# THE EFFECT OF EARLY CONDITIONING EXERCISE ON THE CROSS SECTIONAL AREA OF THE SUPERFICIAL DIGITAL FLEXOR TENDON OF YOUNG THOROUGHBRED HORSES.

**A thesis presented in partial fulfilment of the requirements for the degree of Master of Veterinary Science at Massey University, Palmerston North, New Zealand**

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## ABBREVIATIONS

| | |
|---|---|
| **ADG** | Average daily gain |
| **AL-DDFT** | Accessory ligament of deep digital flexor tendon (distal check ligament) |
| **AL-SDFT** | Accessory ligament of superficial digital flexor tendon |
| **BAPN-F** | Beta-aminoproprionitrile fumarate |
| **BCS** | Body condition score |
| **CDET** | Common digital extensor tendon |
| **COMP** | Cartilage oligometric matrix protein |
| **CSA** | Cross sectional area |
| **CV** | Coefficient of variation |
| **DACB** | Distal to accessory carpal bone |
| **DDFT** | Deep digital flexor tendon |
| **GERA** | Global equine research alliance |
| **GEXA** | GERA exercise trial A |
| **GRF** | Ground reaction force |
| **HA** | Hyaluronic acid |
| **IGF-1** | Insulin-like growth factor |
| **MAD** | Mass average diameter |
| **MCPJ** | Metacarpophalangeal joint |
| **MSCs** | Mesenchymal stem cells |
| **NSAIDS** | Non-steroidal anti-inflammatory drugs |
| **PIP** | Proximal interphalangeal joint |
| **PMMA** | Polymethylmethacrylate |
| **P1** | Proximal phalanx |
| **P2** | Second phalanx |
| **PQCT** | Peripheral quantitative computed tomography |
| **PSGAG** | Polysulphated glycosaminoglycans |
| **rEGH** | Recombinant equine growth hormone |
| **SDFT** | Superficial digital flexor tendon |
| **SL** | Suspensory ligament |
| **TIOM** | The inter-osseous muscle (suspensory ligament) |

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**ABSTRACT**

The effect of conditioning exercise on the ultrasonographic cross sectional area (CSA) of the superficial digital flexor tendon (SDFT) of young Thoroughbred horses was investigated. Two groups of pasture-reared foals were matched for age and sex, and allocated into conditioned (n=18, 6 colts, 12 fillies) and control groups (n=15, 4 colts, 11 fillies). The conditioned group were exercised over 1030m on a purpose-built 515m oval grass track, for five days per week, from ten days of age until completion of the study (eighteen months of age). Conditioning exercise was in both a clockwise and counter-clockwise direction, with the initial velocity being 4.20 $ms^{-1}$, which was increased to 5.56 $ms^{-1}$ at five months of age, and to 6.66 $ms^{-1}$ at eight months of age, with the addition of a 250m sprint at 12 $ms^{-1}$.

All foals underwent a thorough clinical examination and conformation assessment at four days of age, which was repeated monthly throughout the study period. The SDFT at the mid-metacarpal level of both left and right forelimbs were examined clinically and ultrasonographically in all animals at five, eight, twelve, fifteen and eighteen months of age. All ultrasonographic images were obtained using a Sonosite® 180 ultrasound machine with a linear 10-5 MHz transducer and a LA5 HRS acoustic stand-off. Captured images were exported to a Pentium computer and the CSA measured with Scion image, using the average of three measurements for statistical analysis. Twelve animals were euthanased at eighteen months of age (6 conditioned, 6 control), and CSA measurement from digital images of transected SDFT at mid-metacarpal level were used to validate ultrasonographic CSA measurements.

At no time during the course of the study were palpable tendon abnormalities detected in either conditioned or control groups, nor was there any ultrasonographic evidence of tendonitis in the SDFT at the mid-metacarpal level in any of the animals. There was no statistically significant difference in mean CSA between conditioned and control animals at any age, nor between colts and fillies. No relationship between mean CSA, bodyweight or body condition score could be established. There was a good linear correlation between *in-vivo* ultrasonographic CSA obtained prior to post-mortem and *in-vitro* CSA obtained at post-mortem ($R^2 = 0.8881$), with the *in-vitro* CSA being 10% larger.

In this novel conditioning programme, early conditioning exercise did not induce a change in the ultrasonographic CSA of the SDFT of the conditioned group, when compared to that of the control animals. When measured ultrasonographically, the ability of the immature SDFT to undergo an adaptive response to conditioning exercise appears to be limited. With the sensitivity of current *in-vivo* measurement techniques, if there are any subtle changes in SDFT CSA in response to conditioning exercise, such changes are likely to remain undetected. Histological and biochemical assessment of harvested tissue was not performed for the purpose of this thesis (these are cur ently being analysed for another study) and may reveal changes in the SDFT induced by conditioning exercise, at a cellular or molecular level.

# 1. INTRODUCTION

## 1.1 Background

### 1.1.1 Superficial digital flexor tendonitis - its significance in the racing Thoroughbred

The wastage of Thoroughbred racehorses on training tracks and racecourses is a feature of racing worldwide. For example, it is estimated that over one third of the Victorian racehorse population in Australia is replaced annually (Bailey *et al,* 1998). A high level of this wastage occurs at the end of the first or second seasons of racing. While "poor performance" contributes to this, a significant number of horses are retired due to injury or disease associated with training or racing (Bailey *et al,* 1997). Jeffcott *et al* (1982) found that 53% of horses experienced some period of lameness during the racing season, and in 20% of cases the lameness was sufficient to prevent the horse racing again. Similarly Rossdale *et al* (1983) showed 23.3 - 62.2% of individuals had some degree of lameness during the racing season.

Superficial digital flexor tendonitis is a common injury in horses that are required to work at speed and is therefore a significant cause of lameness in racing horses. Of the training days lost due to diagnosed causes of lameness, SDFT tendonitis has been estimated to be responsible for between 9% and 5.7% days (Jeffcott *et al,* 1982); Rossdale *et al,* 1983). The incidence of flexor tendon injury is higher in horses competing over fences or hurdles, compared with those competing in flat races (Marr *et al,* 1993a). The risk of SDFT injury is higher in older horses, particularly horses aged five years and older compared to two year olds (Perkins *et al,* 2004a).

The first report for a sex predilection for injury to the SDFT was reported by Perkins *et al* (2004a), who found males had a 3-fold higher risk for injury compared to females, in a survey of New Zealand Thoroughbred horses.

After injury to the SDFT the overall rate of return to work is 46% and there is a high incidence of recurrence associated with this type of injury, (18% to 50%). The average time out of training can vary from six to 24 months while recuperating (Marr *et al,* 1993a). There is an increasing public awareness for animal welfare and the use of animals for racing. It is in the equine industries best interests to reduce the wastage of horses, through owner education and commitment to the diagnosis, treatment, and prevention of lameness through investment in research.

## 1.2 Literature Review

### 1.2.1 The equine superficial digital flexor tendon (SDFT)

The SDFT originates from the body of the superficial digital flexor muscle which arises proximal to the medial epicondyle of the humerus. This muscle has strong tendinous intersections that progressively continue as the SDFT in the distal forelimb. The tendon has contributions from the accessory ligament of the SDFT, (superior check ligament) which is a fan-shaped fibrous band that has its origin on the caudomedial aspect of the distal radius some seven to 11 cm proximal to the carpus. This fibrous band courses distocaudally, to fuse with the SDFT just proximal to the carpus.

Proximal to the carpus, the tendon is round in shape and passes distally through the carpal canal and down the palmar-most aspect of the metacarpal region.

Here the tendon becomes more flattened and half-moon shaped, with the lateral border of the tendon becoming sharp and the medial border more rounded.

Dorsal to the SDFT is the deep digital flexor tendon (DDFT), which originates from the three heads of the deep digital flexor muscle in the distal antebrachium. Together these two tendons extend distally down the palmar most aspect of the metacarpal region to a level proximal to the proximal sesamoid bones. Here the SDFT forms a fibrous ring, the manica flexoria, which encircles the DDFT as it passes through it. At the level of the proximal phalanx (P1) the SDFT branches to form medial and lateral branches that insert distally onto P1 and proximally on the second phalanx (P2) (Denoix, 1994). In the distal metacarpal and proximal pastern regions the SDFT and DDFT are contained within the digital flexor sheath, a synovial structure that facilitates their movement against each other when the metacarpophalangeal joint is flexed and extended. In the carpal region the SDFT and DDFT pass through the carpal canal in a common carpal synovial sheath (Denoix, 1994).

The flexor tendons are held in place by three annular ligaments, which are local thickenings of the deep fascia. The palmar annular ligament arises from the abaxial borders of the proximal sesamoid bones and it adheres to the SDFT restricting the movement of the tendon and proximal sesamoid bones. The proximal digital annular ligament resembles an "X" when viewed from behind. The proximal margins of the "X" originate at the level of proximal P1 and the distal end insert distally onto P1, the body of the ligament fuses with the SDFT. The distal digital annular ligament originates from the medial and lateral borders of P1 and together with the abaxial palmar ligaments of the pastern joint form a sling that fuses with the DDFT (Dyce *et al,* 1987).

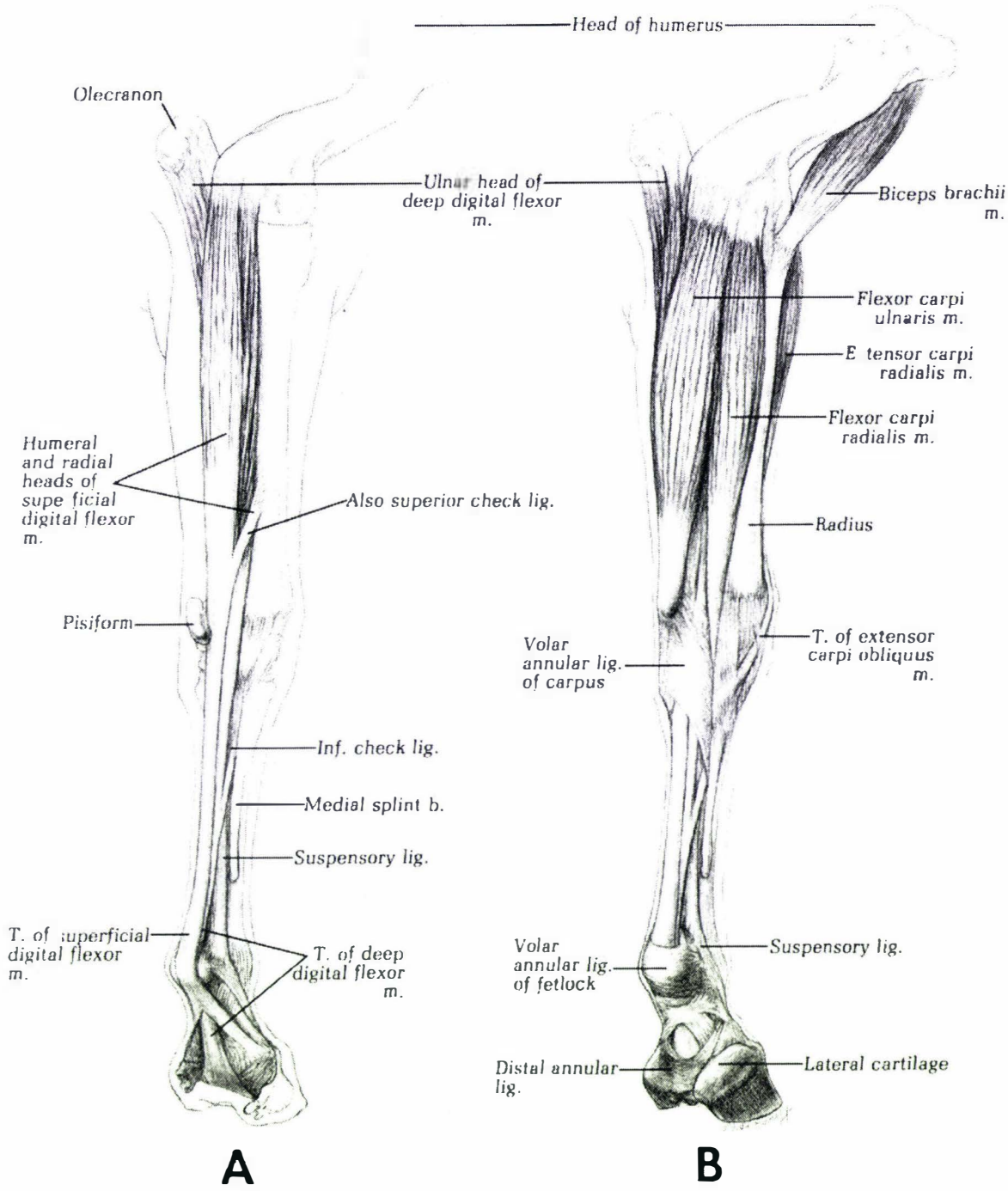


**Figure 1.1 Palmomedial view of structures of the equine forelimb**

**Diagram A depicts the deeper structures. Diagram B depicts the superficial structures.**

***From Horse Shoeing Theory and Hoof care, Lea & Febiger. Philadelphia (Emery, Miller, Van Hoosen 1977)***

#### *1.2.1.1 Blood supply and innervation of the SDFT*

The vascular and microvascular anatomy of the normal equine SDFT has been determined by dissection of vinyl-perfused specimens and by microangiography on high detailed film (Stromberg and Tufvesson, 1977), (Kraus-Hansen *et al,* 1992a). A branch of the median artery described as a "nutrient artery" is closely associated with the accessory ligament of the SDFT and enters the tendon proximally at the transition between muscle and tendon, branching to form an intratendinous network. Distally the SDFT receives its blood supply from the distal metacarpal branch of the digital artery at the level of the proximal border of the palmar annular ligament. Near the distal border of the palmar annular ligament the proximal digital branch of the digital artery also contributes to the blood supply of the distal extent of the SDFT. The tendon also receives complementary blood supply from the body of the superficial digital flexor muscle, the peritendon in the metacarpal region, the adhesion between the SDFT and the proximal annular ligament, and at its distal insertion, the periosteum. (Denoix, 1994). All of these branches contribute to the medial and lateral vessels of the SDFT in the metacarpal region. Within the tendon there is an extensive intratendinous arterial network, with longitudinal arterioles coursing between fibre bundles and anastomosing with fine perpendicular branches.

Innervation to the metacarpal region of the SDFT is from the medial and lateral palmar nerves. The medial branch of the palmar nerve originates from the median nerve while the lateral branch originates from both the median nerve and the ulnar nerve. The digital region of the SDFT is innervated by the digital nerves (Denoix, 1994).

### *1.2.1.2 The function of the SDFT in relation to the stay apparatus*

The horse has the ability to remain standing for long periods of time. This is due to most of the horse's weight being supported by the tendons, ligaments and deep fascia of the stay apparatus.

The bones of the forelimb help support the weight of the horse and are attached to the cranial end of the trunk at the attachment of the serratus ventralis muscle to the medial surface of the scapula. The shoulder joint is prevented from flexion by the biceps tendon that connects the supraglenoid tubercle of the scapula with the proximal radius. At the distal end of the radius the pull of the biceps is transmitted via the lacertus fibrosis and extensor carpi radialis muscles to the dorso-proximal aspect of the third metacarpal bone (Dyce *et al,*. 1987). This pull prevents the carpal joint from flexing while standing.

In the standing horse the distal radial condylar facets are located palmarly, this tends to fix the carpus spontaneously in extension. The strong palmar ligament of the carpus, the palmar wall of the carpal canal, and the accessory carpal bone ligaments limit over-extension of the carpus. The close packing of the carpal bones also helps to maintain the joint in extension. Over-extension of the carpus is limited by the tension of the flexor tendons and the accessory ligament of the SDFT. The fetlock joint is prevented from over-extension by the suspensory ligament, proximal sesamoid bones and distal sesamoidean ligaments. This is reinforced by tension in the accessory ligaments and distal parts of the SDFT and DDFT.

Flexion of the proximal interphalangeal joint is prevented by the insertion of the SDFT onto the abaxial parts of the thick scutum medium. Tension in the distal sesamoidean ligaments and the extensor branch of the suspensory ligaments also limits flexion of this joint (Denoix, 1994).

Extension of the proximal interphalangeal joint is controlled by the DDFT and the palmar ligaments of the joint. Over-extension of this joint is also prevented by the straight sesamoidean ligament and the SDFT.

During the stance phase and during propulsion the SDFT acts as an extensor on the proximal interphalangeal joint, which is opposite to its role during the swing phase of the stride. The distal branches of the SDFT, the extensor branches of the suspensory ligament and the collateral and palmar ligaments of the proximal interphalangeal joint all contribute to the stabilisation of this joint in a frontal plane. (Denoix, 1994)

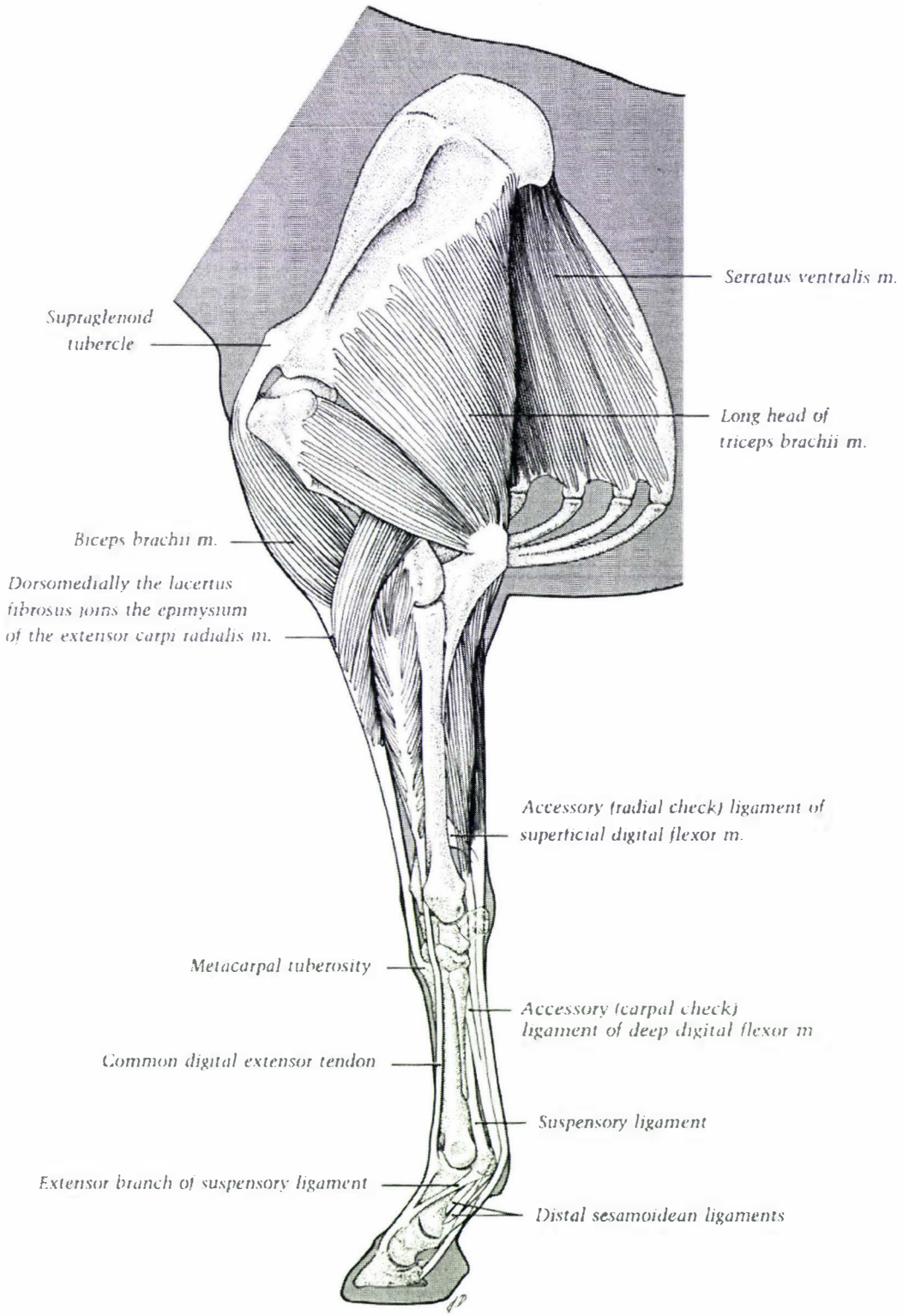


**Figure 1.2 Stay apparatus of the equine forelimb**

***From Adam's Lameness in Horses, Lea & Febiger, Philadelphia, Fourth Edition (Stashak 1987)***

### *1.2.1.3 The function of the SDFT in relation to locomotion*

At the beginning of the swing phase of the stride, tension and elasticity of the SDFT contributes passively to the flexion of the carpal, metacarpophalangeal and proximal interphalangeal joints. Flexion of these joints and the distal limb is increased by active muscle belly contraction, which induces relaxation in the AL-SDFT. These movements are accompanied by a proximal sliding of the SDFT with respect to the carpal canal (Denoix, 1994). The elastic energy stored in the SDFT under stress, is used to reduce the energy cost of locomotion and to increase the efficiency of locomotion. To maximise energy storage the tendon elongates to a point very close to failure (Goodship, 1993).

During the stance phase the fetlock joint is supported by the SDFT. Extension of the metacarpophalangeal joint induces tension in the AL-SDFT. The AL-SDFT prevents overstretching of the SDFT muscle belly by carrying the load during metacarpophalangeal hyperextension, especially at the end of a race when muscles are fatigued. SDFT strain increases significantly after desmotomy of the AL-SDFT(Denoix, 1994).
The proximal insertion of the superficial digital flexor muscle on the medial humeral epicondyle also limits flexion of the elbow. During full weight bearing, high tension in the SDFT and DDFT is responsible for stabilisation of the proximal interphalangeal joint (PIP). The two distal branches of the SDFT that insert onto the proximal aspect of the middle phalanx are essential for stabilisation of the PIP joint in both the frontal and transverse plane. (Denoix, 1994)

### 1.2.2 The morphology of the equine SDFT

The SDFT is composed of parallel collagen fibres arranged longitudinally and sparsely interspersed with tenocytes and extracellular matrix. Small blood vessels and loose connective tissue are present between fascicles of collagen fibres (Crevier-Denoix *et al,* 1995).

Collagen composes approximately 30% of the wet weight of the tendon, with water and the extracellular matrix comprising approximately 60% and 10% respectively (Smith and Webbon, 1996). The predominant collagen in the normal adult SDFT is type I, (95%) with a small proportion of type III and type V associated with the intra-tendinous vasculature (Goodship, 1993).

Various glycosaminoglycans have been isolated within the equine SDFT, including chondroitin sulphate, dermatan sulphate, keratan sulphate, heparin, heparin sulphate and hyaluronic acid (Dowling *et al,* 2000). Of the glycoproteins of the extracellular matrix, cartilage oligomeric matrix protein (COMP) is one of the most abundant (Smith *et al,* 1997).

### *1.2.2.1 Collagen arrangement within the SDFT*

There is a complex structural hierarchy within the tendon. The molecules of collagen are built into a series of progressively larger subunits that combine to form the definitive tendon. It is collagen that imparts the main tensile strength to the tendon. Tropocollagen molecules are arranged in triple helix form, and make up microfibrils, subfibrils, and fibrils. The collagen fibrils are arranged in bundles called fascicles that vary in size and cross-sectional shape. Within the fascicle, the fibrils are separated by loose fibrous tissue, forming interfascicular septa (Goodship, 1993).

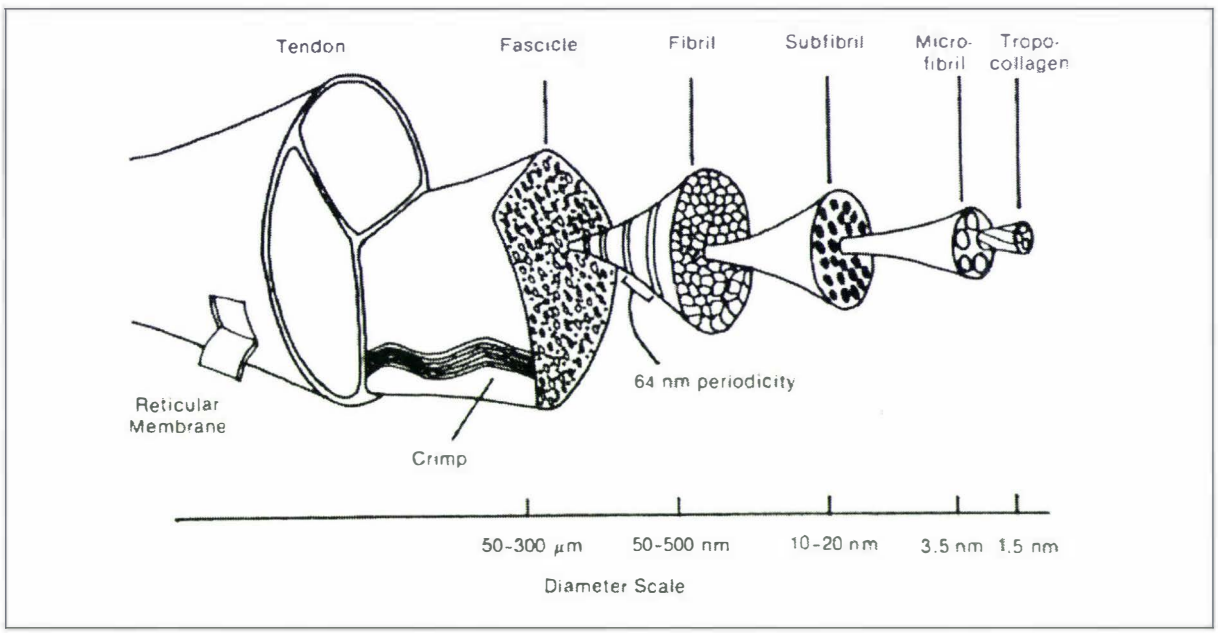


**Figure 1.3 The complex structural hierarchy of tendon**

***From Superficial digital flexor tendonitis in the horse, Equine Veterinary Journal, (Dowling et al, 2000).***

### *1.2.2.2 Collagen cross-linkage*

A biomechanical feature of collagen that influences the mechanical strength of the tendon is the formation of cross-linkages between adjacent longitudinal collagen molecules. These cross-linkage account for the mechanical stability and tensile strength of collagen (Crevier-Denoix *et al,* 1995).

The cross-linking of type I collagen in the SDFT involves the covalent bonding of hydroxylysine and lysine. Hydroxylysine and lysine are converted to their respective aldehydes by the action of the enzyme lysyl oxidase. These products can then form a number of different types of cross-links, being either reducible or non-reducible. The reducible cross-linkages decrease with age.
In the mature animal the proportion of reducible cross-linkages is less than 10% of the level in the foal (Silver *et al,* 1983). Other non-covalent cross-links are provided by the proteoglycans of the tendon matrix (Smith and Webbon, 1996).

### *1.2.2.3 Tendon fibre crimp pattern*

Tendons have a crimped, waveform appearance when viewed in longitudinal histological sections. The crimping is observed at the level of the collagen fibril. The crimp pattern contributes to the complex mechanical behaviour of the tendon, unfolding during initial loading of the tendon, allowing the tendon to stretch by 3-5% without causing structural damage (Crevier-Denoix *et al,* 1995;Wilmink *et al,* 1992).

