0.05. For each parameter, the coefficient of variation (CV) was calculated as a measure of variability. The velocities of each group were compared by ANOVA. Results will be presented in the following sections.

3.5. Results and discussion

When digitising the same point ten times, the standard deviation in the horizontal direction was ± 0.8 pixels (from a mean of 473.0), and ± 0.6 pixels in the y direction (from a mean of 34.2), indicating the technique was highly repeatable. For the Basler camera, the horizontal spatial resolution was 640 pixels, giving a ratio of around 2.1 pixels/cm for a 3m field of view. This means that the SD reported of 0.8 pixel equates to a manual digitising error of about 0.4cm. For the Canon camera, with a horizontal resolution of 720 pixels, the error is around 0.3cm. In the vertical direction, the resolution for the Basler camera was 480 pixels for a field of view of around 2m and so the SD of 0.6 pixels equates to an error of around 0.3cm. For the Canon, the spatial resolution of 526 pixels equates to an error of around 0.2cm. Therefore, the effect of measurement error on lengths was considered negligible. These results are also very similar to the 0.5cm maximum accuracy of marker placement reported by Clayton and Schamhardt (in Back and Clayton, 2001), indicating the method was accurate.

Due to difficulties in controlling data capture at evaluation events, data could not be obtained for all horses. This was especially the case for the youngest horses, where data for six of the sixteen horses were not of sufficient quality to be included. Therefore, results for this group should be interpreted with caution.

3.5.1. Growth

Since stride parameters are related to leg length (Section 3.2), changes with age are likely as the leg grows. This means that stride parameters are not likely to show adult values until skeletal growth is complete, and therefore it is essential to know when growth has ceased. MC length, indicative of leg length, showed a significant strong increase with age (0.827, $p=0.000$) until around 36 months (Figure 3.8, Table 3.1),

consistent with a previous study that reported a cease of radius growth at 36 months (Fretz *et al.* 1984). There was minimal growth in adult horses (0.378, $p=0.05$) and no significant change in the oldest horses ($p=0.153$). The apparent decrease in length in the oldest horses (coefficient = -0.315) may be spurious due to the high variability of data in this group.

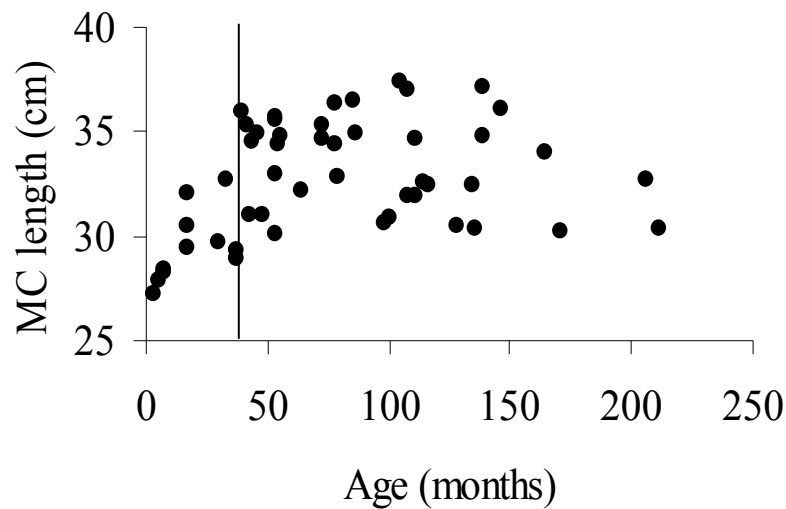


Figure 3.8 Metacarpus (MC) length increased until around 36 months, indicated by the vertical line

Group	Correlation coefficient (p value)
Young (<35 months)	0.827 ($p=0.000$)
Adults (36-99 months)	0.378 ($p=0.050$)
Old (>100 months)	-0.315 ($p=0.153$)

Table 3.1 Metacarpus length, representative of leg length, showed a strong increase in young horses and no significant change in older horses.

These results suggest that stride parameters during growth will be influenced by the change in leg length. Stride variables are therefore discussed in both their raw form, and once normalised to leg length.

In horses older than 36 months, MC length showed a fair degree of variation (approximately 6cm), and therefore results for non-normalised step length and frequency are discussed in the light of this.

As expected, step length showed a significant positive correlation with MC length (Figure 3.9), with a correlation coefficient of 0.937 ($p=0.000$).

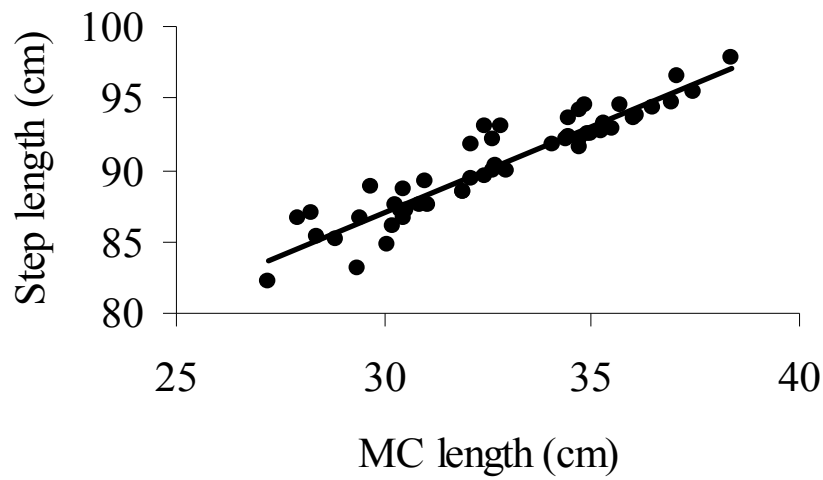


Figure 3.9 Step length showed a strong positive correlation with MC length

This was expected, given that leg length is a major factor in determining step length (Section 3.2.1), and is consistent with previous findings in both humans (Ganley and Powers 2005; Vaughan *et al.* 2003) and horses (Back *et al.* 1995b).

3.5.2. Step length

In general, step length increased in the growing horse and showed a gradual decrease towards old age (Table 3.2, Figure 3.10). The high correlation in Group 1 (0.806, $p=0.009$) and minimal correlation with age in Group 2 (0.273, $p=0.208$) and 3 (-0.264, $p=0.290$) indicated that step length increase stopped once limb growth became minimal at around 36 months.

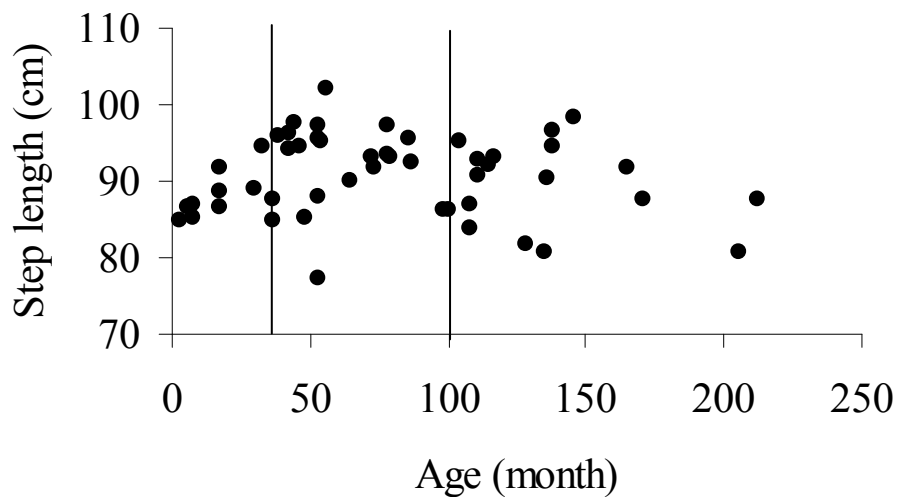


Figure 3.10 Step length showed an increase with growing leg length, and a decrease towards old age.

Group	Correlation coefficient (p value)	CV
Young (<35 months)	0.806 (p=0.009)	3.56
Adults (36-99 months)	0.033 (p=0.880)	5.89
Old (>100 months)	-0.216 (p=0.390)	5.98

Table 3.2 Step length showed an increase in the growing horse and a decrease in the older horses

In the youngest horses, the Pearson' correlation coefficient of 0.806 (p=0.009) indicates a strong positive correlation between step length and age. Step length is a product of limb range of motion (RoM) and length; and since RoM shows no significant change in the horse (Back *et al.* 1995b; Cano *et al.* 2001), the major determinant of step length is leg length (Section 3.2.1). Therefore, the increase in step length with age is expected, and is consistent with results from the human field (Beck *et al.* 1981; Cano *et al.* 2001; Leach and Cymbaluk 1986; Sutherland 1997) and the equine field (Back *et al.* 1994b; Back *et al.* 2002; Cano *et al.* 2001). Although horses in the studies by Cano *et al.* and Back *et al.* were in trot, and therefore not directly comparable, the general trend of an increase in step length with age is consistent with the current study.

Step length showed no significant change in the middle-aged horses (p=0.880). There was, however, a small but significant increase when data for Groups 1 and 2 were combined (0.352, p=0.048). This continuation of step length increase after limb growth had ceased may be an effect of training; one of the factors correlated with superior gait quality is a longer stride length (Holmström *et al.* 1994).

Beyond 100 months, step length decreased slightly with age, but the correlation was not significant (p=0.390). Although there is limited information on changes to step length in the older horse, this result is consistent with work into senescence from the human field. A decrease in step length has been well documented in ageing humans (Cofré *et al.* 2011; DeVita and Hortobagyi 2000; Judge *et al.* 1996a; Judge *et al.* 1996b; Kerrigan *et al.* 1998; McGibbon and Krebs 2004; Winter *et al.* 1990), and is likely to be due to both physiological degeneration and altered motor patterns (Judge *et al.* 1996b; Kerrigan *et al.* 1998; Winter *et al.* 1990), although the relative contribution of each has yet to be established.

The CV was smallest in the youngest horses and largest in the eldest horses, although the value was not particularly high. In elderly humans, step length variability has been

found to increase in older subjects (Beauchet *et al.* 2003; Kang and Dingwell 2008). Since increase in step length variability is also a factor in prediction of falls, it may represent a decrease in balance with age (Judge *et al.* 1996b). As a quadruped, however, the horse may have different methods of balancing from the bipedal human. Therefore ageing may not affect equine balance to the same extent, and the minimal increase in CV is expected. The increase in step length variability in the eldest horses may also have been due to the higher variability of leg length, indicated by MC length. However, variability is still relatively high once step length had been normalised to leg length (see below), indicating that there may have been an effect of decreased balance over and above the effect of leg length variability.

The general trend of step factor was a decrease in the young horse to a plateau at skeletal maturity (Figure 3.11, Table 3.3). Similarly, Back *et al.* (Back *et al.* 1994b; Back *et al.* 2002; Back *et al.* 1995b) found that adult stride length, once normalised to wither height, was relatively shorter than in foals.

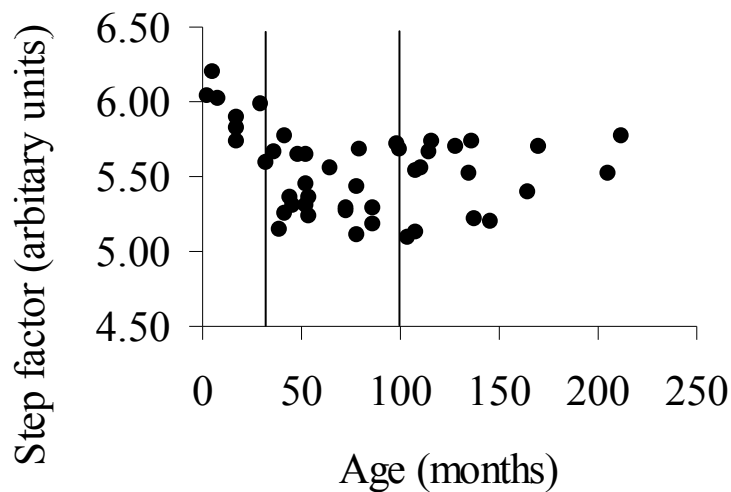


Figure 3.11 Step factor showed a decrease with age until 36 months, followed by a plateau

Group	Correlation coefficient (p value)	CV
Young (<35 months)	-0.71 (p=0.05)	14.13
Adults (36-99 months)	-0.052 (p=0.821)	8.97
Old (>100 months)	0.22 (p=0.420)	4.22

Table 3.3 Step factor showed a significant decrease in the young horse, indicating an effect of CPG maturation

In the growing horses, the correlation coefficient of -0.71 ($p=0.05$) indicated a significant decrease with age. However, the CV in young horses was much greater (14.13) than in either adult (8.97) or old horses (4.22) therefore these results should be interpreted with caution.

Since step length is highly dependent on leg length, step factor should remain constant and any deviation indicates the influence of another factor, such as CNS maturation (Sutherland 1997). The significant decrease in step factor in the youngest horses indicates that CNS maturation, as well as leg growth, affects step length.

Walking efficiency in humans has been found to be greatest when stride length is directly proportional to body height (Weyand *et al.* 2010). The onset of this directly proportional relationship occurs when there is no further change with age. As shown in Figure 3.8, the age of no further change of step factor is around 36 months, when limb growth ceases. This suggests that horses' step length is optimised for energy efficiency at this age.

There was no significant correlation with age in either adult ($p=0.821$) or old ($p=0.420$) horses, which was expected since leg growth had ceased.

3.5.3. Frequency

The general trend was a slight decrease in step frequency until around 100 months (Figure 3.12, Table 3.4).

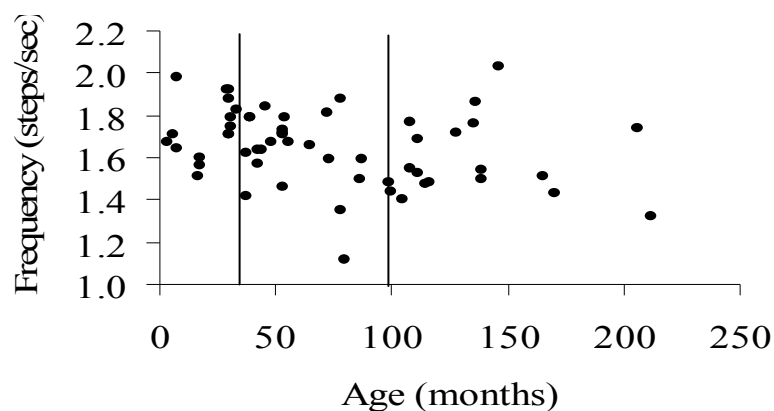


Figure 3.12 Frequency showed a significant decrease up to 100 months, followed by more scattered data

Group	Correlation coefficient (p value)	CV
Young (<35 months)	0.34 (p=0.223)	8.01
Adults (36-99 months)	-0.34 (p=0.100)	10.76
Old (>100 months)	-0.04 (p=0.880)	11.57

Table 3.4 There was no significant effect of age on frequency within each group. However, each group shows a high degree of variability.

In the youngest horses, there was a non-significant ($p=0.223$) increase with age. This increase may be spurious since the CV is large (8.01) and previous studies have reported a decrease in frequency in both the horse (Back *et al.* 1994b; Back *et al.* 2002; Cano *et al.* 2001) and the human (Beck *et al.* 1981; Ganley and Powers 2005; Sutherland *et al.* 1980; Vaughan *et al.* 2003). When the data for young and adult horses are combined, frequency does show a significant decrease (-0.38 , $p=0.02$) up to 100 months. This is consistent with the literature cited above, and also with the idea that frequency will decrease as the pendulum-like leg increases in length (Section 3.2.2.). The continuing trend of frequency decrease after limb growth has ceased may also be an effect of training, since one of the desired results of training a horse is a longer, slower stride (Back *et al.* 1994a; Holmström *et al.* 1994).

In the oldest horses, the minimal correlation indicates there was little influence of age. This may be because frequency is influenced mainly by leg length (Section 3.2.2), and since this shows no change towards old age, there is likely to be no resulting change in frequency. In humans, frequency does not usually change in the older subject (Judge *et al.* 1996b; Kerrigan *et al.* 1998; Winter *et al.* 1990).

The large CV in all three groups is possibly due to the relatively high variability of MC length, although since this remained high in the normalised data (see below), another factor may have caused the high CV. The CV may have been influenced by the three different surfaces that the horses walked on (compacted sand, concrete and tarmac topped with rubber matting). Surface type has been found to influence stride frequency in trot (Chateau *et al.* 2010; Chateau *et al.* 2009a) and although approximately similar numbers of horses walked on each surface within each age group, the different surfaces may have been a source of variability. In contrast to the current study, variability of frequency was found to be highest in the young human (Sutherland *et al.* 1980; Vaughan *et al.* 2003), probably due to the immaturity of the CNS. CV was largest in the

eldest horses, consistent with data from the human field that found gait variability increased towards old age, possibly due to deterioration of the CPGs (Beauchet *et al.* 2003; Hollman *et al.* 2007; Menz *et al.* 2003).

Once normalised to leg length, frequency showed a change only in the youngest horses (Figure 3.13, Table 3.5).

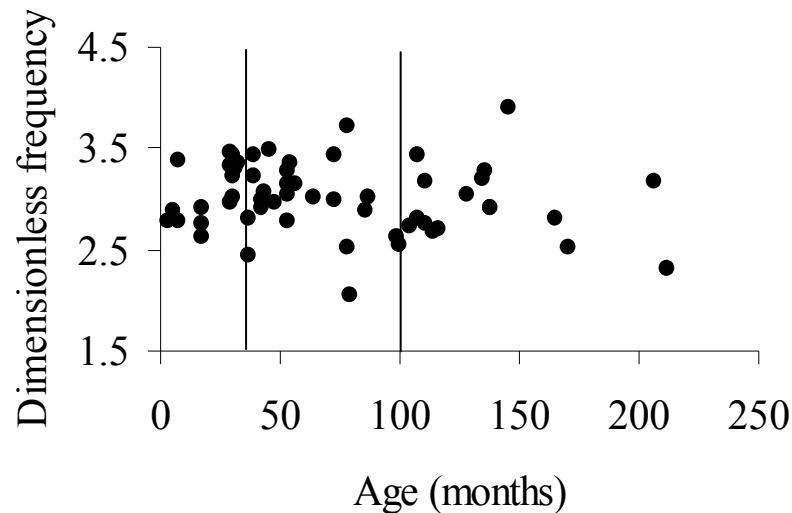


Figure 3.13 Dimensionless frequency showed no great trend with age

Group	Correlation coefficient (p value)	CV
Young (<35 months)	0.584 (p=0.02)	9.29
Adults (36-99 months)	-0.21 (p=0.325)	12.24
Old (>100 months)	-0.102 (p=0.687)	12.81

Table 3.5 Dimensionless frequency showed a significant increase with age in the young horse, indicating an effect of CNS maturation, but no further significant change.

The significant increase with age 0.584 (p=0.02) in the youngest horses could indicate an effect of CNS maturation once leg length had been taken into account. There was no further change with age beyond 36 months, possibly due to the variability of the data. The largest CV was found in the eldest horses, in line with data from the human field (Beauchet *et al.* 2003; Hollman *et al.* 2007; Menz *et al.* 2003).

3.5.4. Stance percentage

There was no significant correlation with age in the youngest ($p=0.158$), adult ($p=0.132$) or oldest ($p=0.537$) horses (Figure 3.14, Table 3.6). However, there was a small but significant positive correlation of 0.31 ($p=0.05$) when data for young and adult horses were combined.

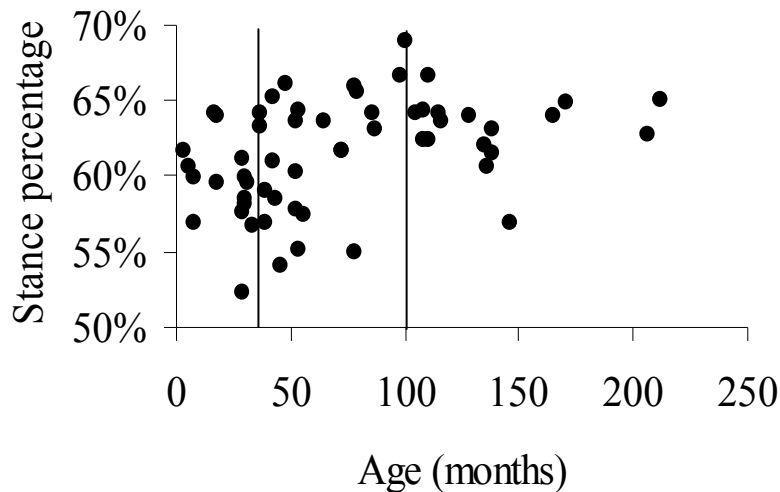


Figure 3.13 Once results for groups 1 and 2 were combined, stance percentage showed an increase up until 100 months

Group	Correlation coefficient (p value)	CV
Young (<35 months)	-0.38 ($p=0.158$)	4.99
Adults (36-99 months)	0.32 ($p=0.132$)	6.25
Old (>100 months)	-0.16 ($p=0.537$)	3.94

Table 3.6 There was no significant change in stance percentage within each group, possibly due to the stability of the quadrupedal horse.

