INTRODUCTION - THE TRAINING ENVIRONMENT—WHY IS THE SDFT MOST AT RISK?

The majority of superficial digital flexor tendon (SDFT) injuries occur during the fettting process of training regimes. Previous studies have established that there is a lack of evidence-based practices in current equine conditioning programmes, which may increase incidences of injury during training and limit the validity of previous research findings in the training environment. Variations in training frequency, training intensity, training effects, surfaces and equipment have all been identified as potential risk factors for SDFT injury. In addition to this, individual risk factors such as age, breed and sex, as well as individual variations in tendon vascular supply, flexibility and strength may also contribute to the initiation of acute/chronic tendon injuries and longer recovery rates. With so many factors reported to influence injury rates in vivo (and considering the ethical factors involved with conducting such studies), the use of biomechanical and biothermal tests in vitro has elucidated the basic physiological response of SDFT tissues and cells to the strains experienced during locomotion. Furthermore, comparing the structure-function relationship of SDFT (which provides the role of an energy-storing tendon) with the anatomically opposing common digital extensor tendon (CDET) (which is a positional tendon) provides a valuable insight into how the material properties of the SDFT are optimised for energy storage and how these properties are affected by ageing and injury.

Abstract

The forelimb superficial digital flexor tendon (SDFT) is an energy-storing tendon that is highly susceptible to injury during activities such as galloping and jumping, such that it is one of the most commonly reported causes of lameness in the performance horse. This review outlines the biomechanical and biothermal effects of strain on the SDFT and how these contribute to the accumulation of microdamage. The effect of age-related alterations on strain response and subsequent injury risk is also considered. Given that tendon is a slowly healing and poorly regenerative tissue, prompt detection of early stages of pathology in vivo and timely adaptations to training protocols are likely to have a greater outcome than advances in treatment. Early screening tools and detection protocols could subsequently be of benefit in identifying subclinical signs of degeneration during the training programme. This provides an opportunity for preventative strategies to be implemented to minimise incidences of SDFT injury and reduce recovery periods in elite performance horses. Therefore, this review will focus on the modalities available to implement early screening and prevention protocols as opposed to methods to diagnose and treat injuries.

KEYWORDS

horse, SDFT, tendinopathy, tendonitis, injury prevention
The predominant function of all appendicular tendons is to transfer muscle-generated force to the skeleton. However, tendons can be classed as either positional or energy storing due to the differing in vivo load environments, with energy-storing tendons experiencing particularly high stresses or strains during exercise. These tendons are stretched and recoil with each stride, storing and releasing energy which decreases the energetic cost of locomotion by as much as 26% in the human and 36% in the horse. The principal energy-storing tendons are the forelimb SDFT in the horse, and the Achilles tendon in the human. Due to such extreme loading environments in these energy-storing tendons, they are highly prone to overload-induced injury, with 75%-95% of tendon injuries in horses occurring in the forelimb SDFT, and the majority of human tendon injuries occurring to the Achilles tendon. There is also an age-associated increase in injury risk to the energy-storing tendons of both species, which is discussed in a subsequent section.

In addition to the extreme loading environments experienced by energy-storing tendons, repeated loading of the tendon without sufficient recovery time between exercise sessions makes it more susceptible to failure, even when submaximal loads are applied. Significant changes in the SDFT have been observed after racing, with maximal changes observed 48 hours post-exercise, and returning to baseline values at 72 hours. This would suggest that exposing the tendon to high strains within a 72-hour period (ie before it can re-achieve a state of homeostasis) may further predispose the tendon to injury. Elevation of tendon temperature during high-intensity exercise is also a well-established result of mechanical overloading, and its effect on tenocyte function may have a significant impact on the onset of tendinopathy.

Several extraneous factors predispose the SDFT to injury during exercise. Guidance has been published on the effect of artificial surfaces on the musculoskeletal system, possibly due to the rise in reported musculoskeletal injuries since the increased use of artificial surfaces. Additionally, increasing both fence height and the frequency of jump training sessions within a training programme have shown to increase chances of flexor tendon injuries. Particularly with regard to the disciplines of National Hunt racing and the cross-country phase of eventing, abnormal mechanical loading resulting from varying terrain is likely to lead to erratic changes in magnitude, frequency, duration or direction of forces placed upon the limbs.