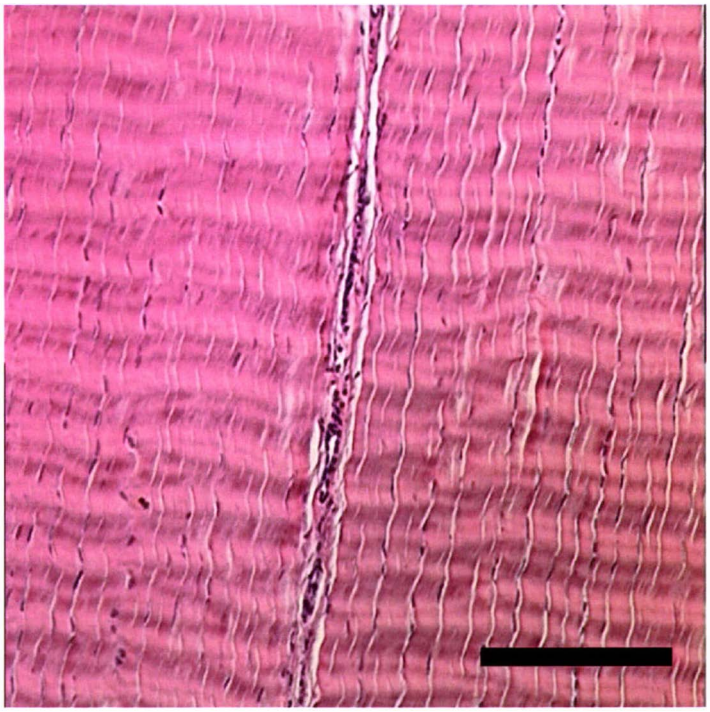

**Figure 1.4 Hematoxylin and eosin histological section of equine SDFT viewed under polarised light depicting the crimp pattern of collagen fibres.**

**Bar = 0.2mm.**

***From Sports Medicine and Arthroscopic Review (Firth 2000).***

When sections of tendon are examined microscopically under polarised light, there is an alternating light and dark banding due to the tendon being in a relaxed state. The wavelength and angle of the crimp may vary between fibrils within a fascicle. Consequently when a tendon is stretched some fibrils will straighten out before others. When a tendon is stretched to failure, some fibrils fail before others, resulting in a partial rupture as seen in many cases of tendonitis (Goodship, 1993).

The crimp morphology is related to the mechanical behaviour of the tendon. Collagen fibre bundles with a smaller crimp angle will experience higher levels of stress at a given level of strain, and will fail before those with a larger crimp angle (Wilmink *et al,* 1992).

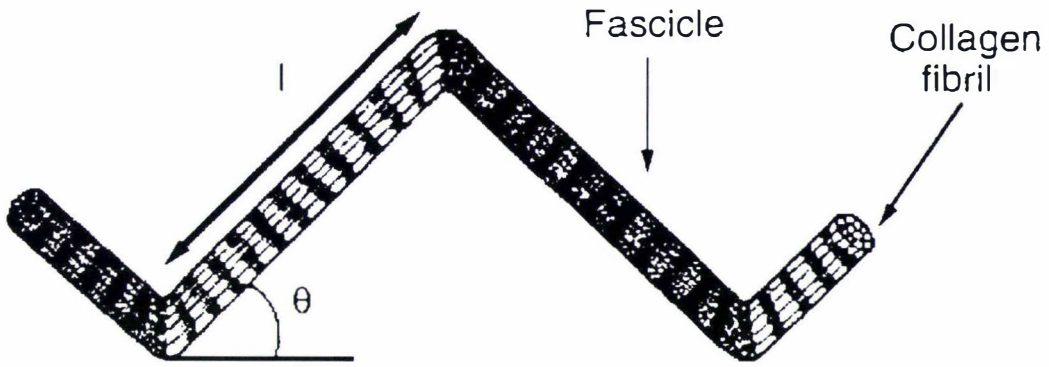


**Figure 1.5 Collagen fibril crimp angle and length.**

**θ = Crimp angle, l = True crimp length.**

***From Sports Medicine and Arthroscopic Review (Firth 2000).***

As horses age, there is a decrease in the crimp characteristics between fibrils in the central and peripheral regions of the SDFT (Patterson-Kane *et al,* 1997a). When the tendon is subjected to high loads the central fibrils fail before those in the periphery. This finding correlates well with the ultrasonographic findings of tendon injury in horses (Wilmink *et al,* 1992).

### *1.2.2.4 Collagen fibril diameter*

Electron micrographs of the equine SDFT show the cross sectional shape of the collagen fibrils to be circular. In the horse, a bi- or tri-modal pattern is apparent at birth, which is in contrast to other species where a bi- or tri-modal pattern develops with ageing.

In the adult horse fibrils can be classified into three categories according to their diameter: small (<40 nm), medium (40-120 nm), and large (>200 nm). The diameter of the type I collagen fibre in the foal is uniformly small. As growth proceeds, the fibril diameter increases, and there is more variation in the size of the fibrils in the adult (Goodship *et al,* 1994).

Parry *et al* (1978) hypothesised that large diameter fibrils have greater tensile strength due to the higher density of intrafibrillar covalent cross linkages between collagen molecules. This prevents slippage between the collagen molecules when the fibril is loaded. Therefore at the same level of increasing strain, a smaller diameter fibril will fail before one with a larger diameter. In contrast it has been suggested that the smaller diameter fibrils, having a greater surface area per unit mass, would have a proportionately greater interaction with the connective tissue matrix than the larger diameter fibrils. This attribute may allow the tissue to resist plastic flow more readily and prevent slippage between fibrils (Parry *et al,* 1978).

It is most likely that the orientation and proportion of the small and larger diameter fibrils determine both the strength and elasticity of the structure (Patterson-Kane *et al,* 1996).

The mass average diameter (MAD) has been positively correlated with the in vitro ultimate strength of the tendon. The MAD is the mean of the fibril diameter-area distribution and allows for the fact that a small number of large diameter fibrils can occupy a large proportion of the mass of collagen in a tendon cross section (Parry and Craig, 1988).

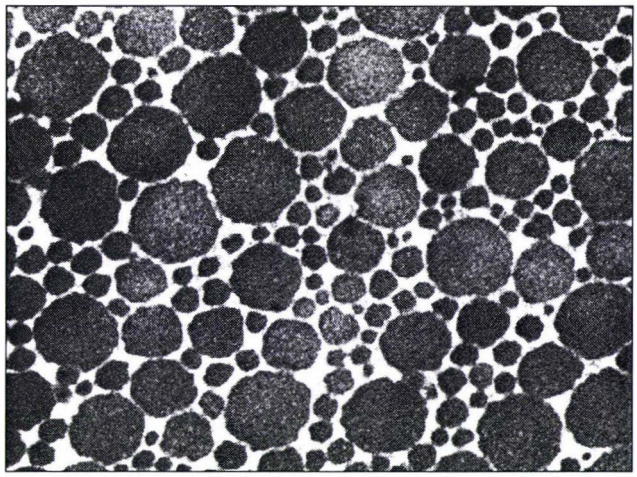

**Figure 1.6 Transmission electron micrograph from the mid-metacarpal region of the SDFT of a non-trained horse depicting small and large diameter collagen fibrils.**

***From Sports Medicine and Arthroscopy Review, (Firth 2000).***

### *1.2.2.5 The tenocytes*

The tenocytes are responsible for the production and maintenance of the surrounding tendon tissue and extra-cellular matrix. The distribution and density of cells in relation to matrix appears to differ between the two flexor tendons and suspensory ligament of the forelimb. The significance of the specific cell distribution is not yet known but may be associated with the functional roles of the different collagenous structures (Goodship, 1993). There is little work quantitatively describing tenocytes populations in normal or diseased SDFT.

Webbon *et al* (1996) identified distinct populations of tenocytes within the normal equine tendon based on nuclear morphology and viewed with 2D light and electron microscopy. Type I cells have a thin spindle shaped nuclei. Type II cells are groups of cells with more rounded, cigar shaped nuclei. Type III cells are cartilage-like cells with rounded nuclei and visible nucleoli.

Very large cells with round nuclei and surrounding extracellular matrix which part the collagen fibrils, known as chondroids, are only identified in the metacarpal region of the flexor tendons in older horses. The significance of these cells is not clear (Smith and Webbon, 1996; Goodship, 1993).

Immature tendon has considerably larger numbers of type II cells than mature tendon. With ageing, type I cells predominate. Type III cells are found in regions of the tendon subjected to compressive forces (Webbon, 1978).

Doube (2001) developed a staining technique using propidium iodide, enabling equine tenocyte nuclear morphology to be studied using confocal laser scanning microscopy and 3D image analysis. He was able to demonstrate two types of tenocyte populations within the mid-metacarpal region of the SDFT using nuclear length and width measurements, which is in general agreement with observations made by previous researchers. He found the cellularity of a small volume of SDFT to be 62,000 ± 25,000 cells/$mm^3$, indicating that larger volumes of SDFT must be sampled in order to accurately quantify SDFT cellularity.

To fully understand the pathogenesis of tendonitis a better understanding of the role of the tenocyte, in both normal and diseased tendon, needs to be determined.

Zeichen *et al* (2000) found that cultured tenocytes will align parallel to the direction of externally applied biaxial strain. Doane and Birk (1991) discovered that tenocytes taken from a 14 day old chick embryo and cultured in random orientation aligned in a uniaxial manner, despite the absence of external mechanical stimulation.

The tenocytes cytoplasm contains smooth muscle actin (Shulz Torres *et al*, 2000) and myosin (Ippolito *et al*, 1977). It is possible that the tenocyte is an active and contractile cell, that actively controls tension within the tendon extracellular matrix (Ippolito *et al*, 1977).

Murphy and Nixon found that insulin-like growth factor (IGF-1) stimulates tenocyte proliferation and collagen synthesis. This is similar to work by Banes *et al* (1995) who found that IGF-1 in combination with platelet derived growth factor and mechanical stimulation, stimulated tenocytes to multiply. TGF-β has been found to induce collagen synthesis and fibroblast proliferation (Fenwick *et al*, 2000). Chan *et al* (1997) found tenocyte proliferation was significantly enhanced by basic fibroblast growth factor.

Further understanding of the mechanisms that control tenocyte orientation, tenocyte proliferation, and crimp formation may lead to therapies that manipulate the repair processes of healing tendon and enhance the quality of the regenerating tissue.

### 1.2.3 The biomechanical properties of the SDFT

Tendons transmit forces between muscle and the tendon's osseous insertion, in order to move the skeleton. The tensile strength of tendon is the highest of the soft tissues in the body, because collagen is the strongest fibrous protein and these fibres are arranged parallel to the direction of the tensile force of the muscle.

### *1.2.3.1 Tension in the SDFT during the stance phase*

Just prior to the foot making contact with the ground there is contraction of the SDF muscle, which tenses the SDFT before high loads are placed on the tendon, thus preventing sudden elongation and vibration of the tendon. Sudden hoof impact with the ground during landing leads to vibration of the SDFT, which is limited by the palmar metacarpal fascia and annular ligaments, along with contraction of the flexor muscle, removing laxity from the distal limb joints and pre-stiffening the tendons.

During the mid-stance phase high tension is induced in the SDFT and the AL-SDFT by fetlock extension. Fetlock extension produces high stresses in all components of the suspensory apparatus. The flexor tendons and accessory ligaments are strongly elongated during the mid-stance phase.

During propulsion, the last period of the stance-phase, the passive elastic behaviour of the flexor tendon, suspensory apparatus and associated accessory ligaments induces fetlock elevation and brings the pastern forward vertically. Active muscle contraction of the flexor muscles contributes to fetlock flexion and induces proximal displacement of the SDFT therefore relaxing the AL-SDFT (Denoix, 1994).

Riemersma *et al* (1993) found a biphasic peak of *in-vivo* tendon force strain in the forelimbs of ponies at the walk. Strains peaked during the first part of the stance phase in the SDFT and DDFT, and in some individuals, the inter-osseous muscle (TIOM). The AL-DDFT was loaded during the second part of the stance phase.

The total load for the flexor tendons was not as great as that of the TIOM and AL-DDFT. Stephens *et al*, (1989) demonstrated that the pattern of strain in the flexor tendons changed between the walk and faster gaits. At the trot and gallop the peak of strain occurs near the mid-stance phase and the peak of the strain curve is higher.

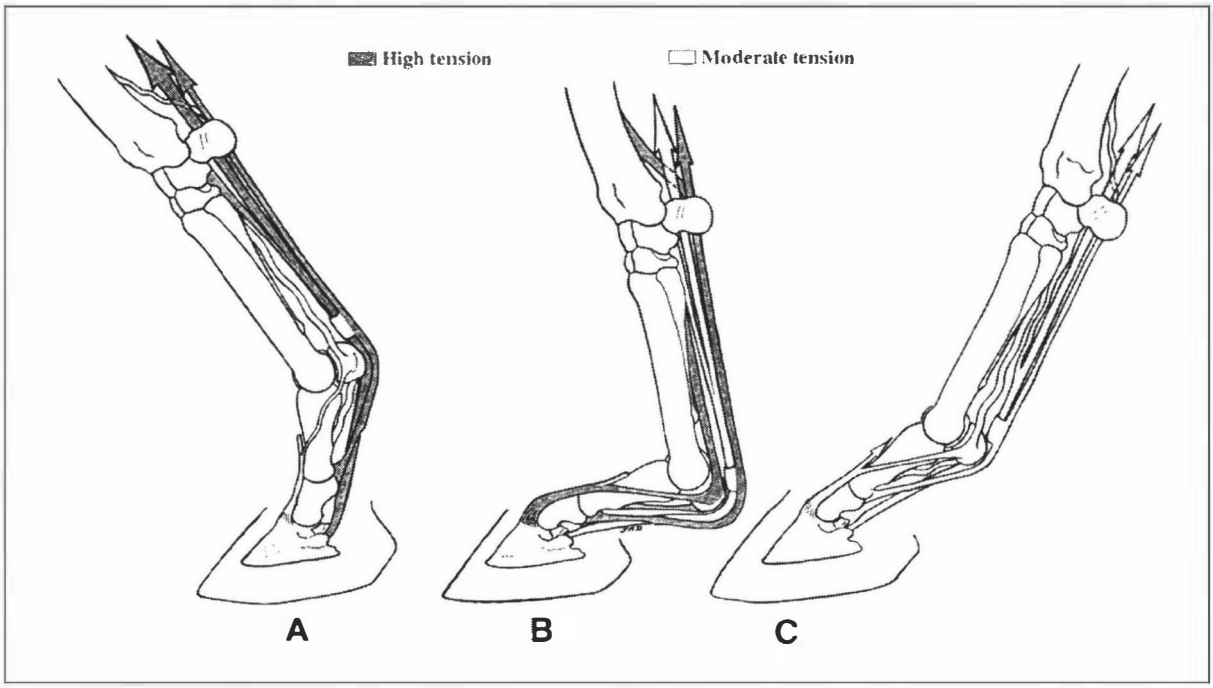


**Figure 1.7 The Functional anatomy of the flexor tendons and suspensory ligament during the stance phase.**

A = Propulsion B = Mid-stance phase C = Landing.

***From Veterinary Clinics of North America, Functional anatomy of tendons and ligaments of the distal limb (manus & pes), (Denoix 1994).***

### *1.2.3.2 Stress strain curves*

Stress strain curves have been calculated for the equine SDFT, where the percentage elongation (strain) is plotted against the force per unit area (stress). There is variation in the curve between tendons and tendon sites (Riemersma and Schamhardt, 1985).

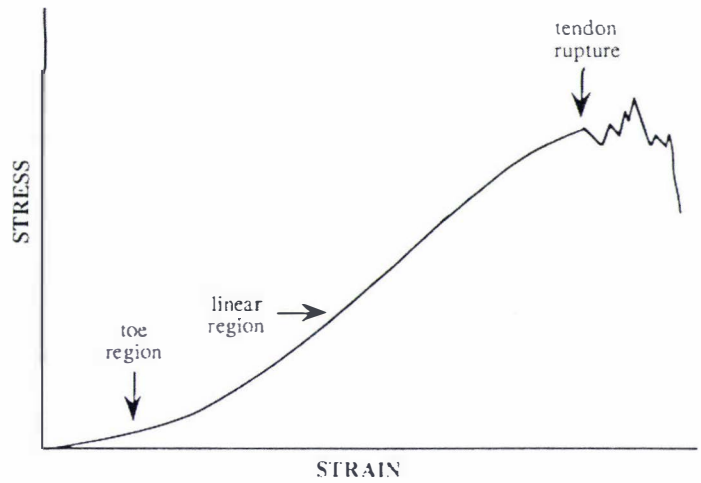


**Figure 1.8 Stress strain curve for tendon**

***From Veterinary Clinics of North America, The Pathobiology and Repair of Tendon and Ligament Injury. (Goodship, Birch, Wilson 1994).***

The "toe" region is associated with fibril crimp pattern elongation, and there is a non-linear stretch to the tendon. The region of "linear deformation" is the area of the curve from which the modulus of elasticity is determined and characterises the elastic stiffness of the tendon. The region of "rupture" is where the stress strain curve quickly returns to zero as collagen cross-linkages and fibrils are ruptured.

From the stress strain curve a number of biomechanical parameters of the tendon can be determined, such as the ultimate tensile stress (the force per unit area at the point at which the tendon breaks), the modulus of elasticity (E), (a constant derived from the ratio of stress to strain for the linear part of the stress/strain curve), and the ultimate tensile strain (Goodship *et al,* 1994).

The ultimate tensile strain of the tendon is the percentage of extension of the tendon at its breaking point. The equine SDFT can extend up to 10-12% without rupture. In the galloping thoroughbred strains in the SDFT can reach up to 16%, indicating that the tendon is at or close to its ultimate tensile strain (Stephens *et al*, 1989).

Crevier *et al* (1996) showed a greater ultimate tensile strain in the metacarpal region of the tendon, in comparison to the fetlock and phalangeal regions (12% vs 8.5%).

It has been suggested that tendon injuries occur at the mid metacarpal region because this region has the tendon's smallest CSA, and therefore stress will be highest at this site (Webbon, 1973; Kraus-Hansen *et al*, 1992).

Riemersma *et al* (1985) showed that in the hind limb, the SDFT load-strain characteristics were more or less homogeneous in spite of a large variation in tendon CSA, which indicated the strength of a tendon site was independent of its CSA at that site. He also demonstrated that the tendon sites with the smallest CSA contain the highest content of collagen, tendon fibres, and dry substances. His work clearly showed that the CSA of the hind limb SDFT is not representative of the strength of the tendon, and within a tendon, the CSA is inversely proportional to the collagen content and proportional to the modulus of elasticity (Riemersma and De Bruyn, 1986).

While Riemersma's work has led to a better understanding of the relationship between tendon CSA, composition, and mechanical function, the work must be interpreted with caution. The tendons used in the studies were dissected from the hindlimbs of adult horses weighing between 400 –700 Kg, and were of various breeds. Therefore direct extrapolation of this work may not reflect the relationship between SDFT CSA, collagen content and tendon strains in the forelimb of young athletic Thoroughbreds in conditioning exercise regimens or race training.

The SDFT is preferentially loaded at the early stage of the stride (Goodship *et al*, 1994). When poor hoof conformation exists, (such as lowering of the toe with respect to the heels, or raising of the heels with respect to the toe), there is reduced loading of the DDFT and increased loading of the SDFT (and AL-SDFT). This can also occur in soft ground, which allows the toe to sink. Factors such as the weight of the rider, poor hoof conformation or hard ground will increase these peak forces (Denoix, 1994).

#### *1.2.3.3 Hysteresis*

Hysteresis is a property of the tendon that results from cyclic loading and unloading of the tendon. It is best demonstrated by the area between the two curves on the stress-strain curve, and represents the energy lost during the loading cycle, which is approximately 5% in equine tendons. Some of the rise in tendon core temperature during exercise can be attributed to this energy loss (Riemersma and Schamhardt, 1985).

### 1.2.4 The growth and development of the equine SDFT

The SDFT of the foetus and neonate is histologically indistinguishable, but age leads to changes that are more or less characteristic for each tendon at particular sites along its length (Webbon, 1978).

#### *1.2.4.1 The growth rate of the long bones of the equine forelimb*

Fretz *et al* (1984) determined the growth rates of the long bones in Thoroughbred cross foals by placing radiographic markers into predetermined sites in the distal ends of the radius, third metacarpal and metatarsal bones, and the distal end of the proximal phalanx. The growth of these bones was monitored for 300 days and a rapid linear growth rate was observed from birth to ten weeks of age in all of the bones. Campbell and Lee, (1981) found a similar pattern of bone growth for the long bones of the forelimb in crossbred ponies.

In the radius, growth rate declined exponentially to 60 weeks of age, after which growth at the distal end of the radius virtually ceased. In the third metacarpus/metatarsus, growth decreases abruptly and nearly plateau's by ten weeks of age. The total bone growth determined to occur at the distal end of the radius, distal third metacarpal/metatarsal bones, and the proximal ends of the first phalanx were 87.2mm, 18.5mm, 23mm, and 13 mm respectively (Fretz *et al*, 1984).

If the work of Fretz *et al* (1984) could be applied to growing Thoroughbred horses, it is possible that most of the increase in length of the SDFT and SDFT muscle has occurred by ten weeks of age, and the increase in length of the tendinous-muscle unit from birth to 300 days is approximately 118 mm.

### *1.2.4.2 Changes in SDFT cellularity in relation to maturation*

In the foetus the SDFT is composed of numerous longitudinally arranged cells with plump granular nuclei between collagen fibres. As gestation progresses the cellular component of the tendon decreases and the fibrillar component increases (Webbon, 1978).
Webbon (1978) described elongation of the tenocyte cell nucleus as the foal ages, and observed that after six months of age the number of tenocytes is higher in the lateral part of the tendon than the medial part.

As a horse matures there is a significant decrease in the cellularity of the SDFT and the acellularity is more prominent in the metacarpal region, than the proximal and intrasynovial regions of the tendon (Bailey *et al*, 1992 ;Crevier-Denoix *et al*, 1998). These acellular zones can be observed from two years of age onward and do not appear to increase significantly as the animal ages (Webbon, 1978). The importance of this acellularity is not clear. Stromberg (1971) ascribed acellular areas as signs of necrosis or degeneration in macroscopically abnormal tendons. However Bailey *et al* (1992) found no relationship between discolouration of the core of the SDFT observed in degenerative lesions, and the tendon cellularity.

There is some evidence that aged horses are susceptible to spontaneous rupture of the SDFT (J. Patterson-Kane, unpublished observation).

### *1.2.4.3 Focal chondroid metaplasia of the SDFT*

Focal chondroid metaplasia is defined as the development of a fibrocartilaginous matrix made of non-orientated collagen fibres containing chondrocytes, either isolated or in small groups. The SDFT of foals are characterised by the total absence of focal chondroid metaplasia. By five years of age the distal metacarpal, sesamoidean and distal regions of the SDFT are characterised by the presence of focal chondroid metaplasia and this increases with age. Focal chondroid metaplasia is not a feature of the proximal metacarpal and middle metacarpal regions of the SDFT at any age.

The presence of focal chondroid metaplasia is probably subsequent to the specific stresses that these tendon segments are subjected to during locomotion. The SDFT slides against the palmar aspect of the proximal sesamoid bones during extension of the metacarpophalangeal joint, subjecting this portion of the SDFT to longitudinal traction and transverse compression. Together with age, these forces induce the differentiation of tendinous tissues (Crevier-Denoix *et al,* 1998).

Focal chondroid metaplasia can occupy up to 30% of CSA from the distal and sesamoidean regions of the SDFT. This localised differentiation has been described in the flexor tendons of other species (Crevier-Denoix *et al,* 1998).

### *1.2.4.4 Diffuse chondroid metaplasia of the SDFT*

Diffuse chondroid metaplasia is defined as chondrocytes disseminated among the collagen fibres, and not contained in a fibrocartilage type matrix, as is the case in focal chondroid metaplasia (Crevier-Denoix *et al,* 1998). Webbon (1978) described the presence of diffuse chondroid metaplasia in the distal portion of the SDFT. This histologic observation may

be correlated with the proximity of the insertion site of the SDFT on the phalanges. (Webbon, 1978).

Crevier-Denoix *et al* (1998) observed diffuse chondroid metaplasia was only present in horses over six years of age and principally in the digital regions of the SDFT. In horses aged 14 -23 years there was evidence of diffuse chondroid metaplasia in some metacarpal and sesamoidean segments. The presence of chondrocytes within a tendon close to its osseous insertion may indicate transition to the bone on which the tendon inserts. Crevier-Denoix *et al* (1998) found that lesions to the distal portion of the SDFT in horses are most frequent in the oldest age category of horses (16-20 years) of whatever activity.

#### *1.2.4.5 Fibrocartilaginous changes in the distal segment of the SDFT in relation to age*

Crevier-Denoix *et al* (1998) found that the dorsal border of the distal SDFT tended to differentiate into fibrocartilage in association with ageing while the palmar border generally differentiated as non-fascicular dense connective tissue. The fibrocartilaginous tissue observed on the distal borders of the tendon was characterised by an intercellular substance consisting of collagen fibres longitudinally orientated, with chondrocytes disseminated in this matrix.

In adult horses a fibrocartilaginous band was present on the dorsal border of some segments of the distal SDFT, which was between 0.5 to 1.0 mm thick. Bands of focal chondroid metaplasia leave the fibrocartilage and go deep into the tendon, from the dorsal border and continuing into the medial and lateral borders, and partially into the palmar border (Crevier-Denoix *et al*, 1998).

The authors consider the focal chondroid metaplasia and fibrocartilage of the dorsal border to be a normal feature of the distal SDFT, and is related to compression stresses in the sesamoidean region of the tendon. Differentiation of fibrocartilage is not a feature of the proximal or middle segments of the SDFT (Crevier-Denoix *et al*, 1998).

#### *1.2.4.6 Age-related changes in collagen fibres*

The equine SDFT attains maturity at approximately two years of age as collagen fibril diameter, mature collagen cross-links and crimp morphology have stabilised by this age (Patterson-Kane *et al*, 1997b).

In older horses the central zone of the SDFT has a significantly higher proportion of type III collagen than the peripheral zone. This may represent the early stages of degenerative change and that ageing in the SDFT results in a significant increase in collagen-linked fluorescence (Birch *et al*, 1999a).

#### *1.2.4.7 Collagen fibril diameter in relation to ageing*

Davankar *et al* (1996) and Parry *et al* (1978) have reported the collagen fibril diameter distribution in the SDFT as a function of age. In the foetus at nine months of gestation, the collagen fibril diameter distributions were unimodal. With maturity the distributions varied markedly from one another with bimodal forms becoming apparent. At senescence (19 years), the number of large diameter fibrils was significantly less than those seen at maturity. The apparent breaking down of the large diameter fibrils with increasing age may be a consequence of a fatigue mechanism within the tissue (Patterson-Kane *et al*, 1996).

#### *1.2.4.8 Fibre fascicle CSA in relation to ageing*

Gillis *et al* (1997) evaluated the middle metacarpal region of the SDFT in horses aged from 2 to 23 years old and found that the fibre fascicle CSA significantly decreased with age.

#### *1.2.4.9 Changes in interfascicular connective spaces in relation to ageing*

The interfascicular connective spaces are areas of loose connective tissue and blood vessels, grouped into thin bands between the fibre fascicles, and are longitudinally orientated. Crevier-Denoix *et al* (1998) determined that there was no significant increase in the number of interfascicular connective spaces with age. However the size of the interfascicular connective spaces decreased with age, this being more prominent in aged animals. This is in contrast to Gillis *et al* (1997) who found that complete septa (equivalent to interfascicular connective spaces) between the fascicles increased in older horses.

The interfascicular connective spaces were significantly larger in number and size in inactive adult horses, compared to those of active adult horses, suggesting that exercise results in a decrease in the interfascicular loose connective tissues (Crevier-Denoix *et al*, 1998).

### *1.2.4.10 Age-related changes in collagen crimp pattern, crimp angle and crimp length*

In the foetus the tendon fibril crimp angle and length are significantly greater than those of adult animals. It is likely that the crimp angle and the crimp length decrease rapidly from birth to maturity (Patterson-Kane *et al*, 1997a). Collagen crimp morphology varies in relation to the site within the SDFT, and the greatest age-related changes are observed within the central regions of the tendon.

In older horses the crimp angle of the central fibres was significantly lower than that of the peripheral fibres, these differences were not apparent in horses less than ten years of age. In younger animals the central fibre crimp angle was greater than that of the older group. There was a difference in crimp angle between younger and older animals for the peripheral fibres, but this was less marked than that of the central fibres (Wilmink *et al,* 1992).

The change in peripheral crimp angle found by Wilmink *et al* (1992) is in contrast to that of Patterson-Kane *et al* (1997a), who found that the peripheral crimp angle did not change significantly with age. This was in wild horses that had not been subjected to any imposed exercise regimens, therefore these changes may be indicative of differences in levels of exercise between the two studies.