Previous studies also reported a significant increase in stance percentage between four and 26 months (Back *et al.* 1994b; Back *et al.* 1995b) and between 12 and 36 months (Cano *et al.* 2000), although this increase was not significant. The increase in stance phase percentage is in contrast to the likely effect of training; horses judged as having a good trot have been found to have a significantly smaller stance percentage than those with a poor trot (Holmström *et al.* 1994), indicating that training would decrease stance percentage. The increase in stance percentage is also in contrast to work in the developing human, where both stance phase percentage and single-limb support increase as double-limb support decreases (Sutherland *et al.* 1980) - although horses make use of a different method of support from the human as described in Section 3.2.3.

The explanation offered by Back *et al.* (Back *et al.* 1995b) for the increase in stance *duration* is that the CoM of the adult horse, with longer limbs, covers a greater distance between the pro- and retracted positions. These authors therefore conclude that, at the same speed, the time taken to complete the stance phase will be greater in larger horses. However, the longer limbs will also increase the time taken to complete the swing phase. Therefore stance *percentage*, as a proportion of the total stride cycle, would remain the same. The increase in stance percentage seen in both the current study and in two studies by Back *et al.* may be because the CoM moves through a greater distance during the stance phase than the limb moves during swing, taking a longer time and therefore increasing stance percentage. To investigate this, however, requires further data capture that is outside the scope of this thesis.

In the eldest horses, there was no significant correlation between age and stance percentage ($p=0.537$). This result is contrary to studies from the human field, which report an increase in both stance percentage (Judge *et al.* 1996b; Winter *et al.* 1990) and double support time (Kerrigan *et al.* 1998) towards old age. However, as a quadruped, the horse has a larger base of support and therefore may not need to make use of increased stance percentage for gait stability. The similarity of CV across all three age groups indicates that age did not greatly influence the variability of stance percentage.

To separate the effect of limb growth from CPG maturation, the percentage of stance was normalised to leg length. After a decrease with age in the youngest horses, there was no significant correlation with age (Figure 3.15, Table 3.7).

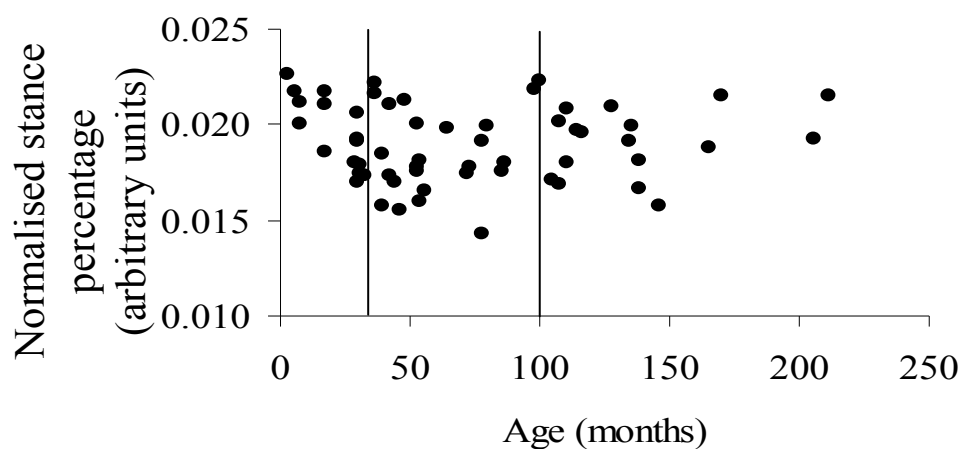


Figure 3.15 Normalised stance percentage showed a significant decrease up to 36 months, followed by no significant change

Group	Correlation coefficient (p value)	CV
Young (<35 months)	-0.791 (p=0.000)	9.44
Adults (36-99 months)	-0.027 (p=0.901)	11.69
Old (>100 months)	0.137 (p=0.587)	9.66

Table 3.7 Stance percentage as a function of leg length showed a significant negative correlation in the youngest horses

The significant negative correlation seen in the youngest horses (-0.791, p=0.000) indicates an effect of CNS maturation and, possibly, of training. The CV was fairly similar across all three age groups, although slightly higher in Group 2.

3.5.5. Velocity

The overall trend was an increase with age in the young horse, which reached a plateau at skeletal maturity, followed by more scattered data (Figure 3.16 and Table 3.8).

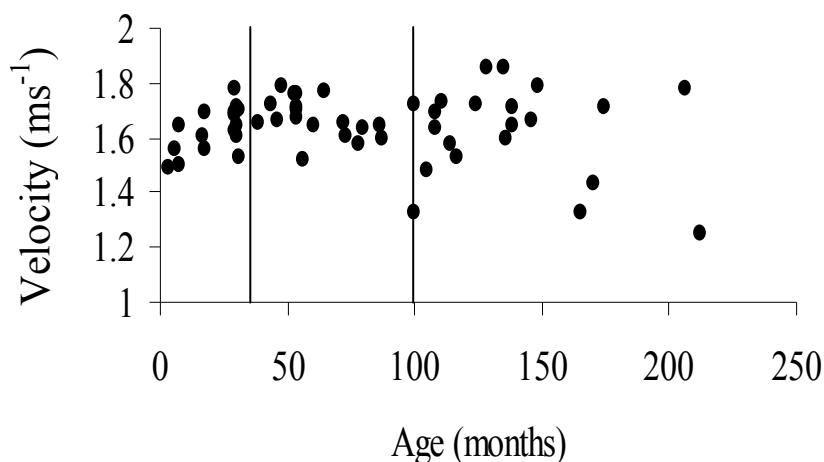


Figure 3.16 Velocity increased until skeletal maturity, with increasing variability towards old age

Group	Correlation coefficient (p value)	CV
Young (<35 months)	0.58 (p=0.02)	5.04
Adults (36-99 months)	-0.20 (p=0.301)	6.43
Old (>100 months)	-0.45 (p=0.164)	12.07

Table 3.8 As expected, velocity increased in the youngest horses and showed no significant change towards old age.

Velocity, a product of step length and frequency (Section 3.2), showed a significant increase in young horses (0.58, $p=0.02$). This is expected, since step length increased and frequency showed minimal change (Sections 3.5.2 and 3.5.3). In the human, velocity has been found to increase from one to seven years old, with the most rapid increase occurring between three and five years old (Sutherland *et al.* 1980) alongside the most rapid increase in leg length. However, the difference between child and adult velocity was reported to be not significant (Ganley and Powers 2005).

There is a large body of evidence to suggest that walking at an optimum speed minimises energy costs in both humans (Peterson and Martin 2010; Weyand *et al.* 2010) and horses (Hoyt and Taylor 1981; Minetti *et al.* 1999; Wickler *et al.* 2000), because this speed allows maximal interchange of potential and kinetic energy (Biewener 2006; Cavagna *et al.* 1977). Therefore, the cease in change of velocity at 36 months indicates that horses reach their optimum walking speed at this age, which is consistent with the finding that step length is optimised for energy efficiency at this age (Section 3.5.3).

There was no significant correlation between velocity and age in adult horses ($p=0.301$), which is expected since there is no change in either step length or frequency (Sections 3.5.2 and 3.5.3).

Towards old age, the correlation coefficient of -0.45 indicated that velocity decreased with age, although this was not significant ($p=0.164$). A decrease in velocity has been widely reported in elderly humans (Hollman *et al.* 2007; Judge *et al.* 1996b; Kang and Dingwell 2008; Kerrigan *et al.* 1998; Priest *et al.* 2008), mostly due to a decrease in step length (Judge *et al.* 1996a) since cadence is unchanged. The decrease in velocity may also be to maintain contractility of a weaker muscle (Section 3.3.4).

The CV of the oldest horses was also approximately twice as large (12.07) as in either the youngest (5.04) or adult (6.43) horses and is reflected in the scattered data points for this group in Figure 3.15. This high variability is consistent with previous studies in the human field and is indicative of gait instability (Hollman *et al.* 2007; Menz *et al.* 2003). Maintaining stability in the quadrupedal horse may be less challenging than in the bipedal human (Section 3.2.3), so the decrease in velocity and increase in variability may instead be due to degeneration of motor control of gait (Judge *et al.* 1996b) and loss of strength (Kang and Dingwell 2008).

An ANOVA test revealed that there was no significant difference in velocity ($p=0.4$) between the three groups, suggesting that changes to stride parameters and joint angles (Chapter 6) are independent of speed. Previous studies have also found no significant difference in velocity between foals and adults (Back *et al.* 1994b; Back *et al.* 1995b; Cano *et al.* 2001).

Dimensionless velocity showed no overall change with age (Figure 3.17, Table 3.9). This may be because dimensionless velocity was calculated as a product of two parameters that show an inverse relationship to each other (Figure 3.11 and 3.13). Also, in the growing horse, the increase in limb length may have had a greater influence on velocity than CNS maturation. The CV did show a general increase with age, consistent with changes due to deterioration of gait as described above.

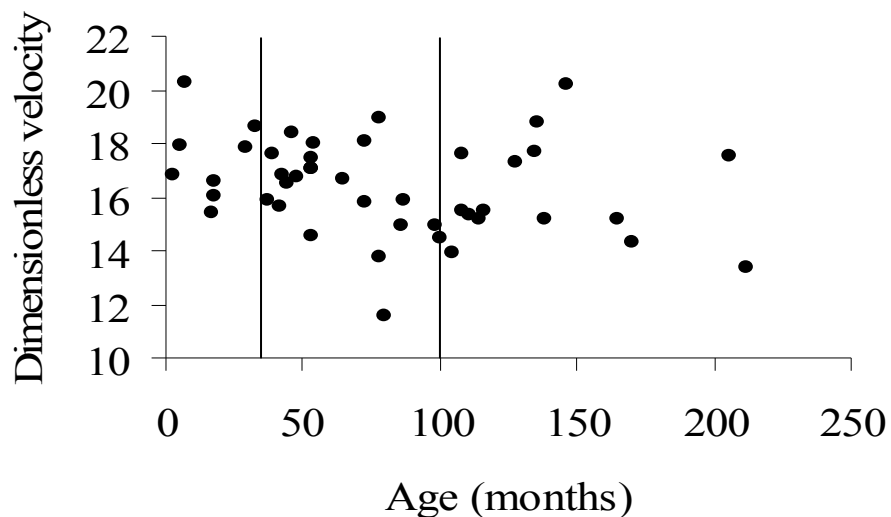


Figure 3.16 Age did not influence dimensionless velocity

Group	Correlation coefficient (p value)	CV
Young (<35 months)	0.335 (p=0.223)	8.01
Adults (36-99 months)	-0.344 (p=0.100)	10.76
Old (>100 months)	-0.038 (p=0.88)	11.57

Table 3.9 Dimensionless velocity showed no significant change with age

3.6. Conclusions

The overall aim of this chapter was to investigate the effect of age on equine stride parameters, and was split into three main areas. The first was to investigate the development of stride parameters; including separating the effects of the growing leg from the effect of CPG maturation and training by normalising stride parameters to leg length. The second aim was to provide values for stride parameters during degeneration of gait, since these have not previously been reported in the literature. The third aim was to compare stride parameters to previously-reported values to validate the main results of this thesis contained in Chapter 6. The close match between the stride parameters reported here with previously-reported data and trends indicates the validity of results given in Chapter 6. The conclusions from the other two aims will now be presented.

3.6.1. Development of stride parameters

As expected, step length and velocity increased in the youngest horses, probably as a direct result of the increase in leg length. Stance percentage and frequency did not show a difference in Group 1 alone, but did show a change when data for Groups 1 and 2 were combined. This indicates that training may continue to have an effect once leg growth has ceased after skeletal maturity. These results are consistent with (Morales *et al.* 1998) and (Holmström *et al.* 1994), who also found these results only in older horses, suggesting that these changes only occur after a certain amount of training.

Once normalised to leg length, all parameters except velocity showed a change with age. A lack of change in the normalised parameter with age would indicate that it is determined by leg length alone (Sutherland 1997), so the further change with age indicates the influence of another factor such a development of the CPGs or training. The low variability of gait in the young horses indicates that the CPGs are mature at a young age, consistent with the horse's need to escape from predators from the moment of birth. The change seen in normalised parameters could therefore represent a fine-tuning of the CPGs and the effect of training. Various studies by Back *et al.* (Back *et al.* 1994b; Back *et al.* 1995b) into the developing horse also reported a low CV and similar angle-angle diagrams between the four month-old foal and the adult, and concluded that equine kinematics are mature at a very young age. This decrease in variability with age

has also been reported in human studies (Gorton *et al.* 1997; Lasko-McCarthy *et al.* 1990; Sutherland 1997; Sutherland *et al.* 1980).

In conclusion, growth of the limb pendulum leads to an increase in step length and velocity. Stance percentage and frequency change beyond the cease of leg growth, possibly due to training. The low variability of gait indicates that CPGs are mature when the horse is just months old, and the change seen in most normalised parameters was possibly due to the fine-tuning of the CPGs through training.

In practical terms, the horse owner should understand that gait changes are likely to occur with leg growth up until skeletal maturity at around three years old. After this time gait is likely to depend more on the type and quantity of training given, and therefore owners can choose the most appropriate training regime for their horse.

3.6.2. Degeneration of gait parameters

Towards old age, step length and velocity decreased - although not to a significant degree - and the variability of velocity increased. These changes were probably as a result of the deterioration of both physiological and neural parameters (DeVita and Hortobagyi 2000; Judge *et al.* 1996b; McGibbon 2003; Winter *et al.* 1990). Since methods of balance in the horse are different from in the human, the changes are more likely to represent physiological deterioration. The increase in variability may also have been due to the variability of leg length in the older horses, but since the normalised parameters also showed a high variability, this is more likely to be an effect of degeneration of balance.

There was minimal change in frequency or stance percentage. The negligible change in frequency is consistent with data from the human field and is probably because leg length, the primary influence, does not change towards old age. The minimal change in stance percentage is in contrast to human studies, where an increase was seen that may be in compensation for reduced stability. However, the quadrupedal horse can exploit static equilibrium in walk and therefore its stability may be less affected by age. It could also be that instability occurs in only the very eldest horses, and that a change would have been seen if very old horses had been included in the study.

Normalised parameters showed no change with age in the eldest horses, indicating that leg length alone was influential. This is expected, since CPGs were mature.

In conclusion, ageing affects step length and (therefore) velocity but has little effect on frequency and stance percentage, possibly because the horse is relatively stable. In practical terms, owners are unlikely to be able to alter the gait of an older horse to a great degree in terms of training, but could possibly track velocity and stride length to indicate possible deterioration of the CPGs and therefore be aware of stability issues, especially in the very eldest horses.

3.6.3. Limitations of the study

There were four limitations to this part of the study: limited step length data for young horses, a relatively young upper age limit, inconsistent surface type and the assumption that no horse was suffering from a musculoskeletal disorder.

The first limitation of this study is that stride parameters could not be obtained for all horses due to difficulties in controlling data capture conditions. The worst-affected parameter was step length in Group 1, where data for six out of sixteen horses were not of sufficient quality to be included. This means that step length and factor for this group should be interpreted with caution and the conclusions drawn take this into account. A future study would control data capture to ensure all parameters were of sufficient quality.

A second limitation was that the oldest horse in this study was 17.6 years old, over ten years younger than the average lifespan of a horse. Ideally, the effects of ageing could have been studied through to the very old horse. However, since horse passports only came into force in 2004³, accurately determining date of birth is difficult, especially for the older horses which would have provided useful ageing information.

A third limitation is that horses walked on three different types of surface. Although frequency was shown to be influenced by surface type in trot (Chateau *et al.* 2010; Chateau *et al.* 2009a), it is unlikely to have a major impact in walk. The discussion of

3 Source: <http://www.legislation.gov.uk/uksi/2004/1397/regulation/1/made>

influence of surface type on joint angle (Section 6.4.1) concludes that there was no significant difference, indicating that surface type was not influential.

A final limitation was that although each horse underwent regular clinical examination and those filmed at evaluation events were also presented for veterinary inspection, none were subjected to full assessment due to the high cost. Therefore, the effect of impairment could not be separated from the effect of ageing. These two factors affect gait in different ways in humans (McGibbon and Krebs 2004) and therefore it cannot be confirmed that the older horses were free from impairment that was not clinically evident. A future study could examine the stride characteristics of horses with a known impairment, such as arthritis, to provide a database of distinguishing features of pathological gait, especially when these changes are subtle.

Due to these limitations, the data from this chapter should possibly be considered as preliminary, especially in the growing horse. Future studies could correct these and possibly compare the data to the results presented here.

3.6.4. Further work

In humans, ageing has been found to cause a redistribution of the motor pattern compared to children and adults (Cofré *et al.* 2011; DeVita and Hortobagyi 2000). This altered motor pattern is reflected in redistributed joint moment and power, and changes the contributions of muscle groups at various joints. Future work using inverse dynamics could combine the joint angle data described in Chapter 6 with kinetics to obtain joint moment and power, to investigate how these are redistributed with age. This would provide an insight into the changes in motor patterns and serve as a more sensitive indication of the deterioration of CPGs with age.

A further extension of this work could be to obtain step width as an indication of gait stability (Judge *et al.* 1996b) during maturation and degeneration of gait. The recently-developed marker-free system that operates in the coronal plane (described in Section 5.1.1) may be suitable to detect changes in stride width.

4. The current state of the art of motion analysis

4.1. Introduction

Motion analysis is necessary to provide an objective measurement of the quality of gait and allows quantitative comparison, for example evaluation of surgical intervention (Baker 2006). Movement analysis is also used for assessment of athletic performance as well as having widespread use in research.

The technique selected will depend on the nature of the data required and on the complexity of the application. For example, whilst it is unlikely that a veterinary surgeon will benefit from a finite element model of the bones of a joint that requires surgery, an objective measurement of the motion of that joint before and after surgery could be useful to determine the success of the intervention.

This chapter provides an overview of the current state of the art of motion capture, including techniques to capture kinematic and kinetic data.

4.2. State of the art of kinematic analysis

Kinematic analysis describes how body segments move through space during a given time, without reference to the associated forces (Barrey 1999). Scientific analysis of equine kinematics began in the 1870s with the work of Muybridge and Marey (Barrey 1999), who paved the way for modern gait analysis and, as a consequence, modern cinema (van Weeren, in (Back and Clayton 2001)), Figure 4.1.

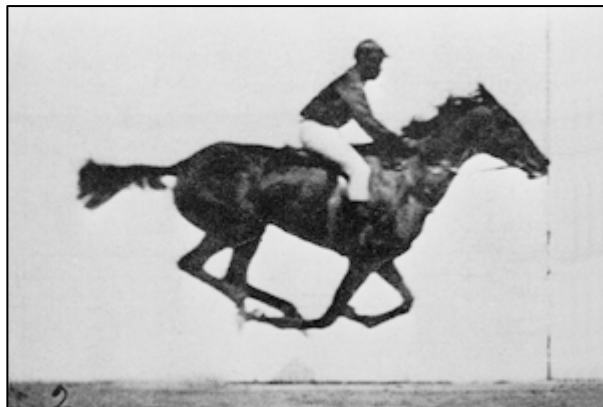


Figure 4.1 In the late 1800s, Eadweard Muybridge produced a series of stop-motion photographs of human and animal locomotion. This technique was the precursor of modern kinematic gait analysis.

In the early part of the 20th century, the field of kinematic study was dominated by the Germans, primarily by anatomists responsible for investigating muscle function and the link between conformation and locomotion (van Weeren, *ibid.*). After the Second World War, the use of the horse as a working animal declined in favour of mechanisation. With the increasing economy from the 1960s onward, greater prosperity allowed much wider access to equestrian sports. This changed the role of the horse from a military and agricultural tool to an athlete. Assessment of athletic performance requires objective and sensitive measurement techniques, and the advances in computing technology throughout the 1970s allowed gait analysis to progress to the sophisticated techniques of modern times (Barrey 1999).

Several techniques currently exist to record kinematics, from a simple video camera to more complex inertial and opto-electronic systems (OES). Simple systems can track gait in two dimensions (2D), which is sufficient for most equine applications since movement occurs primarily in the sagittal plane (Chateau *et al.* 2004). However, for more detailed analysis, a more expensive three-dimensional (3D) system may be required. The current state of development of these systems is discussed in the following sections.