Due to the high incidence and multifaceted nature of SDFT injuries, this review summarises the current understanding of the structure-function relationships in the SDFT and identifies the biomechanical and biothermal effects of repeated high strains on the SDFT. Age-related alterations which result in increased injury risk are also reviewed, and we identify important areas for future study regarding the use of early screening tools and preventative strategies for minimising incidences and severity of SDFT injury.

2 | STRUCTURE-FUNCTION RELATIONSHIPS AND THE SPECIALISED ROLE OF THE SDFT

The tendon is an integral part of joint movement and stability, as this intermediate tissue structure transmits the force generated from muscle contraction to bone, facilitating movement around the joint. Tendons contain approximately 50%-60% water, and the remaining dry matter is composed of type I collagen (approximately 70%-85% DM), arranged in a hierarchical structure. This structure is immersed in a noncollagenous matrix containing a small, but crucial, population of tenocytes which maintain the tendon’s structural stability. As shown in Figure 1, collagen molecules accumulate to form subunits of increasing diameter, forming longitudinally aligned fibrils—the fundamental load-bearing material of structural tissues within the body. Each fibril is composed of an arrangement of triple-helical collagen molecules, which are covalently cross-linked to one another, conferring strength to the fibril and overall stability to the molecules. These fibrils band together to form fibres and ultimately fascicles, which occupy most of the tendon volume. As such, these tendon subunits are commonly analysed as linear elastic structures. The interfascicular matrix (IFM) that surrounds and links the fascicles is a loose connective tissue that is comprised of type III collagen, elastin and proteoglycans, and is produced and maintained by a small population of interfascicular cells.

The composition and organisation of the extracellular matrix in different tendons provide optimised mechanical properties for different functions. Understanding the complex interactions between varying proportions of tendon components to maintain a state of homeostasis is essential in understanding mechanisms by which injury occurs in different tendons. However, these differences in composition and mechanical properties at the different hierarchical scales have historically been challenging to determine due to high variability in the experimental data and lack of techniques to investigate differences, particularly at the lower levels of tendon hierarchy. A significant body of research has more recently focused on elucidating the mechanisms by which tendon functional specialisation occurs and also identifying the age-related alterations which result in increased injury risk. A common approach to investigate tendon structure-function relationships is by the comparison of energy-storing tendons with their anatomically opposing positional counterparts (the equine common digital extensor tendon (CDET) for the SDFT, and human anterior tibialis tendon for the Achilles tendon). These positional tendons are ideal comparators as they experience much lower stresses and strains during locomotion, such that they are not susceptible to overload-induced injuries. Functional differences between positional and energy-storing tendons can be explored by examining the structural composition of the whole tendon, macrostructural and microstructural structures. Due to ethical and technical constraints placed upon in vivo studies, the use of in vitro studies has helped to experimentally elucidate the biomechanical and biothermal thresholds for cell viability, which have resulted in findings that are applicable to both equine and human studies.
By initially elucidating the compositional specialisation of these differing structures in vitro using the methods described below, researchers can subsequently begin to understand the varying effects of biomechanical and biothermal stresses induced during intense exercise on the SDFT in vivo and establish threshold values for injury risk. The quantification of a strain threshold value of irreversible damage at a microscopic and macroscopic level could subsequently help to predict extraneous factors that influence such mechanical parameters.\(^{37}\)

However, it is important to note that testing cells or tissues in an in vitro setting is not completely analogous to the environment in vivo. There are several important differences which may influence cell viability, which are well recognised in the literature.\(^{38}\) Furthermore, previous reports investigating mechanisms of tissue physiology and damage often involve various laboratory species, which may not accurately reflect the equine system due to factors including genetic variation and metabolic differences. However, current research in vitro does provide insight into the physiological mechanisms that underpin general tendon function and the mechanism by which injury occurs in vivo.

### 3 | BIOMECHANICAL/STRUCTURAL EFFECTS OF STRAIN

The effect of locomotory strain on the microstructure of tendons is measured in vitro through mechanical testing. As tendon injury relates to the straining/tearing of tendon tissues, tensile testing is used to determine the maximum stress (force per unit area, measured in MPa) and strain (percent elongation) that the tendon can withstand while being stretched before breaking. Tensile tests can also be used to measure elastic modulus, which is a method of quantifying 'stiffness' or the ability of the tendon to undergo reversible deformation during tensile loading. The elastic strain limit of the tendon is an important strain threshold at which maximum elastic modulus is reached, after which microdamage starts.