The reduction in crimp angle in the central region was apparent with ageing in feral horses. Therefore the reduction in crimp angle of the central region of the SDFT appears to be a normal age-related change, occurring in the absence of imposed training regimes (Patterson-Kane *et al*, 1997a).

In older horses the mean crimp length is shorter for the central fibres than the peripheral fibres, while in the younger horses there is no difference between the two sites (Wilmink *et al*, 1992). The mean crimp length of central and peripheral fibres is shorter in older horses than for equivalent sites in younger horses.

#### *1.2.4.11 The modulus of elasticity in relation to ageing*

Gillis *et al* (1995a) found an increase in the modulus of elasticity with age in the mid-metacarpal region of the SDFT. The diffuse chondroid metaplasia of the sesamoidean and distal portions of the SDFT may explain the lower modulus of elasticity of this region compared to that of the metacarpal segments (Crevier-Denoix *et al*, 1998).

Wilmink *et al* (1992) demonstrated that the modulus of elasticity was the same for fibres in both the central and peripheral regions of the SDFT in both young and older horses.

### *1.2.4.12 The CSA of the SDFT in relation to ageing*

Birch *et al* (1999b) reported that the ultrasonographic CSA of the SDFT has reached maturity in terms of size at two years of age.

Kasashima *et al* (2002) found that the ultrasonographic CSA increased as a function of time in Thoroughbred foals from 2-15 months of age.

Smith *et al* (1994) determined the normal ultrasonographic CSA range for the SDFT using three distinct populations of horses (22 Irish Draught crossbreds, 15 Thoroughbreds, 15 Ponies). There was no statistically significant difference in the data obtained from the Irish Draught crossbreds and the Thoroughbreds, therefore these two groups were combined for further statistical analysis. They found no statistically significant difference in the CSA of the tendon between left and right limbs in the same horse.

In Ponies there was a linear relationship between physical parameters (weight, height and mid-metacarpal circumference) and SDFT CSA, but this was not apparent in either the Thoroughbred or Irish Draught crossbred horses. They postulated that this was due to the larger variation in the physical parameters of the ponies. The mean CSA of the SDFT at the mid-metacarpal level in adult horses was 1.255 $cm^2$ (s.d. 0.146 $cm^2$), with the range of 0.894 – 1.617 $cm^2$ (Smith *et al*, 1994).

### 1.2.5 Changes in the SDFT in relation to exercise

The architecture of the SDFT appears to be variable and it can evolve with age and be modulated by the activity of the horse (Crevier-Denoix *et al*, 1998).

#### *1.2.5.1 Changes in collagen fibrils in relation to exercise*

Patterson-Kane *et al* (1997c) examined the effects of controlled treadmill exercise on the collagen fibrils of the SDFT in Thoroughbred horses, and observed that the mass average diameter (MAD) of the central region fibrils was significantly lower than that of the same region in the control horses.

The changes in the mean central region MAD were attributed to the reduction in numbers of larger fibrils and an increase in the number of smaller fibrils. In three of the five exercised horses the MAD of the central fibrils was significantly lower than that of the peripheral region and they postulated that the small diameter fibrils could have been newly synthesised by stimulated tenocytes, or could be subunits of degenerating larger fibrils. They interpreted the reduction in MAD of the tendon core as microdamage (Patterson-Kane *et al*, 1997c).

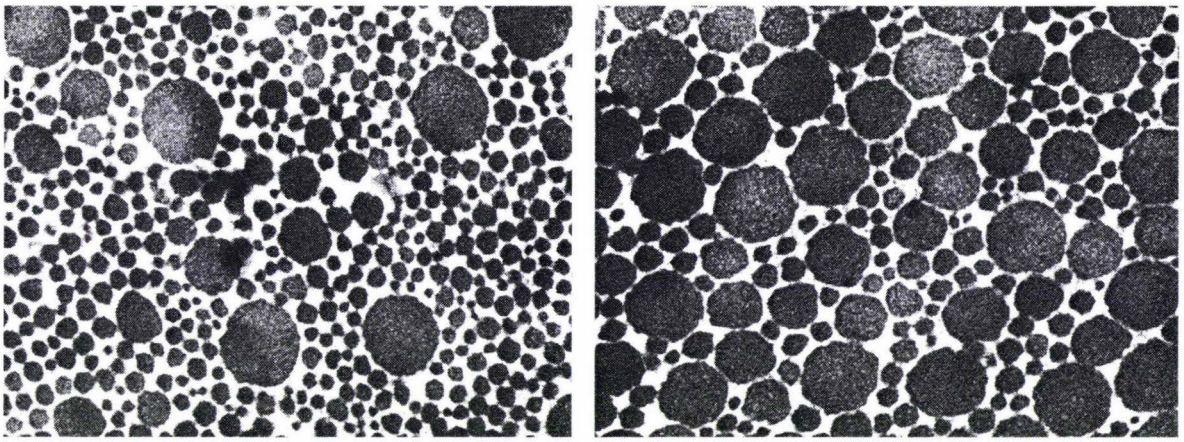

**Figure 1.9 Transmission electron micrographs of the mid-metacarpal region of the SDFT of Thoroughbred horses.**

**The picture on the left is from a horse trained for 18 months. The picture on the right is from a non-trained horse. The exercised tendon has a trained reduction in numbers of larger fibrils and an increase in the number of smaller fibrils.** ***From Sports Medicine and Arthroscopic Review, (Firth 2000).***

### *1.2.5.2 Changes in collagen fibre crimp pattern in relation to exercise*

A reduction in collagen fibril crimp angle and length has been demonstrated in response to both ageing and exercise (Patterson-Kane *et al*, 1997b). These authors studied the SDFT of young Thoroughbred horses that underwent a controlled treadmill exercise regimen. In the central region of the exercised animals, they found that the mean collagen fibre crimp angle and the mean crimp length were significantly lower than that of the control group.

In the peripheral region the mean crimp angle was higher in the exercised group, than that of the controls. However the mean crimp length was not different between the two groups (Patterson-Kane *et al*, 1998).

In the exercised horses, there were no differences in the mean crimp angle, and mean crimp length between the central and peripheral regions of the SDFT. The increase in the crimp angle in the periphery of the SDFT in exercised horses was an unexpected finding in this study. It would suggest that it is a response to exercise and functional adaptation to galloping exercise that has not previously been demonstrated in the equine SDFT.

Rapid high strain cycling experienced by the SDFT of young Thoroughbreds subjected to a uniform galloping exercise regimen causes reduction of crimp angle in the tendon core, as well as reduction of crimp period length in some horses. These changes may be evidence of microtrauma, which predispose the tendon to further overloading and damage (Patterson-Kane *et al*, 1998).

#### *1.2.5.3 Changes in interfascicular connective spaces in relation to exercise*

The interfascicular connective spaces were significantly larger in number and size in inactive horses when compared to active horses. This suggests that exercise results in a decrease in the interfascicular loose connective tissues, and possibly a proportional increase in the fascicular collagen fibres, as well as coalescence of the fibre fascicles (Crevier-Denoix *et al*, 1998).

#### *1.2.5.4 Tendon weight, volume and density in relation to exercise*

Firth *et al* (2004) assessed the tendons from young Thoroughbred horses that were in early race training for 13 weeks. They found that the weight and volume of SDFT segments from the trained group were greater than those of the controls. In the trained group the volume increase was greater than the weight increase, resulting in a significant reduction in density. A possible explanation was that the water content of the tendon matrix in the trained horses was slightly greater, reducing the density when compared to that of the control group. Although this difference was not statistically significant, it was concluded that the changes in size and density of the SDFT segments were adaptation to training, or training and growth, and it is possible that early training might produce fluid imbibition or matrix changes that lead to an increase in SDFT CSA with reduced tendon density (Firth *et al*, 2004).

### *1.2.5.5 The CSA of the SDFT in relation to exercise in mature horses*

Gillis *et al* (1993) observed that the mean CSA of the SDFT increased over time (four months) with race training in mature animals, whereas the mean echogenicity of the same tendon decreased over the same time period. They postulated that the decrease in echogenicity could be attributed to three processes: an increase in the size of fibre fascicles; an increased septal or vessel size; and newly formed synthesised collagen with fewer cross- links. An increase in the size of fibre fascicles offers fewer reflective surfaces for ultrasound waves, thus forming a darker or more hypoechoic ultrasound image.

Two of the horses in the study of Gillis *et al* (1993) exhibited mild heat, pain and lameness, (which are clinical signs of tendonitis), with only one of these horses having ultrasonographic evidence of tendonitis. The increase in SDFT CSA in the study may have been due to tendonitis and/or peritendinous soft tissue inflammation.

In contrast, Birch *et al* (1999b) found no difference in the CSA of the SDFT in two groups of young Thoroughbred horses undergoing low and high intensity treadmill exercise over a five and eighteen month period (Birch *et al*, 1999).

Perkins *et al* (2004) examined the effect of early race training over a thirteen-week period on the SDFT of two-year-old Thoroughbred fillies, and found no significant difference in ultrasonographic CSA between trained and control groups at either the beginning or end of the trial. For pooled data there was a significant difference at 8 cm DACB between weeks 0 and 14. They postulated that there appeared to be a slight increase in CSA after early

training despite the difference not being significant, and that the effects of growth and development may play a role in this increase.

Firth *et al* (2004) examined the SDFT from two-year-old Thoroughbreds trained over a 13-week period on a racetrack and found a 5% difference in mean ultrasonographic CSA between exercised and control animals when all levels were pooled, with the difference at 8 and 16 cm DACB was 13% and 20% respectively.

The difference was due to the mean weight and volume of tendon per cm length in the conditioned group being greater, however the average density of the tissue in each tendon segment was less in the trained horses. There was no evidence of pathology on histological examination of the SDFT tissue, and a possible explanation for the reduction in density was that the water content of the tendon matrix in the trained horses was slightly greater, thus reducing the tendon density when compared to the control tendons.

If the work of Firth *et al* (2004) were true and could be applied to the study of Gillis *et al* (1993), it is possible that the observed ultrasonographic changes were due to an increase in the weight and volume of the SDFT resulting in a significant reduction in tendon density. Therefore the observed increase in CSA and reduced echogenicity ultrasonographically might have been an adaptive response of the SDFT to early training.

### *1.2.5.6 The CSA and biomechanical properties of the immature SDFT in relation to exercise*

Kasashima *et al* (2002) studied the effect of treadmill exercise in young Thoroughbred foals from two to fifteen months of age and demonstrated a significant increase in SDFT CSA in the treadmill-exercised group over and above the normal increase in CSA due to growth (P=0.04). There was no statistical difference in CSA between the left and right limb. They suggested that this was evidence that tendon development can be modulated by exercise during the growth period in the horse.

Cherchutham *et al* (2001) determined the effects of exercise on the biomechanical properties of the SDFT in Dutch warmblood foals. From one week until five months of age the foals were managed in three treatment groups, being either housed in stalls and not exercised, housed in stalls and exercised daily, or maintained at pasture. Eight foals from each group were euthanased at eight months of age and the remaining animals were all housed together in a stall and paddock until euthanasia at eleven months. At five months of age mean CSA and normalised force at rupture were significantly greater for the pasture reared group, and stress at 4% strain was significantly less, when compared to the other groups. At eleven months of age mean CSA and normalised force at rupture were not significantly different between treatment groups.

They concluded that exercise significantly affected the biomechanical properties of the SDFT in foals and that evenly distributed low to moderate intensity exercise at a young age may be more effective in developing a strong flexible tendon, than single episodes of high intensity exercise super-imposed on stall rest.

### *1.2.5.7 The molecular composition of immature tendons in relation to exercise*

Cherdchutham *et al* (1999) examined the effects of conditioning exercise (as described in section 1.2.5.6) on the SDFT of three groups of Dutch Warmblood foals at five months of age. After which all groups received light exercise for six months and the tendons were again examined at eleven months of age. They found no statistically significant differences in any of the biomechanical parameters from each of the tendon regions examined. At five months of age the DNA content was significantly higher in the pasture group than the box-rested or box-rested and exercised groups. At eleven months of age the DNA content of the former exercised group was significantly lower than the other two groups.

Hyaluronic acid (HA) was significantly higher in the pasture group at five months of age. At eleven months of age there was no difference in HA levels between the box-rested or pasture groups, while the exercised group levels were significantly lower. They concluded that HA content increases in the SDFT with ageing. There was a reduced accumulation of Cartilage oligometric matrix protein (COMP) in the foals given enforced exercise, when compared to the box-rested and pasture managed foals. They also found that ageing significantly increases hydroxyline content (Cherdchutham *et al*, 1999).

#### *1.2.5.8 Exercise induced changes in other equine tendons*

Birch *et al* (1999b) examined the common digital extensor tendons (CDET) from groups of young Thoroughbreds in a high and low intensity exercise training regimen over a five and eighteen month period. They found that the CDET of the high intensity exercise groups had a larger CSA than that of the low intensity exercise group.

The hypertrophy of the CDET in response to high intensity training and not the SDFT of the same horses in the study may be related to the difference in energy storing capacities and mechanical functions of the two tendons. The CDET functions as a relatively inextensible link between the extensor muscles and the distal limb, whereas the SDFT acts as an energy store and an increase in CSA would increase tendon stiffness and reduce its energy storing capacity (Birch *et al*, 1999b).

### 1.2.6 The influence of gender on tendon behaviour

Perkins *et al* (2004) found that in New Zealand Thoroughbred racehorses the risk of injury to the SDFT was 3-fold higher in male horses compared with females. Other authors have found that male horses have an increased risk of serious or catastrophic injury (Cohen *et al*, 1999; Estberg *et al*, 1996; Estberg *et al*, 1998). However the management of seriously injured horses may have influenced the perception that there is an increased risk of injury for male horses, as female horses were more likely to be salvaged for a breeding career, with male horses being euthanased. The findings of Perkins *et al* (2004) were not influenced by treatment or management of cases and appears to represent a gender associated predisposition to SDFT injury in the New Zealand Thoroughbred racehorse.

### *1.2.6.1 Oestrogen and progesterone*

Anterior cruciate ligament injury in woman athletes is higher than in men. Yu *et al* (2001) established the presence of oestrogen and progesterone receptors in the human anterior cruciate ligament. They observed a dose-dependent decrease in the fibroblast proliferation and procollagen type I synthesis with increasing oestradiol concentrations, and the effect was attenuated with increasing progesterone concentrations. The effect was more pronounced at lower oestrogen concentrations, suggesting that oestrogen levels were the dominant factor. The changes in fibroblast proliferation and type I procollagen synthesis may explain the increase in anterior cruciate ligament injury rate observed in female athletes, suggesting the acute cyclic hormonal variations in the female athlete during menstruation may predispose her to ligament injury.

No research into the effect of sex hormones on the SDFT has been performed in horses.

### *1.2.6.2 Androgens*

Postacchini *et al* (1975) studied the effect of testosterone on rabbit Achilles tendon with a view to using androgens as a possible therapy for tendinopathy in human athletes. Testosterone propionate was injected intramuscularly into the gluteal muscles of three groups of nine to twelve month old male rabbits, every three days for a total of two, four, and six injections respectively. There was no difference in the Achilles tendons between treatment and control rabbits. However a faint hyperplasia of the tendon cells which had nuclear and cytoplasmic dimensions greater than normal were observed in the treatment groups.

In rabbits that received androgens injected directly into the right Achilles tendon, (either Testosterone propionate, Testosterone Bisulphate or Testosterone hemisuccinate, dissolved in a solvent) there was a proliferation of tendon cells and differentiation of tenocytes into cells with the appearance of fibroblasts, and these cells where surrounded by newly synthesised intercellular matrix.

### 1.2.7 Equine superficial digital flexor tendonitis

#### *1.2.7.1 The aetiology of equine SDFT tendonitis*

Tendonitis can be caused by trauma, infection, and adverse reactions to chemicals applied to the skin, however the most common cause of tendonitis in the horse is over stretching of the tendon during athletic activity. Many factors contribute to tendon injury such as hoof imbalances, conformational defects, improper or excessive training, hazardous training surfaces and poor rider skills. Tendonitis due to intrinsic overloading occurs in the mid-metacarpal region, between the most distal aspect of the carpal canal and the proximal aspect of the digital flexor sheath (McCullagh *et al*, 1979).

Numerous theories have been proposed for the aetiology of tendonitis such as excessive biomechanical forces, exercised-induced hyperthermia, ischaemia and reperfusion injury, and fibroblast anoxia (Birch *et al*, 1997; Gillis *et al*, 1997).

At present there is little experimental evidence to support or refute these potential mechanisms, and it is likely that the aetiology of tendonitis is multifactorial (Dowling *et al*, 2000). Degenerative changes occur within the central region of the SDFT and are thought to precede clinical evidence of tendonitis (Webbon, 1978).

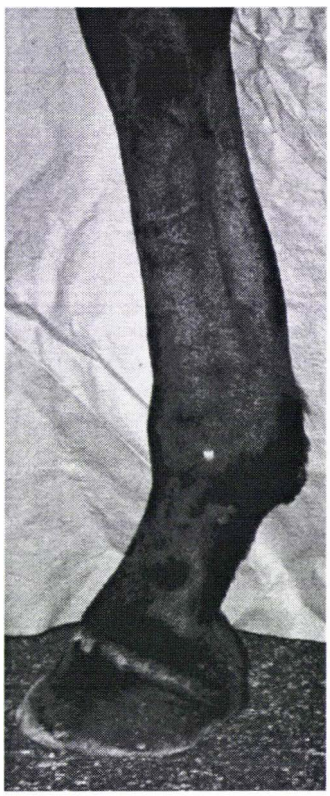

**Figure 1.10 Clinical tendonitis of the mid-metacarpal SDFT**

**There is marked palmar "bowing" of the tendon.**

***From Sports Medicine and Arthroscopic Review, (Firth 2000).***

### *1.2.7.2 Degenerative changes of the equine SDFT*

Gross post-mortem examination of the SDFT from apparently normal horses has revealed an abnormal reddish brown discolouration to the central core region in many instances (Webbon, 1977).

Most tendons in mature horses exhibit a patchy cellularity, although there is no overall decrease in tenocyte number with age (Birch *et al,* 1999a). On the basis of DNA counts, Birch *et al* (1999a) found that the central degenerative changes within the mid-metacarpal region of the SDFT are associated with an increased cellularity.

However this must be interpreted with caution, as there was no differentiation made between tenocytes and other cell types in that study.

Further degenerative changes become more evident in horses over three years of age and include matrix fibrillation, chondroid metaplasia, chondrone formation, neovascularisation, and fibroplasia (Pool, 1996).

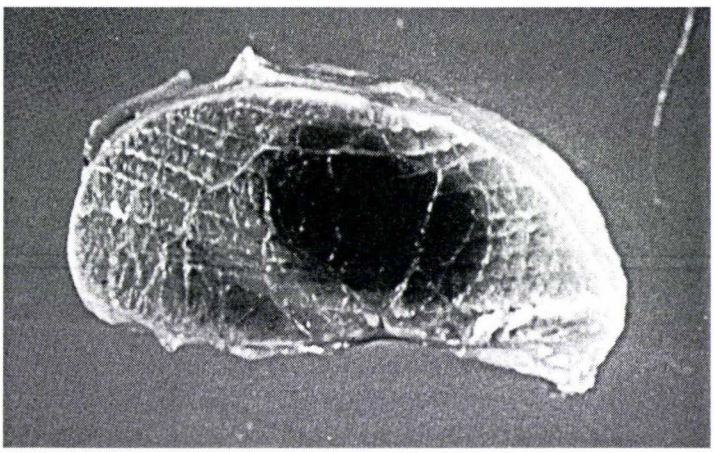

**Figure 1.11 Transverse mid-metacarpal section of SDFT from a clinically normal horse at necropsy. Gross discolouration of the central core region is evident.**

***From Journal of Biomechanics. Exercised induced hyperthermia as a possible mechanism for tendon degeneration. (Wilson and Goodship 1993).***

There appears to be an age-related trend towards tendon matrix degeneration and the gross abnormal appearance of the tendon is accompanied by changes in the composition of the extracellular matrix of the tendon. Birch *et al* (1998) demonstrated that the central core tissue of degenerate SDFT had an increased total sulphated glycosaminoglycan content, a high proportion of type III collagen, a decrease in collagen-linked fluorescence and high cellularity relative to the peripheral region of the same tendon. Dry matter content and total collagen content were not significantly different between zones.

They postulated that the findings suggest a change in cell metabolism and matrix turnover in the central core of the tendon, that is likely to contribute to a decrease in the mechanical properties of the tendon, thus predisposing the SDFT to characteristic partial rupture.

During exercise the repeated loading of the tendon generates a rise in the core temperature within the tendon. Peak intra-tendinous temperatures range from 43-45 °C and this has been postulated as a possible cause of the degenerative changes that precede SDFT tendonitis (Wilson and Goodship, 1994). However tenocytes recovered from the centre of the SDFT remain viable when subjected to rises in temperatures of this magnitude *in-vitro*, while those recovered from the periphery of the tendon do not, suggesting that the tendon has adapted to this physiological process (Birch *et al*, 1993-1994).

#### *1.2.7.3 The pathology of tendonitis and tendon healing*

Acute tendonitis is characterised by collagen fibre damage and an increase in the CSA of the tendon due to intra-tendinous haemorrhage and oedema. The response of the tendon to injury follows a sequence of repair similar to that of other vascularised connective tissues (Watkins *et al*, 1985). Initially a fibrin clot fills the deficit at the site of injury and mononuclear cells remove necrotic tissue and debris from the lesion. Monocytes stimulate migration of fibroblasts into the fibrin clot, which deposit a collagenous framework along the fibrin strands. Tissue macrophages and activated platelets induce neovascularisation, transforming the fibrin clot into fibrovascular granulation tissue. The early granulation tissue filling the tendon deficit is a poorly organised mass of immature collagen that has little tensile strength. The tendon lesion contains this fibrovascular mass for the initial eight weeks after tendon injury (Watkins *et al*, 1985).

Examination of histological sections of acutely (2 days after onset of clinical signs) injured SDFT reveal loosely packed and irregularly organised collagen fibres infiltrated with exuberant granulation tissue (fibrin strands, leucocytes, angioblasts) and haemorrhage. The tenocytes within the affected area have extensively developed rough endoplasmic reticulum in their cytoplasm, indicative of increased protein synthesis. The average diameter of fibrils is significantly reduced when compared to that of the control tendon (contralateral non-affected SDFT) (Kobayashi *et al*, 1999).

In the remodelling phase of repair the immature collagen is replaced with type III collagen fibrils contained within fibre bundles, which become orientated longitudinally along the lines of tension. The tensile strength of the healing tendon increases threefold from week eight to week twelve. By 24 weeks after injury the repair tissue has developed into a mature scar with type I collagen fibres aligned longitudinally (Auer and Stick, 1999)

Normal tendon is composed almost exclusively of type I collagen whereas the repairing tendon contains up to 30% of type III collagen, suggesting that the repairing tendon tissue is not derived from proliferating tenocytes, but from mesenchymal cells migrating from peritendinous tissues or blood vessels (Williams *et al*, 1980). Although the repair tissue has restored the tendon continuity, it does not restore tendon elasticity and normal tensile strength. The resultant scar tissue has substantially shorter crimp length than the surrounding normal tendon. The reason for this is not fully understood, however it appears likely that the damaged collagen fibres are replaced with new collagen that exhibits a different crimp periodicity. The observation that fibroblasts follow the zig-zag course of the fibres supports the concept that new collagen deposition occurs and crimp is formed at the early stage of fibrillogenesis (Wilmink *et al*, 1992).

Adhesion formation leading to compromised gliding of the injured tendon can occur. The eventual result may be a long-term disability or an increased risk of re-injury when athletic activity is resumed.

In most tendon injuries extrinsic tendon healing predominates, with the majority of the wound constituents derived from the peritendinous tissues. Extrinsic tendon healing is characterised by type III collagen production produced by fibroblasts. Intrinsic tendon repair such as type I collagen synthesis by differentiated tenocytes appears to be overshadowed by the extrinsic components. Methods to stimulate tenocyte production of type I collagen in lieu of type III collagen would be advantageous (Auer and Stick, 1999).

In the early stages of tendon healing after injury, the fibrils are predominantly of small diameter, and change towards the normal adult pattern as healing progresses. The larger fibrils provide more tensile strength, while the smaller fibrils provide elasticity but are mechanically weaker than the larger diameter fibres (Crevier-Denoix *et al*, 1995).

#### *1.2.7.4 Treatment of SDFT tendonitis*

Many surgical and medical procedures have been advocated for the treatment of tendonitis, however there is little objective evidence that any have consistent and enduring beneficial effects (Dowling *et al*, 2000).

A methodical approach to the treatment of tendonitis has been described by dividing the periods of tendon injury, healing and rehabilitation into acute (inflammatory), subacute (repair) and chronic (remodelling) phases (Bramlage, 1996). These categories allow application of specific therapies based on an understanding of tendon pathology and repair. The various therapeutic therapies can be classified as physical, pharmacological and surgical groups (Bramlage, 1991; Bertone, 1996; Bramlage, 1996)

### *1.2.7.5 Physical therapy during the acute stage of SDFT tendonitis*

Physical therapies during the acute stages of injury have been the cornerstone of treating tendonitis, where reducing the inflammation is indicated to limit the action of proteolytic enzymes on the remaining intact tendon matrix (Bramlage, 1991). These therapies include cold hosing, ice application, supportive bandaging and box rest. Elevation of the heel has previously been advocated, however this increases strain on the SDFT and is no longer recommended (Riemersma *et al*, 1996a).

### *1.2.7.6 Pharmacological therapy for the treatment of equine SDFT tendonitis*

The use of anti-inflammatory drugs (NSAIDS) is controversial for their effects on inflammation, however they are commonly used and provide analgesia. The use of corticosteroids systemically has been shown to inhibit the fibroblastic response required for tendon healing, and intra-lesional use is detrimental, causing collagen necrosis and hyalinisation (Dowling *et al*, 2000).

Sodium Hyaluronate (HA) has been administered by both intralesional and peritendinous injection in the treatment of acute tendon injury with conflicting results. Initial studies reported that HA improved tendon healing based on subjective ultrasonographic interpretation, and significant reductions in post-surgical adhesion formation were observed in intrasynovial tendons (Spurlock, 1989; Gaughan *et al*, 1991). Further studies have failed to show any significant beneficial effects of intralesional HA on collagenase-induced tendonitis and had no significant beneficial effect on the recurrence of equine SDFT tendonitis when compared to untreated cases (Dyson, 1996).

Polysulphated glycosaminoglycans (PSGAG) have been used intralesionally in the acute stages of tendonitis for their inhibition of macrophage activation, and collagenase and metalloproteinase activity (Foland *et al*, 1992). There has been some suggestion that PSGAGs improve collagen fibril organisation and stimulate tenocytes to produce collagen, HA and glycosaminoglycans. To date there is no conclusive proof of significant improvement of prognosis over other treatments (Dowling *et al*, 2000).

Beta-aminoproprionitrile fumarate (BAPN-F) has been used in the acute stages of tendonitis for its inhibition of excessive cross-linking in the early stages of tendon repair. BAPN-F binds to the enzyme lysyl oxidase inhibiting the deamination of lysine, the first step in the formation of collagen fibre covalent cross-linking. Under the influence of controlled exercise, the use of BAPN-F promotes the alignment of collagen fibres. Initial results showed that 80% of equine SDFT treated with intralesional BAPN-F had at least a 75% improvement in ultrasonographic measurements (Genovese *et al*, 1996). However long term data on the return to racing of horses treated with BAPN-F has been less convincing with 45 -50% of horses returning to maximal activity, which is similar to the results of confinement and a controlled exercise regimen (Genovese *et al*, 1996).

Dowling *et al* (2002) investigated the effect of recombinant equine growth hormone (rEGH) on both normal and healing SDFT. In normal adult horses administered intramuscular rEGH for a six-week period there was no difference in CSA, maximal load to failure, yield load, ultimate and yield tensile strain, ultimate and yield tensile stress, or tendon stiffness when compared to the control animals.

The use of intramuscular rEGH in treating collagenase-induced tendonitis had a negative effect on the biomechanical properties of the SDFT in the early phase of tendon healing, with the treated group having a significant increase in CSA, a concomitant reduction in ultimate and yield tensile stress, and reduced tendon stiffness when compared to the control animals (Dowling *et al*, 2002a; Dowling *et al*, 2002b).