4.2.1. Video-based systems

One of the simplest and least expensive methods to capture equine locomotion is with a video camera. This technique has been used in applications such as characterising motion in the forelimb (Hodson *et al.* 2000), the effect of trotting speed and incline on hindlimb parameters (Hoyt *et al.* 2002) and investigating the stride parameters associated with fatigue (Wickler *et al.* 2006). Video can often be input straight into a PC (Figure 4.2) to track the motion of interest. The subject can be captured either with markers or marker-free, which overcomes the limitations of marker-based tracking described in Section 4.2.5. This thesis makes use of a marker-free tracking technique, whose development and validation is described in Section 5.2.



Figure 4.2 Simple video-based systems can yield useful data

As well as requiring little specialised equipment, video capture is not susceptible to sunlight interference that can affect infra-red systems. This means that capture can take place away from a laboratory environment, for example at competition or evaluation events. A video-based system can often be used in conjunction with an OES to provide information on the validity of a trial, for example if the subject's gait patterns were abnormal.

The main drawback of a video-based system is that the post-processing is required, and therefore gait data is not available in real-time. Obtaining 3D data requires more complex techniques, such as triangulation to obtain co-ordinates in all three dimensions.

4.2.2. Opto-electronic systems

An OES records the position of skin-mounted markers that are either active, emitting their own signal, or passive, reflecting light emitted by an external source. Infra-red light is often used in passive systems to maximise the contrast between markers, that reflect the light, and the background, which absorbs it.

First, the cameras are set up to ensure that the field of view is optimal for the required application, and their positions are identified by a calibration process. Markers are placed on the subject, whose movement is then captured. The position of each marker, which is usually spherical to maintain a circular outline from any viewpoint, is

identified using edge detection routines. Once the markers have been identified, usually in real-time, their co-ordinates within the capture volume are determined automatically. Since the position of each camera is known, the 3D location of each marker can be found using triangulation. If a marker is lost through occlusion, it can be reconstructed by the user. Finally, the segmental movement can be reconstructed and viewed from any perspective.

OES are commonly used in analysis of equine gait, including assessment of the longitudinal development of locomotion (Back *et al.* 1994b; Back *et al.* 1995b), the effect of training (Back *et al.* 1999), the influence of size (Bullimore and Burn 2006), changes with injury (Clayton *et al.* 2000a) and the behaviour of the joint under load (McGuigan and Wilson 2003). Despite their popularity, there are two main drawbacks of OES: skin displacement artefact, which is discussed fully in Section 4.2.5, and sunlight artefact. In outdoor or sunlit conditions, superfluous light can make it difficult to determine the edges of the markers and may create artefact when reflected from background objects. Even recent studies have raised the issue of sunlight interference; for example being limited to overcast or twilight conditions (Ren *et al.* 2010), or advising the use of a blind to minimise the effects of sunlight (Heike *et al.* 2010). Filters can be used to reduce this effect, and outdoor systems have started to become available, but both of these solutions are expensive. An alternative is to conduct the trials in an indoor laboratory environment where sunlight levels can be controlled. However, laboratory-based trials are usually expensive to run (Barrey 1999; Simon 2004) and have only limited space for movement. Although treadmills can be used for continuous capture of gait, treadmill-based motion does not necessarily represent normal overground gait in either humans (Mündermann *et al.* 2006) or horses (Buchner *et al.* 1994), and the equipment is expensive to install and maintain.

4.2.3. Accelerometer-based systems

The use of accelerometry to analyse gait began as far back as the 1930s (Liberson 1936), but only began to find widespread use in the 1970s (Morris 1973) when computing power became sufficient to process the raw signal efficiently.

There are two basic types of accelerometer: the mass-spring and the bending beam system. The mass-spring system detects compressive or tensile forces that occur during movement. The displacement, x , of the mass causes a directly proportional restoring force in the spring, and since both spring stiffness k and mass m are known, the acceleration of the mass component can be calculated from $a = kx/m$ (Kavanagh and Menz 2008; Mathie *et al.* 2004). The methods of measuring the displacement, and therefore acceleration, of the mass depend on the class of sensor used. Classes include piezo-electric, piezo-resistive, variable capacitance and the more modern thermocouple sensor (Godfrey *et al.* 2008; Mathie *et al.* 2004). In the second type of accelerometer, two charged plates are held apart, one supported on a cantilevered beam and the other attached to an independent structure. An acceleration causes the beam to deflect from its starting position, changing the distance between the two plates and therefore the capacitance. Since capacitance is proportional to applied acceleration (Godfrey *et al.* 2008), acceleration can be calculated from the change in capacitance (Sidek *et al.* 2009).

Advantages of accelerometry include direct measurement of acceleration, which reduces the inaccuracy associated with the differentiation of displacement data (Kavanagh and Menz 2008). The small size of the sensors and the insensitivity of the system to sunlight interference allow its use outside of a laboratory. Modern systems are comparatively inexpensive and can record continuously over a long period of time (Godfrey *et al.* 2008; Mathie *et al.* 2004). The technique has been successfully applied in an equine environment, for example when assessing lameness (Keegan *et al.* 2004), trunk movement (Pfau *et al.* 2005) and the influence of track surface (Chateau *et al.* 2009a; Leleu *et al.* 2004).

The main disadvantage of accelerometry sensors is that they are susceptible to skin and soft tissue displacement artefact as described in Section 4.2.5. Furthermore, this technology requires periodic calibration to reduce signal drift due to gain fluctuations or changes in temperature (Kavanagh and Menz 2008). Although calibrating the system once before a trial is acceptable, periodic calibration produces more accurate results. To eliminate the influence of rotational motion on linear acceleration, the sensors must be carefully placed or a gyroscope can be added to the system (Godfrey *et al.* 2008; Kavanagh and Menz 2008). Additional instruments, which can include a magnetometer

to provide direction, transform the sensor into an inertial measurement unit, combining data from all components to improve accuracy (Boonstra *et al.* 2006).

This technology has been found to give similar results to an OES for sagittal plane kinematics (Boonstra *et al.* 2006; Cloete and Scheffer 2008; Mayagoitia *et al.* 2002). However, recent attempts to validate accelerometry have been hampered by inaccuracies introduced when the displacement of marker data is differentiated (Kavanagh and Menz 2008).

4.2.4. Goniometers

A final type of kinematic assessment technique is the goniometer, which consists of two mechanical arms connected by a potentiometer (Clayton and Schamhardt, in Back and Clayton 2001). The centre of this device is placed over the joint's centre of rotation, with each arm aligned along a limb segment. There are two types of goniometer: manual and electronic. Manual goniometers are used for static measurements of a joint angle but are not in common use in horses due to low inter-operator reliability (Lijebrink and Brink 2010). In an electronic goniometer, rotation of the joint causes a change in the goniometer's electrical resistance, recorded by the potentiometer as a voltage which can be converted to joint angle through calibration. These are occasionally used to track equine kinematics (Alexander 2001; Weishaupt 2002, 2004), but require careful fixation to ensure alignment with the segments to be tracked.

4.2.5. Limitations of skin-mounted markers in motion capture

The system described above - OES, inertial systems and goniometers - use skin-mounted markers or sensors. Although this is the most common method of kinematic analysis (Mündermann *et al.* 2006), skin-mounted apparatus carries inherent limitations. This will now be explored, using the term "marker" to denote a marker used in OES capture, a sensor used in inertial capture or a goniometer.

One of the primary limitations of the study of deformable tissues is that markers placed on the skin do not necessarily represent the movement of the underlying bone (Alonso

et al. 2007; Günther *et al.* 2003). For example, skin displacement artefact has been found to be as great as 40mm at the human hip joint (Cappozzo *et al.* 1996), and up to 115mm at the cranial part of the greater trochanter in horses (van Weeren *et al.* 1990). Artefact can be composed of several independent factors, including skin deformation and sliding, muscle contraction, inertial effects and the interference of adipose tissue and non-contracted muscles (Fuller *et al.* 1997; Mündermann *et al.* 2006). As mentioned by Leardini *et al.* (Leardini *et al.* 2005), the frequency content of this type of artefact is very similar to genuine bone movement, making it difficult to separate the two. Although skin movement in horses is more pronounced in the proximal limb than in the distal limb, a skin displacement of 5mm has been recorded at the distal metacarpus (van Weeren *et al.* 1988). Pins inserted directly into the bone remove the interference of skin displacement and therefore provide a more accurate representation of bone movement, but this technique is highly invasive.

A second consideration is the high-frequency, low-amplitude noise caused by vibration of the markers with respect to the underlying bone. This leads to error when raw OES displacement signals are differentiated to provide velocity and acceleration data (Alonso *et al.* 2007), although the authors report a smoothing technique to produce acceptable results. Additionally, extensive filtering may eliminate components of the genuine signal (Kavanagh and Menz 2008).

A third disadvantage of current marker-based technology is that the physical markers can interfere with motion, especially on small subjects (Mündermann *et al.* 2006; van sint Jan *et al.* 1997). This problem is decreased with the use of modern cameras, whose greater resolution can track smaller markers. Markers can also self-occlude (Mündermann *et al.* 2006) and although this can be overcome with the use of multiple cameras, this is an expensive solution.

A final consideration is marker placement error, in terms of both inter- and intra-operator repeatability. Locating landmarks is affected by three factors: the difficulty of reducing a large surface to a single landmark; the influence of the soft tissue that covers the landmark; and the effect of palpation technique (Della Croce *et al.* 2005). Inter-operator error has been found to be the primary cause of variability of measurements in humans (Gorton *et al.* 2009) and can lead to variations of up to 25mm (Della Croce *et*

al. 1999). Intra-operator variability of placement has been found to be as large as 21mm in the human pelvis and lower limb (Della Croce *et al.* 1999). In horses, the maximum accuracy of marker placement is around 5mm (Back and Clayton 2001), leading to difficulties in marking exactly the same landmark on multiple occasions. Another factor is the length of time between assessments; whereas inter-session repeatability has been found to be low in the frontal and coronal planes, intra-session repeatability has been found to be much higher (Kadaba *et al.* 1989). Inaccuracy of marker placement could mean that useful information is lost within noisy data (Schwartz *et al.* 2004), especially if the data are on a small scale (Della Croce *et al.* 1999). Although the technique used within this thesis is marker-free, the first stage of the tracking process involves manual identification of the points to be tracked (Section 5.1.1), and the influence of manual placement is discussed in the same section.

4.2.6. Summary of kinematic analysis

Kinematic gait analysis quantifies the positions and orientations of anatomical segments in space, over time. Skin-mounted, infra-red, systems currently suffer from drawbacks including soft tissue displacement artefact and sunlight interference, although further developments in computing technology may help to overcome these limitations.

4.3. Current state of the art of kinetic analysis

Kinetic analysis is the study of forces, which can either be measured directly using force plates or shoes, or indirectly with strain gauges. Although it provides useful information, kinetic analysis can be more complex than kinematic analysis (Clayton 2005; Godfrey *et al.* 2008).

In the 1880s, Marey was one of the first to attempt to measure the forces produced by a moving horse, using instruments such as an air-filled rubber ball attached to a horse's shoe (van Weeren, in Back and Clayton 2001). In the 1930s, Fenn (Fenn 1930) and Elftman (Elftman 1939) developed more sophisticated force platforms, and Manter designed a platform that could record forces in all three dimensions (Manter 1938),

serving as the precursor for modern force plates (Figure 4.3). The 1960s and 1970s saw development of this technique (Bertram 2007; Cavagna 1975) with advanced computing power, and also the introduction of the force shoe (Fredricson 1970), which today can accurately measure forces in all three dimensions on a variety of surfaces (Chateau *et al.* 2009b). Both force plates and shoes can be used to measure 3D ground reaction force (GRF) and centre of pressure (CoP).



Figure 4.3 Modern force plates can record useful data but require specialised installation

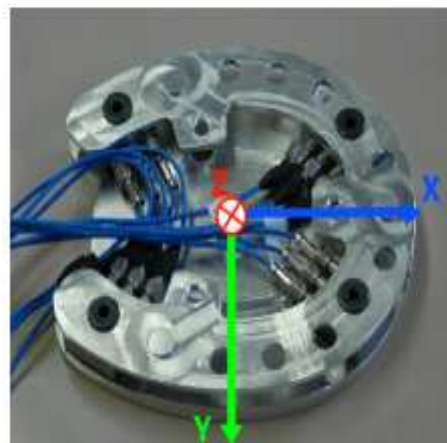
Analysis of GRF, especially the vertical component, provides valuable information on the changes in locomotion associated with lameness. Since lameness is an adaptation of normal gait to reduce limb loading and therefore pain (Clayton 2000; Weishaupt 2006, 2004), force plates have been widely used to detect changes in the kinetic characteristics of the lame horse (Buchner 1996; Clayton 2000; Dow 1991; Merkens 1988; Weishaupt 2006, 2001; Williams 1999), including the asymmetry of loading and a decrease in peak vertical force. This type of analysis is valuable for objective measurement of change that may be too subtle for detection by traditional human observation (Merkens and Schamhardt 1988).

The disadvantages of force plates are that they are expensive and require specialised installation (Oosterlinck *et al.* 2009) and it can be difficult to persuade some animals to step on the plate (Chateau *et al.* 2009b). Kinetic variables have been found to depend on factors such as linear velocity, habituation and body shape in horses and dogs (Gillette and Angle 2008; Khumsap *et al.* 2002; McLaughlin 1996). To limit the chance of more than one hoof striking the force plate at a time, the plate must be relatively small.

However, this limits the frequency of “clean” strikes - where the hoof hits the plate sufficiently far from the edges for the recording to be accurate. The strike rate can be as low as once in every six passes, especially at faster gaits (Merkens *et al.* 1993); a major drawback when assessing a potentially lame horse whose movement should be kept to a minimum. The problem of the low strike rate can be resolved using an instrumented treadmill, which has been used to measure equine ground reaction forces since the early 2000s (Weishaupt *et al.* 2002; Weishaupt *et al.* 2001). The disadvantages of this system are that it is expensive, it may not be suitable for measuring the effect of different ground conditions and treadmill locomotion may not represent overground movement (Buchner *et al.* 1994). To overcome some of these issues, instrumented force shoes can be used. These have been in development since the 1970s (Frederick and Henderson 1970), but until recently they were not able to record forces in all three dimensions accurately. Although a force shoe now exists that yields results comparable to force plate data (Chateau *et al.* 2009b), it exists only in prototype form, must be modified to suit hoof shape, and its height of 20mm may influence locomotion (Figure 4.).



Figure 4.4 A prototype force shoe has been developed that shows comparable results to a force plate (Chateau *et al.* 2009b)



An alternative method to determine GRF has been developed that exploits the linear relationship ($r=0.95-0.99$) between MCP joint angle and limb force (McGuigan and Wilson 2003). This method is limited to measurement of the vertical GRF, but since it is this component that is correlated with musculoskeletal damage (Clayton 2000; Dow

1991; Merckens 1988a, b), results would be relevant. An alternative method proposed by Witte *et al.* (Witte *et al.* 2004), using the inverse relationship between stance time and peak vertical force, found a relatively good prediction of peak vertical GRF in trot but considerably overestimated the force in walk and canter. This may have been due to assumptions about the shape of the GRF curve and the distribution of body mass between the front and hind limbs. A more complex method, developed by Bobbert *et al.* (Bobbert *et al.* 2007), gave a close match to GRF measured by force plate in both walk and trot. This method used the linear and angular velocities and accelerations of individual segments to calculate the acceleration of the centre of mass (CoM) of the trunk. The CoM acceleration is then combined with rate of change of angular momentum to determine the moment arm of the total GRF with respect to the hooves in contact with the ground. The ratio of these moment arms indicates the relative contribution to GRF from each supporting limb for calculation of individual limb forces. In summary, vertical GRF can be predicted from stance duration and kinematics, either using a simple method or a more complex but more accurate system.

A final tool used in kinetic analysis is a pressure mat, which can record pressure distribution, vertical force and the timing of stance phase (Gillette and Angle 2008). The main advantage of pressure mats is that they can measure consecutive foot strikes during a single pass (Gillette and Angle 2008) as they are composed of many sensors. However, studies have found significant differences between pressure mat and force plate measurement of critical variables such as peak vertical GRF and vertical impulse (Oosterlinck *et al.* 2009).

In conclusion, kinetic analysis often requires specialised equipment but can yield useful data that may be too subtle to detect by eye.

4.4. Other measurement techniques

4.4.1. Strain gauges

Strain gauges are used to measure strains in tissues under an applied load. Deformation of the tissue also causes the strain gauge to deform, which changes its electrical resistance and produces an output voltage. Since the degree of strain is proportional to

the load applied, assuming ideal elastic properties, the output can be used to calculate the load applied in a given direction. Three gauges are often combined in a “rosette” to measure strains in all three dimensions, each requiring wiring and strain amplifiers (Thomason 1998).

Strain gauges can be applied to hard tissues such as bone and hoof, or to soft tissue such as tendon and ligament (Clayton and Schamhardt in Back and Clayton 2001, Barrey 1999). Hoof deformation has been measured under various conditions, such as during heel elevation (Bellenzani *et al.* 2007) and to measure the effect of different therapies for laminitis (Hansen *et al.* 2005). The deformation of long bones such as the third metacarpus has also been quantified using strain gauges (Davies 2005; Merritt *et al.* 2010). The limitations of measuring strain in bones is that it is invasive, the site must be carefully prepared, measurement is limited to subcutaneous bone to minimise soft tissue trauma, and the initial length of the bone is hard to determine (Clayton and Schamhardt in Back and Clayton 2001). In both bone and hoof, the relationship between surface strain and load is complex (Clayton and Schamhardt in Back and Clayton 2001), but accurate finite element modelling has recently been used to characterise principal strains in the hoof (Salo *et al.* 2010).

Strain gauges can also be applied to soft tissue, for example to measure the effect of toe wedges on tendon load (Takahashi *et al.* 2006) or to investigate the contribution of muscle to locomotion (Butcher *et al.* 2009). Beyond the initial “toe” region of the stress-strain curve, the flexor tendons display a linear relationship between load and elongation until rupture (Riemersma and Schamhardt 1985). Since tendon fibres are aligned along the tendon axis, unidirectional strain gauges are usually sufficient. The disadvantage of using strain gauges to measure soft tissue deformation is that the insertion procedure is by necessity invasive and can damage the tendon fibres (Jansen *et al.* 1998). The data will also be limited to the specific site (Dowling and Dart 2005), and, as with hard tissue, determination of the initial length can be difficult (Clayton and Schamhardt in Back and Clayton 2001).

4.4.2. Electromyography (EMG) analysis

EMG records the electrical activity that precedes muscular contraction and therefore provides information on muscle activity. This technique has been used to diagnose neuromuscular problems (Wijnberg *et al.* 2004), assess the characteristics of muscle activity in the lame horse (Zaneb *et al.* 2009) and to investigate back function (Licka *et al.* 2009).

The electrodes that detect electrical potentials can be surface-based or fine-wire. The advantage of surface electrodes is that they can provide useful information on surface muscle activity and are non-invasive. However, since the electrodes can cover a wide area, unwanted signal from neighbouring muscles (or cross-talk) is common (De Luca and Merletti 1988; Koh and Grabiner 1993; Lowery *et al.* 2003; Türker and Miles 1990). Surface EMG can also be affected by impedance caused by hair, oil and dead skin cells (Gillette and Angle 2008). Fine-wire EMG offers greater specificity, but its main disadvantage is its invasive nature (Gillette and Angle 2008). Not only does an invasive technique lead to concerns for equine welfare, but the insertion process causes superfluous electrical activity (Wijnberg *et al.* 2003).

4.5. Summary

Equine gait has traditionally been assessed by human eye, which may be unable to detect important but subtle changes (Dow *et al.* 1991; Merkens and Schamhardt 1988), can be subjective (Merkens and Schamhardt 1988; Weishaupt *et al.* 2001) and can lack consistency (Arkell *et al.* 2006; Keegan *et al.* 2010). Quantitative analysis of motion allows objective measurement of gait in a range of applications from assessment of the success of surgery to analysis of sport performance. Motion analysis includes kinetics, which is the analysis of force, and kinematics, which is the analysis of a body segment moving through space.

Methods of kinetic analysis often involve the use of specialised equipment such as force plates and force shoes. Force plates require specialised installation and can suffer from a low strike rate. Force shoes or instrumented treadmills can be used to overcome this limitation, but both are still in the developmental stage.

Kinematics describes the movement of body segments, and includes joint angles and angular acceleration. The most commonly-used method of tracking body segments involves the application of reflective markers whose position is detected by specialised cameras. More recently, accelerometry systems have become available, which can be used outside of a laboratory environment. Both of these systems, however, use skin-mounted markers or sensors which are sensitive to skin movement relative to the underlying bone, marker vibration and physical interference of the markers. To overcome some of these limitations, and for use in an environment where marker application is impractical, a marker-free system has been developed. This system will be described in Chapter 5.