However, the majority of injuries are generally thought to occur as a combined result of continuous loading cycles above a certain intensity rather than due to a single overloading event. Matrix microdamage accumulates, overwhelming the capacity of cells to repair structural defects before subsequent loading cycles,\(^{39}\) ultimately leading to clinical injury. Cyclic loading tests are subsequently used to apply continuous and repeated tensile loads to the tendon tissues to observe the progressive degradation of the material and ultimately tendon failure, and so would be more representative of the nature of stresses applied in a practical setting.\(^{39}\) By using a combination of tensile and cyclic tests, studies have established how the SDFT is specialised for energy storage at different levels of the tendon hierarchy, and how these specialisations are affected by ageing.

### 4 | SPECIALISATION AT THE WHOLE TENDON LEVEL

When considering tendons as a whole, energy-storing tendons are more elastic than their positional counterparts, with lower elastic modulus (more compliant) and higher strain to failure, which allows...
them to withstand their high strain environment.\textsuperscript{40,41} During high-speed galloping, for instance, the equine flexor tendon has typically shown to stretch by 10\%-15\%.\textsuperscript{13} These specialised mechanical properties are accompanied by several reported compositional differences between tendon types, including greater cellularity, glycosaminoglycan (GAG) and elastin content, and lower collagen content in the SDFT compared with the CDET.\textsuperscript{12,40,42-44} There are also differences in collagen cross-link profile and collagen fibril diameter between tendon types, with a lower mass average fibril diameter in the SDFT.\textsuperscript{12}

While it is difficult to directly relate the differences in matrix composition with specific alterations in mechanical properties, it is likely that the higher water and GAG content and lower collagen content in the SDFT result in a more compliant material, while the higher elastin content confers superior recoil. Indeed, it has been demonstrated that water content in the SDFT is negatively correlated with elastic modulus, while fibril diameter shows a positive correlation with elastic modulus.\textsuperscript{12} Differences in protein turnover rate have also been identified between tendon types, with a longer collagen half-life in the SDFT, indicating slower turnover of this protein than in the CDET. By contrast, turnover of noncollagenous proteins occurs more rapidly in the SDFT than in the CDET, which may act to repair any damage to the noncollagenous matrix.\textsuperscript{44} Similarly, it appears that collagen turnover in the human Achilles tendon is also slow or even absent in the adult, with studies indicating minimal collagen turnover in this tendon once maturity is reached.\textsuperscript{45} However, comparative studies of protein turnover in functionally distinct human tendon are yet to be conducted, and turnover rates of specific proteins within tendons from large species remain unknown.

5 | SPECIALISATION AT THE MACROSCALE

Recent studies have further elucidated the structure-function relationships in the tendon at the macroscale by assessing mechanical and compositional specialisations of subunits of functionally distinct tendons. Unexpectedly, fascicle mechanical properties do not reflect those observed at the whole tendon level, with fascicles from the SDFT failing at lower strains than those from the CDET, despite the SDFT as a whole demonstrating significantly greater extensibility than the CDET.\textsuperscript{41} This greater extensibility is provided by the IFM, which exhibits low stiffness behaviour in the SDFT, allowing sliding and recoil between fascicles.\textsuperscript{29,41} Recent work demonstrates similar capability for interfascicular sliding in the human Achilles tendon, with the presence of discontinuities within the tendon observed by ultrasound indicating a complex strain environment within the IFM.\textsuperscript{46} The IFM in energy-storing tendons also exhibit greater ability to resist and recover from cyclic loading, with less energy loss (hysteresis) and stress recovery compared with the IFM in positional tendons, both in horses and humans;\textsuperscript{29,47} as well as increased fatigue resistance in the SDFT compared with the CDET.\textsuperscript{48} This behaviour is likely provided by the specialised composition of the IFM, which is rich in lubricin and elastin.\textsuperscript{32,43,49} Lubricin is a large mucopolysaccharide, which enhances joint lubrication, and mouse knockout studies have demonstrated that lubricin promotes interfascicular sliding in the mouse tail IFM.\textsuperscript{50} It is likely that elastin provides the IFM with efficient recoil. Indeed, the elastin content in the IFM is greater in the SDFT than in the CDET,\textsuperscript{43} and enzymatic depletion of elastin in the subunits of the SDFT reduces IFM fatigue resistance and the ability to recover from loading, but does not affect fascicle mechanical properties.\textsuperscript{51} Studies have also shown that protein turnover rate is greater in the IFM than within the fascicles, which is likely a mechanism to repair damage to this region.\textsuperscript{52,53}