#### *1.2.7.7 Surgical intervention for the treatment of equine SDFT tendonitis*

Transection of the AL-SDFT was previously performed to allow lengthening of the SDFT-complex and therefore reduce the peak load on the SDFT during full weight bearing when the horse returned to work. Subsequent *in vitro* investigations into the biomechanical effects of AL-SDFT desmotomy revealed that SDFT strain and metacarpophalangeal joint hyperextension increased following transection of the AL-SDFT (Shoemaker *et al*, 1991). After transection of the AL-SDFT, horses were 1.3 times more likely to race on five or more occasions, but these horses were 5 times more likely to experience suspensory ligament injury (Alexander *et al*, 2001).

Percutaneous tendon splitting has been used in both the acute and chronic stages of tendonitis. It was originally thought that the procedure improved vascularisation of the chronically injured tendon. However later investigation revealed that tendon splitting resulted in increased trauma and granulation tissue production, no alterations in collagen production, and continued lameness (Silver *et al*, 1983).

More recently the procedure has been used in acute tendon core lesions to allow evacuation of the intratendinous haematoma and oedema, thereby reducing lesion size and improving collagen fibre alignment. The long-term benefits of tendon splitting are controversial as the quality of the repair and the return to racing is variable, and not superior, to other methods of treatment (Henninger, 1994).

#### *1.2.7.8 Miscellaneous therapy for the treatment of equine SDFT tendonitis*

The application of topical blister ointments, line firing, or pin firing, was commonly used for the treatment of chronic musculoskeletal injuries in the horse (McCullagh *et al*, 1979). It was believed that the intense inflammatory reaction induced by such treatments would improve the healing of the tendon due to increased vascularity and inflammatory cell exudate. It has now been shown that such procedures have no direct benefit on tendon healing, in fact healing is often delayed and there is an increased risk of peritendinous adhesions (Silver *et al*, 1983).

Other therapies that have been employed for the treatment of SDFT tendonitis include therapeutic low intensity ultrasound, low frequency infrared laser therapy, extracorporeal shock wave therapy and electromagnetic field therapy. Results from these treatments are varied and results obtained are not better than those obtained by using conservative methods (Dowling *et al*, 2000).

Current research into the effects of bone marrow derived stem cells on the healing of tendons and ligaments is showing some promise. Young *et al* (1998) implanted autologous marrow-derived mesenchymal stem cells (MSCs) suspended in a collagen gel, into 1cm long gap deficits in rabbit Achilles tendons, and evaluated the tendons, both biomechanically and histologically at four, eight and twelve weeks post implantation. In the treated tendons there was a significantly greater load-related structural and material properties at all time intervals, when compared to the control tendons. The values were typically twice that of the control tendons, and the load related material properties of the treated tendons increased significantly over time. The treated tendons had a significantly larger CSA and the collagen fibres appeared to be better aligned. They postulated that the mesenchymal stem cells contracted and organised collagen implants to large tendon deficits and significantly improved the biomechanics, structure and probably function of the tendon after injury.

Smith *et al* (2003) recently described a technique of implanting autologous MSCs into a damaged SDFT of an eleven-year-old pony. MSCs have the potential to differentiate into tenocytes and regenerate tendon matrix, however clinical trials and data are lacking in horses and further research is warranted to determine whether MSCs are more effective than other current treatment methods (Smith *et al*, 2003). Other researchers are currently investigating the implantation of adipose derived stem cells into SDFT lesions, however data and controlled studies are lacking on the efficacy of this treatment method (Dykgraaf, S. personal communication).

The use of pig urinary bladder mucosal lining to manufacture an acellular matrix which acts as "scaffold" allowing migration and proliferation of angioblasts and mesenchymal cells in healing tissues has recently been developed (ACell Vet™), and claims excellent results in healing SDFT lesions, however scientific data and controlled studies are lacking to prove if this new treatment obtains better results over conservative methods (Mitchell *et al,* 2003).

#### *1.2.7.9 The use of ultrasonography in relation to tendon healing*

Quantitative ultrasonographic analysis of equine SDFT injuries has become the most common technique used to diagnose and monitor tendon healing. The ultrasonographic and histological findings in tendonitis are closely correlated throughout the inflammatory, repair and remodelling phases (Marr *et al*, 1993b). Quantitative data of tendon injuries allows decisions to be made with regards to prognosis, appropriate therapy, and the introduction of a controlled exercise programme. Serial ultrasonographic examinations during the exercise programme can be used to assess healing and for detecting re-injury of the tendon, if the exercise is level is too intense for the stage of tendon repair.

#### *1.2.7.10Equine SDFT rehabilitation*

After injury to the SDFT, tendon cellularity, collagen type, and tendon fibre crimp pattern require a minimum of six months to return substantially towards normal (Genovese *et al*, 1986; Reef 1998). Therefore a minimum of six months of restricted athletic activity is required for the majority of tendon healing to occur.

The acute and painful period of tendonitis ranges from three to eight weeks in horses, with clinical lameness resolving long before substantial tendon healing occurs (Gillis, 1996). Several tendon rehabilitation protocols have been developed and all involve strict confinement of the horse to prevent re-injury, and controlled exercise. The following protocol, based on clinical experience and the ultrasonographic findings of 2800 cases of tendon and ligament injury at the University of California, Davis, is shown as an example of such a programme:

At the initial examination the clinical signs and ultrasonographic changes within the tendon (increases in size, echogenicity, fibre alignment, extent of the lesion, and lesion CSA) are recorded. An initial period of stall confinement is initiated, and based on the severity of the lesion, a period of hand walking is commenced (usually for 6-12 weeks).

At the next examination the clinical signs of lameness are assessed along with the ultrasonographic findings. It would be expected that the lesion has an increased echogenicity and the CSA of the lesion and the tendon are either the same or reduced. The incremental increase in exercise prescribed is based on the degree of improvement seen ultrasonographically, generally trotting is added into the exercise regimen at this stage if the lesion has not deteriorated.

At the third examination healing is regarded as good if clinical signs are absent, the lesion is hard to visualise ultrasonographically, the CSA is stable or reduced, and the fibre pattern is good. Again the amount of exercise and duration of the exercise programme are based on the degree of improvement with the tendon lesion. During the rehabilitation programme the horse is not allowed free or uncontrolled exercise.

Table 1.1 Example of a controlled exercise regimen for SDFT rehabilitation.

| Injury | 0-30 days | 30-60 days | 60-90 days |
|---|---|---|---|
| mild | Hand walk 15min twice daily | Hand walk 40 min. daily | Ride at walk 20-30min. daily |
| moderate | Same | Same | Hand walk 60 min. daily |
| severe | Same | Hand walk 30 min. daily | Hand walk 40 min. daily |

**Exercise protocol following the first examination (0 to 90 days). Horse is confined to a stall or equivalent size paddock.**

| Progress | 90-120 days | 120-150 days | 150-180 days |
|---|---|---|---|
| good | Ride at walk 30 min daily | Ride at walk 45-60 min daily | Add 5 min. trotting every 2 weeks |
| fair | same | same | Ride at walk 60 min. daily |
| poor | Hand walk 60 min. daily | Ride at walk 20-30 min daily | Ride at walk 60 min. daily |

**Exercise protocol following the second examination (90 to 180 days). Horse is confined to a stall and equivalent size paddock.**

| Progress | 180-210 days | 210-240 days | 240-270 days |
|---|---|---|---|
| good | Add canter 5 min every 2 weeks | same | Full flat work; no racing, speed work or jumping |
| Fair | Same | Same | Same |
| poor | Re-evaluate case & discuss further treatment options | | |

**Exercise protocol following the third examination (180 to 270 days). Horse is confined to a stall and equivalent size paddock.**

| Progress | 270-300 days | 300-330 days | 330-360 days |
|---|---|---|---|
| good | Begin work at racing speed; jumping | competition | same |
| Fair | Same | Same | same |
| Poor | Re-evaluate case & discuss further treatment options | | |

**Exercise protocol following the fourth examination (270 to 360 days).**

### 1.2.8 Ultrasonographic examination of the SDFT

The first report of quantitative analysis of SDFT injuries in racehorses appeared in 1990 (Genovese *et al*, 1990), and from this early work, tendon monitoring and rehabilitation programmes have been developed. Ultrasound is a safe and non-invasive method of determining the anatomical structure of the limb and has revolutionised the diagnosis, treatment and management of tendon injuries. The presence, type and severity of a tendon lesion can be determined ultrasonographically. The response of the injured tendon to controlled exercise can be evaluated by ultrasound at regular intervals allowing an objective assessment of the rehabilitation programme.

#### *1.2.8.1 The principles of ultrasound*

Diagnostic ultrasound utilises sound waves, which are described in terms of their frequency, period wavelength, propagation speed, amplitude and intensity. The wavelength, frequency, amplitude, intensity and period of the sound wave generated is determined by the sound source. The tissue through which the sound waves travels determines the propagation speed and also has some effect on the wavelength of the sound wave emitted (Reef, 1998).

Ultrasound transducers (probes or scan heads), convert electrical voltage into electrical energy via piezoelectric crystals, which are excited by electrical energy into emitting ultrasound. These sound waves enter tissues and are reflected from the interfaces between tissues (Rantanen, 1993).

The acoustic impedance of the tissues, and the angle from which the ultrasound beam was directed determines the strength of the reflected ultrasound wave. The acoustic impedance of the tissue is determined by the tissue's density, and only small differences in acoustic impedance occur between various soft tissues of the body, however large differences occur between soft tissues, bone and structures containing air.

The reflected ultrasound beam from a tissue interface returns to the transducer head and is converted back to electrical energy by the piezoelectric crystals, which in turn is processed by the ultrasound machine into a series of dots that form an image (Reef, 1998). The brightness of each dot corresponds to the amplitude of the returning sound wave (echo), and the location of the dot corresponds to the anatomic location of the echo-generating structure. Assigning a different scale of grey to varying echo strengths produces a grey scale image.

Recently van Schie and Bakker (2000) examined the effects of structure-related echoes in ultrasonographic images of equine SDFT and observed that the echo pattern on the ultrasound screen is derived from a combination of echoes that are directly related to the tendon, and from those that result from interference. This misrepresentation of the tendon is due to propagation, refraction and reflection artefacts.

Propagation artefact is due to most ultrasound scanners calculating the speed of the sound waves in water, whereas the velocity of sound waves in tendons is higher. The higher velocity in tendon leads to a miscalculation of the position of each reflected sound wave, resulting in a misrepresentation of the dorso-palmar thickness of the tendon. The dorso-palmar thickness of the ultrasonographic image is smaller than it is in reality, and results in ultrasound underestimating the size of the tendon.

Refraction artefact is caused by the scanners miscalculation of the position of each reflected sound wave, as the ultrasound machine calculates the position of the reflected sound wave on the assumption that the sound wave travels through the tendon in a straight axial direction along the transducer beam axis. However the ultrasound waves are in fact refracted from the straight axial direction towards the more abaxial part of the tendon, resulting in a false representation of an abaxial located reflector in a more central direction. The clinical importance of this arises when imaging the more convex abaxial part of the tendon, and the phenomenon results in a remarkable deformation in the ultrasonographic image of the medial and lateral parts of the tendon (van Schie & Bakker, 2000).
The effect of the propagation, refraction and reflection artefacts result in the ultrasonographic image of the SDFT being routinely underestimated and this in part explains why the CSA of the SDFT at post-mortem is larger than the ultrasonographic CSA obtained *in-vivo* prior to post-mortem.

#### *1.2.8.2 Patient preparation for ultrasonographic examination of the SDFT*

In most circumstances the horse should be sedated for the ultrasound examination, allowing the ultrasonographer to concentrate on the examination and preventing accidental damage to the equipment.

The skin surface over the palmar/plantar aspect of the limb is prepared by clipping the hair as short as possible and wiping the limb with water, before applying ultrasonic coupling gel to the skin surface. The horse should stand squarely on the limb being examined to ensure proper tension within the SDFT, as laxity in the SDFT can produce ultrasonic artefacts (Rantanen, 1986).

### *1.2.8.3 Ultrasonographic examination of the equine SDFT*

Ultrasonographic examination of the SDFT involves evaluating the size, shape, texture, position and fibre alignment of the tendon. A 7.5 - 10 MHz linear transducer is ideal for imaging tendons, with a display depth of 4-6 cm often used. The smallest depth of field that can display all the flexor tendons should be selected for optimal image quality (Reef, 1998).

An ultrasound standoff pad is used to increase the visibility of superficial structures. This is a soft, 1.5 centimetre thick block of solidified acoustic gel that is secured against the scan head with a plastic housing device. The ultrasound standoff pad is flexible and conforms to the surface being imaged, allowing irregular shaped body parts to be easily imaged with a linear array transducer. Because of the excellent impedance-matching property and low acoustic attenuation of the gel, there is little or no degradation in the ultrasound image in the first two or three centimetres of tissue. The use of a standoff pad improves image resolution of superficial structures, by moving the area of interest into the focal zone of the transducer (Biller and Myer, 1988).

The palmar surface of the SDFT should be scanned with the transducer head perpendicular to the long axis of the tendon for the transverse or cross sectional scan. For the sagittal, or long axis scan, the transducer head is parallel to the long axis of the tendon. Ultrasonographic examination of the SDFT should include an entire survey of the tendon and evaluation of the surrounding subcutaneous tissues and vasculature. Tendon CSA, lesion CSA, tendon echogenicity, and fibre alignment should be graded in each region of the tendon. The signalment of the horse and identification of the leg must be recorded on the ultrasound images saved to hard copy (Reef 1998).

#### *1.2.8.4 Precise tendon level identification*

Two methods for precise level identification of the SDFT have been determined. For one method, a ruler is attached to the lateral aspect of the metacarpus and measurements are recorded in centimetres distal to the base of the accessory carpal bone (DACB). In the hindlimb the measurements are recorded in centimetres distal to the tuber calcaneus.

Genovese *et al* (1990) proposed a system whereby the metacarpus was divided into six zones and the metatarsus into eight zones. Since the development of this classification system others have expanded the metacarpus to seven zones and the metatarsus into nine zones. The zone designation was to assist the practitioner scanning in the field without a ruler.

In the forelimb the most proximal zone is Zone 1A and corresponds to the most proximal 4 cm of the tendon distal to the accessory carpal bone. Zone 1B is approximately 4-8 cm DACB. Zones 2A and 2B span the 8 cm of the middle third of the metacarpal region. Zones 3A and 3B span the distal third of the metacarpal region. The palmar surface of the metacarpophalangeal joint is designated zone 3C, and includes the axial surfaces of the proximal sesamoid bones and the palmar annular ligament.

In the hindlimb the most proximal zones are along the plantar surface of the talus with zone 1A the proximal 7cm and zone 1B the distal 7cm of the plantar surface of the talus. Zone 2A is the proximal 4cm in the metatarsus region, zone 2B is the proximal third of the metatarsus. The middle 8cm of the metatarsus is designated zones 3A and 3B, with zones4A and 4B the distal third. Zone 4C includes the plantar metatarsophalangeal joint surface.

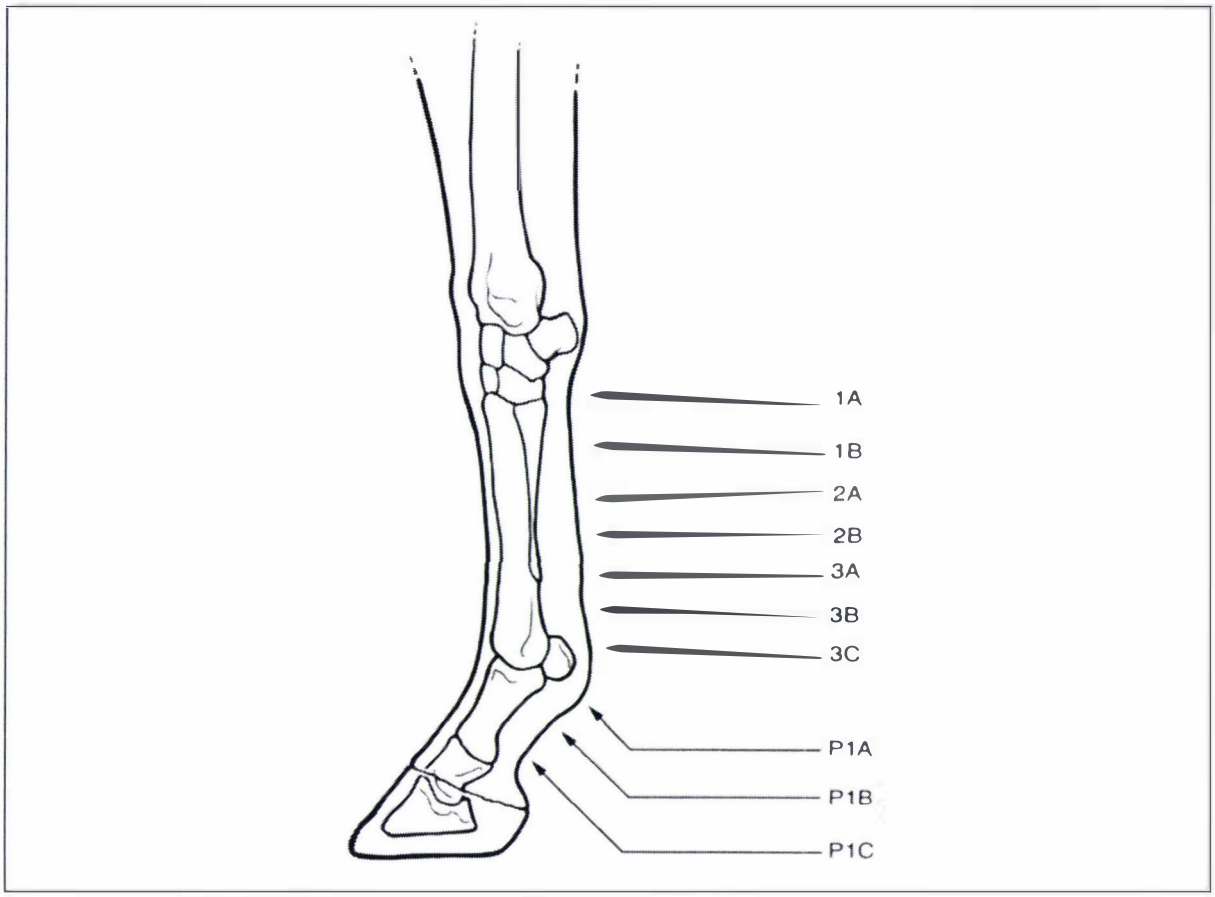


**Figure 1.12 The ultrasonographic zones of the SDFT.**

***From Lameness in Horses (Dyson and Ross 2002)***

### *1.2.8.5 The Ultrasonographic appearance of normal equine SDFT*

When examined with ultrasound a normal SDFT has a uniform echogenicity, parallel fibre pattern, and a CSA that ranges from 0.6 to 1.2 $cm^2$ depending on the breed of horse (Reef, 2001).

The SDFT is usually slightly less echogenic than the DDFT. The echogenicity of the SDFT is significantly brighter proximally than in the middle or distal tendon regions. When viewed in a sagittal plane the SDFT is composed of long parallel bundle fibres that appear as long white echoes. In a transverse plane the image is a uniform distribution of pin-point echoes.

In the proximal metacarpal region the SDFT is round in appearance, becoming flattened in the distal metacarpal region. In the proximal pastern region the SDFT forms a ring around the deep digital flexor tendon (manica flexoria), before bifurcating into a medial and lateral branch, each of which inserts distally onto proximal P1 and proximally onto P2 (Genovese *et al*, 1986).

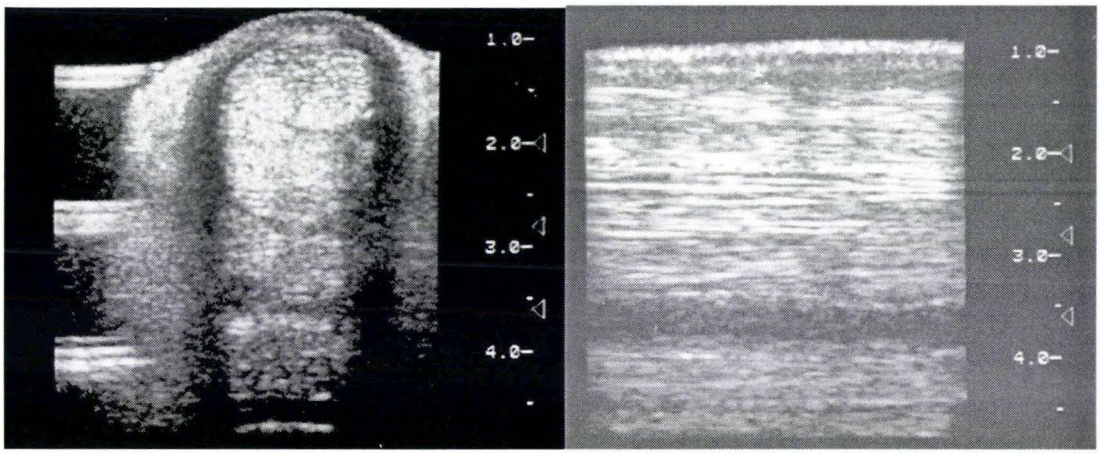


**Figure 1.13 Transverse and longitudinal ultrasonographic images of the SDFT**

### *1.2.8.6 Fibre alignment and echogenicity scoring of the equine SDFT*

Fibre alignment quantitation provides additional ultrasonographic information for assessment of tendon injury and is useful during tendon repair to assess healing and tendon response to gradual increases in exercise.

A fibre score of 0 is given when the fibre alignment in the zone has 76% to 100% parallel fibres. A score of 1 is given when 51% to 75% of the fibres are parallel. A fibre score of 2 corresponds to 26% to 50% parallel fibres and a score of 3 refers to 0% to 25% parallel fibres within the lesion.

A normal SDFT has an echogenicity score of 0. An echogenicity score of 1 represents a lesion that is mostly echogenic, a score of 2 represents a lesion that is 50% echogenic and 50% anechoic, while a score of 3 is given to a lesion that is mostly anechoic (Genovese *et al*, 1996).

The practicality of these grading systems relates to the assessment of the SFDT during the initial examination at the time of injury and subsequent examinations during the repair phase. As the tendon heals its echogenicity increases and short linear fibres (echoes) are detected within the lesion in the sagittal view. With continued healing longer fibres (echoes) can be detected and an improvement in fibre alignment occurs. This is reflected in an improvement in fibre alignment and echogenicity scores. Concurrently a reduction in tendon T-CSA, and CSA at the zone of maximum injury occurs during tendon healing. (Reef, 2001).

#### *1.2.8.7 Ultrasonographic measurement of the SDFT*

Cuesta *et al* (1995) determined the lateral to medial, and palmar to dorsal dimensions of the SDFT at various levels. The SDFT increases in size lateromedially, and decreases in size palmo-dorsally, from proximal to distal.

| Centimetres distal to the Accessory Carpal bone (DACB) | Latero-medial Measurement (cm) | Palmo-dorsal Measurement (cm) |
|---|---|---|
| 2.5 cm | 1.66 (0.19 s.d) | 0.78 (0.13 s.d) |
| 7.5 cm | 1.67 (0.14 s.d) | 0.73 (0.16 s.d) |
| 12.5 cm | 1.78 (0.12 s.d) | 0.70 (0.12 s.d) |
| 17.5 cm | 2.01 (0.20 s.d) | 0.63 (0.14 s.d) |

**Table 1.2 The lateral to medial, and palmar to dorsal dimensions of normal adult SDFT as determined by Cuesta *et al* (1995)**

### *1.2.8.8 The CSA of the SDFT and its clinical significance*

The measurement of the CSA has largely superseded the dorso-palmar and medio-lateral measurements of the SDFT. CSA can be used to confirm subtle tendonitis or tendon injury. (Reef, 2001). An increase in tendon CSA is the first clinically detectable sign of tendonitis (Genovese *et al*, 1986).

| Tendon Level (cm distal to accessory carpal bone) | Tendon Level (zones) | Mean CSA ($cm^2$) | s.d ($cm^2$) | CSA Range ($cm^2$) |
|---|---|---|---|---|
| 4 | 1A | 1.30 | 0.12 | 1.00-1.61 |
| 4-8 | 1B | 1.26 | 0.12 | 0.94-1.58 |
| 8-12 | 2A | 1.23 | 0.13 | 0.89-1.57 |
| 12-16 | 2B | 1.26 | 0.14 | 0.91-1.60 |
| 16-20 | 3A | 1.25 | 0.14 | 0.89-1.61 |
| 20-24 | 3B | 1.35 | 0.17 | 0.93-1.77 |
| 24-28 | 3C | 1.48 | 0.15 | 1.11-1.85 |

**Table 1.3 Normal Adult SDFT CSA measurements**

***From Equine Veterinary Journal, The cross-sectional area of normal equine digital flexor tendons determined ultrasonographically. (Smith, Jones, Webbon 1994).***

A routine approach is to measure the CSA of the seven SDFT regions in the forelimb, (nine in the hindlimb) and compare these to the measurements obtained from the contralateral normal limb.

Disruption to the tendon fibres results in an increase in tendon CSA due to haemorrhage or inflammatory cell infiltration, leading to separation of tendon fibres.

As most SDFT injuries are unilateral, the contralateral limb CSA can be used to serve as a base line for comparison for the injured tendon. However some horses can present with bilateral tendonitis therefore lesions can go undetected if CSA is used as the sole determinant for diagnosis of tendonitis (Reef 1998; Genovese *et al*, 1990).

Ultrasonographically, injury to the SDFT is usually seen in three forms: tendon enlargement without evidence of fibre tearing; fibre tearing without tendon enlargement; and most commonly, fibre tearing with tendon enlargement. The most common SDFT injury is a central core lesion, which can be very small with little or no enlargement of the tendons CSA, or large involving up to 50% or more of the tendons CSA (Reef, 1998). The CSA of a hypoechoic lesion within a tendon and the CSA of the SDFT at the level of the lesion can be used to determine the percentage of abnormal tendon at that zone. The level at which there is the most significant increase in CSA and fibre tearing is the maximum injury zone (MIZ).

#### *1.2.8.9 Ultrasonographic quantification of SDFT injury*

During the mid 1980s the average percentage of injury to the SDFT was 40%. Trainers and horse owners have become increasingly aware of the clinical signs of tendonitis and with advances in tendon diagnostics, and any subtle swelling of the SDFT can be easily evaluated with ultrasonography (Reef, 1998). The ultrasonographic appearance of tendonitis can vary from a minor reduction in tendon echogenicity with normal fibre alignment and little or no increase in tendon CSA, to major tearing of the fibres with hypoechoic or anechoic areas.

The most common lesion of the SDFT is a central core lesion that appears as a black hole in the centre of an echogenic tendon. The core lesion can vary in size from being very small with no increase in tendon CSA, to large, involving 40% or more of the tendons CSA at the level of the injury (Reef, 1991). Palmar lesions of the SDFT are commonly due to external trauma such as a strike from another limb. Tendons that have significant areas of fibre disruption will generally have marked peritendinous oedema in the acute stage of injury, which is seen as a dark hypoechoic region around and between tendons.

Generally, disruption to the tendon fibres results in an increase in tendon CSA and appears as anechoic or hypoechoic separation of tendon fibres, due to haemorrhage or inflammatory cell infiltration. Organised clot has an echogenicity similar to that of normal tendon when viewed in cross section, therefore it is imperative that the tendon be viewed in both cross section and a sagittal plane to allow differentiation of tendon fibre and clot. In a sagittal plane a clot has an amorphous appearance without longitudinal fibre pattern. The more echoes that are present within a lesion, the less fibre tearing, haemorrhage and inflammation there is (Reef, 2001; Genovese *et al*, 1990).