5. Methods development⁴

This chapter covers the new techniques developed and used within this thesis, and also describes the model used to calculate strain (Lawson *et al.* 2007). The development of a new marker-free system (MFS) is explained and its validation against established methods is discussed. Finally, the method of calculating mass for data normalisation is described and its validity is examined.

5.1. Development of the marker-free tracking techniques

Since around a third of the horses were filmed at evaluation events where marker application was not possible, a MFS was developed to track equine kinematics. The MFS can either sagittal-plane kinematics (MFSsa)⁵ which is the principle system used in this thesis, or in the coronal plane (MFSco). The main advantage of the MFS is that it is completely non-invasive as it does not even require contact with the subject. Therefore, the MFS is suitable for use when contact with the subject is impractical (for example motion capture of a wild animal), or when subjects should be unaware that capture is taking place, possibly in a human trial where gait must be as natural as possible. With no need to apply markers, trials are quicker than existing technology. The MFS also overcomes inaccuracies associated with marker-based systems such as skin and soft tissue movement artefact, and it is not affected by sunlight interference (Sections 4.2.1 and 4.2.3).

The MFS was custom-written in Matlab (The Mathworks, Inc., USA) as a fast, repeatable method of tracking the kinematics of the metacarpo-phalangeal (MCP) and distal inter-phalangeal (DIP) joints using input from a single video camera. The MFS tracked the equine distal limb throughout stance phase, based either on the properties of the selected pixels (MFSsa) or using a Sobel edge detection routine (MFSco).

⁴ Part of the contents of this chapter has been submitted to the Equine Veterinary Journal

⁵ The initial development of the MFSsa was carried out by James Lord.

Investigative trials were carried out to determine the optimum tolerances, but are these adjustable to suit the applications.

Pixel intensity: All pixels whose colour intensity (levels of red, blue and green) was within $\pm 4\%$ of that defined during initial manual registration of co-ordinates were located.

Pixel velocity: Pixels located further than a defined distance from the previous frame were eliminated, constraining the programme to select only those pixels whose velocity was less than the user-defined level. Since biological motion is characterised as having a smooth acceleration profile (Kilner *et al.* 2007), this constraint effectively prevented the programme from selecting pixels that did not fit biological motion, decreasing the chance of inaccurate tracking. Additionally, the programme was able to calculate the direction of travel and searched only for pixels in this direction. The programme took into account the inverse-pendulum nature of the distal limb, allowing a greater range of movement for the proximal points (pos1 and pos2) than the more distal points (pos3 and pos4).

Segment length: Pixels that returned a segment length that differed by more than $\pm 3\%$ from the initial segment length were eliminated, to maintain the limb as a series of rigid bodies and to maintain contact between the segments.

If this process returned more than one pixel location, the average co-ordinates were returned. For each frame, the most distal point (the mid point of the bottom of the hoof) was located first because its minimal movement during stance limits the variability of location.

The co-ordinates of these four landmarks were then used to define three segment vectors; *P1*, from pos1 to pos2; *P2*, from pos2 to pos3; and *P3*, from pos3 to pos4 (Figure 5.2).

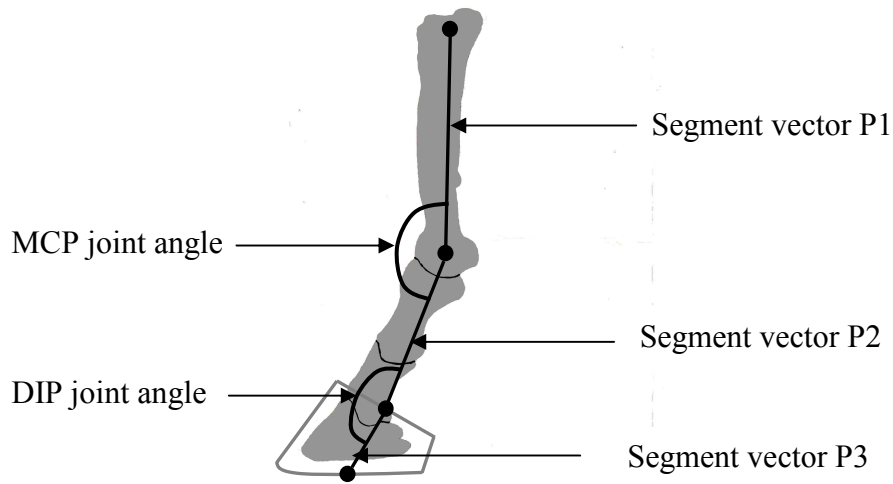


Figure 5.2 Trigonometry was applied to the segment vectors defined by the four co-ordinates to calculate MCP and DIP angles

MCP angle was calculated from segment vectors P1 and P2, and DIP angle from P2 and P3 using the following equation:

$$\text{Joint angle} = \text{inverse cosine} \frac{(\text{segment vector 1} \cdot \text{segment vector 2})}{(\text{length of segment 1}) \times (\text{length of segment 2})}$$

Joint angles were used for two purposes. Initially, they were used to investigate the change in joint angle with age (Chapter 6); and secondly as input into a model that calculated strains in the superficial (SDFT) and deep digital flexor tendons (DDFT) (Lawson *et al.* 2007b), described in Section 5.2, for comparison to previously-published *in vitro* studies.

Coronal plane MFS (MFSc0)

The protocol of the MFSc0 begins with defining the area of interest at the level of the MCP and the top of the metacarpus (Figure 5.3a). Next, a Sobel edge detection routine determines the co-ordinates of the medial and lateral boundaries (Figure 5.3b), and a velocity constraint eliminates those co-ordinates that did not fit smooth biological motion (Kilner *et al.* 2007). The remaining co-ordinates on each side are averaged to provide medial and lateral co-ordinates. Finally, a virtual centroid is calculated as the average of the two edge co-ordinates (Figure 5.3c). As the hoof remains stationary throughout stance (Clayton *et al.* 2007b), its co-ordinates are fixed. These points are

tracked throughout stance phase and MCP and DIP angles calculated through trigonometry, as described earlier.



Figure 5.3a
The area of interest was defined



Figure 5.3b
A Sobel edge detection routine was carried out



Figure 5.3c
A virtual centroid was calculated

5.1.2. Validation of MFSsa and MFSco

To validate this new technology, data found by the MFS were compared to bone-pin data (BPD), the gold standard. Since the MFSsa was the system primarily used in the current study, it was further validated against an opto-electronic system when tracking a mechanical linkage.

Validation of the MFS against equine bone-pin data

The input into the MFS was of horses led in walk either parallel to or towards a digital video camera at 100Hz, as detailed in Section 6.2.2. This input was then tracked using the MFS and compared to previously-published results captured using bone pins (Chateau *et al.* 2004; Clayton *et al.* 2007a; Clayton *et al.* 2007b). Since four horses were employed in the previously-published studies, the same number was used in the present study to allow comparison of intra- and inter-horse standard deviation (SD).

The age of the horses was 6 ± 2 years to age-match the study by Chateau *et al.*, but the age of the horses was not reported by Clayton *et al.* For both joints, the range of motion (RoM) was compared to both previously-published studies, allowing for the different method of calculating RoM for each study. Whereas Chateau *et al.* separate RoM into

extension (initial impact to maximum extension) and flexion (maximum extension to toe-off), Clayton *et al.* calculate RoM as the difference between maximum flexion and maximum extension. Therefore, the validation results are presented separately. Maximum joint angles found by the MFSsa were also compared to data by Chateau *et al.*, the only study to report this variable.

The sagittal-plane MFS

The mean maximum MCP and DIP angles found by the MFSsa were within 4° and 3°, respectively, of that found by Chateau *et al.*, and mean MCP and DIP RoM were within 2° and 4°, respectively. In each case the inter-horse SDs were similar (Table 5.1). The mean intra-subject SD for the MCP RoM was 3.2°, similar to the 4.2° reported by Chateau *et al.* Although the intra-subject SD of 6.1° for the DIP RoM was slightly higher than the reported 3.4°, it is comparable.

	MCP		DIP	
	Maximum	RoM	Maximum	RoM
MFSsa	140.5 ± 2.9°	54.5 ± 5.1°	157.5 ± 4.2°	55.0 ± 3.7°
Chateau <i>et al.</i>	136.8 ± 2.6°	52.5 ± 6.4°	160.4 ± 6.8°	58.8 ± 7.5°

Table 5.1 Joint angle data found by the MFSsa was comparable to data reported by Chateau *et al.*

The MFSsa found mean MCP and DIP RoM to be within 1° and 5°, respectively, of that reported by Clayton *et al.* and inter-horse SDs were very similar (Table 5.2). The intra-horse SDs were 3.9° and 4.6°, respectively, but these were not reported by Clayton *et al.*

	MCP RoM	DIP RoM
MFSsa	32.0 ± 2.9°	41.2 ± 2.2°
Clayton <i>et al.</i>	31 ± 3°	46 ± 3°

Table 5.2 Joint angle data found by the MFSsa was comparable to data reported by Clayton *et al.*

Typical graphs of stance phase joint angle were very similar to that previously reported for both MCP (Figure 5.4) (Clayton *et al.* 2007a) and DIP (Figure 5.5) (Clayton *et al.* 2007b) joint angle.

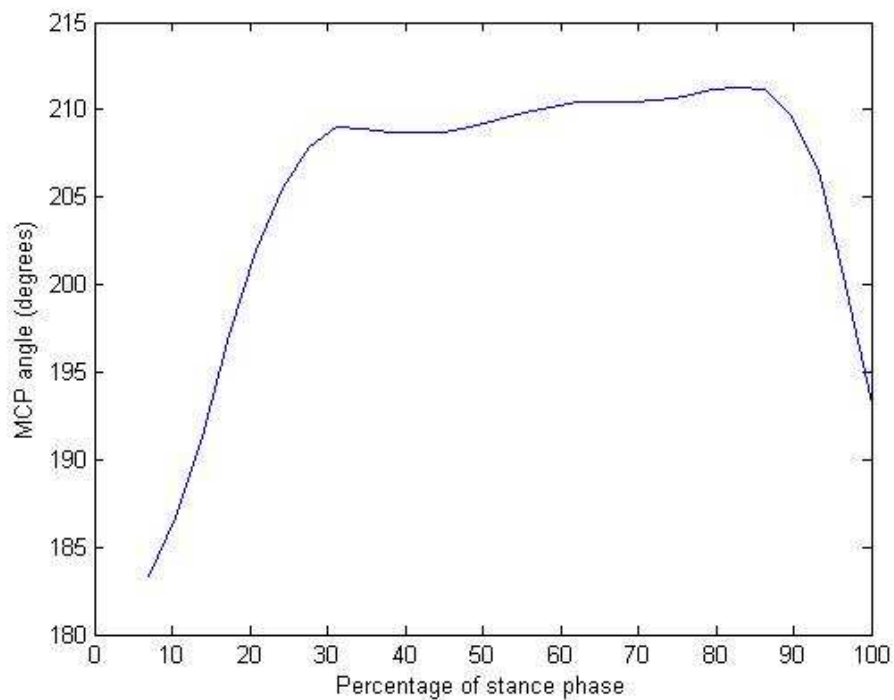


Figure 5.4 A typical graph of sagittal-plane MCP joint angle during the stance phase

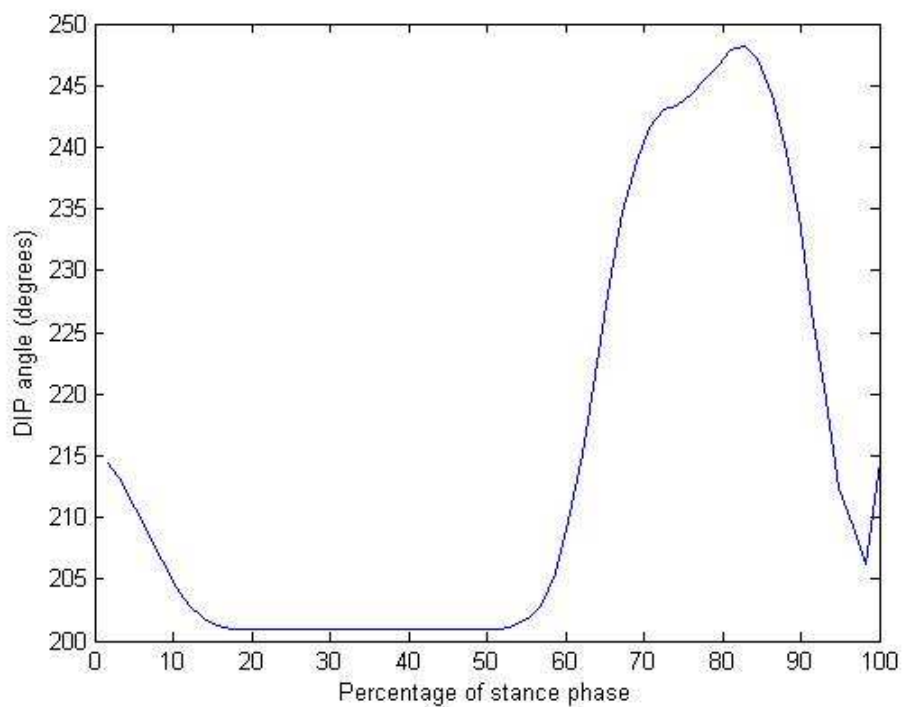


Figure 5.5 A typical graph of sagittal-plane DIP joint angle during the stance phase

The coronal-plane MFS

For the MFScO, the mean RoM of both MCP and DIP fell within the values reported by the two BPD studies.

The RoM for MCP data reported by the MFScO was higher than that reported by Chateau *et al.* (Table 3). However, the SD given by Chateau *et al.* is much larger than for the MFScO, indicating a lack of repeatability of results, especially since the intra-horse SD found by the MFScO was 1.3° and 1.2° for MCP and DIP data, smaller than the 4.1° and 3.1° reported by Chateau *et al.*

	MCP RoM	DIP RoM
MFScO	7.0 ± 1.2°	4.0 ± 1.3°
Chateau <i>et al.</i>	1.3 ± 4.8°	2.6 ± 3.7°

Table 5.3 Joint angle data found by the MFScO was comparable to data reported by Chateau *et al.*

The MFScO also found comparable results to those found by Clayton *et al.* (Table 4). The RoM for MCP and DIP were within 2° and 1°, respectively and the inter-horse SDs were smaller (Table 4). Mean intra-horse SDs were also small, being 1.5° and 1.1° for MCP and DIP, respectively, although these were not reported by Clayton *et al.*

	MCP RoM	DIP RoM
MFScO	5.3 ± 0.9°	3.3 ± 0.4°
Clayton <i>et al.</i>	7 ± 3°	4 ± 2°

Table 5.4 Joint angle data found by the MFScO was comparable to data reported by Clayton *et al.*

Typical graphs of stance phase joint angle were very similar to that previously reported for both MCP (Figure 5.6) (Clayton *et al.* 2007a) and DIP (Figure 5.7) (Clayton *et al.* 2007b) joint angle.

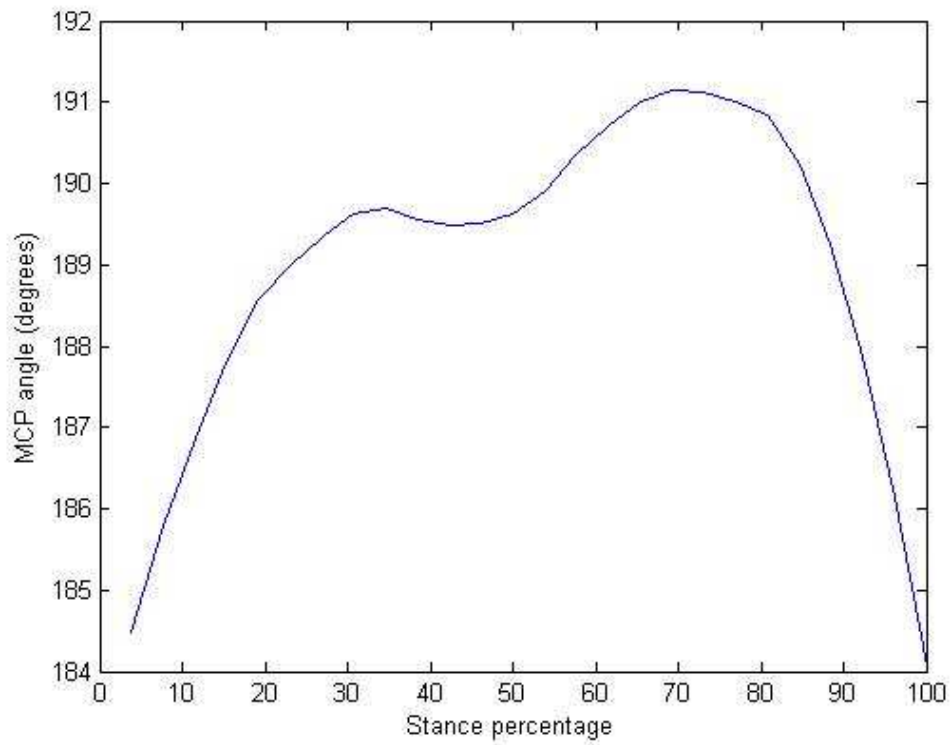


Figure 5.6 A typical graph of coronal-plane MCP joint angle during the stance phase

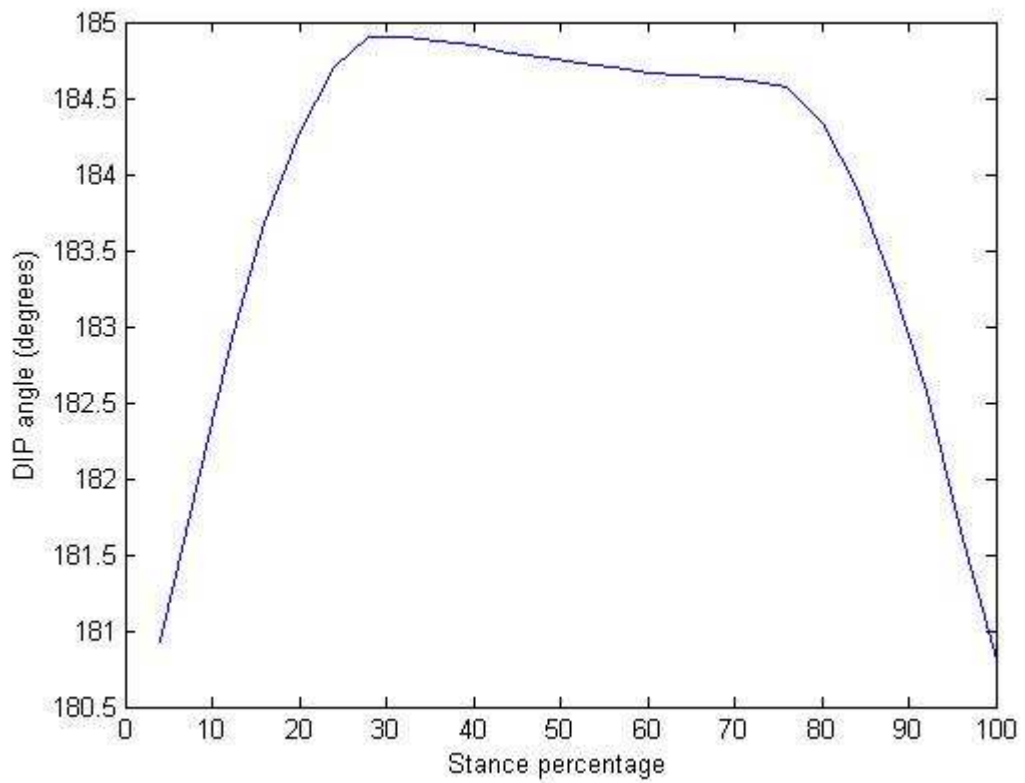


Figure 5.7 A typical graph of coronal-plane DIP joint angle during the stance phase

There was a difference in mean walking velocity between horses from the current study (1.67 ms^{-1} , range 1.61 to 1.76 ms^{-1}) and from the study by Chateau *et al.* (1.28 ms^{-1} , range 1.18 to 1.37 ms^{-1}) and Clayton *et al.* (1.51 ms^{-1} , range 1.44 to 1.60 ms^{-1}). The higher walking speed of the horses in the current study compared to the other two could be expected to cause a greater RoM in the sagittal-plane kinematics of the passive distal limb. And although mean MCP RoM reported by the MFSsa was 2° higher than BPD, both RoM and maximum angle in the DIP were smaller than BPD. This indicates that the difference in walking velocity did not influence joint angle.

There was also a difference in frame rate, which was 60 Hz in the Chateau study, 120 Hz in the Clayton study and 100 Hz in the current study. However, no difference was found between camera systems set at 25 Hz and 100 Hz (Section 6.4.1), thus it is unlikely that frame rate was influential.