While the fascicles in the SDFT do not appear to be specialised to enhance tendon extensibility, they are optimised for improved energy-storing capacity, with a helical structure providing greater compliance and enabling more efficient extension and recoil, as well as enhanced fatigue resistance of the fascicles, properties that are likely conveyed to the whole tendon.\textsuperscript{48,54,55} By contrast, fascicles from the CDET, which do not have a helical structure, rely on sliding between collagen fibres to allow fascicle extension, and show a much poorer ability to recover from loading, resulting in decreased fatigue resistance.\textsuperscript{48,55} While these structural differences in fascicles from functionally distinct tendons have been identified, any compositional differences in the fascicular compartment of functionally distinct tendons are yet to be determined.

6 | SPECIALISATION AT THE MICROSCALE

Very little is known about any differences in structure and/or composition between energy-storing and positional tendons at the microscale. However, recent work has shown that collagen fibrils from functionally distinct bovine tendons respond differently to applied elongation, with fibrils from energy-storing tendons demonstrating high strain stiffening and ability to resist molecular disruption, while fibrils from positional tendons are able to extend further, but suffer increased damage characterised by formation of kinks.\textsuperscript{28,56} These properties may be conferred by differences in cross-link profile, with more thermally stable and greater total cross-link density in flexor tendons compared with extensors.\textsuperscript{28} Differences in collagen crimping have also been observed, with shorter crimp and greater crimp angle in the SDFT compared with the CDET, which likely confer the superior recoil ability in the SDFT.\textsuperscript{57}

7 | EFFECT OF AGEING ON FUNCTIONALLY DISTINCT TENDONS

Previous studies have found that the risk of SDFT injury increases with age.\textsuperscript{8,9} As such, a significant body of work has been undertaken to establish how tendon properties alter as a function of age, and identify differences in ageing response in functionally distinct tendons.\textsuperscript{13,58-61} While most of these studies have focused on the effect of ageing on mature tendons, a few studies have investigated
alterations in tendon mechanics during development, demonstrating that energy-storing and positional tendons have similar mechanical properties at birth, which then diverge. More recent work has identified the importance of post-natal IFM development, particularly in energy-storing tendons, demonstrating divergence of IFM mechanical properties between functionally distinct tendons during development which is accompanied by increases in IFM protein abundance.

8 | EFFECT OF AGEING AT WHOLE TENDON LEVEL

When considering age-related changes in tendon mechanical properties after maturation, data in the literature are contradictory, with studies variously reporting no alterations or decreases in mechanical properties with ageing, particularly stiffness, both in human and equine tendons (65 and references therein). Few studies have directly compared the response of functionally distinct tendons to ageing, reporting no alterations in modulus, failure stress or failure strain with increasing age in either the SDFT or the CDET. However, there are large variations in tendon mechanical properties between individuals, which may mask any effect of ageing on tendon mechanics. A few alterations in tendon composition with ageing have also been identified specifically in the SDFT, including a decrease in elastin content, as well as accumulation of partially degraded collagen. In human tendon, decreased GAG and collagen contents have been reported with ageing, but this response varies between different tendons and the implications of these alterations on tendon mechanical properties remain unclear (65 and references therein).

A small number of studies have also investigated the effect of ageing on rodent tendons, demonstrating a decrease in tendon mechanical properties with age in the rat Achilles and increased anterior tibialis stiffness in mice. Gene and protein levels of lubricin and elastin in the rat Achilles and anterior tibialis tendons are also decreased with age; this is accompanied by a decrease in collagen expression at the gene, but not the protein level. Interestingly, in this study, no differences were observed between different tendons with ageing; however, the degree to which the Achilles tendon in the rat is specialised for energy storing remains unknown. In addition, mechanisms of ageing are likely to differ between larger animals and rodents, in which growth plate closure does not occur and skeletal growth continues slowly after puberty, such that care must be taken when translating these findings to larger animals.