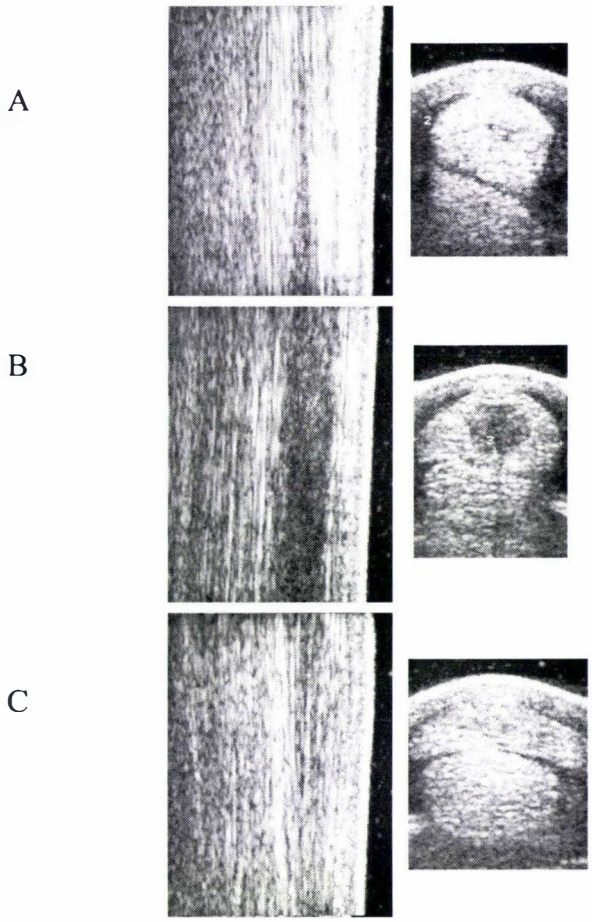


**Figure 1.14 The ultrasonographic appearance of the central core lesion**

Image A (8cm DACB) proximal extent of the lesion
Image B (20cm DACB) maximal extent of the lesion
Image C (24cm DACB) distal extent of the lesion

***From Sports Medicine and Arthroscopic Review (Firth 2000)***

### *1.2.8.10 Quantitative assessment of equine SDFT lesions*

Several methods for assessing the severity of a tendon lesion have been described (Genovese *et al*, 1986; Gibson *et al*, 1997; Gillis *et al*, 1995b). Generally, the length of a lesion, tendon CSA, lesion CSA, lesion echogenicity and fibre alignment within the area of injury determines the severity of a tendon lesion (Reef, 2001).

Measurements of the tendon and lesion at the area of maximal injury only partially describe the injury severity. The most complete assessment to date incorporates the tendon and lesion CSA in each zone, and the lesion echogenicity and fibre alignment. Tendon CSA (TCA) and lesion cross sectional area (LCA) at the most severe area of injury in each zone, are added to obtain a total measurement score for each tendon. The lesion echogenicity and fibre alignment are graded using a scale from 0 to 3.

Mild injuries involve 15% or less of the total tendon CSA, moderate injuries involve more than 15%, but less than 25% of tendon total CSA, and severe injuries involve greater than 25% of tendon total CSA (Reef, 2001).

Quantitative assessment of an injured tendon improves the objectivity of SDFT injury, allowing the practitioner to determine the prognosis for return to athletic activity, a suitable rest period and an appropriate rehabilitation programme. Objective monitoring of the tendon lesion during the recovery period can determine when the horse is ready to resume training.

#### *1.2.8.11 Ultrasonographic assessment of equine SDFT healing*

As tendon healing progresses the CSA of the lesion and the tendon usually decreases. The ultrasonographic appearance of the tendon lesion becomes hypoechoic and amorphous as the deficit fills with granulation tissue. As the fibroblasts in the granulation tissue produce collagen the echogenicity of the lesion gradually increases and short linear echoes appear.

The fibre pattern of these newly formed fibres is initially random and becomes more parallel as healing progresses, however a conservatively treated core lesion rarely returns to a completely normal ultrasonographic appearance, as random fibre pattern, short linear echoes and demarcation of the original core lesion from the surrounding uninjured tissue usually remain (Reef, 2001).

Ultrasonographic evaluation of tendon lesion healing is an important aspect of the rehabilitation programme, with several studies highlighting the importance of imaging a good fibre pattern throughout the entire SDFT before the onset of cantering or galloping exercise. Fibre pattern is a critical factor in determining success during the rehabilitation of horses with tendon injuries. (Dyson, 1996; Gillis *et al*, 1995b; Reef, 2001).

Ultrasonographic assessment of a tendon lesion should be performed eight weeks after injury or treatment is instituted. Additional follow up examinations should be performed every eight weeks, or before any increase in the level of the horses exercise. Each increase in the level of exercise should only occur when ultrasonographic improvement has been detected, and the level of exercise should not be increased if there has been an increase in total tendon CSA by 10%, or if the CSA at one injury zone has increased by 20% compared to the previous examination. Increases in total tendon CSA or increases in CSA at any one injury zone, are indicative of excessive loading of the tendon for the current level of healing, and may indicate possible re-injury. These horses require their level of exercise to be reassessed and reduced until there is ultrasonographic evidence of improvement in the lesion, and should not begin cantering or galloping before six months after treatment commenced or re-injury is likely (Reef, 1998).

### 1.2.9 *In-vitro* methods of determining SDFT cross sectional area (CSA)

Calculation of tendon CSA is necessary to determine stress from mechanical loading of the tendon, allowing a comparison of properties from tendons of various sizes and from different species. Methods reported for determination of tendon CSA include ink blot morphometric digitised measurement, use of dry and wet displacement volumes, vernier callipers, area micrometer, laser micrometers and digital photograph analysis (Firth *et al,* 2004; Birch *et al*, 1999b; Gillis *et al*, 1995b). Volumetric displacement methods require destruction of the tendon tissue and calculation of the tendon length to compute CSA. Vernier callipers and micrometers compress the tendon during measurement. Laser micrometry measures the CSA under tension and is accurate, but requires reconstruction of the tendon shape from multiple uniaxial measurements. These are all *in-vitro* techniques requiring isolation of the tendon (Ellis, 1969).

The SDFT is usually subjected to tensile loads and methods that measure tendon CSA under compression may contribute to error in CSA measurements, resulting in inaccurate calculation of stress values (Ellis, 1969).

#### *1.2.9.1 Photographic determination of the SDFT CSA*

The section of SDFT from the region of interest is recovered at post-mortem and inserted into a hole, approximately the same size as the tendon section, made in wedge of black foam rubber. The black background provides a contrast against the tendon. The cut surface of the tendon is manoeuvred so that the proximal surface is flush with the foam rubber. A plastic calibration disc with a 30mm diameter hole is placed over the tendon, and a digital photographic image is obtained of the tendon and disk, perpendicular to the

cut tendon surface. The CSA of the tendon image is obtained in an image analysis programme (Scion Image), using the diameter of the hole in the plastic disc as calibration for the image analysis programme (Firth *et al,* 2004).

Birch *et al* (1999b) determined the SDFT CSA obtained photographically at post-mortem, by printing the image onto photographic paper at an enlargement of eight times. Print enlargements were cut out, weighed and CSA calculated.

#### *1.2.9.2 Inkblot morphometric measurement of tendon CSA*

At post-mortem a 1cm thick transverse section of SDFT is removed from the region of interest and three inkblot impressions are made from each end of the tendon section. The impressions are digitised, and using a digitising tablet and software, six corresponding CSA values are calculated. The CSA values are averaged to generate a value for the inkblot morphologic CSA of the tendon at that level (Gillis *et al*, 1995).

#### *1.2.9.3 Mould technique for CSA determination*

At post-mortem the tendons are dissected from the limb and moulds are made of the tendons using an aqueous based precision dental impression material. After casting the moulds are removed from the tendons and cut transversely at the required level. The cut mould sections from various levels are photographed digitally and the CSA determined using image analysis software. An advantage of the mould technique over other methods of *in-vitro* CSA analysis is that CSA at multiple sites can be determined without transection of the tendon, allowing the tendon to be used for tensile testing (Kasashima *et al*, 2002).

### 1.2.10 The repeatability of ultrasonographic image acquisition and image analysis

Pickersgill *et al* (2001) used the CSA as an objective measurement to quantify the variability occurring during the course of the ultrasonographic assessment of the equine SDFT. The effects of three variables on the CSA measurement were determined; image acquisition by two different operators, image analysis by two different operators calculating the tendon CSA, and the use of two different sets of equipment during calculation of CSA values. They found that there was no statistical difference ($P>0.05$) in the image acquisition between operators and between the equipment used in all but the most distal levels of the tendon, however a significant difference ($P<0.01$) was reported between operators during image analysis. They concluded that to avoid confounding results during ultrasonic investigations, only one operator should undertake image analysis of tendon CSA, although different operators could undertake image acquisition.

#### *1.2.10.1 Validation of ultrasonographic CSA and post-mortem CSA*

Smith *et al* (1994) demonstrated excellent agreement between the ultrasound-generated CSA obtained prior to euthanasia and the photographic image CSA obtained at post-mortem. The average percentage difference between areas measured ultrasonographically and photographically was 4.59% (s.d. 3.67%). In the same study they found there was no statistically significant difference in measurement of CSA between left and right limbs of the same horse.

Gillis *et al* (1995) found a good correlation between inkblot CSA and *in-vivo* ultrasonographic CSA for standing horses, with the mean inkblot CSA being 15% larger. The difference between the two CSA measurements may be attributed to the tension in the SDF muscle while standing, and compression of the tendon section and fibre separation while obtaining inkblot impressions, resulting in a larger CSA value.

Birch *et al* (1999b) found a difference of 9% between CSA measurements of the SDFT obtained ultrasonographically prior to post-mortem and values obtained at post-mortem using photography. Images were printed onto standard photographic paper and enlarged eight times. Print enlargements were cut out, weighted and CSA calculated.

Similarly Kasashima *et al* (2002) found a good correlation between ultrasonographic CSA measurements prior to post-mortem and cast based post-mortem measurements ($R^2$=0.78) in young Thoroughbred horses.

### 1.2.11 The effects of growth and exercise on the biomechanical, biochemical and structural properties of tendons in other species

While the economic significance of tendon disease is generally confined to horses, cattle and dogs, the majority of research into the effects of exercise and growth on tendon behaviour has been carried out on laboratory species. The response of the tendon to exercise can be measured at the structural, mechanical, and chemical level. However most studies have been limited to measuring only one or two of these variables, and therefore comparisons between species are often difficult.

### *1.2.11.1 Tendon biomechanics in other species*

Bennett *et al* (1986) investigated the mechanical properties of tendons from several mammalian species (Red necked Wallaby, Common Porpoise, White sided Dolphin, domestic Pig, Arabian Camel, Roe Deer, Fallow Deer, Sheep, Donkey and Horse). Dynamic tensile tests were performed using physiologically relevant frequencies, (2.2 Hz similar to stride frequency) and stress ranges (over 30 MPa) on various tendons from legs and tails. No consistent differences were found between tendons from different species or different anatomical sites. All tendons approached a straight line on the plot of Young's modulus (GPa) over Stress (MPa) at about a modulus of 1.5 GPa at stresses above 30 MPa. Percentage energy dissipation of 6% to 11% was measured for the different species. Similar findings were reported by Ker (1981) when investigating the tensile properties of the Plantaris tendon of sheep.

### *1.2.11.2 The effect of exercise on tendon biomechanics in other species*

Several studies suggest that endurance training results in an increase in tendon strength and stiffness. Viidik (1967) reported an increased stiffness of approximately 10% in the Achilles and tibialis posterialis tendons of rabbits trained for 40 weeks on a treadmill. The tensile strength of these tendons increased by 5%.

Woo *et al* (1980) found that the digital extensor tendons of swine subjected to a twelve-month training regimen increased in stiffness and the ultimate strength increased by 62%. Interestingly the same training programme had no effect on the digital flexor tendons of the same animals. Simonsen *et al* (1995) compared the response of rat Achilles tendons to a

strength training and endurance training regimen. No differences were found in force at ultimate tendon failure between strength trained and control rats, however in endurance-trained rats the average force at ultimate tendon failure was significantly higher than that of the control animals.

Vilarta and Vidal (1989) also reported an increase in stiffness and tensile strength in the Achilles tendon in rats following a thirty-day exercise programme.

Kiiskinen (1977) reported that the tensile strength of the patellar tendons from mice trained for seven weeks on a treadmill did not differ significantly from that of the control mice. However the training was initiated before the mice had reached maturity, while the work of Woo *et al* (1980) and Viidik (1967) used mature animals.

Although studies into the effects of long-term exercise on the tensile strength of tendons are limited some patterns are beginning to emerge and there may be a correlation between increases in tendon strength and stiffness associated with long term exercise (Buchanan and Marsh, 2002).

#### *1.2.11.3 Tendon growth and development in other species*

Ippolito *et al* (1980) studied the Achilles tendons from newborn, two-month-old and four-year-old Albino Rabbits. Their results suggested that Achilles tendon undergoes morphological and biochemical changes during ageing. The tenoblast became more slender and longer with age and there was an increase in extracellular matrix, causing a relative decrease in the number of cells per unit of tissue surface. With ageing, collagen

fibres increased in diameter and varied more in thickness. This is similar to the changes observed in Equine tendon cell morphology and fibril diameter with ageing (Smith and Webbon, 1996; Goodship, 1993).

Fibrocartilaginous changes have been observed in the tendons and ligaments of humans in response to loading. Formation of a fibrocartilaginous matrix is at sites where the tendons or ligaments are under compression. This occurs where they change direction by wrapping around bony pulleys (e.g. Achilles tendon), threading through fibrous retinacula, or at insertion to bone (Benjamin *et al*, 1998). Similar changes occur with focal and diffuse chondroid metaplasia in Equine SDFT (Crevier-Denoix *et al*, 1998).

#### *1.2.11.4 The effects of exercise on tendon biochemistry in other species*

Because collagen is the most abundant protein in tendons, it would be expected that changes in biomechanical properties would be associated with changes in collagen concentration. Reports from various studies are conflicting with regards to the effect of exercise on tendon collagen concentration.

Woo *et al* (1981) observed that the effects of long term exercise had a positive effect on the extensor tendons of miniature swine, but not on their flexor tendons. The extensor tendons showed hypertrophy, improved mechanical properties, and increased collagen concentration and total weight. Whereas training improved the strength of the insertion of the flexor tendon onto bone, it had no effect on the substance of the flexor tendon. Woo *et al* (1981) postulated that during exercise non-collagenous materials are diminished in extensor tendons, thus increasing the collagen concentration and correspondingly changing

the mechanical properties of the tendon attributed to collagen. However since flexor tendons already have a low amount of non-collagenous materials, the concentration is not further reduced and therefore changes in collagen concentration are not detected. The differences in results between the extensor and flexor tendons may be attributed to the different loads to which the two types of tendons are subjected. The appropriate stimulus required to induce remodelling of the flexor tendons may not have been provided in the study.

Heikkinen and Vuori (1972) showed that the degree of physical activity influences the metabolism and structure of connective tissues in aged mice. Exercise increased the relative amount of collagen in Achilles tendons, and accelerated the turnover of collagen and other proteins. Physical inactivity had an opposite effect. These changes were regarded as adaptation responses by the tendon to physical stress.

Suominen *et al* (1980) investigated the effects of exercise on the metabolism of connective tissues in young mice. They observed that exercise accelerated the metabolism of collagen in Achilles tendon, whereas the metabolism of glycosaminoglycans was unaffected.

Curwin *et al* (1988) randomly assigned 3 week old White Leghorn Roosters to exercise or control groups. Exercised birds were subjected to a progressive treadmill-training programme for eight weeks. The exercise programme induced a 46% increase in Achilles tendon collagen deposition, without changes in DNA, proteoglycan, and collagen concentrations or tendon dry weight. Tendon collagen from exercised birds contained 50% less pyridinoline cross-links. They postulated the results suggest that high-intensity exercise induced a greater matrix-collagen turnover in growing chickens resulting in

reduced maturation of tendon collagen. This is in contrast to the work of Viidik and Vailas, who did not find an increase in collagen concentration in the Achilles tendons of trained adult rats or rabbits (Viidik, 1967; Vailas *et al*, 1985).

#### *1.2.11.5 The effects of exercise on tendon weight and CSA in other species*

Viidik (1967) found no differences in either the fresh weight or the dry weight of the tendons from exercise rabbits when compared to that of the control group. Similarly Vailais *et al* (1985) found that exercise did not alter the dry weight of the patella tendon of trained rats, while Curwin *et al* (1988) found no difference in the dry weight of Achilles tendons from exercised chickens.

Woo *et al* (1980 and 1981) reported that exercise had no effect on the dry weight of swine digital flexor tendons, however an increase in dry weight of the digital extensor tendons was recorded. The CSA of the digital extensor tendons following long-term exercise in adult swine, was observed to increase, in contrast to the digital flexor tendons in the same animals (Woo *et al,* 1980; Woo *et al,* 1981). Buchanan and Marsh (2002) found that the Achilles tendon of guinea fowl did not hypertrophy in response to long-term training.

## 1.3 Summary

Superficial digital flexor tendonitis remains a significant cause of wastage in racehorses. It is a debilitating, and possibly career-ending, injury. The occurrence of tendonitis is not restricted to racehorses, as horses used for other equestrian activities such as eventing, polo and showjumping, may suffer career-ending tendonitis. While the incidence of tendonitis is relatively low in two and three-year-old horses, the incidence increases as the horse ages.

Since its introduction into veterinary medicine in the early 1980's, ultrasonography has revolutionised the diagnosis and management of tendon injuries, allowing any subtle swelling of the SDFT to be evaluated early in the disease process, before the clinical signs of tendonitis are advanced and significant tendon damage has occurred.

The exact pathophysiology of clinical SDFT tendonitis is yet to be determined. It is known that strains in the SDFT of galloping Thoroughbreds can reach up to 16%, which is at, or close to the tendon's ultimate tensile strain. The reddish discolouration of the tendon core, found at post-mortem in clinically-sound horses supports the concept of pre-existing degenerative changes within the SDFT. It is possible that cumulative microtrauma from repetitive cyclic overloading leads to degenerative changes within the SDFT, and clinical signs of tendonitis becomes apparent when the weakened and degenerate tendon fails under the appropriate mechanical load.

Many treatments have been advocated for SDFT tendonitis but evidence is lacking to show that any of these significantly improve the prognosis. This may well reflect the current level of understanding of the pathophysiology of tendonitis.

Currently, strict confinement of the horse in conjunction with a controlled exercise programme, has been found to offer the best prognosis for return to athletic activity. Such rehabilitation programmes are costly, time-consuming, and require long-term commitment and compliance on the horse owner's behalf.

In Thoroughbred racehorses with superficial digital flexor tendonitis the chances of returning to racing are less than 50%, and of those that do return to racing, nearly half will re-injure the tendon. Clearly prevention of tendonitis would significantly reduce the wastage of horses. Therefore research into tendon injury and repair, and the education of horse owners and trainers are critical in tendon injury prevention.

Many skeletal tissues respond to changes in functional demand with an adaptive response, and the mechanisms for adaptation in muscle and bone have been well established, but the factors controlling the response of tendon to training are poorly documented. This may be in part due to any change within the SDFT being so subtle that detection of such a change could be difficult, or impossible, with current in- vivo measurement techniques.

Most studies have investigated the effect of exercise on tendons in mature animals. Tendons have been shown to adapt to exercise training in a number of species with alterations in morphological, biomechanical and biochemical characteristics. Flexor and extensor tendons respond differently in mature animals to the effect of exercise, with adaptive hypertrophy seen in the extensor tendons, but not in the flexor tendons. There are a small number of studies to date examining the effect of exercise on the CSA of the SDFT in mature horses, and these studies give conflicting results. It is most likely that exercise does not induce an increase in the ultrasonographic CSA of the SDFT.

The one published study to date that reported an increase in the CSA of the SDFT in mature animals, also observed the horses having clinical evidence of tendonitis, which may explain the increase in CSA (Gillis *et al*, 1993).

There is only one published study examining the effect of treadmill exercise on the CSA of the SDFT in young Thoroughbred horses (Kasashima *et al,* 2002). Several differences exist between the treadmill-exercised study and the over-ground conditioning exercise of the present study. Treadmill exercise alters the biomechanical forces and kinematics of locomotion, therefore the results of Kasashima *et al* (2002) may be confounding.

During growth when collagen is being synthesised, applying early controlled exercise may result in a stronger tendon that may be accompanied by an increase in CSA. If immature flexor tendons could be modified in a favourable manner during growth to make the tendon bigger and thus stronger, then preventing or reducing the incidence of tendonitis may be attainable.

The factors described above was motivation for a novel approach to modify tendon structure. The study described in this thesis, concerned conditioning exercise imposed on Thoroughbred foals from ten days to 18 months of age, and was aimed to determine if a particular exercise programme would modify the CSA of the SDFT of the immature horse, without the development of tendonitis. The use of ultrasonography to measure CSA throughout the course of the study enabled the SDFT to be closely monitored for early evidence of tendonitis, evident as an increase in CSA, with or without disruption to fibre pattern and alignment. Therefore the injury effect of conditioning exercise on the SDFT CSA, if any, could be determined, and separated from any developmental effect that conditioning exercise may have on the immature SDFT.

## 1.4 Hypotheses

**Hypothesis 1**

The CSA of the SDFT at the mid-metacarpal level in conditioned and control foals will increase with age.

**Hypothesis 2**

There will be no significant difference in mean CSA of the SDFT at mid-metacarpal level between conditioned and control foals at any age.

**Hypothesis3**

There will be no significance difference between the mean CSA of the mid-metacarpal left and right SDFT in the conditioned and control foals

**Hypothesis 4**

The CSA of the SDFT determined ultrasonographically *in-vivo* will be significantly correlated with the ex-*vivo* CSA determined photographically.

### 1.4.1 Objectives of this study

The aims of this study were to:

1. To test the hypotheses by serially measuring the ultrasonographic cross sectional area of the superficial digital flexor tendon in 33 Thoroughbred foals (18 exercised and 15 control)
2. Analyse and interpret the findings in relation to the influencing variables, (sex, growth, exercise and leg) and the possibility of interaction between these variables.
3. Discuss the findings of the study in relation to prevention of injury to the superficial digital flexor tendon and differences in the CSA in relation to early conditioning.

# 2 MATERIALS AND METHODS

## 2.1 Overview of the study

This study was part of the GEXA project, a collaborative study involving the four participants of the Global Equine Research Alliance (GERA); Massey University, Colorado State University, USA; Royal Veterinary College, UK, and Utrecht University, NL.

The mission statement of the GERA research group is " To reduce the incidence of injury in the equine athlete through modification of tissues by application of appropriate conditioning regimens in the young animal" (McIlwraith, 2000)

The primary purpose of the GEXA project was to evaluate the effect of early conditioning on the quality of the tissues of the musculoskeletal system of typical pasture-reared New Zealand Thoroughbred foals.

Within the GEXA project, several studies were undertaken with the animals used for this thesis, and data from theses studies included:

- Biochemical and immunological markers of articular cartilage, bone and tendon disease or injury.
- CSA, shape and bone mineral density of the distal forelimb bones using pQCT at four days, two, four, twelve and sixteen months of age.
- Radiographs and scintigraphy scans of carpi and metacarpophalangeal joints at sixteen months of age.
- Comparison of cortisol concentrations, growth characteristics and the onset of puberty across groups.

## 2.2 The animals

The animals consisted of thirty one Thoroughbred foals, sired by fifteen different thoroughbred stallions, born in the 2000 breeding season at Flock House Thoroughbred Research stud, in the Rangitikei region of New Zealand. At birth, the foals were allocated into a conditioned or control group on the basis of birth date, sire and sex. The conditioned group consisted of 18 foals, (6 colts, 12 fillies), the control group of 15 foals, (4 colts, 11 fillies), with a foaling spread of 89 and 95 days respectively *(Appendix 1)*

All the foals were reared at pasture, in paddocks of approximately two hectares, which is typical of New Zealand foal management. The foals' diet was predominantly ryegrass/ clover pasture, with supplementary meadow hay fed during winter. All foals were weaned at 4 months of age.

Two horses (G32 and G33), were purchased as weanlings and allocated into the control group.

## 2.3 Exercise regimen

The exercise foals received controlled exercise over 1030 meters, for five days per week, beginning when each foal was ten days old. All exercise was conducted on a 515m grass exercise track. The track surface was turf. A layer of sand was added to the track surface during June 2001, to improve the consistency of the surface and prevent slipping during wet conditions.

Initially the foals were exercised in a group of four foals with their dams. A four-wheel motorcycle with a barrier to prevent the horses from passing the vehicle, led the group and controlled the pace, and another four-wheel motorcycle followed behind the group to encourage the horses to exercise. After weaning at four months of age, the foals exercised in groups of eight foals. The foals were exercised in both a clockwise and counter clockwise direction, with equal frequency.

The distance and velocity at which the foals were exercised increased with age. Initially the foals were exercised at a constant velocity of 4.20ms$^{-1}$. This was increased to 5.56 ms$^{-1}$ in February 2001, and then to 6.66 ms$^{-1}$ in May 2001, with the addition of a sprint over 250m at 12 ms$^{-1}$, after 500m of base exercise, (6.6 ms$^{-1}$).

For every conditioning session, the actual times and distances of each group of foals were recorded by the stud manager and data were analysed (Huitema 2001).

## 2.4 Monitoring

The stud manager observed the foals daily as part of the stud's routine farm management practice. Any abnormalities were recorded and reported to the stud veterinarians for advice or treatment. Each foal was weighed and had condition score assessed by the stud manager every two weeks.

The behaviour patterns of the foals at pasture were monitored during the day (Stolwijk, 2001). The observations recorded the duration of grazing, resting and play periods.

### 2.4.1 Clinical examination

All foals underwent a thorough clinical examination at four days of age, including conformation assessment. This was repeated monthly throughout the study period until the foals were 18 months old. As part of the monthly clinical examination, and prior to each ultrasonographic examination, both the left and right fore limb SDFT, DDFT and suspensory ligaments were examined. Palpation was performed with both the foal bearing full weight on the limb, and with the limb flexed. Observations were made for any heat, swelling, enlargement of the tendon or ligament, or pain on palpation.

### 2.4.2 Ultrasonographic examination

The SDFT at the mid-metacarpal level, of both the left and right forelimbs, were examined ultrasonographically in all foals at five, eight, twelve, fifteen and eighteen months of age. Examinations were performed during the specific month that the animals were to be examined, therefore the age of examination was a mean age and shall be referred to as five, eight, fifteen and eighteen months of age.

## 2.5 The ultrasonographic equipment

All ultrasonographic images were obtained using a Sonosite® 180 ultrasound machine (Sonosite Inc., SE Bothell, WA, USA), a Sonosite L38, 10-5 MHz linear transducer, with an LA5 HRS acoustic stand-off (ATL Professional Medical Supply, Bothell, WA).

A standard phantom was constructed using a metal staple embedded in Polymethylmethacrylate (PMMA). The ends of the staple prongs projected 2 mm above the PMMA surface. The distance from the outside of one staple prong to the inside of the other staple prong was determined to be 11.95 mm using 150mm Vernier callipers.

The standard phantom was imaged before and after each tendon imaging session. The captured phantom images of a particular day were used for calibration when the image analysis programme was used for determination of SDFT CSA of tendons scanned on that day.

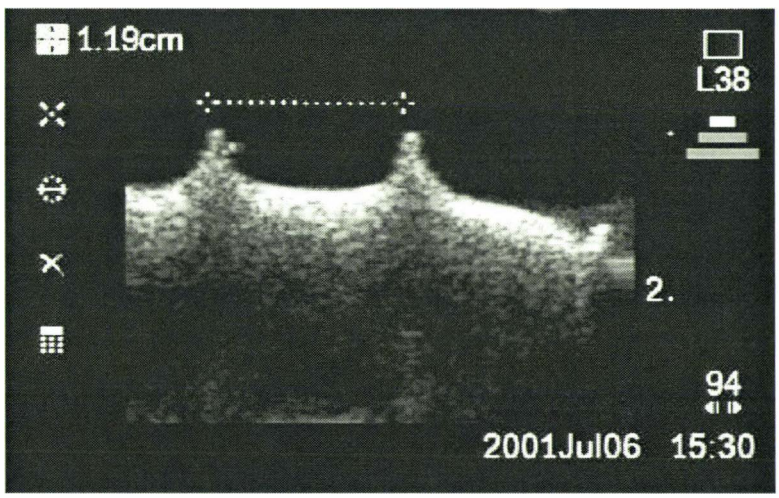


**Figure 2.1 Ultrasonographic image of standard phantom obtained prior to tendon imaging session.**

## 2.6 The scanning protocol

All examinations were performed in a covered barn with a level concrete floor. The foals were held by an experienced assistant and were not sedated. A region on the palmar aspect of the mid-metacarpus of both forelegs was clipped over the SDFT.