Ideally, a greater number of horses would have been compared. However, since bone pins are invasive, the number of subjects was minimal for ethical reasons, and the current study had to match the population size to allow comparison of standard deviation. Each MFS provided a fast method of tracking anatomical landmarks throughout stance, processing each trial in less than a minute. The automated nature of the program ensured repeatability; since it gives the same result each time, there is no error associated with the manual placement of markers discussed in Chapter 4.

Validation of the MFSsa against an opto-electronic system⁶

To validate the MFSsa, the precisely repeatable motion of a four-bar linkage mechanism (Figure 5.8) was tracked with both the MFSsa and a widely accepted opto-electronic system (OES), and the angles reported by each system were compared. The OES used was a Vicon 512 system (Vicon Ltd., UK), consisting of six cameras. The frame rate for both systems was set at 50Hz, the maximum rate possible for the Vicon system.

⁶ The validation of the MFSsa against the OES was carried out by Oliver Warlow

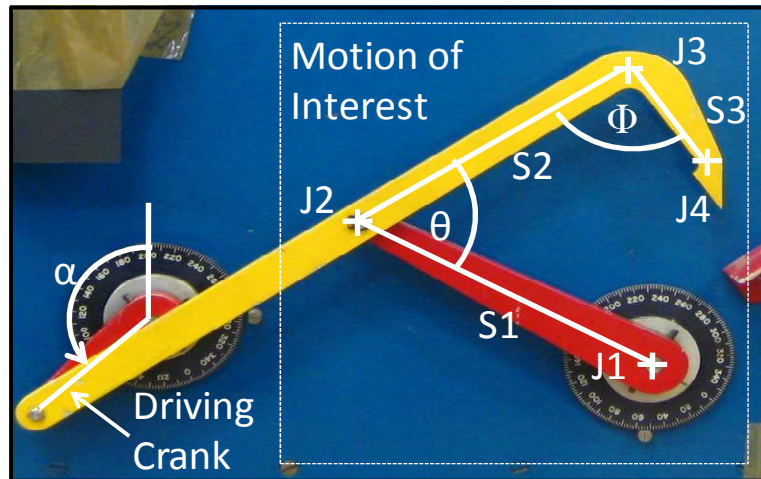


Figure 5.8 The precisely repeatable motion of a four-bar linkage was captured with both the MFS and an OES for comparison of the two systems.

To compare the systems, two angles on the four-bar linkage were tracked: a fixed angle Φ and a varying angle θ . The magnitudes of both angles and the range of θ were recorded and compared. For the OES, two markers were placed on the centre line of each of the three segments of the linkage for calculation of θ and Φ . Six trials were performed, removing and replacing the markers between trials to assess the influence of marker placement variation. For the MFSsa, the linkage was covered in a felt material to simulate the non-uniform surface found in a practical application of this tool. Two sets of videos were recorded, each of five different trials to assess the importance of joint centre placement.

	Varying angle θ		Fixed angle Φ
	Minimum	Maximum	Mean
OES	$56 \pm 1.0^\circ$	$128 \pm 0.5^\circ$	$91 \pm 1.2^\circ$
MFSsa	$51 \pm 0.6^\circ$	$123 \pm 0.6^\circ$	$96 \pm 1.5^\circ$

Table 5.5 The MFSsa was comparable to an opto-electronic system

In general, the MFSsa was comparable to the OES (Table 5). Mean maximum and minimum values of the varying angle θ found by the MFSsa were within 5° of those found by the OES and the range of motion was identical. The mean SDs of maximum and minimum values for θ were very similar between the two systems, indicating that the variability introduced by manual re-placement of joint centres was similar. For the

fixed angle Φ , the intra-trial SD was slightly higher than that found by the OES, ranging from 1.2° to 2.2° from a mean of 96° whereas for the OES it was 0.4° to 0.5°.

The major difference from the OES was that the MFSsa found a range of motion of up to 11° in Φ - a fixed angle. This variation indicates that the joint centres that define the angles were drifting, which occurs when the motion of the segment was in line with its central axis. However, the motion of interest for the present study was the inverse pendulum-like motion of the distal limb, where the limb never moves along its axis. For this reason the MFSsa was accepted for tracking the cohort of this thesis.

Conclusions of validation study

Since it was not possible to attach markers to all horses to track their kinematics, a new marker-free tracking system (MFS) were developed that overcame some of the limitations of existing systems such as marker vibration and skin displacement artefact (see Sections 4.2.1 and 4.2.3).

When tracking the precisely repeatable motion of a four-bar linkage, the MFSsa was found to be comparable to an existing opto-electric system, reporting exactly the same range of motion, similar minimum and maximum angles and a similar SD. Although the MFSsa reported a range of motion of 11° in a fixed angle, this drift only occurred during axial translation, which does not occur during stance phase, and therefore was not considered a limitation for this application.

The MFS reported similar ranges of motion, maximum joint angle and SD to BPD, indicating that the MFS was accurate. Mean maximum MCP angle found by the MFSsa was similar to that found by Hodson *et al.* (Hodson *et al.* 2000), whose techniques also involved automatic digitisation of 2D video data.

Although the MFSsa does track surface landmarks, it fits a model of expected movement to the data, for example limiting hoof movement whilst allow a larger range of motion of the more proximal points, to minimise the effect of skin displacement.

The main advantage of a marker-free system is that the user does not have to come into contact with the subject, reducing the time required for subject preparation and making the system completely non-invasive. The MFS is suitable for use when marker application is impractical, for example at competition or for capture of a wild animal.

The subject may even be unaware that gait analysis is taking place, ensuring that gait is representative of normal movement; making the MFS consistent with the premise that the technique should not interfere with what it is measuring (Baker 2006). Since the video used by the MFS records reflected visible light, it is not susceptible to sunlight interference that can affect infra-red based opto-electric systems. This allows trials to be conducted outside, eliminating the need to capture within a laboratory environment, decreasing cost and allowing natural gait. Finally, the MFS overcomes some of the limitations of marker-based capture than can be affected by soft tissue movement and marker interference (Section 4.2.5).

The repeatability, accuracy and high throughput speed of the MFS could make it suitable for digitisation of video-based kinematics, especially in an outdoor environment.

5.2. Strain calculation

Since longitudinal development studies of tendon stiffness are more numerous than those of kinematics, especially into senescence, joint angles were used to calculate strain in the flexor tendons for comparison to previously-published studies.

Maximum MCP and DIP joint angles were used as input into an anatomically accurate, scaled link-segment model written in Matlab (Lawson *et al.* 2007b). This model calculates strain values in the suspensory ligament (SL), the SDFT and DDFT, and produced a 3D animation of the limb in motion. For each horse, the mean maximum strain in the SDFT and DDFT over three trials was calculated.

The model consisted of the distal limb skeleton (the three phalanges, proximal and distal sesamoid bones and metacarpal bones), extracted from Computed Tomography (CT) scans and rendered as 3D objects in Matlab (Figure 5.9). Bone sizes were scaled to the subject to ensure that the model was as subject-specific as possible; full subject specificity would have required taking computerised tomography (CT) scans of every horse. Since all horses were of the same type, variation in bone allometry was considered to be negligible. The positions of the phalanges and metacarpal bones were determined from locations and orientations recorded *in vivo*, whereas the positions of the sesamoid bones were simulated. Virtual ligaments tied the proximal and distal sesamoids to the proximal and distal phalanges, respectively. The movement of the

proximal sesamoids was calculated using a fixed-length attachment to the proximal phalanx and maintaining optimal contact with the third metacarpus. The position and orientation of the sesamoid bones was determined using an optimisation routine that provided the most amount of contact between the articular surfaces in three dimensions.

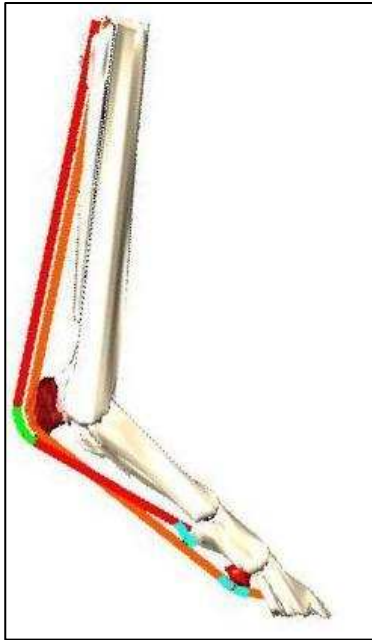


Figure 5.9 A scaled link-segment model was used to calculate strain in the SDFT and DDFT.

The paths of the SDFT, DDFT and SL were overlaid on the underlying skeleton and prevented from penetrating the bones. This involved applying a least-squares fitting algorithm to minimise the distance between the meshes of the surfaces, without penetrating the bone surface. The tendons wrapped at three locations: at both the proximal and distal sesamoid bones and at the proximal palmar extremity of the middle phalanx. Tendon paths depended on the position of the bones and, for the SDFT, the strain and therefore diameter of the DDFT. Maximum tendon strain was calculated as the maximum change in length (ΔL) divided by the initial length at the start of stance phase. ΔL was calculated as the difference between initial and maximum length, which occurs in the SDFT at around 60% of stance phase duration (Lawson *et al.* 2007a) and in the DDFT just before heel-off at about 90% of stance

phase duration (Lawson *et al.* 2007a). For the purposes of this study, the PIP joint was fixed at 180°. The influence of this on tendon strain is described in Section 6.4.5. The calculated strains, representative of MCP and DIP angle, could then be compared to abundant existing literature on changes to tendon strain with age.

5.3. Mass calculation

Given that the distal limb is a passive system (McGuigan and Wilson 2003), the degree of joint flexion depends not only on the stiffness of the spring-like tendon but also on the magnitude of the mass it supports. To isolate the effect of tendon stiffness, joint angles were normalised to the mass of the horse.

Since no weighbridge was available at the venues where filming took place, it was not possible to determine the mass of each horse. Instead, abdominal depth was measured to

represent girth because heart girth size shows a linear relationship with mass, with an r^2 value of 0.97 (Rodriguez *et al.* 2007). Abdominal depth in lateral view was digitised from an image of the horse at rest, on a vertical line from just behind the withers to the ventral abdomen, just behind the olecranon process (Figure 5.10). Since abdominal depth depended on the distance from the camera, a known length on the same still image was also digitised and used for calibration. The calibration object was most commonly a showjumping pole, and horses were positioned the same distance from the pole as during filming. The horse in Figure 5.10 is behind the pole for illustrative purposes. The calibration distance was then used to calculate the real-world depth of the abdomen and hence estimate mass. The values of abdominal depth are presented in Appendix A.

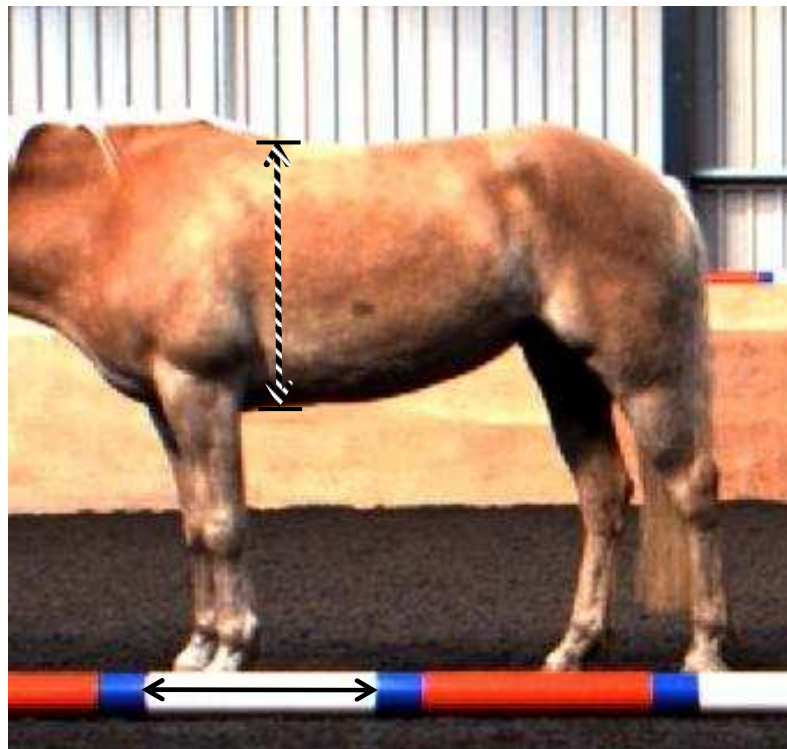


Figure 5.10 The abdominal depth in lateral view (hatched line) was digitised to indicate mass. A known length, of 60cm here, (solid line) was also digitised for calibration.

This method was validated by finding the Pearson's correlation coefficient between calculated abdominal depth and actual mass on a dedicated capture day, where a weighbridge was available. The mass of thirteen horses aged 10-19 years was obtained using an Eziweigh-2 equine weighbridge, and photographs taken for measurement of abdominal depth. The correlation between mass and chest depth was 0.809 ($p=0.001$), supporting the use of digitisation of chest depth as a method for estimating mass.

To determine the influence of age on the relationship between abdominal depth and actual mass, the gradient of this relationship was compared between young and old horses. The older horses were the same as described above and data for young horses were taken from a previously-published study in horses of a similar breed of horse to the current study (Thompson 1995). Since the population was of similar Sport Horse type, the effect of breed was considered negligible. The gradient of the relationship between abdominal depth and mass in older horses (0.12) fell within one standard deviation (± 0.05) of this gradient in young horses (0.07).

5.4. Summary of method and materials

This chapter presented the new methods developed and used within this thesis. To overcome some of the limitations of current marker-based motion capture, a new marker-free system (MFS) was developed to track the kinematics of the equine distal limb, in both the sagittal (MFSsa) and coronal (MFSCO) planes. The MFS was compared to bone-pin data (BPD) and MFSsa was also validated against an opto-electronic system. Data produced by the MFS was generally comparable to BPD and the graphs were similar to those previously reported. However, the MFSsa did report a range of motion within a supposedly fixed angle. This was due to limited ability to track axial translation, but since this type of motion does not occur during the stance phase of equine locomotion, the MFSsa was accepted for this study. The MFSCO was comparable to previously-reported bone-pin data.

The MFSsa is the main system used in this thesis since most joint rotation, and therefore tendon elongation and strain, occurs in the sagittal plane. However, the MFSCO could be useful to determine ad-/abduction in the equine distal limb, since this type of rotation may lead to interphalangeal joint injury (Chateau *et al.* 2001, 2002). For this thesis, the kinematic data found by the MFSsa serves two purposes. First, data are used to assess the influence of age on equine kinematics, and secondly as input into a model developed by Lawson *et al.* (Lawson *et al.* 2007b) to calculate strains in the SDFT and DDFT. These strains could then be compared to existing literature on the development of strain with age, since these studies are more numerous than those that investigate kinematic development.

Since the equine distal limb is a passive system (McGuigan and Wilson 2003), joint angles will not only depend on the stiffness of the tendon, but also on the mass of the horse. Therefore for accurate comparison, joint angles must be normalised to mass. In this thesis, chest depth was calculated to indicate mass since no weighbridge was available where motion capture took place. Calculated chest depth was found to be closely linked to mass (Pearson's correlation coefficient = 0.809), similar to results found by Rodriguez *et al.* (2007). The influence of age and breed was deemed to be negligible. These methods were applied to a population of 57 horses, to track how equine kinematics change with age, which is presented in Chapter 6.

6. The influence of tendon stiffness on the development and degeneration of locomotion with age⁷

6.1. Introduction

Tendon injury is common in the equine athlete, leading to criticism of the equine industry on welfare ground and economical loss (Section 1.1). Injury to the tendon occurs if loads placed on it exceed its mechanical integrity (Smith *et al.* 2002) and since loss of mechanical integrity is typically a gradual process, degeneration does not normally present clinical signs until the point of rupture. When, and if, rupture occurs depends on both the quality of the tendon and on the number and magnitude of the loading cycles experienced, and is therefore subject-specific. It is thus important to maximise the quality of the tendon during development and also to minimise tendon loading during degeneration. Detection of these degenerative changes is difficult since they are usually subclinical and gradual. Traditional human observation may not be able to detect these subtle changes (Dow *et al.* 1991; Merkens and Schamhardt 1988), especially since this method can be subjective (Merkens and Schamhardt 1988; Weishaupt *et al.* 2004) and intra-observer reliability has been reported to be low (Arkell *et al.* 2006; Keegan *et al.* 2010).

A simple, non-invasive method to detect these two important changes in the stiffness of the tendon could be through a study of the kinematics of the distal limb. The three joints of the distal limb are primarily supported during the stance phase by the superficial (SDFT) and deep (DDFT) digital flexor tendons, although other structures such as the suspensory ligament play a role. The tendons wrap around and passively support the metacarpo-phalangeal (MCP) and distal inter-phalangeal (DIP) joints by limiting flexion (Section 1.3.2, (Denoix 1994; McGuigan and Wilson 2003). Since the distal limb is passive, the angles of these two joints could be expected to reflect tendon stiffness. The hypotheses were therefore that in the youngest horses, with increasing tendon stiffness, maximum joint angle would be negatively correlated with age; in middle-aged horses, with constant tendon stiffness, there would be no significant correlation; and towards old age, where tendon stiffness decreases, there would be a positive correlation between joint angle and age.

⁷ Part of the contents of this chapter has been published in the *Equine Veterinary Journal* (Addis and Lawson (2010) 42: (Suppl. 38): 556-560.

Knowledge of the development of equine locomotion is at present limited to horses up to 36 months old (Back *et al.* 1994b; Back *et al.* 2002; Back *et al.* 1995; Cano *et al.* 2001), and there is limited information on the degeneration in the older horse. This chapter therefore investigates the development and degeneration in equine locomotion across a range of ages from three months to 212 months. This age range covers the suggested cease of synthesis at around three years old, as well as the degeneration of tendon towards old age.

Although there are limited studies on the development and degeneration of kinematics, studies into the change in tendon stiffness are more numerous. Joint angles can be used to calculate strain (Lawson *et al.* 2007) as a measure of stiffness, for comparison to previously-published longitudinal data on the development of stiffness.

Since the distal limb is passive, the range of motion of the joints will depend not only on the stiffness of the spring-like tendons but also on the mass of the horse; Back *et al.* (1995) concluded that the larger MCP joint angles shown in the adult horse may be due to the higher mass. This means that the angles of the MCP and DIP joints must be normalised to mass before meaningful conclusions can be drawn about the influence of tendon stiffness. Each horse's mass was therefore calculated to allow comparison of normalised kinematic results.

The chapter begins with the materials and methods used to determine the age-related change in kinematics. Next, the results section presents statistical evidence for a lifelong change in kinematics and tendon strain. Finally, the results obtained are discussed with reference to previously-published literature. The methods employed are compared to the gold-standard of invasive data, and finally conclusions are drawn and possible future directions are suggested.

In summary, this chapter focuses on the development and degeneration of equine locomotion with age and the possible influence of the changing mechanical properties of the tendon on the passive equine distal limb.

6.2. Methods and materials

This section describes the horses studied, the motion capture protocol and the statistical methods used to assess the influence of age and a discussion of the limitations and assumptions. Chapter five described the development of the marker-free systems used to define MCP and DIP joint angles (Section 5.2), how mass of the horses was calculated for normalisation (Section 5.4) and how the joint angles were used to calculate strain in the SDFT and DDFT (Section 5.3) using a subject-specific model (Lawson *et al.* 2007).

6.2.1. Study population

The population of 57 Sport Horses consisted of mares, geldings and stallions. Horses were of amateur standard and therefore subjected to approximately similar exercise regimes. Data was captured on seven different occasions; at two evaluation events (the Anglo-European Studbook and at the Glenwood Stud, both during November 2008) and at five stud or competition yards in Northumberland from November 2008 to September 2009. All of the population was subject to frequent clinical examination, and those captured at the evaluation events also subject to veterinary examination. None showed signs of lameness at data capture. Each horse's age, determined from the date of birth in their passport, ranged from young foals of three months old to older horses of 212 months.

There are two ages of particular interest; the first is when tendon synthesis ceases at skeletal maturity between 24 and 36 months (Smith *et al.* 2002), and the second is when tendon degeneration is manifest. Since the average age onset of tendon degeneration is varied (Section 2.6), a mean age of 100 months was chosen for convenience of comparison. These two defining ages allowed the subjects to be divided into three age groups for statistical analysis:

- Group 1: 16 young horses from three to 35 months old, whose tendon stiffness was assumed to be increasing
- Group 2: 20 adult horses from 36 to 99 months old, whose tendon stiffness was assumed to remain constant
- Group 3: 21 mature horses from 100 to 212 months old whose tendon stiffness was assumed to be decreasing.