9 | EFFECT OF AGEING ON FASCICLES AND INTERFASCICULAR MATRIX

The effect of ageing on tendon subunits has also been investigated in the horse, with almost all of observed changes occurring to subunits of energy-storing tendons, and many of these occurring specifically to the IFM. The IFM in the aged SDFT exhibits alterations in a range of quasi-static and viscoelastic mechanical properties, including increased stiffness, decreased fatigue resistance and ability to recover from loading. By contrast, no age-related changes to the mechanical properties of the CDET IFM have been identified. Despite the age-related alterations in mechanical properties observed in the SDFT IFM, few age-related alterations in IFM composition have been identified. There is little change in the abundance of proteins in the SDFT IFM with ageing, with the exception of fibromodulin and elastin, which are both decreased in the aged SDFT. The remaining elastin in the IFM becomes disorganised, and there also appears to be a decrease in matrix turnover specifically within the IFM, based on the number of cleaved proteins detected, which may result in a decreased ability to repair microdamage to this region.

Age-related changes have also been observed in fascicles from energy-storing tendons, with those from the aged SDFT exhibiting increased stress relaxation, with altered response to loading leading to decreased fatigue resistance. This is likely due to a loss of helix structure in these fascicles, meaning that when load is applied, extension occurs due to fibre sliding, leading to decreased efficiency and ability to recover from loading. By contrast, few age-related changes have been identified within fascicles from the CDET, although studies have demonstrated an increase in fascicle failure strain with ageing in the CDET. While several mechanical and structural alterations to the fascicular matrix in the SDFT have been identified with ageing, there are few apparent changes in fascicle composition, with the exception of fibromodulin and collagen type III, which are decreased in fascicles from the aged SDFT. Fibromodulin and collagen III both act as regulators of collagen type I fibrillogenesis; therefore, an age-related decrease in these proteins may reduce the capacity for fibril formation and repair. In contrast to the IFM, there are no alterations in protein cleavage with increasing age in the fascicular matrix, indicating no change in protein turnover in healthy tendon. However, the decreased levels of fibromodulin and collagen III may impair tendon healing, leading to chronic tendinopathy.

10 | BIOTHERMAL EFFECTS OF STRAIN

Adaptive processes succeeding biomechanical strains in vivo involve complex and intricate interactions between tendon tissue and vascular, nervous and immune systems, and involve inflammatory agents which play a key role in the early stages of healing. It has been suggested that recurring short-term thermal insults may stimulate stress protein production, protecting tenocytes from damage in later exercise bouts. These proteins are produced in response to sublethal chemical, metabolic or thermal insults and protect the cells from subsequent, otherwise lethal, conditions. However, prolonged or repetitive exposure to hyperthermic conditions damages tendon cells that play an important role in maintaining the extracellular matrix. In vitro tests can be
used to measure the biothermal effects of strain by exposing explanted SDFT cells to varying intensities of hyperthermic conditions and quantifying the percentage of cell survival under such conditions. Measuring the responses to varying intensities of temperatures may help to elucidate a threshold value that ultimately leads to impaired functioning of the SDFT.

In terms of establishing a temperature threshold value, inducing localised hyperthermia on explanted SDFT cells has shown that tendon fibroblast activity and cell survival rate are negatively correlated with increasing temperatures and time of exposure to higher temperatures.36,38 A local temperature rise above 42.5°C in particular appears to be above the viability threshold of fibroblast cells,34 which initiate degenerative processes in the central core of the tendon, subsequently predisposing it to mechanical failure.35 No in vitro studies have specifically compared tendon responses between energy-storing and positional tendon tissues. However, tendon fibroblasts from the SDFT are significantly more resistant to increasing temperatures than dermal cells when subjected to temperatures of 45°C for 30 minutes.38 Exposure beyond 10 minutes still results in a decline in survival rate (91 ± 4%). Exposure for the same amount of time to 48°C has reported a survival rate of just 22 ± 4% in tendon fibroblasts. Furthermore, exposing tenocytes to 43°C for 30 minutes induces the death in a small population of normal primary tenocytes and upregulates the expression of catabolic and proinflammatory genes by surviving cells.24

11 | PATHOPHYSIOLOGY OF SDFT INJURIES

As previously explained, the viscoelastic properties of healthy tendon ideally allow it to extend without damage and recover its macroscale elastic properties after loading. Therefore, damage can be defined as a threshold value at which irreversible changes occur due to application of strain,73,78 causing structural disruption to the site. This threshold value is referred to as the ‘metabolic tipping point’,79 where the metabolic demands of the tendon during mechanical stress exceed the rate at which the nutrient supply can reach the structure for repair. Although the complex mechanisms that precede the accumulation of irreversible damage in vivo are still poorly understood, human and animal studies on tendon repair after injury give some indication of the healing process.59,77,80-82