To determine the mid-metacarpal site for ultrasonographic examination of the SDFT, the length of the third metacarpal bone was measured with a 40 cm precision engineering steel ruler, by identifying the carpometacarpal and the metacarpophalangeal joint spaces on the lateral aspect of the limb. The level at which the image of the SDFT was to be captured was marked with an alcohol-based marker pen (Vivid, Stephens, Bic, Auckland, N.Z.) on the lateral aspect of the SDFT midway between the two joints. Hair debris was wiped from the skin with a cotton towel, and water-soluble ultrasound transmission gel (Vibragel ultrasound gel, Crown Dental & Medical Ltd, CHCH, N.Z.) applied to the clipped region. The assistant positioned the foal to stand squarely on both forelimbs at the time of scanning and image capture. Prior to image capture, the foal's identification number, age in months at the time of scanning, and leg identification was entered into the ultrasound machine to identify the image.

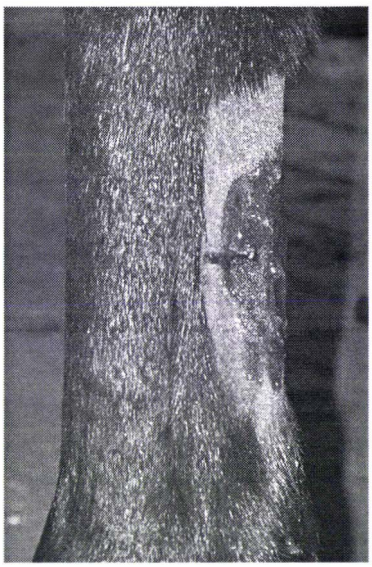

**Figure 2.2 Image depicting the mid-metacarpal site prepared for ultrasonographic examination of the SDFT**

## 2.7 The image transfer process

The captured images were exported via an S-VHS port from the ultrasound machine to a Pentium computer with a Mico DC10 capture card (Pinnacle Systems). The exported images were saved as bitmap files for later image analysis.

## 2.8 Ultrasonographic *in-vivo* measurement of the SDFT CSA

The CSA of the SDFT was measured with Scion image (Scion Corp, Frederick, Maryland 21701, USA), prior to which the scale was set using the calibration image of the phantom standard collected on the same day as the tendon images to be analysed. The CSA was calculated by drawing around the perimeter of the SDFT with the area tool. CSA was calculated three times, to three decimal places, and each value (in $cm^2$) was displayed on screen and subsequently recorded in a MS Excel spreadsheet (Microsoft Corporation). The average value of the three measurements was used for statistical analysis.

## 2.9 Euthanasia and tissue harvest

To quantify the effect of early exercise on the musculoskeletal system and determine that no harm was being done with this novel tissue conditioning regimen, twelve horses (six exercised and six control animals) were humanely euthanased at eighteen months of age. The selection of horses for euthanasia was based on age and sex to ensure three colts and three fillies represented both the exercise and control groups. The remaining horses (n= 20) entered race training.

At tissue harvest the forelimbs were removed through the distal scapula 150 mm proximal to the shoulder joint. The skin was removed from the right fore limb. The carpometacarpal and metacarpophalangeal joints (MCPJ) were identified, and the length of the third metacarpal bone was measured in centimetres on the lateral aspect of the leg with a 40 cm precision steel ruler. The tendon was transected at the mid point of the length of the lateral aspect of the third metacarpal bone, with the MCPJ at the normal standing flexion angle of 55 degrees.

## 2.10 Photographic *in-vitro* measurement of the SDFT CSA

The transected ends of the right SDFT were prepared for photography by placing the proximal surface of each section in a piece of black foam rubber, which contained holes of the approximate size of the tendon sections. The end of the tendon was manoeuvred so that the proximal cut surface was flush with the foam surface. The side surface was hidden from view by the conforming of the black rubber, which did not distort or compress the tissue. A perspex calibration ring, machined to 20mm diameter, was placed over the tendon end. Each segment was identified by a label in the frame. A recording was made for several seconds, at incident angle at 90 degrees, and at several degrees either side of normal, using a hand-held video camera (Sony hi8 camera) approximately 40cm above the rubber surface. The images were captured manually as single frames, using a Pentium computer with micro CD10 capture card, and saved under their horse, tendon and segment level code.

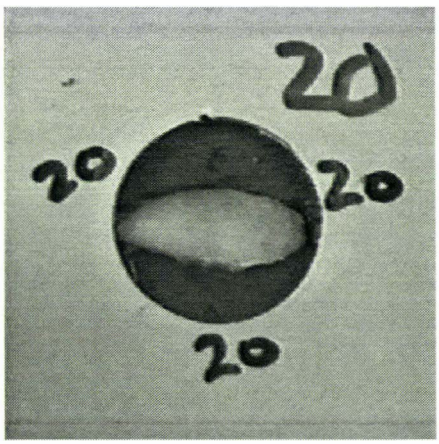


**Figure 2.3 The transected end of mid metacarpal SDFT in black foam rubber photographed at post-mortem.**

## 2.11 Quantification of variation in repeat tendon images and measurements of CSA of tendon images

The CV of repeated measurements of the same tendon image and the variation in repeated images of the same tendon are shown in Appendix 2.

## 2.12 Statistical analysis

Image analysis data were collated and analysed in SPSS 10.1.3 (SPSS Inc, Chicargo, IL, USA). The following data points were missing: G32 and G33 (bodyweight, BCS, SDFT CSA, from birth to six months of age, as these control fillies were purchased at six months old), and G21 at eighteen months (bodyweight, BCS, SDFT CSA, due to euthanasia after femoral fracture in the paddock, at seventeen months).

The repeated measures general linear model procedure (SPSS 10.1) was used to examine the effect of variables of tendon CSA in relation to age. Within the model the treatment groups, (conditioned or control), sex (filly or gelding), and limb (left or right forelimb) were tested as fixed effects. To correct for differences in bodyweight, birth weight was included in the model as a co-variant, as birth weight explains the majority of variance in bodyweight in New Zealand Thoroughbred foals (Morel, P. – unpublished data).

To investigate any association between the size of the horse and SDFT CSA, and that perhaps one treatment group may contain larger horses than the other, the percentage difference in the average bodyweight between the exercise and control horses was calculated for each age group.

Linear regression models were used to test the repeatability of imaging the same level of the SDFT (Pilot study 2), and to test the relationship between the ultrasonographic *in-vivo* and *in-vitro* CSA of the right fore SDFT.

For all analyses the significance level was set at $p<0.05$. The results were presented using box plots and scatter plots. In the box plots, the top and bottom of the box represent the $25^{th}$ and $75^{th}$ percentile. The middle horizontal line indicates the median, and the open circles are outliers.

# 3 RESULTS

## 3.1 Clinical examination

Minor ailments of the animals that were euthanased, that required medical treatment and/or a rest period during the study are displayed in Table 3.1. The conditions included subsolar abscessation, abrasions and lacerations. During June 2001 the conditioned group did not exercise for two weeks due to mild respiratory disease.

| Horse ID. | Treatment Group | Clinical Examination Findings | Weeks Prior to Euthanasia |
|---|---|---|---|
| G3 | Control | Swelling RF proximal cannon.<br>Bilateral swelling forelimb over region of medial and lateral PSB.<br>Bilateral swelling forelimb over region of medial and lateral PSB. | 20<br>12<br>8 |
| G17 | Control | RF foot abscess.<br>RH femoropatellar joint effusion.<br>RH hoof wall crack/abscessation at toe. | 20<br>14<br>8 |
| G1 | Conditioned | LH foot abscess.<br>Recurrence of LH foot abscess. | 24<br>8 |
| G16 | Conditioned | Bilateral swelling of forelimb PSB with pain on flexion of right distal limb.<br>Superficial shoulder laceration. | 15<br>14 |
| G20 | Conditioned | LH lameness – undiagnosed.<br>Sustained trauma during transportation to slaughterhouse. Mainly superficial abrasions and contusions. | 16<br>0 |
| G31 | Conditioned | RF foot abscess.<br>Superficial grazing to left thorax.<br>RH fetlock skin abrasions and swelling of distal limb.<br>RH fetlock joint effusion and swelling over fetlock region. | 16<br>20<br>10<br>8 |

**Table 3.1 Injuries and musculoskeletal findings occurring within six months prior to euthanasia.**

**RF –right fore, LF –left fore, LH -left hind, RH –right hind, PSB –proximal sesamoid bone**

### 3.1.1 Tendon examination by palpation

During the course of the study, at no time during the monthly clinical examinations, or prior to U/S scanning, were palpable tendon abnormalities detected. There was never evidence of heat, swelling, enlargement, or pain on palpation with the SDFT, DDFT, or suspensory ligament in either the left or right forelimb of any of the conditioned or control animals at any examination time.

No ultrasonographic abnormalities were detected in the mid-metacarpal region of the left and right fore legs in any of the animals, while imaging the tendon to obtain an image for CSA calculation.

During the course of the study, it was observed that the echogenicity of the SDFT increased considerably as the animals aged. At five months of age the SDFT appeared hypoechoic when compared to an image of a normal adult SDFT, and it was not until the horses were fifteen to eighteen months of age, that their SDFT had the echogenicity consistent with a normal adult SDFT (Moffat, personal observation).

## 3.2 Ultrasonographic *in-vivo* measurement of the SDFT CSA

### 3.2.1 The increase in mean SDFT CSA with growth

The mean SDFT CSA increased with growth in both groups. The largest increase in mean CSA was between five and eight months after which there was minimal increase in mean CSA.

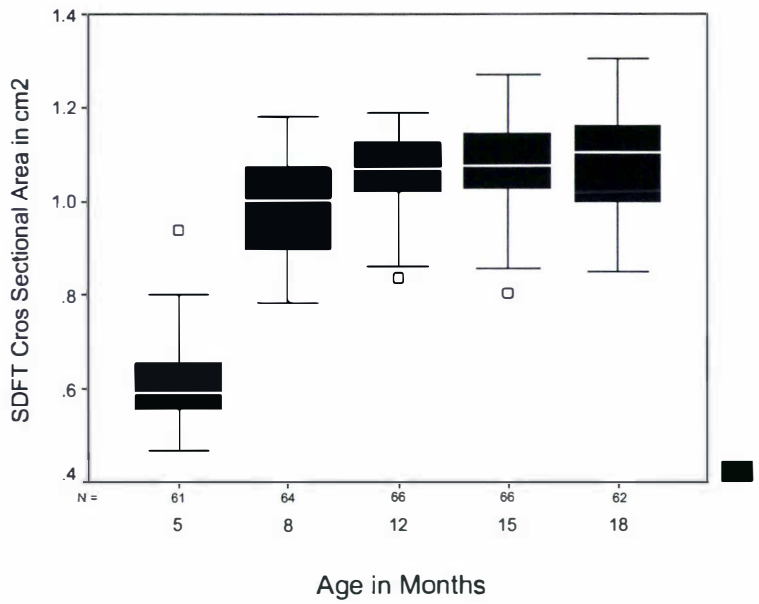


**Figure 3.1 Box and whisker plot of the mean CSA of the SDFT of all the foals over the course of the study.**

### 3.2.2 The difference in mean CSA between the left and right forelegs

Across all age categories, there was no significant difference in the mean CSA of the SDFT between the left and right limb (P = 0.667). Figure 3.2 shows the box and whisker plots for both left and right foreleg CSA.

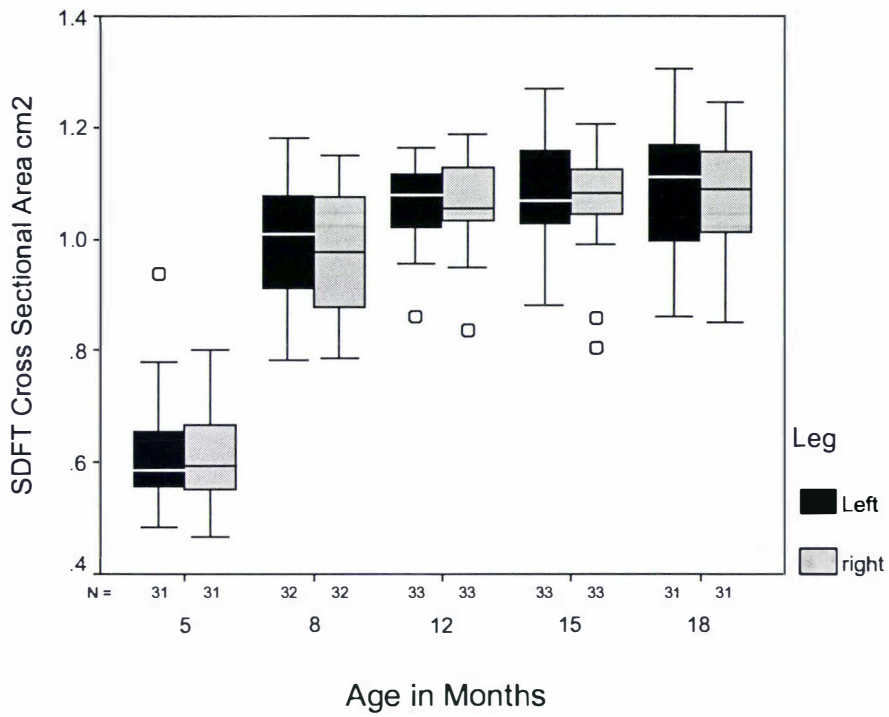


**Figure 3.2: Box and whisker plot of the mean CSA of the SDFT of the right and left leg**

### 3.2.3 CSA of SDFT in relation to conditioning exercise

The effect of conditioning exercise on the mean CSA at each measurement interval was examined. There was no significant difference in the mean CSA of the SDFT between conditioned and control animals at any age (P = 0.058).

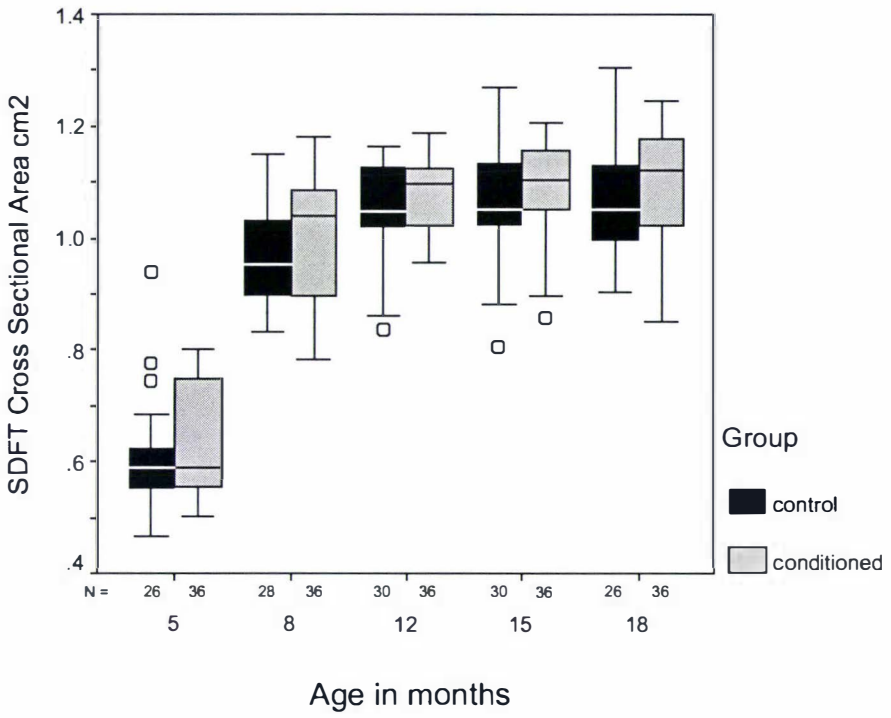


**Figure 3.3: Box and whisker plot of the mean SDFT CSA in conditioned and control animals**

There appeared to be a greater variance in the CSA in the conditioned animals at five and eight months of age, which was confirmed by calculating the mean percentage difference in CSA for each age group (Table 3.3). The greatest percentage difference between conditioned and control animals was at eight months of age. The percentage difference at the remaining measurement intervals was 3% or less.

| Age in Months | Mean Percentage difference in CSA between Treatment Groups | Treatment Group with largest Mean CSA |
|---|---|---|
| 5 | 0.768% | Conditioned |
| 8 | 5.93% | Conditioned |
| 12 | 3.25% | Conditioned |
| 15 | 1.68% | Conditioned |
| 18 | 2.8% | Conditioned |

**Table 3.2 The mean percentage difference in SDFT CSA in all ages that measurements were recorded.**

The mean SDFT CSA was calculated for conditioned and control groups at each age interval (Table 3.3).

| Treatment Group | Age in months | Mean CSA ($cm^2$) | Standard deviation | Intra-group CSA Range | % Difference over the lowest CSA measurement |
|---|---|---|---|---|---|
| **Conditioned** | 5 | 0.629 | 0.096 | 0.50 – 0.81 | 62% |
| **Control** | 5 | 0.625 | 0.094 | 0.46 – 0.77 | 67% |
| **Conditioned** | 8 | 0.997 | 0.119 | 0.78 –1.19 | 52% |
| **Control** | 8 | 0.942 | 0.083 | 0.83 – 1.18 | 42% |
| **Conditioned** | 12 | 1.079 | 0.062 | 0.95 – 1.19 | 25% |
| **Control** | 12 | 1.045 | 0.088 | 0.84 – 1.13 | 34% |
| **Conditioned** | 15 | 1.086 | 0.084 | 0.89 – 1.20 | 34% |
| **Control** | 15 | 1.068 | 0.107 | 0.81 – 1.20 | 48% |
| **Conditioned** | 18 | 1.098 | 0.107 | 0.91 – 1.25 | 37% |
| **Control** | 18 | 1.068 | 0.098 | 0.90 – 1.21 | 34% |

**Table 3.3 The CSA of the SDFT in conditioned and control horses at each age.**

**The right hand column is the range expressed as a percentage over the lower CSA value as used by Kasashima *et al* (2002).**

The between-horse variation in SDFT CSA was largest at the onset of the study and decreased with age.

### *3.2.3.1 Relationship of SDFT CSA to body weight*

To examine if bodyweight had an effect on SDFT CSA, the data was pooled across all ages, irrespective of treatment group. There was no correlation between SDFT CSA and bodyweight using the linear regression model. The best result was R=0.1158

There was no significant difference in the average bodyweight between the two groups at any age. The range for the percentage difference in mean bodyweight was between 0.02%-0.78% over the ages. On four of five occasions the conditioned group was heavier than the control group.

### *3.2.3.2 Influence of body condition score on the SDFT CSA*

There was no statistically significant difference in body condition score between the conditioned and control horses at any age. At fifteen and eighteen months the fillies had a significantly higher condition score than the geldings (7.204 vs 6.725; p=0.01) and (7.294 vs 6.813; p=0.043) respectively. At eighteen months the body condition score of the control horses was greater than that of the conditioned horses, however this was not statistically significant.

### 3.2.4 Difference in SDFT CSA between sexes

The influence of gender on the SDFT CSA at each measurement interval was examined. There was no significant difference in mean SDFT CSA between colts and fillies $P = 0.559$.

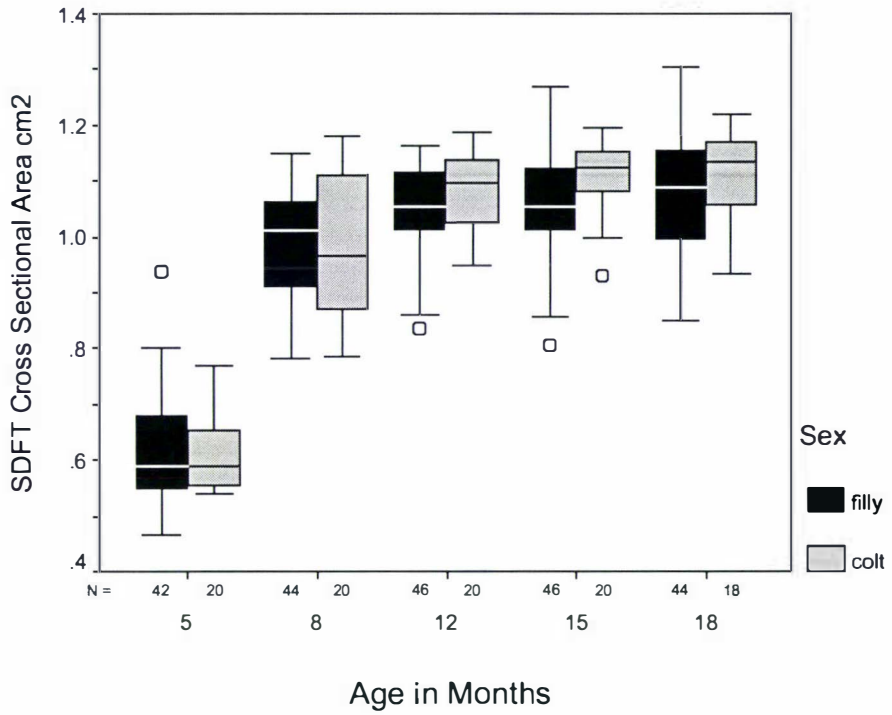


**Figure 3.4: Box and whisker plot of the mean SDFT CSA of fillies and colts**

Visually there appears to be a greater variance in the CSA in the colts at eight months of age. The mean CSA in the colts at eight months was 0.983 $cm^2$, s.d. 0.137 $cm^2$ and the mean CSA in the fillies at the same age was 0.973 $cm^2$, s.d. 0.096 $cm^2$.

## 3.3 Correlation between *in-vivo* and *in-vitro* SDFT CSA

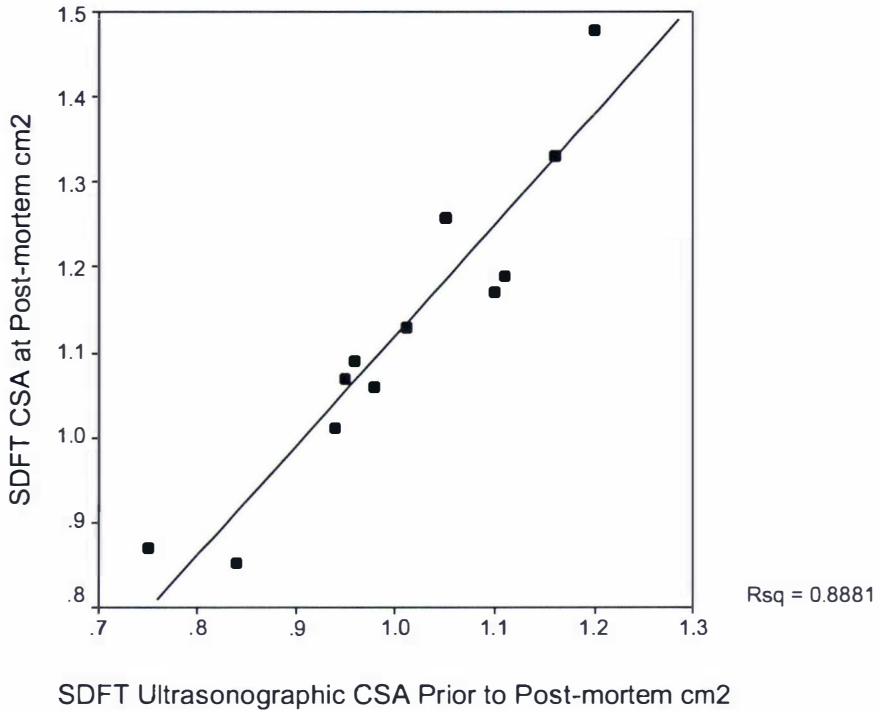


**Figure 3.5: Regression of the Ultrasonographic *in-vivo* SDFT CSA just prior to post-mortem and the *in-vitro* SDFT CSA obtained at post-mortem**

The individual animal *in-vitro* SDFT CSA was consistently larger than the CSA value obtained *in-vivo* just prior to post-mortem, regardless of which treatment group the individual came from. On average the *in-vitro* CSA was 10.3% larger than the ultrasonographic *in-vivo* CSA.

# 4 DISCUSSION

## 4.1 The Animals

### 4.1.1 Behaviour at pasture

A major assumption of this study was that the conditioned animals received a greater total exercise load than the control group. Stolwijk (2001) examined the behavioural effects of exercise on the foals by quantifying the duration of grazing, rest, and play periods of the two groups while at pasture. There was no significant difference in activity at pasture between the two groups of foals and imposed exercise did not induce a compensatory reduction in natural paddock activity in the exercised animals. However the foals were observed for only a restricted time.

The normal activity of the conditioned foals at pasture in this study was similar to the behaviour of foals in the study of Kasashima *et al* (2002), in which exercised foals received conditioning exercise (high-speed treadmill) from 55 days to 15 months of age. They found no statistically significant difference in the level of activity at pasture of the exercised group and imposed exercise did not induce a compensatory reduction in the natural exercise of the exercised animals.

Caanitz *et al* (1991) observed the effect of high-speed treadmill exercise on the behaviour of adult Standardbred horses. The greatest influence on behaviour was seen immediately after exercise, where the horses spent more time drinking and standing (resting) than they did on non-exercise or rest days. They postulated that the changes in behaviour might be related to the physiological changes that accompany exercise. Eating, walking and self-grooming were not significantly influenced by exercise.

In the present study there was no observed difference in the time spent drinking or standing (resting) in the period after exercise between the conditioned and control animals and this may be related to the lower intensity of exercise that the conditioned foals received compared to the high speed treadmill exercise in the study of Caanitz *et al* (1991).

### 4.1.2 Growth rate

The growth rate and average daily gain (ADG) of the foals from birth to eighteen month of age was not significantly different between conditioned and control animals, or between fillies and colts (Brown-Douglas *et al*, 2003). The body weights of both the conditioned and control animals were similar to those recorded by the stud in previous seasons. Between birth and four months of age the overall mean ADG reduced from 1.5kg/day to 1.0 kg/day. From five months of age until the completion of the study, ADG ranged between 0.5 and 0.7 kg/day. The bodyweights of the horses in this study were similar to those of grain-fed Northern Hemisphere Thoroughbreds (Pagan *et al*, 1996; Thompson, 1995).

During the course of the study there was no statistically significant difference in body condition score between the conditioned and control animals as assessed fortnightly by the stud manager, nor between colts and fillies at five, eight and twelve months of age. At fifteen and eighteen months of age the fillies had a significantly higher body condition score than that of the colts.

The effect of conditioning exercise from ten days of age was not detrimental to the average daily gain, bodyweight, or body condition score of the conditioned foals. The growth rate data are displayed in Appendix 3.

### 4.1.3 Exercise regimen

Huitema (2001) quantified the distance and velocity of the conditioning exercise during the study. The distance over which the foals exercised (1030m) remained constant and the average velocity was increased with age. The initial velocity was 4.20ms$^{-1}$, which was increased to 5.56 ms$^{-1}$ in February 2001, and in May 2001 to 6.66 ms$^{-1}$, with the addition of a sprint over 250m at 12 ms$^{-1}$, after 500m of base exercise, (6.6 ms$^{-1}$). This contrasts with the only study to demonstrate an increase in SDFT CSA in response to conditioning exercise (Kasashima *et al,* 2002), in which the conditioned foals commenced exercise on a horse walker alongside their mares (1.6 ms$^{-1}$ for 10 min), over a distance of 960m at 55 days of age. At 2 months of age an ascending exercise regimen of walking, trotting and cantering on a treadmill began, consisting of trotting for 4 minutes, interspersed with five, 15 second cantering periods. Trotting speed was 2.5ms$^{-1}$ initially, and from 150 days was 3.3ms$^{-1}$. Cantering speed was increased from 5 ms$^{-1}$ at 60days to 11 ms$^{-1}$ at 360 days. (Appendix 4 summarises the distances covered during the conditioning exercise regimen of Kasashima *et al,* 2002).