6.2.2. Data capture

The data capture protocol was designed to focus on the kinematics of the MCP and DIP joints. Since the DIP is contained within the hoof, an approximation of this joint was calculated using the geometric centre of the MCP, the mid point of the coronet band and the mid point of the hoof base.

Data were captured at both 25Hz and 100Hz to determine if 25Hz is sufficient to detect age-related changes in locomotion. During each trial, a single camera was used; a Canon XL-H1 DV camera (Canon Inc., Tokyo, Japan) at 25Hz with a resolution of 720x576 pixels, or a Basler piA640-210gc camera (Basler AG, Ahrensburg, Germany) at 100Hz with a resolution of 640x480 pixels. To compare these systems, the maximum MCP and DIP angles recorded for the same ten strides were compared by paired t-test.

Horses were led in walk at a self-selected speed on a line of progression perpendicular to the camera at a distance of approximately four metres and the field of view was approximately three metres, sufficient to record at least one complete stride per trial (Figure 6.1). The height of the camera was approximately 0.5m from the ground.



Figure 6.1 Still showing the camera's eye-view of a subject. The striped pole was used to calibrate distances using the red and white sections, each of 60cm width.

Horses were led within 20cm of a calibration object of known size, most commonly a showjumping pole, for calculation of lengths such as chest depth. Walking next to this pole also ensured that horses walked in a straight line, which not only minimised out-of-plane motion but also minimised deviation of distance from the calibration object for calculation of lengths. The absolute distance between the horse and camera was only considered important to ensure that one complete stride was recorded. Where possible, handlers were requested to walk several paces in front of the horses so as not to interfere with capture.

A minimum of three trials were recorded per horse, and MCP and DIP angles for the same forelimb were obtained using a marker-free system in Matlab (The Mathworks, Inc., USA) (Section 5.2). Mean maximum angles were used for statistical analysis. Since kinematics are dependent on speed (Khumsap *et al.* 2002; Robert *et al.* 2002), the mean walking speeds of the three age groups were compared by ANOVA to determine if there was any significant difference between the groups. Speed was determined by calculating the distance travelled by a point with constant velocity, such as the top of the withers, during a given time and over at least one complete stride, as described in Section 3.2.

Since data were captured at various stud farms, competition yards and evaluation events, horses walked on a variety of surfaces that fell into three categories; concrete, tarmac covered with dense rubber matting approximately 12 mm thick, and an indoor school with a covering of packed sand and rubber particles approximately 50 mm thick. Surface deformation was visually assessed, and was found to be negligible on concrete and rubber matting, and minimal (approximately 5 mm) on the school surface. To test the influence of these surfaces on maximum joint angle, the mean maximum MCP and DIP angles of seven age-matched controls were compared by ANOVA.

6.2.3. Data capture limitations and assumptions

There were four main limitations to the data capture protocol; the first is that the kinematics of the proximal inter-phalangeal (PIP) joint were ignored; the second was that the DIP joint was approximated; the third was that horses were recorded in 2D; and lastly, the exercise regimes of the horses was not known. These will now be discussed along with other, less important, limitations.

Exclusion of PIP kinematics

The kinematics of the PIP joint were ignored for three reasons. Firstly, the joint's limited range of motion (RoM) contributes minimally to the change in length and therefore strain in the SDFT and DDFT. Secondly, variability in this joint is large, indicating a lack of repeatability. Thirdly, during the stance phase the SDFT and DDFT have opposing action on the PIP due to a difference in insertion points. These limitations will now be discussed in further detail.

First, the PIP shows a limited sagittal-plane RoM; for an isolated specimen loaded in a press, the RoM was found to be only 8.1° (Chateau 2002). Similar results of 10.0° (Chateau *et al.* 2004) and 13.0° (Clayton *et al.* 2007) have been found *in vivo* at walk. Although there is possibly sufficient mobility to warrant including the PIP, its RoM is small when compared to the other two distal joints; for example the range of motion in the walking horse has been found to be 52.5° in the MCP and 58.8° in the DIP (Chateau *et al.* 2004).

Secondly, the variability of PIP RoM has been found to be relatively high. For example, an *in vitro* study using a limb loaded into a power press found a standard deviation of $\pm 4.6^\circ$, over half of the mean RoM of 8.1° (Chateau 2002). Results *in vivo* at walk are similar; Chateau *et al.* (2004) reported a standard deviation of 2.5°, a quarter of the 10.0° mean RoM. This same study found intra- and inter-subject variability to be 10% and 24% of the RoM respectively. As a final example, Clayton *et al.* (2007) found a standard deviation of $\pm 4.0^\circ$ from a mean of 13.0° at walk. This is in comparison to the MCP, which showed an intra-subject variability of 4.2°, an inter-subject variability of 5.6° and a standard deviation of $\pm 6.4^\circ$ from a mean RoM of 52.5°. Similarly, the DIP has been found to show an intra-subject variability of 3.4°, an inter-subject variability of 7.6° and a standard deviation of $\pm 7.5^\circ$ from a mean RoM of 58.8° (Chateau *et al.* 2004). This variability indicates a lack of repeatability in PIP measurement.

Thirdly, during weight bearing, the action of the SDFT is antagonistic to that of the DDFT. Whereas the SDFT insertion spans the PIP joint, the DDFT inserts more distally on the flexor surface of the distal phalanx. The result of this is that, during weight bearing, the SDFT limits PIP flexion, whereas the DDFT facilitates PIP flexion (Denoix 1994). This makes it difficult to separate the action of the two tendons on this joint.

Finally, a recent study recommended that the pastern segment be modelled as a single rigid segment due to the difference in skin displacement between the fetlock and pastern regions (Clayton 2010), effectively excluding PIP joint kinematics.

Although ideally the PIP joint would have been included, it was ignored in this study due to its low range of motion, high variability and the conflicting actions of the flexor tendons that support it. Future studies of this nature could include the PIP joint.

Approximation of DIP joint

Since DIP joint angles cannot be measured directly, angles at this joint were calculated using the geometric centre of the MCP, the mid point of the coronet band and the mid point of the hoof. Although an approximation, this method was accepted for two reasons. Firstly, a recent article recommended that the DIP is measured between the hoof and a rigid pastern segment (Clayton 2010). Secondly, this method tracked repeatable points and therefore allowed comparison between horses. To ensure the validity of this method, DIP joint kinematics are compared to those found by invasive bone-pin data (Chateau *et al.* 2004), which is discussed in section 6.4.

Investigation of only sagittal-plane kinematics

The second main limitation of this study is that only the sagittal plane kinematics were investigated. However, since the distal joints are primarily designed for flexion and extension (Chateau 2002), most joint rotation and therefore tendon elongation occurs in this plane. For example, sagittal plane rotation was far greater than rotation in either the coronal or transverse plane in walk for the MCP, PIP and DIP in a bone-pin study by Chateau *et al.* (2004). These authors report mean MCP sagittal plane RoM as 52.5°, whereas in the coronal plane this was 1.3° and in the transverse plane 3.7°. This has been found to be similar for the DIP joint in walk; whereas sagittal plane RoM was 46.0°, the RoM in both the coronal and transverse plane was only 5.0° (Clayton *et al.* 2007). For this reason, 2D capture was accepted for this section. Future work could combine joint angles found by the sagittal-plane and coronal-plane marker-free systems described in Chapter 5, to give 3D values.

Exercise regimes of horses unknown

Exercise regime influences tendon development, especially in the young horse, (Section 2.6.9) and therefore may influence joint angles. The precise exercise regime of individual horses was not known since a cross-sectional population was used. To ensure that exercise intensity across the population was approximately similar, only amateur-level horses were chosen for the study and no horse was included that had undergone intensive training, for example racing. Level of exercise of horses at the stud farms or competition yards was obtained from the trainer or owner, and since evaluation events were aimed at amateur horses, their level of exercise was assumed to be average. Future studies could follow the population longitudinally, but would require a longer timescale and larger budget than was available for the current project.

Comparison of 25 Hz and 100 Hz camera systems

The suitability of a 25Hz camera system for detecting age-related changes in kinematics was assessed by comparing the maximum joint angles of the same ten stance phases reported by 25Hz and 100Hz camera systems. There was no significant difference between the camera systems for either maximum MCP ($p=0.8$) or maximum DIP ($p=0.5$) angle.

The influence of surface type

Horses were filmed at a variety of locations on surfaces that fell into one of three categories; concrete, tarmac covered in rubber matting and packed sand. To test the influence of surface, the maximum MCP and DIP angles of seven age-matched controls were compared by ANOVA. No significant difference was found for either MCP ($p=0.811$) or DIP ($p=0.111$) joint angle between the three surfaces.

Influence of the handler

The handler was different for almost every horse. Ideally, horses' gait could have been recorded on a treadmill to ensure identical conditions for each horse; however motion captured on a treadmill is not necessarily representative of over-ground locomotion (Buchner *et al.* 1994). Additionally, there are substantial time and transport costs associated with this protocol, including training the horses that would only have been filmed once.

6.2.4. Statistical methods

To determine the effect of age on joint angle and tendon stiffness, statistical tests were applied to four variables; maximum normalised stance phase MCP and DIP angle, and maximum strain in both the SDFT and DDFT. The mean maximum value of three trials was taken to ensure repeatability.

Horses were divided into three age groups based on the assumed mechanical properties of the tendon within each group (Section 6.2.1). The influence of age was analysed by three methods, each carried out in Minitab (Minitab Ltd., UK). The first method was calculation of the Pearson's correlation coefficient between the variable and age, to indicate the strength and direction of the assumed linear relationship. Significance was set at $p=0.05$. Secondly, the r^2 value of the relationship was calculated as a measure of how well age could predict the variable. As well as these two statistical tests, the gradient of the slope of joint angle against age was calculated in the youngest (Group 1) and oldest horses (Group 3) to indicate rate of change.

In addition to the intra-group analysis, the average age of change between groups was calculated. The age at which developmental kinematic changes cease was found by calculating the intersection of the assumed linear trend of Group 1 with the mean value of the assumed plateau of Group 2. The age of apparent onset of degeneration was found by calculating the intersection between the mean of Group 2 with the assumed linear trend of Group 3 (Figure 6.2).

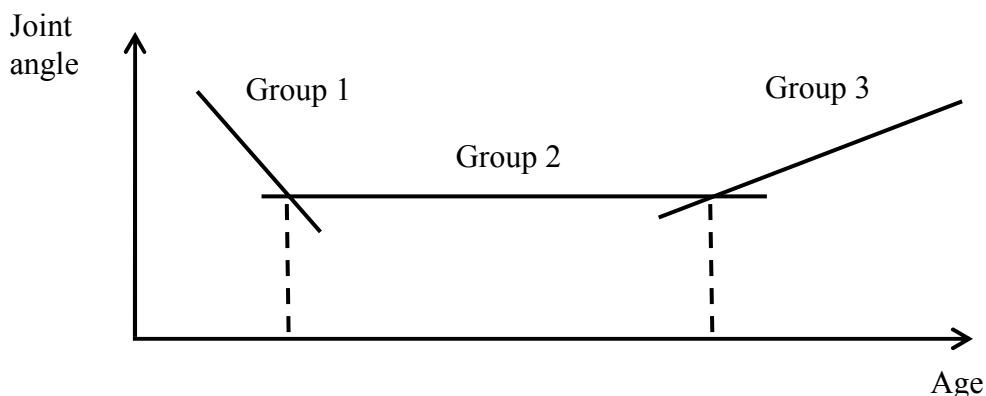


Figure 6.2 The intersections between the assumed linear trends of Group 1 and 3 with the mean of the assumed plateau of Group 2 were used to indicate the average age of cease of developmental change and onset of degenerative change.

6.2.5. Summary of method and materials

The distal limb kinematics of 57 horses led in hand in walk were captured with a video camera. Videos were processed in Matlab using a novel automated marker-free tracking system to calculate angles in the MCP and DIP joints (Section 5.2). Since joint angle depends on both stiffness of the tendon spring and the mass of the horse, angles were normalised to mass to allow separate investigation of the influence of tendon stiffness (Section 5.4). Raw joint angles were used to calculate strain in the two flexor tendons (Section 5.3), for comparison of tendon stiffness to previously-published work. The PIP joint was ignored due to its low range of motion, high variability and the opposing influence of the two flexor tendons.

The influence of age on the four variables (maximum normalised MCP and DIP angle, and peak SDFT and DDFT strain) were analysed by several methods. Firstly, the Pearson's correlation coefficient was calculated between the variable and age to indicate the strength and direction of the assumed linear relationship. Secondly, the r^2 value of the linear regression was found as a measure of how well age could predict the variable. Thirdly, the gradient of the trend in Group 1 and 3 horses was found to indicate the rate of change. Finally, the intersection of the assumed linear trend in Groups 1 and 3 with the plateau of Group 2 was calculated to determine the average age of change within the population. The results will be presented in the following section.

6.3. Results

6.3.1. Kinematic changes with age

This section presents evidence of a change in joint angle with age in both the MCP and DIP joints. Mean walking velocity was not significantly different between the three groups ($p=0.4$) and therefore unlikely to be influential on kinematics. The range of speeds was 1.25 ms^{-1} to 1.85 ms^{-1} and the mean value was $1.64 \text{ ms}^{-1} \pm 0.03 \text{ ms}^{-1}$.

The general trend in maximum normalised stance phase joint angle was an initial significant decrease with age, no change in the adult horses and finally a significant

increase towards old age (Figure 6.3 and Figure 6.4). The intersection of Group 1 and 2 horses was found to occur at 29 ± 12 months for MCP data and 31 ± 13 months in DIP data; and between Group 2 and 3 horses at 136 ± 53 months for MCP data and 127 ± 68 months for DIP data.

Due to time and financial constraints, a cross-sectional population was used in this study. A longitudinal study, where the same horses were tracked over a long period of time, could have reduced the scatter of data points, and therefore could help to identify of inflection points in the angle/age or strain/age curves.

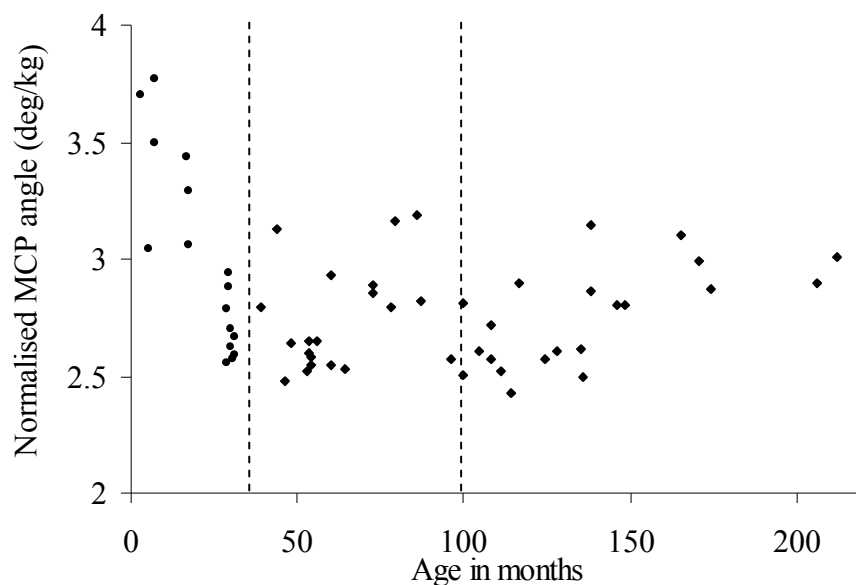


Figure 6.3 Maximum normalised MCP angle shows a change with age. The vertical lines represent the age group boundaries of 36 and 100 months.

In Group 1 horses between three and 35 months old, the Pearson's correlation coefficient was -0.88 ($p=0.000$), equivalent to a rate of change of 0.41% /cm girth depth per year. The r^2 value of the relationship was 0.77 .

In Group 2 horses between 36 and 99 months old, the Pearson's correlation coefficient was not significant ($p=0.16$), and the minimal gradient of 0.5% indicated a negligible change with age. The r^2 value of the relationship was 0.10 .

In Group 3 horses between 100 and 212 months old, the Pearson's correlation coefficient was 0.62 ($p=0.003$), equivalent to a rate of change of 0.05% /cm girth depth per year. The r^2 value was 0.39 .

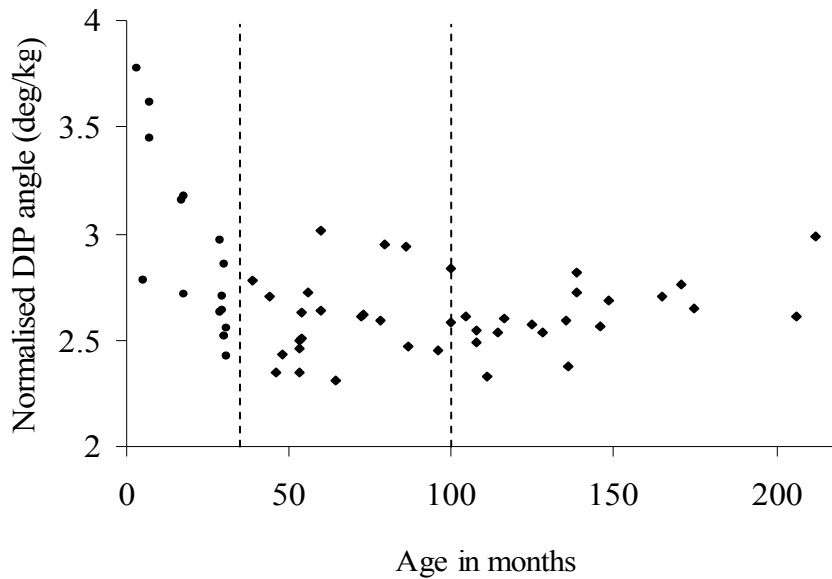


Figure 6.4 Maximum normalised DIP angle shows a change with age. The vertical lines represent the age group boundaries of 36 and 100 months.

In Group 1 horses, the Pearson's correlation coefficient was -0.81 ($p=0.000$), equivalent to a rate of change of $0.37^\circ/\text{cm}$ girth depth per year. The r^2 value of the relationship was 0.65 .

In Group 2 horses, the Pearson's correlation coefficient was not significant ($p=0.5$), and the minimal gradient of 0.1% indicated no change with age. The r^2 value was 0.02 .

In Group 3 horses, the Pearson's correlation coefficient was 0.48 ($p=0.03$), representing a rate of change of $0.02^\circ/\text{cm}$ per year. The r^2 value was 0.22 .

6.3.2. Change in tendon strain with age

Strains in the superficial (SDFT) and deep (DDFT) digital flexor tendons were calculated from un-normalised MCP and DIP joint angles using a scaled link-segment model (Lawson et al. 2007), as an indication of tendon stiffness. As expected, strain showed a significant decrease with age in young horses, followed by a plateau then a significant increase towards senescence (Figure 6.5 and Figure 6.6). The intersection of Group 1 and 2 horses was found to occur at 19 ± 13 months for SDFT data and 16 ± 14 months in DDFT data; and between Group 2 and 3 horses at 77 ± 64 months for SDFT

data and 69 ± 64 months for DDFT data. The very large standard deviation of intersection age is discussed in section 6.4.2.

Change in SDFT strain with age

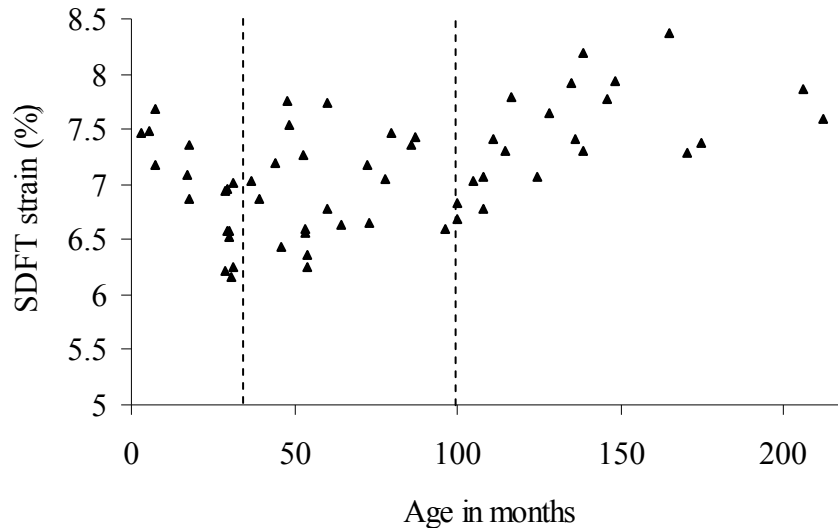


Figure 6.5 Maximum SDFT strain shows a change with age. The vertical lines represent the age group boundaries of 36 and 100 months.

In Group 1 horses, the Pearson's correlation coefficient was -0.81 ($p=0.000$), equivalent to a 1.2% change in strain across the group age range of 2.7 years. The r^2 value of the relationship was 0.59.

In Group 2 horses, the Pearson's correlation coefficient was minimal (0.11) and non significant ($p=0.6$), and the gradient of 0.3% was minimal. The r^2 value was 0.01.

In Group 3 horses, the Pearson's correlation coefficient was 0.53 ($p=0.01$), representing a 0.9% change in strain of over 9.3 years. The r^2 value was 0.28.

Change in DDFT strain with age

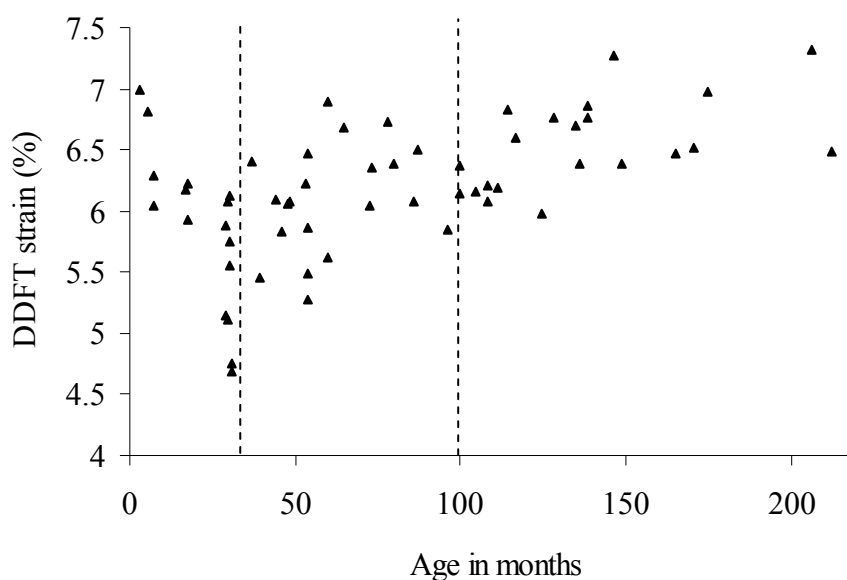


Figure 6.6 Maximum DDFT strain shows a change with age. The vertical lines represent the age group boundaries of 36 and 100 months.

In Group 1 horses, the Pearson's correlation coefficient was -0.77 ($p=0.000$), equivalent to a 1.5% change in strain across the group's age range of 2.7 years. The r^2 value of the relationship was 0.59.

In Group 2 horses, the Pearson's correlation coefficient was non significant ($p=0.22$), and the gradient of 0.7% was minimal. The r^2 value was 0.08.

In Group 3 horses, the Pearson's correlation coefficient was 0.54 ($p=0.01$), representing a 0.7% change in strain over 9.3 years. The r^2 value was 0.28.

6.3.3. Summary of results

Evidence for an age-dependent change in kinematics and tendon strain has been presented, with trends showing a significant negative correlation with age in the young horse, levelling off to no significant correlation in the middle aged horse, before showing a significant increase towards old age. These data will now be discussed.

6.4. Discussion

This chapter investigated the role of changing tendon stiffness on the development and degeneration of equine joint angles with age. During development, maturational changes occur within the flexor tendons that result in an increase in their stiffness. Since these tendons wrap around and support the joints of the distal limb, the stiffening tendon could limit range of motion of the joint. Therefore, a decrease in maximum joint angle with age could be expected. During degeneration, the weakening tendon decreases in stiffness and could therefore allow a larger range of joint motion. Therefore, an increase in maximum joint angle with age could be expected in older horses. To this end, the distal limb kinematics of 57 Sport Horses from young foals of three months old to older horses of 212 months old were studied. Since joint angles of the passive distal limb depend not only on the stiffness of the tendon spring but also on the mass they support, maximum joint angle was normalised to mass, represented by abdominal depth. The results of the influence of age on kinematics and tendon strain will now be discussed, starting with a discussion of the data capture methods and possible limitations.

6.4.1. Discussion of data capture methods

Comparison of two camera systems

Two camera systems, at 25Hz and 100Hz, were compared to determine if a simple video camera was sensitive enough to detect changes in joint angle with age. No significant difference was found between the two systems for either MCP or DIP maximum angle, suggesting that a 25Hz system may be suitable for tracking distal limb kinematics in walk. This may be an incentive for horse owners to film their horses to detect subtle changes in kinematics indicative of subclinical tendon injury, before catastrophic injury occurs. However, at faster gaits it is unlikely that 25Hz would be sufficient.

The novel automated marker-free tracking system

The new marker-free tracking system used has certain advantages over existing marker-based technology, including lack of susceptibility to skin displacement artefact (Section 4.2.5). Its repeatability, accuracy and high throughput speed could make it suitable for digitisation of video-based kinematics, especially in an outdoor environment. The similarity of results to those found by current systems is encouraging (Section 5.1.2).

Calculation of mass

The high Pearson's correlation coefficient (0.809, $p=0.001$) between actual and calculated mass indicated that the method used was suitable for the ages and breeds studied (Section 5.3). This is consistent with (Rodriguez *et al.* 2007), who report a 0.97 r^2 value between heart girth size and mass.

Influence of surface type

There was no significant difference between the three surfaces used in either MCP or DIP data, indicating that surface type was not influential in this study. This is consistent with (Chateau *et al.* 2009) who found that maximum vertical ground reaction force (GRF_z) was similar on three different surfaces. Since angle of the passive MCP joint is highly correlated to vertical force (McGuigan and Wilson 2003), a similarity of GRF_z across different surfaces would result in similar MCP angles. However, surface type has been found to affect the kinematics of faster gaits, with a stiffer surface leading to increased GRF_z , DIP joint force (Reiser 2001) and decreased stance phase duration (Burn 2005; Reiser 2001). This would be likely to result in an increase in MCP and DIP maximum angle. The absence of a significant difference between groups in the current study was probably due to the low speed and also the low deformability of all surfaces (Section 6.2.2). For a similar test on faster gaits, use of a consistent, non-deformable, surface is suggested.

6.4.2. Discussion of the age-related change in joint angle

The general age-related trend in normalised joint angle was a relatively rapid decrease with age in Group 1 horses up until about 30 months old. This was followed by a plateau showing no change with age in Group 2 horses up until about 131 months, and finally an increase towards old age in Group 3 horses. These changes will now be discussed in further detail.

Young horses

In Group 1 horses, the significant negative correlation between joint angle and age for both MCP and DIP data is consistent with the proposed theory that the increase in stiffness of the flexor tendon limits the range of motion of the distal joints that they wrap around and support; and provides evidence in support of the hypothesis that there

is an effect of age on joint angle above and beyond the influence of increasing mass in the young horse.

In contrast to the current study, maximum MCP angle has been found to increase with age between four and 26 months in Dutch Warmblood horses (Back *et al.* 1995) and between four and 30 months in ponies (Back *et al.* 2002). However, since these data were not normalised to mass, an increase is expected as the increase in weight increases the flexion of the passive distal limb joints. Once normalised to mass, maximum MCP angle from both studies by Back *et al.* was found to decrease with age (Table 6.1), which is consistent with the current study. Care should be taken with interpretation of the results of the 2002 study since it also included the effects of feeding regimes. A third study (Cano *et al.* 2001) shows a similar increase in metatarso-phalangeal joint angle but since masses were not given, normalised joint angles cannot be compared.

	Younger		Older	
	Non-normalised	Normalised	Non-normalised	Normalised
Back <i>et al.</i> 1995	52.0°	0.25°/kg	55.6°	0.1°/kg
Back <i>et al.</i> 2002	38.5°	0.49°/kg	43.4°	0.27°/kg

Table 6.1 MCP data from previous studies also showed a decrease with age once normalised to mass. Masses were as follows: Back *et al.* 1995: young horses 211kg, older horses 540 kg. Back *et al.* 2002: younger ponies 78kg, older ponies 162kg.

The high r^2 values for MCP (0.77) and DIP (0.65) data indicate that a substantial degree of the variability in the age-angle model is accounted for by the age of the subject. Both mass and tendon stiffness change with the age of the horse, but since mass is accounted for, the remaining factor that could alter joint angle is tendon stiffness. The similarity in rate of change in MCP (-0.41°/cm girth depth/year) and DIP (-0.37°/cm/year) indicates that the kinematics of these joints mature at the same rate.

The average age of the intersection between the assumed linear trend of Group 1 horses and the mean of Group 2 horses was found to be 30 months (29±12 months for MCP data and 31±13 months for DIP data), although this will vary in the individual horse. This is consistent with the theory that matrix production ceases at skeletal maturity between 24 and 36 months old (Smith *et al.* 2002), therefore altering the mechanical properties of the tendon from increasing stiffness to constant stiffness. This change in stiffness is reflected as a change in joint angle trend from a decrease to a plateau. The

standard deviation of around one year is consistent with the theory that the cease of matrix synthesis occurs at skeletal maturity, which also occurs over about a year.

Adult horses

In Group 2 horses, the minimal correlation between joint angle and age in both MCP and DIP data supports the hypothesis that age has little effect on kinematics in the adult horse. This is consistent with the theory that once tendon synthesis has ceased after skeletal maturity, there is little capacity for adaptive change (Buckwalter *et al.* 1993; Smith *et al.* 2002).

The low r^2 values of 0.10 (MCP) and 0.02 (DIP) indicate that only a small proportion of the variability of the age-angle model was accounted for by age, suggesting that other factors are influential. One such factor could be how the horse is trained. All horses were of amateur standard and therefore likely to experience a similar intensity of exercise; however the type and quality of training could influence the kinematics. Trainers aim to produce a horse with a high quality of gait, which is significantly correlated with kinematic variables including maximum MCP angle (Back *et al.* 1994a; Holmström *et al.* 1994), and therefore type of training may be influential.

Older horses

In Group 3 horses, the significant positive correlation between joint angle and age for both MCP and DIP data supports the hypothesis that age is again influential on kinematics towards old age. Repeated loading cycles, combined with minimal matrix synthesis (Smith *et al.* 2002), lead to a weaker, more compliant tendon (Gillis *et al.* 1995) and the decreasing stiffness in the flexor tendons allows a greater range of motion of the joints that they wrap around.

The relatively low r^2 values of 0.39 (MCP) and 0.22 (DIP) do however indicate that not all of the variability of the age-angle model was accounted for by the age of the horse. This could be because each horse would have been subjected to different exercise regimes throughout its life. Tendons of Group 3 horses would therefore have all experienced a different quantity and intensity of loading cycles, leading to a variation in rate of degeneration. Therefore, the kinematics of the joints they support could be expected to show a greater degree of variability with age.

This variability is illustrated by the high standard deviation (SD) of the intersections between the plateau of Group 2 and the assumed linear trend of Group 3 horses; the average age of intersection between Group 2 and 3 horses was 136 months for MCP data, with a SD of ± 53 months, and for DIP data the intersection was 127 months, with a SD of ± 68 months. These high SDs reflect the wide age range at which the onset of degeneration can occur due to the different exercise regimes experienced by individual horses.

The rate of change ($0.05^\circ/\text{cm}$ girth depth/year for MCP data and $0.02^\circ/\text{cm}/\text{year}$ for DIP data) was slower than in the youngest horses, indicating that degeneration is a slower process than maturation.

Summary of the age-related changes in kinematics

Evidence has been presented for a lifelong change in the joint angles of the distal limb. After an initial rapid decrease during maturation, maximum MCP and DIP joint angles then show a plateau after skeletal maturity between two and three years old, which continues until about 8 years old. After this time, maximum joint angle shows an increase towards old age. These kinematic changes are consistent with alterations in the structure of the flexor tendons, which lead to a change in stiffness.

6.4.3. Discussion of the age-related change in tendon strain

MCP and DIP joint angles were used as input into a scaled link-segment model (Lawson *et al.* 2007) (Section 5.2) that calculated strains in the SDFT and DDFT. Since previous longitudinal studies only presented data up until 36 months in horses (Cano *et al.* 2001) and 30 months old in ponies (Back *et al.* 2002), calculation of strain allows comparison to the more numerous studies on the longitudinal change in tendon mechanical properties, for example (Crevier-Denoix *et al.* 1998; Dowling and Dart 2005; Parry *et al.* 1978a, b; Patterson-Kane *et al.* 1997a; Patterson-Kane *et al.* 1997b; Smith *et al.* 2002), and how these changes affect the stiffness (Gillis *et al.* 1995; Shadwick 1990).

Tendon strain is calculated as the change in length divided by the initial length and since strain depends on stiffness, or resistance to deformation, strain and stiffness are

inversely proportional. These changes in strain, as a measure of stiffness, will now be discussed in further detail.

Young horses

The significant negative correlation between age and tendon strain during maturation reflects the age-related increase in stiffness due to structural changes that occur during maturation. These changes are stimulated by circulating growth hormones and include maturation of the intra-fibrillar cross-links (Gillis *et al.* 1997; Parry *et al.* 1978a; Patterson-Kane *et al.* 1997b), an increase in fibril diameter (Parry *et al.* 1978a; Patterson-Kane *et al.* 1997b), and increased concentration of both collagen and cartilage oligomeric matrix protein (COMP) (Buckwalter *et al.* 1993; Smith *et al.* 2002; Smith *et al.* 1997; Sodersten *et al.* 2005). The overall effect of these changes is an increase in stiffness during maturation, and current results are consistent with previously-published work (Gillis *et al.* 1995; Shadwick 1990).

The relatively high r^2 values indicate that a reasonable proportion of the change in flexor tendon stiffness is accounted for by age. The remaining variability may be due to the fact that tendons mature more quickly in some horses than in others; however this is difficult to investigate further without either studying the mechanical properties of the tendon *in vitro* or by analysis of circulating growth hormone concentration, which requires the invasive collection of blood.

The rate of change appeared to be slightly higher in the DDFT (-0.58% strain/year) than in the SDFT (-0.44% strain/year), and since strain is inversely proportional to stiffness, this means stiffness in the DDFT increases at a faster rate than in the SDFT. This could be due to a difference in development of the two tendon's biochemistry, leading to a difference in rates of stiffness change. For example, concentrations of mature cross-links and mass average diameter of collagen fibrils have both been found to be consistently higher in the DDFT (Patterson-Kane *et al.* 1997b), and since these are positively correlated with stiffness (Gillis *et al.* 1997; Parry *et al.* 1978a), it is likely that stiffness is higher in the DDFT. Furthermore, the water content in the DDFT has been found to be less than that of the SDFT (Birch 2007), and since water content is negatively correlated with elastic modulus, this would again indicate a higher stiffness of the DDFT. This is reflected in a higher ultimate strain (12.3%) in the SDFT than in the DDFT (10.0%) in mature tendon (Riemersma and Schamhardt 1985), and may signal a

more important role of the SDFT as an energy store during stance phase (Birch *et al.* 1999).

The rate of tendon development occurs more quickly than the rate of degeneration, possibly so that the tendon reaches optimal stiffness as quickly as possible for maximum efficiency of locomotion. Production of tendon matrix is stimulated by growth factors such as growth hormone and insulin-like growth factor (Dowling and Dart 2005; Dowling *et al.* 2002; Smith *et al.* 2002), and since the peak in growth factor concentration occurs between two and three years old (Champion *et al.* 2002; Fortier *et al.* 2005; Hess and Roser 2001; Malinowski *et al.* 1996; Noble *et al.* 2007), it is reasonable to suggest that the rate of tendon matrix synthesis is also high at this age, leading to a rapid increase in tendon stiffness.

Adult horses

The negligible correlation between age and tendon strain in the adult horse reflects the minimal change in tendon stiffness in the adult horse. After skeletal maturity, circulating growth hormone concentration decreases rapidly (Champion *et al.* 2002; Fortier *et al.* 2005; Hess and Roser 2001; Malinowski *et al.* 1996; Noble *et al.* 2007) and thus can no longer stimulate production of tendon matrix, leading to minimal matrix turnover. This means that stiffness is no longer increasing, but the degenerative changes that lead to a decrease in tendon stiffness have not yet begun to be manifest. This minimal change is also reflected in the negligible rates of change and the low r^2 values, which indicate that other factors such as type of training could influence tendon strain in the adult horse.

Older horses

The significant positive correlation between tendon strain and age in the oldest horses is consistent with previously-published studies that have found an increase in strain towards old age (Gillis *et al.* 1995) as the degenerating tendon weakens. Repeated loading cycles leads to a loss of crimp (Buckwalter *et al.* 1993; Crevier-Denoix *et al.* 1998) and an accumulation of micro-damage, for example a break down of larger collagen fibrils to smaller ones (Parry *et al.* 1978a, b), which both lead to a more compliant tendon and hence an increase in strain.

The fairly low r^2 value indicates that not all of the variability can be explained by age. This is reasonable given that degeneration is a complex process that is possibly accelerated by the mechanical effects of exercise (Birch *et al.* 1999; Birch *et al.* 2008), and thus will depend not only on the initial quality of tendon but also on the type and intensity of exercise given throughout the horse's life, making the degenerative process subject-specific.

The rate of degeneration in both SDFT and DDFT was slower than during maturation, which is reasonable since resistance to degeneration is desirable. There was a small difference in degeneration rate between the SDFT (0.09% strain/year) and DDFT (0.07% strain/year). The greater rate of SDFT strain increase is possibly due to the greater strains and stresses it experiences throughout the horse's life (Birch *et al.* 1999; Denoix 1994), possibly leading to faster degeneration and a greater loss of crimp, which are correlated with a more compliant tendon (Patterson-Kane *et al.* 1997b).

6.4.4. Discussion of the role of the SDFT and DDFT in locomotion

Although the distal limb is primarily supported by the SDFT and DDFT (Denoix 1994; McGuigan and Wilson 2003), other structures have some influence. These structures include the suspensory ligament (SL), and the distal sesamoidean ligaments (DSLs) that are the functional continuation of the SL into the digit. The SL also exhibits an age-related change in structure that approximately parallels the development and degeneration of the flexor tendons in terms of collagen content, cross-sectional area and fibril diameter. Collagen concentration of the SL has been found to increase from birth to maturity and then shows a steady decline into old age, similar to patterns in the SDFT (Parry *et al.* 1978b), although an exercise-related increase in collagen concentration has been found to occur only in the SL (Smith *et al.* 1999). There was no significant reduction in cross-sectional area in SDFT, DDFT or SL after either short- or long-term exercise (Birch *et al.* 2008), indicating a similar response in all three structures. Finally, collagen fibril diameter distributions in SDFT and SL have been found to be similar at both birth and old age (Parry *et al.* 1978b), and no reduction was found in either DDFT and SL fibril diameters after high-intensity training (Patterson-Kane *et al.* 1998). The similarity of age-related distribution patterns indicate that these two structures show similar patterns of degeneration. Since the elastic modulus of the tissue is inversely proportional to the cross-sectional area of the tissue (Gillis *et al.* 1997; Riemersma and

Schamhardt 1985), and proportional to both fibril diameter (Parry *et al.* 1978a) and collagen content (Dowling and Dart 2005; Gillis *et al.* 1997; Parry *et al.* 1978a; Riemersma and Schamhardt 1985), it is likely that the change in SL mechanical properties is approximately parallel to the two flexor tendons. Therefore, the flexor tendons could be seen as representative of the ageing of the SL. Despite the variability introduced by the other supporting structures, the results suggest that the change in mechanical properties of the flexor tendons influences the development and degeneration of gait throughout the horse's life. Given the importance of the SDFT and DDFT in locomotion, it is likely that the mechanical properties of these tendons are influential on locomotion (McGuigan and Wilson 2003).

The rate of change in the MCP in the oldest horses was $0.04^{\circ}/\text{cm}$ per year, which for the average chest depth of 80cm for the group represents a change of 4° per year. Since the SD for the MFSsa is $\pm 2.9^{\circ}$, it is expected that changes in joint angle would be detectable over about a year. As discussed in Section 3.5, the accuracy of the camera and digitizing system is a maximum of 0.3cm, much smaller than the chest depth SD of $\pm 4.3\text{cm}$ within the group. This indicates that the effects of chest depth measurement error were considered negligible. Therefore, deviations from normal degeneration are likely to become apparent over about a year using the methods used in this thesis.

Effect of increase in speed/rider on locomotion

The horses in the current study were analysed in walk due to problems in maintaining control over the younger horses. A faster pace, such as trot, would increase the vertical load on the limb and cause greater flexion of the MCP and DIP. For example, a maximum MCP angle of 136.8° has been found in walk (Chateau *et al.* 2004), whereas in trot this was 152.4° (Chateau *et al.* 2006). A rider's weight would also increase the vertical force on the limb, also increasing flexion of the distal joints. This greater joint flexion would lead to greater strain in the flexor tendons. Therefore, for a given age the strain would be larger than in unriden walk, leading to an upwards shift in the strain/angle curve. Further work would be necessary to determine if this would also lead to a change in the gradient of this curve; if a younger horse would show a proportionately larger degree of strain at higher vertical force than an adult horse.