The major differences in conditioning exercise regimens between the present study and the study of Kasashima *et al* (2002) are summarised in Table 4.1.

| Parameter | Present Study | Study of Kasashima *et al* (2002) |
|---|---|---|
| **Pasture management** | Kept at pasture 24 hrs/day.<br>Cohorts of mixed sexes | At pasture for 4 hrs/day.<br>Conditioning exercise conducted after the time spent at pasture.<br>All foals were stabled overnight.<br>Colts and fillies kept separately |
| **Commencement of exercise** | 10 days of age. | 55 days of age on horse walker.<br>From 60 days of age all exercise was conducted on a treadmill. |
| **Distance of exercise** | Constant distance of 1030m for duration of the study. | Exercise distance varied from 915m at commencement of study and increased to 1689m at end of study. |
| **Duration of exercise** | Duration of exercise varied with the velocity.<br>Exercise was performed 5 days/week. | Total duration of exercise was for 5 minutes/day, regardless of velocity. |
| **Exercise surface** | Oval grass training track, with layer of sand to prevent slipping during wet conditions. | Initially on a horse walker until 60 days of age, then on a high-speed treadmill thereafter. (rubber belt) |
| **Sprint velocity** | Sprint over 250m at 12 $ms^{-1}$ introduced when foals were approximately 6 months old. | Sprint velocity increased to10 $ms^{-1}$ at 9 months of age, over a distance of 750m.<br>At 12 months of age sprint velocity increased to 11.0 $ms^{-1}$, over 825m. |
| **Pattern of exercise** | Commenced exercise at a constant base velocity, which was initially 4.2 $ms^{-1}$ until 4 months of age.<br>The base velocity was increased to 5.56 $ms^{-1}$ between 4 and 6 months of age.<br>At 6 months the base velocity was increased to 6.66 $ms^{-1}$ with the incorporation of a sprint over 250m at12.0 $ms^{-1}$ after 500m of base exercise.<br>From 6 months of age there was one period of acceleration and deceleration during the exercise regimen. | Pattern of accelerating and decelerating between trot and canter phases five times during each exercise regimen. |

**Table 4.1: Summary of differences in conditioning exercise regimen between the present study and the study of Kasashima *et al* (2002).**

In summary, the major differences are a greater total distance from five months of age, the foal were accelerating and decelerating several times during exercise, and the total workload was much greater in the foals of Kasashima *et al* (2002). Strains recorded in bone and tendon rise with speed, presumably due to the increased peak limb force and increased extension of the metacarpophalangeal joint (McGuigan and Wilson, 2003; Stephens *et al*, 1989). It is possible that these differences may have contributed to the increase in tendon CSA associated with conditioning exercise in the study of Kasashima *et al* (2002).

Other factors associated with exercise regimen may also have contributed to this difference. Barrey *et al* (1993) observed the stride characteristics of adult horses at the trot and canter, carrying a rider, firstly on a grass exercise track, then a treadmill. Stride length was significantly longer on the treadmill than on the track, and the stride frequency was greater on the track than on the treadmill.

Buchner *et al* (1994) compared the kinematics of 10 Dutch Warmblood horses at the trot, overground (both asphalt and a rubber ground surface) and on a treadmill. The stance duration of the forelimbs was longer on the treadmill than in either of the overground conditions. Both the forelimbs and hindlimbs were moved more caudally during the retraction phase on the treadmill. The treadmill belt velocity decreased by 9% during the stance phase due to the vertical ground reaction force of the horse increasing friction between the belt and gliding surface and the horizontal ground reaction force exerted by the horse acting in an opposite direction to the direction of belt movement, reducing belt speed. They postulated that this is the reason for the differences in biomechanics and kinematics between treadmill and overground locomotion, and should be kept in mind

when extrapolating data from treadmill exercise to overground conditions (Buchner *et al*, 1994).

Scheffer and Back (2001) investigated the effects of three different surfaces (asphalt, fibre/sand mix, pure sand), on the kinematics of the distal forelimb in eleven sound Dutch Warmblood horses with three different types of shoes (normal shoes, eggbar shoes and shoes with 5° heel wedges). Hoof rotation and maximal extension of the fetlock joint at mid-stance were recorded at the walk and trot. On soft track surfaces there was a 1.5°-4° forward rotation of the normal and eggbar-shod foot, with the most rotation being on the sand surface. The maximal fetlock extension was less on a soft surface, particularly on the sand track. The greater degree of forward rotation of the hoof and decreased maximal fetlock extension when on softer surfaces was suggested to reduce loading of the fetlock joint during mid-stance phase, thus reducing tension within the SDFT (Scheffer *et al*, 2001).

If the work of Barrey (1993), Buchner (1994) and Scheffer (2001) could be applied directly to the foals of the present study and the study of Kasashima *et al* (2002), then several differences in the kinematics of locomotion could be postulated. This may explain the difference in tendon strains at exercise between the two studies, resulting in the observed differences in CSA. The stride length and stance duration of the treadmill exercised foals would have been longer than that of the foals exercised on grass. At the trot and gallop, peak strains occur in the SDFT near the mid-stance phase (Stephens *et al*, 1989). Because the foot was in contact with the ground for a longer period of time during the stance phase in the treadmill exercised animals, peak strains in the SDFT occurred for a longer period of time when compared to that of the foals exercised over ground.

Increases in stride length on the treadmill could be explained by both the forelimbs and hindlimbs being moved more caudally during the retraction phase of the stride, with the caudal movement of the treadmill belt.

If the kinematics of an adult hoof with a normal shoe as determined by Scheffer *et al* (2001), could be applied directly to that of an un-shod foal hoof, then the foals in the present study exercised on grass possibly had more forward rotation of the hoof and less maximal fetlock extension than that of the treadmill exercised foals of Kasashima *et al* (2002). The consequence of this reduced fetlock extension and more forward rotation of the hoof is to reduce the peak strains occurring in the SDFT during the stance phase (Denoix 1994; McGuigan *et al*, 2003). Therefore the foals in the present study may have had less peak strain occurring in the SDFT due to the nature of the exercise when compared to the treadmill trained foals of Kasashima *et al* (2002).

### 4.1.4 Clinical examination

The minor ailments in some of the foals during the study did not interfere with their overall workload (Table 3.1). The mild respiratory disease that prevented the foals from exercising for two weeks in June 2001, when the mean age of the animals was 9 months of age, reduced the overall workload by 2.6%. We do not know if the immature SDFT has sensitive periods during its development and if the missed conditioning exercise coincided with such a period. The mean percentage difference in CSA between conditioned and control animals was largest at eight months of age, and although not statistically significant, occurred before the period of missed conditioning exercise. Therefore it seems unlikely that the period of enforced rest due to respiratory disease was detrimental to the outcome of the study.

During the study there was no evidence of tendonitis on palpation of the tendons (no heat, pain, or swelling of the SDFT was detected in any horse), in either the conditioned or control groups, nor was there any ultrasonographic evidence of tendonitis of the SDFT at mid-metacarpal level. There was no statistically significant difference in the mean CSA between the left and right forelimb SDFT, supporting the limb palpation findings. If tendonitis at the mid-metacarpal region in the exercised animals had been present the mean SDFT CSA would have been expected to be larger than that of the control group, by approximately 10% (Reef, 1998).

The CSA of the SDFT of all animals at eighteen months of age was within the normal adult Thoroughbred CSA measurement range, further supporting the conclusion that clinical tendonitis was not present in either treatment group (Tables 1.3, 3.3). In stage II of the GEXA project, where the horses were broken in and entered race training as two year olds, complete ultrasonographic examination of both the left and right SDFT, DDFT and SL was performed. There was no evidence of tendonitis or desmitis in either the conditioned or control animals, again supporting the normal ultrasonographic findings during stage I of the project (Moffat, P.A. unpublished observations 2003).

The absence of any clinical or ultrasonographic evidence of tendonitis in the conditioned horses provides considerable confidence that the conditioning exercise regimen used in this study was not detrimental to the developing SDFT.

## 4.2 Ultrasonographic *in-vivo* measurement of SDFT CSA

### 4.2.1 Change in mean SDFT CSA in relation to growth

The CSA increased in both the exercised and control animals during the period of the conditioning trial. Visual inspection of the plotted data indicates that the data follows a curvilinear plot with the largest percentage increase in mean CSA in both groups being between five and eight months of age.

The ADG of the foals was greatest in the first month of life (1.75 kg/day). By five months of age the ADG had reduced to 0.5kg/day, after which there was minimal change in the ADG. The period of most rapid body growth was prior to CSA data collection and thus we are unable to analyse a relationship between increasing tendon CSA and growth rate from birth to five months of age. The period of most rapid body growth preceded the time period for the largest percentage increase in mean CSA

The shape of the growth curve for the SDFT CSA shown in the present study is similar to the growth curve of the long bones (radius, third metacarpal bone, proximal phalanx) of Thoroughbred-cross horses (Fretz *et al*, 1984), and ponies (Campbell and Lee, 1981), who found the period of most rapid bone growth to be from birth to ten weeks of age. This precedes the period for the largest percentage increase in mean CSA observed in our study. If the growth data of Fretz *et al,* and Campbell and Lee could be applied directly to the foals in the present study, we can hypothesise that the length of the SDFT increases at the same rate as the growth of the long bones in the forelimb, and the increase in SDFT length precedes the period of largest percentage increase in SDFT CSA.

Using linear regression models no relationship between mean bodyweight, growth and CSA could be established. At eight months the percentage difference in mean body weight between the two groups was 0.65%, with the conditioned group being slightly heavier. The influence of body condition score was investigated because the conditioned group may have developed different mean body composition (gained muscle mass and lost body fat), but, not mean body weight, compared to the control group. However there was no significant difference in body condition score between the conditioned and control groups at any age.

Smith *et al* (1994) determined the ultrasonographic range of CSA for the SDFT in normal horses using three distinct populations of horses (22 Irish Draught crosses, 15 Thoroughbreds, and 15 ponies). There was no statistically significant difference in the data obtained from the Irish Draught crosses and Thoroughbreds, therefore these two groups where combined for statistical analysis. In ponies there was a linear relationship between physical parameters (weight, height, and mid-metacarpal circumference) and SDFT CSA, but not in horses, possibly because the ponies had a larger variation in physical parameters. If the variation of the physical parameters in a group of young growing Thoroughbred foals was similar to that of the group of ponies, rather than that of mature adult animals, then larger foals would be more likely to have a larger SDFT CSA. However in the present study there was no significant difference in mean body weight between conditioned and control animals, perhaps contributing to the lack of statistically significant difference in mean SDFT CSA between conditioned and control foals.

As opposed to the curvilinear change in CSA found in the present study, Kasashima *et al* (2002) found a linear increase in the CSA of the SDFT over time. CSA values taken from graphs of Kasashima *et al* (2002) appear to be smaller than CSA values in the present study. Direct comparisons between the two studies are difficult. Although both studies measured the mid-metacarpal CSA, the present study used anatomical sites to determine the mid-metacarpal level of the SDFT, while Kasashima *et al,* (2002) used the zone system (Genovese *et al*, 1986), which incorporates a 4 cm length of SDFT into the one zone. The significance of using the zone system to measure mid-metacarpal CSA is that the ultrasonographic image captured for CSA measurement could be obtained from any region within the 4 cm zone and the consistency of obtaining an image from exactly the same region at subsequent measurement intervals is reduced.

In the study of Kasashima *et al* the mean CSA at level 2A in the exercised group at 55 days of age was 0.4 $cm^2$ and the greatest CSA increase (approximately 50%) was between 55 days and five months of age. The mean percentage increase in CSA in the exercised group between 5 and 8, 8 and 12, and 12 and 15 months, was 14%, 22%, and 14% respectively. In the present study the mean percentage increase in mid-metacarpal SDFT CSA in the conditioned group between 5 and 8, 8 and 12, 12 and 15, and 15 and 18 months was 37%, 7%, 0.6% and 1% respectively.

Possible explanations for the different pattern of increase in mean CSA between the two studies are the intensity at which the animals were exercised and the surfaces on which the exercise was conducted on.

Staniar *et al* (2004) have established a physiological growth model for the Thoroughbred. They determined that up to 6 months of age, growth rate could be related to age in days. Between 7 and 14 months of age there was constant decrease and subsequent increase in growth rate, which was suggested to be due to environmental influences such as season or nutrition. They found no relationship between sex and bodyweight ($P = 0.97$) or sex and average daily gain ($P = 0.42$). The lack of a sex effect between geldings and fillies on growth in their study diminishes the likelihood that puberty plays a large role in the deviation.

In the study of Kasashima *et al* (2002) and the present study, all of the foals were born in the same season (spring) despite being in different hemispheres and having different months of birth. Therefore the environmental influences of season on growth rate that occur after six months of age as determined by Staniar *et al* (2004) are likely to have been similar. We are unable to make comparisons between growth rates of the animals in the two studies, but it is unlikely that differences in bodyweight could explain the increase in CSA in the study of Kasashima *et al* (2002), as there was no relationship between bodyweight and CSA in the present study, or in adult horses as determined by Smith *et al* (1994).

The Thoroughbred foals of the two studies may have originated from two distinctly different genetic backgrounds, but this is unlikely to explain the distinct different patterns of increase in SDFT CSA.

It is possible that the timing of data collection in this present study may misrepresent the period of most rapid increase in SDFT CSA, and the rapid growth phase of SDFT CSA may have commenced before five months of age and ceased before eight months, but appeared to have occurred within this time period due to the study design.

### 4.2.2 Limb effect

The lack of significant difference between the mean pooled left and right limb CSA data in the present study is consistent with work by Kasashima *et al* (2002), although they found a greater than 20% asymmetry between limbs in some foals in both exercised and control groups, and hypothesised that the asymmetry may have been due to variation in tendon maturation. There was no such CSA asymmetry in the present study. In adult horses Smith *et al* (1994) found no significant difference in the CSA of the SDFT between limbs of the same horse at any level, unless pathology was present within one tendon. Similarly, Gillis *et al* (1995b) found no significant difference between the left and right forelimb SDFT CSA in a group of 50 Thoroughbred horses. Perkins *et al* (2004b) found no significant difference between pooled right and left limb mean CSA at any level in fourteen two-year–old Thoroughbred fillies that where in early race training over a thirteen week period.

In the present study the conditioned horses were exercised on an oval track in both a clockwise and counter-clockwise direction with equal frequency, and theoretically used left and right forelimb leads during the canter and gallop equally. The control animals were obviously free to choose their lead preference.

Meij and Meij (1980) found that in thirty horses used for dressage and show jumping, each horse had a consistent left or right side preference, predominantly the left (25 out of 30 animals). They observed when a horse had a strong preference for one side, much longer periods of training were necessary to obtain equal performance on both reins than in horses that did not have strong side preferences.

They concluded that side preference, a postural correlation of handedness in humans, may indicate hemispheric asymmetry with regard to motor function in the horse. The predominance of left "handedness" in some horses may in fact be a result of horses traditionally being handled from the left hand side (near) by humans from birth, with the left rein or side of the horse being subconsciously conditioned more than the right hand side.

It has been shown that Thoroughbred racehorses can change the leading forelimb up to eight times or more per mile when racing, to avoid excessive muscular fatigue due to asymmetric work of the limb (Leach *et al*, 1987; Back and Clayton, 2000). Merkens *et al* (1993) measured the ground reaction force (GRF) patterns of Dutch warmbloods at the canter and found that the GRF patterns were not symmetric for the four limbs. The least GRF was in the trailing hindlimb and was approximately equal to the bodyweight. The leading hindlimb and the leading forelimb had GFR equal to approximately 1.2 times bodyweight and the trailing forelimb had the largest GFR of approximately 1.5 times bodyweight. The leading hindlimb and trailing forelimb support the bodyweight and provide forward propulsion, with the trailing forelimb having a particularly large propulsive component. Similarly McGuigan and Wilson, (2003), estimated the peak vertical GRF, of Thoroughbred horses galloping on a treadmill, to be 12.79 N $kg^{-1}$ BM for the leading forelimb and 15.23 N $kg^{-1}$ BM for the trailing forelimb, with the peak metacarpophalangeal joint angle in the leading and trailing forelimbs being 237.6 and 245.3 respectively.

If the work of McGuigan and Wilson (2003), Stephens *et al* (1989) and Leach *et al* (1987) could be applied directly to the animals in the present study, and each horse had a predominant side, then the trailing fore limb of the conditioned horses would have had greater peak metacarpophalangeal joint angle and ground reaction force, leading to greater strains within the trailing forelimb SDFT. If the animals in this study had a predominant side and if conditioning exercise were to influence the developing SDFT and induce an increase in CSA, we may have observed a difference in CSA between the left and right limbs of individual animals. This was not apparent in the present study.

### 4.2.3 Effect of conditioning exercise on SDFT CSA

The mean pooled left and right CSA of the conditioned and control animals were not significantly different at any age in response to the conditioning exercise. The CSA did have considerable between-horse variation at each age interval, which was largest at the onset of the study and reduced with age (Table 3.3), in contrast to Kasashima *et al* (2002) who found the between-horse variation in CSA was the least at the onset and increased towards the end of the study. It would be expected that the variation in measuring CSA would decrease over time. Because the echogenicity of the tendon images increased as the foals grew and the outline of the tendon became more easily discernable (Moffat, P.A. personal observation), measurement error would be expected to decrease because of the more obvious definition of the tendon border. As well, the effect of any measurement error would be greater in a smaller tendon than in a larger tendon. These may account for the greater variation found in the earlier stages of our study.

The mean percentage difference in SDFT CSA between conditioned and control groups were consistently low, except at eight months, where the conditioned group mean CSA was 5.93% greater than the control group (Table 3.2). The mean percentage difference in CSA between treatment groups at eight months of age could possibly be attributed to a greater variation in tendon growth in the treatment group, over and above naturally occurring variation within the population, at that time. The mean CSA of the conditioned group had more between-horse variation than the control group at eight months (Table 3.3), which could be attributed to a larger variation in individual animal tendon growth during the period of most rapid increase in mean CSA which was between five and eight months of age (37%).

As the level of statistical significance for mean CSA between pooled conditioned and control groups was just below the threshold (P= 0.058), it is possible that there was an interaction between body growth, conditioning exercise and CSA, during a period when the tendon was sensitive to the effect of conditioning exercise, however we were unable to prove this statistically. It is possible that the conditioning exercise regimen in the present study did induce changes within the extracellular matrix of the developing SDFT, and these changes may have been in part responsible for the 5.93% difference in CSA between the two groups being observed at eight months of age. Cherdchutham *et al* (1999) found that Dutch Warmblood foals exercised from seven days of age had higher cellularity, hyaluronic acid, polysulphated glycosaminoglycans and COMP levels than did box-rested foals, when expressed on dry weight basis. The collagen content of the box-rested group was significantly higher than that of the box-rested/exercised group, and the pasture managed group, which were both similar at five months of age.

It is questionable whether the increased collagen content of the box-rested group was due to a direct increase in metabolism of collagen, or a lower concentration of extracellular matrix within the tendon. The possible relationship of these molecular level changes to the ultrasonographic CSA of the developing SDFT was not investigated and therefore cannot be extrapolated to the present study.

No histological or biochemical parameters were obtained from harvested tendons for the purpose of this thesis, and we are unable to hypothesise whether conditioning exercise induced changes at a molecular level either between treatment groups or individual animals. Biochemical analysis may reveal individual differences in collagen content or extracellular matrix in the tendons from either treatment group that have not resulted in an ultrasonographic increase in CSA. Such study is currently being conducted and from this an interaction between conditioning exercise and tendon development may be recognised.

The small number of studies to date that have investigated the response of adult SDFT to exercise give conflicting results. Birch *et al* (1993b) did not find a significant difference in the ultrasonographic CSA of the SDFT in young Thoroughbreds trained on a treadmill for five and eighteen months. Gillis *et al* (1993) found that in young Thoroughbreds in race training, the ultrasonographic CSA increased with exercise over a 116-day period, with the echogenicity of the tendons initially increasing with training then decreasing. More recently Firth *et al* (2004) examined the SDFT from two-year-old Thoroughbreds trained over a 13-week period on a racetrack. There was a 5% difference in mean ultrasonographic CSA between exercised and control animals when all levels were pooled, and the difference at 8 and 16 cm DACB was 13% and 20% respectively.

The difference was due to the mean weight and volume of tendon per cm length in the conditioned group being greater, however the average density of the tissue in each tendon segment was less in the trained horses. There was no evidence of pathology on histological examination of the SDFT tissue, and a possible explanation for the reduction in density was that the water content of the tendon matrix in the trained horses was slightly greater, thus reducing the tendon density when compared to the control tendons.

In both of the study of Firth *et al* (2004), and Gillis *et al* (1993), the horses were exercised for a relatively short time period. It is possible that the exercise-induced increase in CSA in the study of Gillis *et al* (1993) was due to an increase in water content in the tendon. If this were true and immature tendon responded similarly to exercise as mature tendon does, the 5.93% difference in CSA between conditioned and control groups at eight months of age in the present study, may have been a response of the immature tendon to conditioning exercise, and could be due to an increased water content in the tendon matrix, which was transient and resolved by the next ultrasonographic measurement at twelve months of age.

If Riemersma's (1985, 1986, 1996b) findings (the collagen content of the tendon being inversely proportional to the CSA and proportional to the modulus of elasticity), could be applied directly to the forelimb SDFT of young thoroughbred horses, and if the influence of early conditioning exercise were to produce a stronger tendon by inducing an increase in the collagen content of the tendon during the growth phase, we would not expect to find an increase in the CSA of the SDFT in the conditioned animals. It is possible that there could even be a reduction in CSA in the conditioned animals when compared to that of control animals, however this was not apparent in this study.

### 4.2.4 The effect of gender on the SDFT

There was no statistically significant difference in mean CSA between fillies and colts. The mean percentage increase in CSA from twelve to eighteen months of age in both fillies and colts was negligible. The colts were castrated at a mean age of twelve months, after the period of most rapid tendon growth.

If tendon CSA contributes significantly to the strength of the adult tendon, and there is no statistically significant difference in SDFT CSA between males and females, then there should be no sex predilection for injury to the SDFT. However male Thoroughbreds in training had higher odds for injury of the SDFT than females Perkins *et al* (2004a), while no sex predilection for injury to tendons in other species has been recorded. Thus factors other than CSA must be involved in the development of tendonitis.

In humans a higher prevalence for cranial cruciate ligament rupture in female athletes has been reported (Yu *et al*, 2001). In light of the findings of Perkins *et al* (2004a), investigation into the role of sex hormones and tendon injury in the horse may be warranted.

## 4.3 The correlation between *in-vivo* and *in-vitro* tendon CSA

There was a strong linear relationship between the standing *in-vivo* ultrasonographic SDFT CSA at the mid-metacarpal level conducted just prior to post-mortem, and the *in-vitro* photographic tendon CSA at post-mortem ($R^2$=0.881), with the *in-vitro* CSA being 10.3% larger.

The CV of 2% for CSA measurement contributes to the excellent linear correlation found in this study, and it would be expected that the *in-vitro* post-mortem CSA measurement would be larger than the ultrasonographic standing *in-vivo* CSA measurement, because the *in-vitro* measurement is taken without the tendon being under tension from contraction of the SDF muscle and its contribution to standing load on the SDFT (Gillis *et al*, 1995b). van Schie and Bakker (2000) found that ultrasound routinely underestimates tendon CSA and palmar-dorsal measurements due to propagation and refraction artefacts, and these artefacts would also contribute to the standing *in-vivo* ultrasonographic CSA measurement being smaller than the *in-vitro* CSA obtained at post-mortem.

The excellent linear correlation found in the present study is similar to work from other investigators. Gillis *et al* (1995b) found a good linear correlation between standing *in-vivo* ultrasonographic tendon CSA and inkblot morphologic CSA ($R^2$=0.64,P=0.0001), with the inkblot CSA 15% larger than the mean ultrasonographic standing CSA. The 5% difference in CSA measurements obtained at post-mortem between the study of Gillis *et al* and the present study might be attributed to the slight separation of fibres during compression of the tendon segment while obtaining the inkblot impression (Gillis *et al*, 1995b).

Birch *et al* (1999b) found that the ultrasonographic SDFT CSA obtained prior to post-mortem was 9% smaller than the CSA determined photographically at post-mortem. Smith *et al* (1994) found a 4.59% (s.d. 3.67%) average difference between *in-vivo* ultrasonographic CSA and post-mortem CSA determined photographically. Similarly Kasashima *et al* (2002) found a good linear correlation ($R^2$ =0.78) between *in-vivo* ultrasonographic CSA obtained just prior to post-mortem, and cast based post-mortem measurements CSA, with the CV being less than 2% for the precision of CSA measurement.

## 4.4 Precision of the Measurement Technique

In pilot study 1 (Appendix 2) the calculation of the CV for repeat measurements of CSA from the same ultrasonographic image of the SDFT from an adult horse was between 1.2%-2.75%. For a CV of less than 2%, only two measurements of CSA would have been required. Because the ultrasonographic images for this study were captured in the field and the tendon was smaller and more immature (lower echogenicity with possibly less definition of the borders of the tendon) than that of an adult horse, a conservative approach was taken and we measured the CSA three times and used the average for statistical analysis.

In pilot study 2 (Appendix 2) the repeatability of ultrasonographically imaging the tendon at the same level was 2%. Using a general linear model, repeated CSA measurement of the same tendon image and repeated tendon images of the same tendon were treated as fixed effects. No variation in the tendon level at which the repeated images were captured could be identified. A CV of 2% was calculated for the precision the image capture technique and SDFT CSA measurement.

The point mid-way between two anatomical sites was used to determine the level at which the tendon was imaged rather than the zone system or centimetres distal to the accessory carpal bone. This was to account for differences in tendon and MC III length between horses and to ensure the same relative tendon site was imaged in each horse at sequential scans, irrespective of any growth that had occurred. If a constant point (i.e. cm distal to the accessory carpal bone) had been chosen to determine the level at which the tendon was to be scanned, variation in the length of MC III between individuals would not be able to be accounted for, and in smaller animals, the tendon site imaged would have been more distal than that of taller animals. Using the zone system to identify the mid-metacarpal site for tendon scanning could allow a 40mm region of tendon from which an image could be obtained. Therefore the repeatability of obtaining an image from the same tendon level at sequential scanning sessions would be greatly reduced.

There would have been very little elongation of the long bones of the forelimb from the commencement of this study to its completion (Campbell and Lee, 1981; Fretz *et al*, 1984), and using either centimetres distal to the accessory carpal bone or the site determined by the point mid-way on the lateral aspect of the third metacarpus, as in this study, would have ensured the same relative site of tendon was imaged at each sequential scanning.

The sensitivity of ultrasonography to detect very subtle changes in tendon CSA, coupled with the precision of the measurement technique used may have prevented a statistically significant difference between the conditioned and control groups being attained. The CV of 2% was calculated by using adult tendons in the pilot studies prior to imaging foal tendons. Retrospective CV calculation for foal tendons (Table 5.4), ranged between 1.35%-3.0%, with an average CV of 2.18%, which is consistent with the CV of the pilot studies. Any differences in CSA between treatment groups of 2% or less would not have been able to be detected in this study.

## 4.5 Sources of Error and Limitations of this study

### 4.5.1. Commencement of the study

The CSA of the SDFT was not obtained before five months of age due to equipment not being available during this time. It would have made the study complete to have the CSA of the SDFT at birth as a reference or starting point from which to determine the total increase in SDFT CSA from birth to tendon maturity.

### 4.5.2. Assessment of tendon morphology and composition

No histological or biochemical parameters were obtained from the harvested tendons for the purpose of this thesis. Such work is currently being conducted on tissue harvested at post-mortem. As there was no statistically significant difference in ultrasonographic CSA between treatment groups we are unable to determine if conditioning exercise had any influence on the developing tendon at a cellular or molecular level.

If immature tendon were to be modified by conditioning exercise, the level of mechanical stimulation required to induce such changes in the tendon matrix and collagen composition has yet to be determined, and there may well be a fine line between the stimulus required to induce a positive change and the stimulus required to induce tendonitis or pathology within other musculoskeletal tissues of the immature horse. Histological and biochemical analysis of tendon tissue could prove definitively that the conditioning exercise regimen of this study was not detrimental to the developing SDFT.