6.5. Conclusions

This chapter set out to investigate the effect of age on MCP and DIP joint angles, especially the influence of assumed change in tendon stiffness. A new marker-free technique was developed to track the kinematics of the metacarpo-phalangeal (MCP) and distal inter-phalangeal (DIP) joints, which found maximum and range of joint motion to be very similar to invasive bone-pin data. A technique was developed to estimate the horse's mass, so that the mass-normalised joint angles of the passive distal limb could be compared. The high, significant correlation between actual and calculated mass indicated the technique was accurate (Section 5.3).

Evidence has been presented that suggests that the changing stiffness of the SDFT and DDFT throughout the horse's life influences the kinematics of the distal limb joints that they wrap around and support. Population-wide changes in kinematics occurred at two time points; at skeletal maturity between two and three years old when stiffness increase stops; and at around eight years old, where stiffness begins to decrease towards old age. This first change occurs when tendon synthesis ceases at skeletal maturity, and since this is a permanent change, recovery from injury is limited. It is important that the quality of the tendon is optimised whilst synthesis is ongoing and the synthesis machinery can respond to demands placed on it. Horse owners and breeders could therefore track kinematics longitudinally to detect this characteristic change in kinematics, indicative that matrix is no longer being produced. Beyond this point, exercise regimes aimed at improving tendon quality can in fact have a detrimental effect (Smith *et al.* 2002).

Towards old age, longitudinal tracking could allow trainers to detect subtle changes in their horse's kinematics that could indicate subclinical tendon damage. At this point, trainers could reduce the intensity of exercise to avoid catastrophic tendon injury and the associated issues of cost and concerns for equine welfare. The link between altered kinematics and tendinopathy has already been established for the SDFT in horses (Clayton *et al.* 2000), and in the comparable Achilles tendon in the human field (Azevedo *et al.* 2009; Donoghue *et al.* 2008).

7. Conclusions and future directions

7.1. Overview

The overall aim of this thesis was to investigate the how equine locomotion changes throughout a horse's lifespan, focusing on the effect of age on stride variables and joint angles.

The motivation for the work, as outlined in Chapter 1, was the high prevalence of tendon injury in the sport horse, and whether joint angles can be used to identify characteristic changes in the mechanical properties of these tendons. Chapter 2 described the structure and function of the equine distal limb and the changes to tendon structure and stiffness with age. Chapter 3 described the changes seen in stride parameters during growth such as stride length, separating the effects of maturation of the central nervous system (CNS) from physical growth alone. Also presented in this chapter were results of changes to stride variables towards old age. The similarity of data to trends of published literature also indicates that horses' gait was representative of normal gait, indicating that joint angle data was valid. In Chapter 4, the current state of art of motion capture was reviewed, and included the limitations of current marker-based capture. Chapter 5 presented the new methods used in this study, including a marker-free system (MFS) that is suitable for use where marker application is impractical. This thesis focused on sagittal-plane kinematics, since most rotation (and therefore tendon elongation) occurs in this plane. However, a MFS was also developed to track kinematics in the coronal plane. This new MFS was found to be comparable to existing technology. The sagittal-plane MFS was used to track the kinematics of a population of horses from young foals through to older horses to investigate the influence of age on distal limb joint angles. Joint angles were also used as input into a subject-specific model that calculated strain in the two flexor tendons. The purpose of this was to compare strain to published values, since studies into the influence of age on tendon mechanical properties are more common than those that investigate joint angle - especially towards old age. The data capture protocol, results and conclusions of this work was presented in Chapter 6 and the significance and possible application of results is explained in the next section.

7.2. Contributions, significance and clinical relevance

The contributions of this thesis are in three main areas: stride parameters, marker-free tracking and the link between joint angles and tendon stiffness.

7.2.1. Stride data

Previous work carried out to investigate the development of the young horse did not normalise all parameters to leg length, making separation of the effects of CNS maturation and leg growth difficult. A second limitation of previous work is that data for older horses has not yet been reported, and therefore the normal pattern of degeneration of gait is not known.

This work provides normalised data across all stride parameters, allowing further understanding of the role of CNS maturation in gait development. This thesis has found that CNS maturation appears to be complete by 36 months, and therefore changes in gait beyond this age are more likely to be influenced by training. The variability across all parameters was low, indicating that the central pattern generators (CPGs) that define gait were already mature, and further development represented a fine-tuning of the control systems. This is consistent with the theory that, as a flight creature, the horse must be able to escape predation from a very young age. A failure to reach normal values may indicate pathological development. The contribution of this work is that horse owners can expect a change in gait up to 36 months, and that training should begin after this point once the CNS is mature.

A second contribution of this thesis has been to present gait parameters towards old age, providing a database of normal degeneration towards senescence. A slight decrease was found in velocity and stride length, which could represent a decrease in both physiological and cognitive function. Abnormal or accelerated degeneration of stride variables, and especially an increase of variation, may indicate deterioration of the horse's neuromuscular control and alert the owner that the horse's stability may be compromised and therefore care must be taken to prevent a fall. This is the first study that has reported equine stride parameters towards old age and whilst the dataset is of insufficient size to provide a database of "normal" data, it may encourage future work to establish average data to which owners can compare their own horse's stride parameters.

7.2.2. Marker-free tracking of the equine distal limb

Existing technology to capture motion suffers from various drawbacks, including skin displacement artefact, marker wobble and sunlight interference. To overcome some of these, a marker-free system (MFS) was developed that had the further advantage of being completely non-invasive and therefore being applicable at a competition environment. The MFS, which can operate in both the sagittal (MFSsa) and coronal (MFSCO) planes, gave similar results to existing gold-standard technology. The contribution is that owners could use this simple, cheap system to track horses' movement with the ultimate aim of early detection of injury.

7.2.3. The link between joint angle and tendon strain

The high frequency of tendon injury, coupled with the length of time required for recovery, leads to a requirement for a non-invasive, objective measurement of tendon mechanical properties. It would be especially useful to identify the two time points at which these mechanical properties changed; first when tendon can no longer adapt to loads placed on it, and secondly when degenerative changes start to manifest. This thesis tracked the distal limb kinematics of a range of horses from young foals through to older adults, and then input these data into a model that calculated tendon strain. The final result was a pattern of tendon strain change with age, with the main finding that there were two distinct time points at which the pattern changes. The first was at skeletal maturity at around three years old and the second at about eight years old. The first change is due to the cease of matrix production, and it is important to identify this change since it represents the limit of adaptive ability. Up until this point, the mechanical properties of the tendon can be influenced by exercise regimes and owners can use this opportunity to maximise the quality of the tendon to ensure it lasts the lifespan of the horse. However, owners must be careful not to begin high-intensity exercise regimes before this point, since high loading (especially superimposed on enforced confinement) may be detrimental to mechanical properties in the long term. Young horses' kinematics can be tracked as a subject-specific method of identification of tendon maturity, and abnormal development may be indicative of early disorder.

The second change appeared at the onset of degeneration of the tendon due to repeated loading cycles, although this change was more gradual. Again, deviation from this

change may indicate early-stage injury. Tracking a horse's kinematics throughout its life may allow an owner to detect subtle changes indicative of subclinical injury that may be missed by traditional visual methods. Prevention of tendon injury not only has implications for equine welfare but also for the economics of the horse industry.

Therefore, the overall contribution has been to investigate the role of changing tendon stiffness on distal limb kinematics, and to identify two major time points when these change, which indicate a change in the mechanical properties of the tendon.

Possible application to the ageing human

In the human, whose lifespan is around 80 years, investigation of the best methods to maintain gait function is, by default, long-term (Judge 1996). Since the horse's lifespan is around a third of that in the human, the effect of these interventions could be discovered in a shorter period of time. Using an accelerated model for ageing is especially relevant for the aging human population of modern times.

For example, in humans the modulus of the gastrocnemius tendon has been found to be 14% lower in adults aged 69-80 years old compared to adults 20-26 years old (Reeves 2006). The more compliant tendon leads to two important functional adaptations. The first is that a less stiff tendon allows a greater degree of fibre lengthening, leading to suboptimal overlap of myofilaments. Using the ascending limb of the sarcomere length-tension curve, as in most human muscles, the decrease in overlap causes a reduction in force (Reeves 2006; Narici 2008). The second adaptation is that the more compliant tendon requires a longer period of time to be stretched than a younger, stiffer tendon, decreasing the rate at which muscular contraction is transmitted to bone and therefore potentially increasing reaction time (Reeves 2006; Narici 2008). Although there are functional differences between the equine flexor tendons, which act as energy-storing springs (Biewener 2006), and those that transmit muscular force to bone in the human, knowledge of symptoms of tendon degeneration and weakness could be useful in the human field, especially since human tendon can adapt to altered loading conditions (Narici 2008). Exercise such as resistance training could be therefore increase tendon stiffness towards old age.

Although small animals such as mice are often used as a model for aging due to their low maintenance cost and short life span (Lui 2010), their small size requires sensitive

equipment to detect change in locomotion. Not only is detection of kinematic changes easier in larger animals, but tendinopathy in horses results in a similar change in factors such as activity, ultrasound and MRI to those found in humans (Dowling 2000), making the horse an excellent model to study exercise-induced tendinopathy over a relatively short lifespan (Birch 2008).

7.3. Limitations of the current study

Limitations of the study of age on joint angles

The main limitations of the study were that the PIP joint was ignored, the DIP was approximated, only sagittal plane kinematics were studied, and the specific exercise regimes of the horses were unknown. These limitations are justified in Section 6.2.3, and their implications are described here.

The PIP joint was ignored due to its relatively low range of motion (Chateau *et al.* 2004), high inter- and intra-subject variability (Chateau *et al.* 2004; Clayton *et al.* 2007) and the opposing action of the SDFT and DDFT on this joint during stance phase (Denoix 1994). The main effect of ignoring the PIP joint in this study was on calculation of tendon length and therefore strain. Although the range of motion is fairly small, it is not negligible and therefore future studies could include this joint for more accurate calculation of strain. The likely outcome of modelling the PIP joint at 180° on calculating tendon strain is an overestimation in both the SDFT and DDFT. Since this joint is in extension when strain in both tendons is maximal, the tendon path is shorter than modelled and therefore strain is actually smaller than calculated. This is reflected in the higher strains in both tendons than in reported literature (Lawson *et al.* 2007). The largest SDFT strain was around 8.5%, compared to 6.8% previously reported, and the largest DDFT strain of 7.2% was also higher than the 5.5% found previously. However, since the PIP joint was ignored in all horses, the trends with age can still be assessed.

Since the DIP is contained within the hoof, joint angle was approximated from external landmarks. Range of motion and maximum DIP angle were similar to the gold standard of invasive bone-pin data (Chateau *et al.* 2004), indicating that this method was of acceptable accuracy.

Kinematics were studied only in the sagittal plane since this is the plane in which most joint flexion (Chateau *et al.* 2004; Chateau 2002), and therefore tendon elongation, occurs. Although data capture in 3D would allow a more accurate calculation of the tendon path and therefore strain, significant trends with age were found in tendon strain using 2D data from a simple video system. The result that trends can be detected using a simple system in 2D may encourage horse owners and trainers to track kinematics of their horses for early detection of tendon injury.

The exercise that a horse undergoes, especially when young, influences the mechanical properties of the tendon (Section 2.5.9), and therefore potentially the kinematics. Although all horses were of amateur standard and therefore presumed subject to an approximately similar intensity of exercise regime, variability in results may have been reduced by ensuring that all horses underwent identical training and exercise regimes.

Limitations of the study of age on stride parameters

The major limitation was that several data were of insufficient quality for inclusion, especially from youngest group, due to difficulty in controlling the capture environment. This implies that data from this group should be interpreted with caution.

General limitations

A limitation of this cross-sectional work was that the age distribution was not evenly spread out, with some age points being under-represented. A second limitation is that due to the difficulty in confirming the age of older horses, the oldest horse in this study was 17.6 years old, younger than the expected 30-year lifespan of a horse. Thirdly, horses walked on three different surfaces, but whilst this has been shown to affect kinematics in trot, the effect on walking horses was likely to be minimal. Finally, horses were not subject to a full clinical examination due to cost limitations. Further work could include ultrasound and MRI of the joints and soft tissue to ensure that the study population, especially the older horses, were free from disorders that could have affected locomotion.

Ideally, a project could continue the landmark work begun by Back *et al.* (Back *et al.* 1994; Back *et al.* 1995) in tracking the kinematics of the same horses longitudinally. Such a project could ensure that conditions such as exercise regime were consistent across all horses and would determine if these characteristic kinematic changes are

detectable for a horse and how frequently measurements should be taken. Another advantage would be that each time point would be represented by more than one horse. However, the cost and timescale of such a project would be considerable.

7.4. Future directions

This thesis has presented evidence for a change in joint angle throughout the horse's life due to a change in tendon stiffness. The relationship between joint angle and tendon stiffness is due to the passive nature of the equine distal limb, where joint angle is a function of both mass and tendon stiffness. The passive limb means that MCP angle is directly proportional to the force applied (McGuigan and Wilson 2003). An interesting question would be to ask if this relationship remains the same throughout the horse's lifespan, possibly using the sagittal-plane MFS to measure MCP angle. It is possible that middle-aged horses, with a stiffer tendon, would show a smaller maximum MCP angle for a given force than either a very young or old horse, with a more elastic tendon. Although prediction of limb force from MCP angle is a very useful tool, a further study could determine whether this relationship is age-dependent. Such information could be valuable in any study that investigates the relationship between MCP angle, loading and tendon properties; all of which contribute towards reducing tendon injury in the sport horse.

Appendices

Appendix A: Subject details for joint angle and strain data

Subject	Age (months)	Girth (cm)	Normalised max. MCP angle (°)	Normalised max. DIP angle (°)	Maximum SDFT strain (%)	Maximum DDFST strain (%)
Duchess	3	56	3.70	3.77	7.47	7.00
Imogen	5.5	73	3.05	2.78	7.48	6.81
Stan	7.5	60	3.50	3.61	7.16	6.29
Mary	7.5	57	3.77	3.45	7.68	6.04
Jack	17	66	3.44	3.16	7.08	6.17
Jake	17.5	67	3.29	3.17	7.36	6.22
Jemima	17.5	71	3.06	2.72	6.86	5.94
8A	29	73	2.79	2.96	6.20	5.14
25	29	79	2.55	2.63	6.93	5.89
Isabelle	29.5	73	2.94	2.71	6.96	6.08
17	29.5	79	2.88	2.64	6.57	5.12
23	30	78	2.63	2.85	6.52	6.12
30	30	82	2.70	2.52	6.58	5.74
35	30.5	82	2.57	2.51	6.15	5.55
9	31	82	2.67	2.56	6.25	4.68
3	31	86	2.59	2.42	7.01	4.75
12	39	74	2.80	2.77	7.03	6.41
Boogie	44	73	3.13	2.71	6.86	5.46
7	46	85	2.47	2.35	7.18	6.10
Grace	48	84	2.64	2.43	6.42	5.83
Lexy	53	87	2.52	2.34	7.76	6.05
Westwood	53.5	83	2.60	2.46	7.54	6.08
43	53.5	80	2.65	2.50	7.26	6.23
38	54	84	2.58	2.63	6.55	5.86
47	54	85	2.55	2.51	6.59	5.28
39	56	80	2.65	2.72	6.24	6.46
Hobo	60	76	2.93	3.01	6.36	5.48
Sox	60	79	2.55	2.64	7.72	6.90
Murphy	64.5	85	2.53	2.31	6.78	5.63
Lily	72.5	78	2.85	2.61	6.64	6.69
Buster	73	76	2.89	2.62	7.18	6.05
SMS	78	79	2.80	2.59	6.66	6.35
Up The Order	79.5	73	3.16	2.95	7.05	6.73
William	86	69	3.19	2.94	7.46	6.38
Rosie	87	85	2.82	2.47	7.37	6.07
Billy	96	82	2.57	2.46	7.43	6.50
Shanel	100	77	2.81	2.84	6.60	5.84
Billy	100	83	2.50	2.58	6.83	6.38
Cogolie	104.5	84	2.61	2.61	6.69	6.14
Billy	108	82	2.57	2.55	7.03	6.16
Indy	108	82	2.72	2.49	6.77	6.21
Mickey	111	88	2.52	2.33	7.07	6.07
Barrosa	114.5	86	2.43	2.53	7.41	6.20
Researcher	116.5	82	2.89	2.60	7.31	6.83

Rowena	124.5	85	2.57	2.57	7.80	6.60
Peekaboo	128	82	2.61	2.54	7.07	5.98
Miner	135	81	2.61	2.59	7.65	6.76
Sorrel	136	88	2.50	2.38	7.92	6.71
Piracy	138.5	76	3.15	2.82	7.41	6.39
Gemma's Choice	138.5	77	2.86	2.72	8.19	6.76
Saab	146	83	2.80	2.57	7.30	6.85
Asset	148.5	77	2.80	2.69	7.77	7.27
Briar Rose	165	76	3.10	2.71	7.93	6.38
Adee	170.5	79	2.99	2.76	8.38	6.47
Cobweb	174.5	74	2.87	2.64	7.28	6.51
Lily	206	79	2.89	2.61	7.37	6.98
Henbi	212	74	3.01	2.99	7.86	7.32

Appendix B: Subject details for stride parameters

Subject	Age (months)	Stride length (cm)	Stride factor	Stance %	Frequency (steps/sec)
Duchess	3	164.43	6.04	61.7	1.67
Imogen	5.5	173.14	6.20	60.7	1.71
Stan	7.5	174.09	N/A	59.9	1.64
Mary	7.5	170.61	6.02	56.8	1.98
Jack	17	173.17	5.89	64.1	1.51
Jake	17.5	177.18	5.81	64.0	1.57
Jemima	17.5	183.58	5.72	59.5	1.60
8A	29	N/A	N/A	57.7	1.92
25	29	177.62	5.99	61.1	1.71
Isabelle	29.5	N/A	N/A	52.4	1.88
17	29.5	N/A	N/A	58.4	1.71
23	30	N/A	N/A	60.0	1.92
30	30	N/A	N/A	58.1	1.75
35	30.5	N/A	N/A	59.6	1.79
9	31	180.50	5.58	56.7	1.83
3	31	166.20	5.67	63.3	1.62
12	39	170.23	N/A	64.1	1.42
Boogie	44	187.12	5.14	56.9	1.79
7	46	N/A	N/A	59.0	1.79
Grace	48	185.28	5.26	61.0	1.57
Lexy	53	178.44	5.76	65.3	1.64
Westwood	53.5	184.73	5.36	58.5	1.64
43	53.5	N/A	N/A	N/A	N/A
38	54	185.07	5.30	54.1	1.84
47	54	174.97	5.64	66.0	1.67
39	56	189.03	5.31	63.7	1.72
Hobo	60	179.74	5.45	57.8	1.71
Sox	60	169.55	5.64	60.3	1.73

Murphy	64.5	185.56	5.22	64.3	1.46
Lily	72.5	184.23	5.36	55.2	1.79
Buster	73	188.32	N/A	57.4	1.67
SMS	78	N/A	N/A	N/A	N/A
Up The Order	79.5	N/A	N/A	N/A	N/A
William	86	178.56	5.56	63.6	1.66
Rosie	87	186.31	5.27	61.6	1.81
Billy	96	183.12	5.28	61.7	1.59
Shanel	100	195.70	5.10	55.0	1.88
Billy	100	187.15	5.43	66.0	1.35
Cogolie	104.5	186.02	5.67	65.5	1.12
Billy	108	188.80	5.18	64.2	1.50
Indy	108	185.08	5.29	63.1	1.59
Mickey	111	N/A	N/A	N/A	N/A
Barrosa	114.5	174.48	5.71	66.7	1.48
Researcher	116.5	175.09	5.67	68.9	1.44
Rowena	124.5	N/A	N/A	N/A	N/A
Peekaboo	128	190.67	5.10	64.2	1.40
Miner	135	189.44	5.13	62.4	1.77
Sorrel	136	176.77	5.54	64.3	1.55
Piracy	138.5	177.03	5.54	66.6	1.53
Gemma's Choice	138.5	184.25	N/A	62.5	1.69
Saab	146	184.07	5.65	64.1	1.47
Asset	148.5	186.05	5.74	63.6	1.48
Briar Rose	165	N/A	N/A	N/A	N/A
Adee	170.5	173.35	5.69	64.0	1.72
Cobweb	174.5	179.12	5.52	62.0	1.76
Lily	206	174.27	5.73	60.7	1.86
Henbi	212	189.05	N/A	63.1	1.54

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