### 4.5.3. Limitations due to the number of animals in the study

The number of animals in the present study (exercised n= 18, control n = 15) may not have been sufficient to show a statistically significant difference in CSA between treatment groups at a given age. A retrospective power analysis was performed, and based on the means and standard deviations obtained, the minimum sample size necessary to observe a difference between conditioned and control horses 55% of the time, with a significance level of P=0.05, would have been 30 horses in each treatment group. The level of significance for mean CSA between conditioned and control animals across all ages was

just below the threshold (P=0.058). From the power analysis we can ascertain that if we doubled the number of animals in each treatment group, there would have been limited improvement in the ability to detect a statistically significant difference. Horses are a large species and require different management methods and facilities when compared to other smaller species used for research purposes. The financial costs and managerial issues of conducting such a study, with an increase in horse numbers, would be a major constraint. When compared to other similar studies the present study was the largest of its kind.

### 4.5.4. Sources of error during tendon scanning and image analysis

Errors in measurement of the SDFT CSA can arise from a number of factors at the time of image acquisition, such as accurately determining the correct level of the limb for scanning, the position of the limb during tendon scanning, and if the horse is fully weight-bearing by standing squarely on the forelimbs at the time of tendon scanning. If the horse is not standing square, bearing full weight on the limb at the time of scanning, tendon CSA will be artifactually larger as the tendon will not be under normal weight bearing tension (Smith *et al*, 1994). These potential sources of error were minimised by having an experienced assistant restrain the foals so that they stood squarely on all four limbs, ensuring full weight-bearing during tendon scanning and image capture.

The repeatability of ultrasonographic assessment of adult equine SDFT was investigated by Pickersgill *et al* (2001), who found that different operators undertaking ultrasonographic image acquisition produced consistent results. However when different operators undertook image analysis they returned significantly different CSA values.

They suggested that to reduce the variation in CSA analysis during ultrasonographic investigations only one person should undertake CSA analysis, although different operators may undertake image acquisition without confounding effects. There was only one person involved with image acquisition and image analysis in the present study, therefore one source of potential measurement bias was reduced, as was also the case in the study of Kasashima *et al* (2002).

A potential source of measurement error arises because immature animal tendons are smaller in CSA than adult horse tendons. Any deviation from the actual boundary of the tendon image when calculating the CSA of smaller tendons will contribute to a greater C.V. The mean C.V. was calculated for each age group (Table 5.4) and was found to be between 1.35% and 3.03%, which was similar to the C.V. obtained in pilot study 1 using adult tendons. Therefore in the present study the variation in CSA measurement due to operator inconsistency was low.

While ultrasonography is able to image the tendon in the live horse it has been shown to routinely underestimate CSA because of the depiction of abaxial structures in a more axial position. These propagation and refraction artefacts are most pronounced in the convex medial and lateral borders of the tendon. The effect of amplifier gain setting and transducer-tilt and displacement can give rise to substantial variation in grey scale resulting in "filling in" of the image resulting in a miscalculation of CSA (van Schie *et al*, 1999), (van Schie and Bakker, 2000). These artefacts would have contributed to the 10% difference observed in CSA between standing *in-vivo* CSA and *in-vitro* CSA obtained at post-mortem.

## 4.6 Conclusion

The exercise regimen in this novel-conditioning programme did not induce a statistically significant difference in the SDFT CSA of the conditioned horses, when compared to that of the control group. The number of horses used in this study when compared to other similar studies was large and it is unlikely that further research concerning the response of immature tissues to the influence of conditioning exercise will utilise such numbers of animals in the future.

There was no evidence that conditioning exercise was detrimental to the SDFT, either clinically or ultrasonographically. This was further supported by subsequent ultrasonographic examinations when the horses entered race training as two-year-olds. This is a very important finding, and we can again be certain that no artifactual increase in SDFT CSA due to tendonitis was present in either the conditioned or control groups.

The intensity of the conditioning exercise may not have placed sufficient mechanical stimulus on the SDFT to induce changes in tendon CSA. As limited work has been performed on determining appropriate levels of conditioning exercise for developing horses, the welfare of the animals was paramount, and a conservative level of conditioning exercise was employed in the present study.

The one study to date that demonstrates an increase in CSA in immature tendons to conditioning exercise, Kasashima *et al* (2002), had much greater exercise intensity, when compared to the present study. It is likely that this increased exercise intensity, coupled with the different biomechanical forces and kinematics of locomotion, associated with high-speed treadmill exercise, placed greater strains on the immature SDFT. Therefore it is possible that the increase in CSA of the exercised foals in the study of Kasashima *et al* (2002) was due to either an adaptation of immature tendon to conditioning exercise, or exercised-induced tendonitis.

The precision of the image capture and measurement technique employed in this study was good, validated by the excellent correlation between *in-vitro* and *in-vivo* SDFT CSA. Ultrasonography is a very useful diagnostic tool that enables continued monitoring of tendons in the live horse throughout a training regimen. However the sensitivity of ultrasonography to detect very subtle changes in CSA is limited and histological and biochemical analysis of harvested tendon tissue is required to complement ultrasonographic assessment of tendons, in order to detect any changes in response to conditioning exercise at a cellular and molecular level, and to exclude that any such changes are not in fact pathologic.

The findings of this thesis and the limited number of published studies to date concerning the effects of conditioning exercise in both mature and immature animals, support the theory that the ability of flexor tendons to increase CSA in response to conditioning exercise or training is limited, and unlikely to be detected ultrasonographically, unless pathology is present within the tendon

# 5. APPENDICES

## 5.1 Appendix 1 Horse Identification table

| Horse | Birth date | Birth weight | Cohort | Sex | Exercise | Sire |
|---|---|---|---|---|---|---|
| G1 | 16-Sep-00 | 67 | a | C | x | Wallenda |
| G2 | 24-Sep-00 | 55 | a | F | c | Alto-Vita |
| G3 | 28-Sep-00 | 62.5 | a | C | c | Super Imposing |
| G4 | 29-Sep-00 | 52.5 | a | C | x | Danzalion |
| G5 | 30-Sep-00 | 53 | a | F | x | Super Imposing |
| G6 | 4-Oct-00 | 59 | b | F | c | Mughtanim |
| G7 | 5-Oct-00 | 48 | b | F | c | Super Imposing |
| G8 | 6-Oct-00 | 45 | b | F | x | Super Imposing |
| G9 | 6-Oct-00 | 57.5 | b | F | x | Krona |
| G10 | 7-Oct-00 | 58.5 | c | F | c | Krona |
| G11 | 9-Oct-00 | 62.5 | c | C | c | Krona |
| G12 | 12-Oct-00 | 54 | c | F | x | Sam McGuire |
| G13 | 15-Oct-00 | 54.5 | c | F | c | Alto-Vita |
| G14 | 20-Oct-00 | 52.5 | d | F | c | Sam McGuire |
| G15 | 22-Oct-00 | 62 | d | C | x | Sam McGuire |
| G16 | 24-Oct-00 | 49.5 | d | F | x | Borstal Boy |
| G17 | 27-Oct-00 | 59 | d | C | c | Alto-Vita |
| G18 | 27-Oct-00 | 63.5 | e | F | x | Krona |
| G19 | 7-Nov-00 | 57 | e | C | x | Alto-Vita |
| G20 | 9-Nov-00 | 65.5 | e | F | x | Sam McGuire |
| G25 | 12-Nov-00 | | e | F | x | Chinese Gold |
| G21 | 18-Nov-00 | 63.5 | f | F | c | Tanker Port |
| G22 | 20-Nov-00 | 48.5 | f | C | x | Super Imposing |
| G23 | 21-Nov-00 | 58.5 | f | F | x | Casual Lies |
| G24 | 21-Nov-00 | 55 | f | C | c | Sam McGuire |
| G26 | 25-Nov-00 | 47 | g | F | c | Sam McGuire |
| G27 | 25-Nov-00 | 49 | g | F | x | Bijou d'Inde |
| G28 | 28-Nov-00 | 55 | g | F | x | Chinese Gold |
| G29 | 29-Nov-00 | 62 | g | C | x | Krona |
| G30 | 14-Dec-00 | 56 | h | F | x | Krona |
| G31 | 28-Dec-00 | 58.5 | h | F | c | Krona |
| G32 | 10-Sep-00 | | | F | c | Classic Fame |
| G33 | 2-Nov-00 | | | F | c | Sandtrap |

**Table 5.1 Horse identification**

| Sex | Control | Exercise | Total |
|---|---|---|---|
| Colts | 4 | 6 | 10 |
| Fillies | 11 | 12 | 23 |
| Total | 15 | 18 | 33 |

**Table 5.2 Allocation of animals**

## 5.2 Appendix 2 Pilot Studies

### 5.2.1. Pilot study 1: Repeated measurements of the same tendon image

To quantify the variation in measuring the CSA of a single tendon image, a repeat measurement study was undertaken.

A three-year-old Thoroughbred filly was prepared and scanned using the protocol as described in section 2.6. The image obtained was enhanced and the CSA measurement was repeated ten times using the measurement protocol in section 2.7-2.8.

The coefficient of variation was calculated and subsequently plotted against the number of repeated measurements (Figure 5.1).

### 5.2.2. Pilot study 2: Variation in repeated images of the same tendon of an adult horse

To quantify the repeatability of the ultrasonographic tendon image capture process, three adult horses were prepared, and images of both forelimb SDFT obtained as described in section 2.6.

Three images of the same tendon were taken with a ten-minute interval between each image capture. The images where enhanced, and the CSA was determined on each tendon image three times as described in section 2.7-2.8.

The mean CSA was calculated from the three repeat measurements of CSA of the same tendon image. The CV for each tendon, based on three repeat measurements per tendon image was calculated.

## 5.3 Result of Pilot studies

### 5.3.1. Pilot study 1: Repeated measurements of the same tendon image

The coefficient of variation (CV) for the repeated measurements on the SDFT of the same adult horse was consistently low. Based on the low CV a cut off threshold of 2% was set as the criteria to identify the number of repeat measurements required to obtain an acceptable level of accuracy. Based on the results of pilot study 1, three repeat measurements are required to meet the criteria of a CV of less than 2% (Figure 5.1).

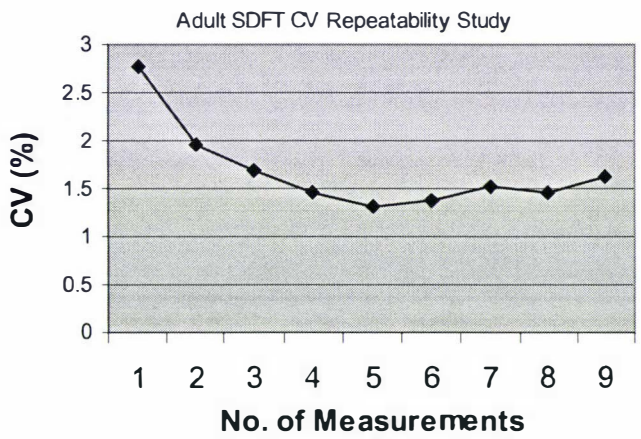


**Figure 5.1 The coefficient of variation for repeated measurements of the same tendon**

### 5.3.2. Pilot study 2: Variation in repeated images of the same tendon of an adult horse

The CV for each tendon image, based on three repeat measurements per image as described in 2.8 was calculated. The repeatability of imaging the tendon at the same level with the tendon scanning technique was tested using a general linear model. Repeated CSA measurement of the same tendon image and repeated ultrasonographic tendon image capture were treated as fixed effects. There was no significant effect of repeat CSA measurement or repeated imaging of the tendon. This implies that there was no significant learning bias in CSA measurement. The model was unable to identify any significant variation between tendon images of the same leg (Table 5.3).

Tests of Between-Subjects Effects
Dependent Variable: SDFT Area

| Corrected Model | Type III Sum of Squares | df | Mean Square | F | Sign |
|---|---|---|---|---|---|
| Corrected Model | 1.108E-02 | 8 | 1.385E-03 | .259 | .976 |
| Intercept | 50.093 | 1 | 50.093 | 9373.623 | .000 |
| AREA_R | 1.781E-03 | 2 | 8.907E-04 | .167 | .847 |
| SCAN_R | 4.693E-03 | 2 | 2.346E-03 | .439 | .647 |
| AREA_R * SCAN_R | 4.693E-03 | 4 | 1.152E-03 | .216 | .928 |
| | | 4 | | | |

a R Squared = .044 (Adjusted R Squared = -.126)

**Table 5.3 The coefficient of variation for each tendon image, based on three repeat measurements per image.**

## 5.4 Mean C.V. at each age interval

| Age in months | Mean CV for CSA measurement |
|---|---|
| 5 | 2.3 |
| 8 | 3.03 |
| 12 | 2.14 |
| 15 | 2.09 |
| 18 | 1.35 |
| Average C.V. | 2.18 |

**Table 5.4 The mean CV calculated for CSA measurement at each age interval.**

## 5.5. Appendix 3 Data collated from the animals by other researchers relevant to this thesis

### 5.5.1. Animal behaviour at pasture (W Stolwijk, 2001)

There was no significant effect of exercise on the foals' behaviour at pasture, and exercise had no significant effect on the overall paddock activity of the conditioned group. The only difference observed between the two groups was due to a temporal shift in behaviour due to the daily exercise routine. While the conditioned group was exercising, the control animals would graze, and therefore rest earlier in the day than the conditioned animals. The conditioned animals would graze for a short period immediately after exercise and then rest.

### 5.5.2. Growth rate of animals during the study (C. Brown-Douglas)

There was no significant difference in growth rate between the conditioned and control horses. There was no significant difference in growth rate between colts and fillies. The bodyweight and average daily gain of the horses in this study were similar to those recorded from the stud in previous seasons and bodyweights of the horses in this study were similar to those of grain-fed Northern Hemisphere Thoroughbreds (C.G. Brown-Douglas *et al* unpublished data)

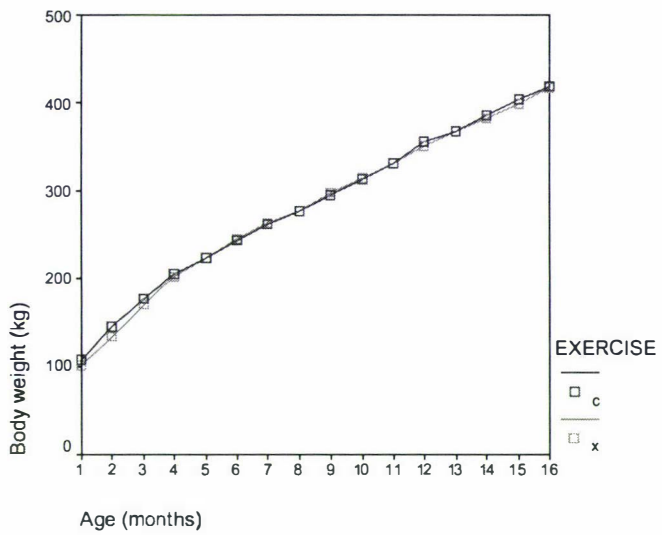


**Figure 5.2: Comparison of body weight from birth to sixteen months of age for conditioned and control horses.**

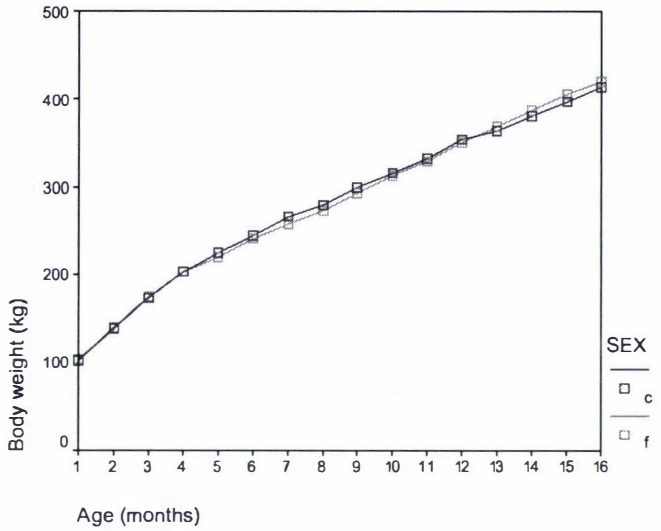


**Figure 5.3: Comparison of body weight from birth to sixteen months of age between fillies and colts.**

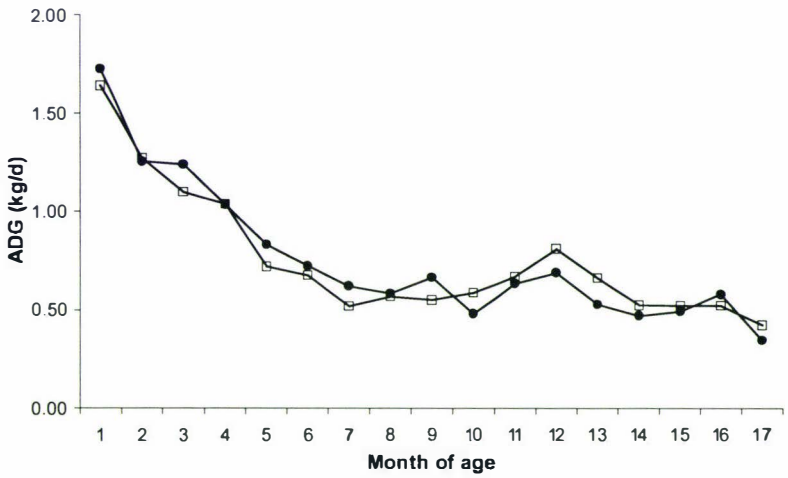


**Figure 5.4:The average daily gain from birth to eighteen months of age for colts (●) and fillies (□)**

### 5.5.3. Body condition score of the animals over the study period

There was no significant difference in the body condition score of the conditioned and control animals over the course of the study. There was no significant difference in body condition score between colts and fillies at five, eight or twelve months of age. At fifteen and eighteen months of age the fillies had a significantly greater body condition score than the colts.

**5 months of age**

There was no significant difference in the body condition score between conditioned and control horses (p=0.91) or between fillies and colts (p=0.66).

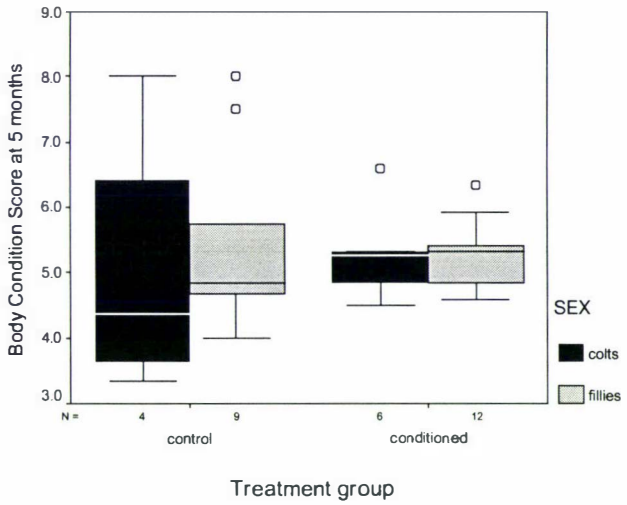


**Figure 5.5 Comparison of body condition score between treatment groups at 5 months of age**

### 8 months of age

There was no significant difference between conditioned and control horses (p=0.41) or between fillies and colts (p=0.68).

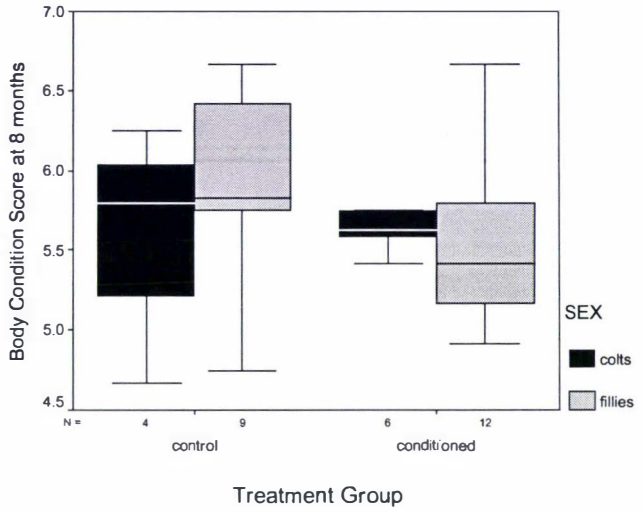


**Figure 5.6 Comparison of body condition score between treatment groups at 8 months of age**

### 12 months of age

There was no significant difference between conditioned and control horses (p=0.14) or between fillies and colts (p=0.12).

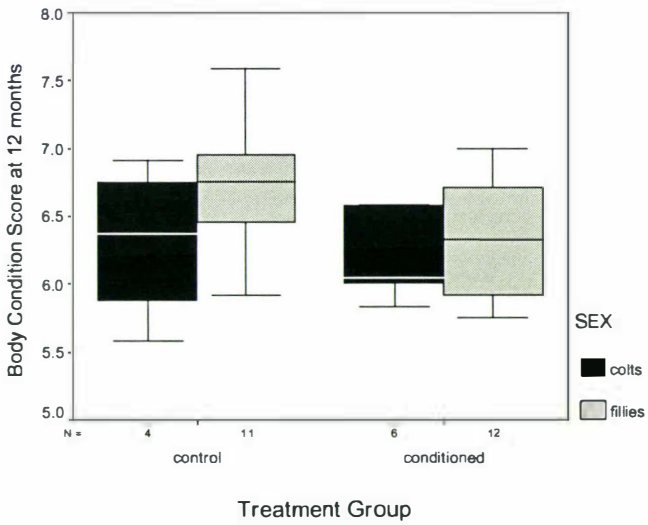


**Figure 5.7 Comparison of body condition score between treatment groups at 12 months of age**

## 15 months of age

There was no significant difference between conditioned and control horses (p=0.18).
The fillies had significantly greater condition scores than colts (7.204 vs 6.725; p=0.01).

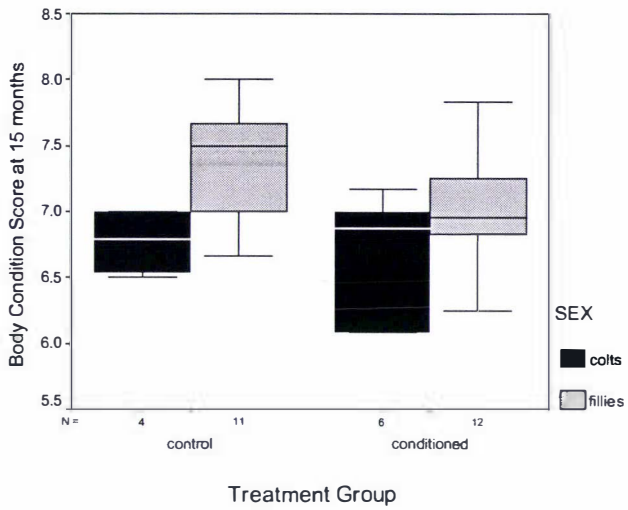


**Figure 5.8 Comparison of body condition score between treatment groups at 15 months of age**

## 18 months of age

The body condition score of control horses tended to be greater than exercise horses, but this was not statistically significant (7.273 vs 6.834; p=0.63).
The fillies had significantly greater body condition scores than colts (7.294 vs 6.813; p=0.043).

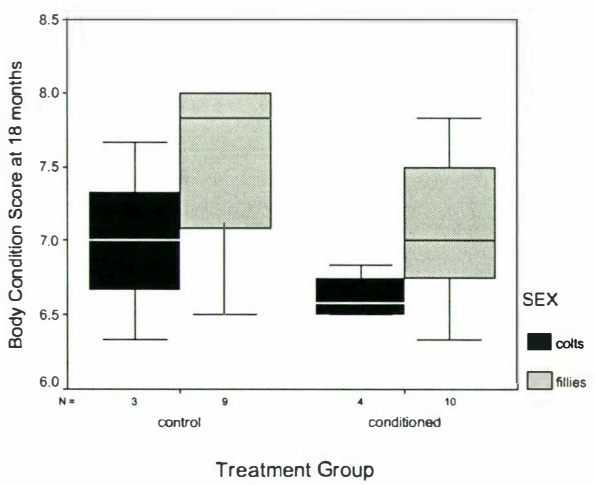


**Figure 5.9 Comparison of body condition score between treatment groups at 18 months of age**

### 5.5.4. Workload of conditioned animals over the study period (L Huitema 2001)

The work load of the exercise group over the course of the study period is displayed below. At the beginning of the conditioning programme the foals were exercised at a constant velocity of 4.20ms$^{-1}$. This was increased to 5.56 ms$^{-1}$ in February 2001, and then to 6.66 ms$^{-1}$ in May 2001, with the addition of a sprint over 250m at 12 ms$^{-1}$ after 500m of base exercise, (6.6 ms$^{-1}$). The velocity at which the foals exercised was constant and repeatable each working day.

A kinematic study was performed to quantify the conditioning exercise between 8$^{th}$ January and 23$^{rd}$ March 2001. The stride length and stride frequency were calculated for each exercise period and validated against the true number of strides recorded on a panning camera. Based on the video observations, it was obvious that many foals had a specific running pattern during their exercise that was consistent across days.

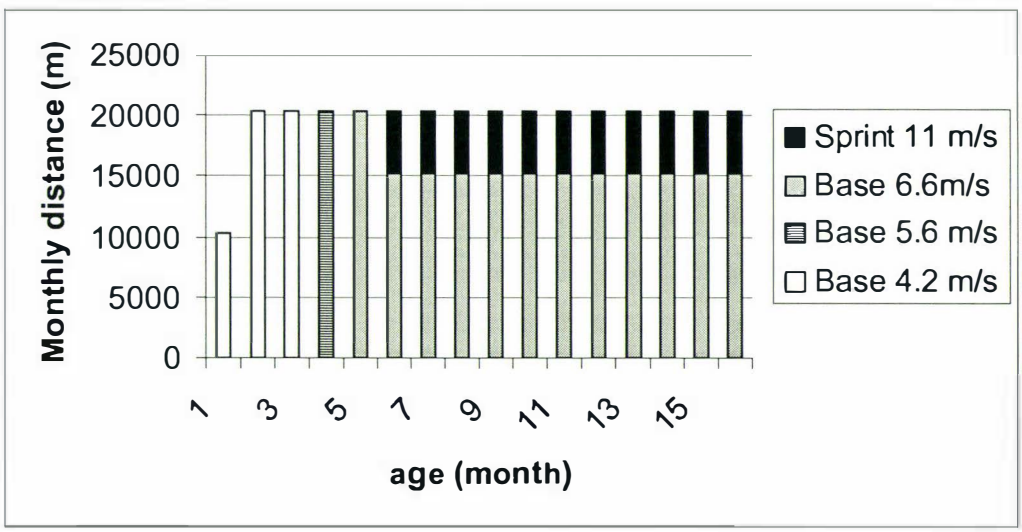


**Figure 5.10 Workload of conditioned animals during the study period**

## 5.6. Appendix 4 Data from similar studies

| Training period | Age (days) | Walk ($ms^{-1}$) | Trot ($ms^{-1}$) | Canter ($ms^{-1}$) | Canter distance (m) | Total distance (m) |
|---|---|---|---|---|---|---|
| 1 | 60-90 | 1.6 | 2.6 | 5.0 | 375 | 915 |
| 2 | 90-120 | 1.6 | 2.6 | 5.5 | 412.5 | 952.5 |
| 3 | 120-150 | 1.6 | 2.6 | 6.0 | 450 | 990 |
| 4 | 150-180 | 1.6 | 3.3 | 6.5 | 487.5 | 1153.5 |
| 5 | 180-210 | 1.6 | 3.3 | 7.0 | 525 | 1191 |
| 6 | 210-240 | 1.6 | 3.3 | 8.0 | 600 | 1464 |
| 7 | 240-270 | 1.6 | 3.3 | 9.0 | 675 | 1539 |
| 8 | 270-300 | 1.6 | 3.3 | 10.0 | 750 | 1614 |
| 9 | 300-330 | 1.6 | 3.3 | 10.0 twice 10.5 three times | 772.5 | 1636.5 |
| 10 | 330-360 | 1.6 | 3.3 | 10.5 | 787.5 | 1651.5 |
| 11 | 360-390 | 1.6 | 3.3 | 10.5 twice 11.0 three times | 810 | 1674 |
| 12 | 390 | 1.6 | 3.3 | 11.0 | 825 | 1689 |

**Table 5.5 Imposed treadmill exercise for the exercised horses in the study of Kasashima *et al* (2002).**

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