In a manner similar to other tissues, healing consists of three phases (for an in-depth review see 83): (a) the inflammatory phase, (b) the proliferative phase (lasting weeks) and (c) the remodelling phase (lasting months/years). This process is complex and requires contributions of both intrinsic and extrinsic cell populations to the healing process.84 The first stage involves initial debridement of damaged materials at the site of injury due to the expression of catabolic and proinflammatory genes,82 followed by scar tissue deposition. The inflammatory phase is important for proper tendon healing to occur,59 and studies suggest that the inflammatory response is better modulated than completely suppressed in humans and canines.35-88 Administration of nonsteroidal anti-inflammatory drugs (NSAIDs) immediately post-injury to decrease acute inflammation could otherwise significantly influence the quality of the subsequent healing response.59,89

In the proliferative phase, a provisional matrix consisting predominantly of type III collagen is synthesised by fibroblasts, which differs structurally and functionally from normal tendon structure.90,91 This helps to restore partial functional continuity and provides a scaffold for the migration of subsequent cells to the injured area. Very little is known about the specific contributions of extrinsic and intrinsic cells to the healing process in the equine tendon, but conditions that promote increased contribution from extrinsic cell populations possibly result in greater scar tissue and adhesion formation in comparison to those that promote intrinsic healing.83 The role of intrinsic and extrinsic cells in healing the tendon is highlighted by studies of tendon healing during embryonic development, whereby fetal and adult fibroblasts display intrinsic differences in tendon tissue regeneration.92 Fetal tendons can regenerate with function restoration of gait and mechanical properties in mice.83 In contrast, histologic studies of tendon removed from mature human patients suffering from tendinopathy have revealed alterations to collagen structure—in place of continuous, well-aligned, crimped collagen fibres, tendinopathic tissue features fragmented, disordered collagen matrix, often lacking clear fibre structure.36 Mechanical changes, such as a decrease in elastic modulus,22 are also characteristics of tendon injury.

While the preliminary formation of scar tissue between tendon ends provides basic functional continuity at the site of disruption, subsequent remodelling during the remodelling phase causes conversion of the provisional matrix to type I collagen (scar tissue) so that it is more capable of contracting as it matures, providing increased mechanical strength. However, studies have found comparatively disordered collagen structure at the fibre level, and a lack of compartmentalisation on a macroscale level.93 Proliferation of the scar between the tendon and adjacent tissues is also undesirable because as these attachments later impede normal tendon gliding and function.94 Adhesion formation results in loss of movement, and consequently higher risk of functional disability,95 as well as promoting a chronic state of tissue inflammation,79 which further compounds the hyperthermic effects of catabolic and proinflammatory genes expressed from surviving cells. Repeated hyperthermic insults induce cell death, leading to compromised metabolism of cell matrix components, subsequently resulting in tendon core degeneration and the development of characteristic core lesions. Unlike in other tissues, such as bone or muscle, mature adult tendons appear to lack the inherent ability to remodel the structure of tendons to their pre-injury state. Instead, tendon healing involves the formation of fibrotic scars, which impairs mechanical strength of tissues, and subsequently tendon injuries can result in significant dysfunction and disability.72 This may explain (in part) why 23%-67% of horses treated using conservative methods re-injure tendons within 2 years of the initial injury.96
12 | APPLIED TECHNOLOGIES TO ASSIST IN EARLY DETECTION OF TENDINOPATHIES

With expensive and time-consuming treatments required post-SDFT injury, there is considerable interest in measures to detect and prevent SDFT injury.97 Prompt detection of early stages of pathology is likely to have a greater outcome than advances in treatment, given that tendon is a slowly healing and poorly regenerative tissue. Conventional techniques for monitoring degenerative changes and screening tools in vivo have come under recent review.98

12.1 | Monitoring biomechanical effects of strain on the SDFT in vivo

Ultrasonography is traditionally used in a clinical setting to monitor live changes to fibre pattern, echogenic intensity and the presence of lesions (i.e., the repair trajectory) in the SDFT post-injury. Predictive values for successful return to productive performance post-injury are widely used in human medicine and have recently been investigated in equine practice.99 However, even minor changes in transducer angle, amplifier gain and displacement can lead to inconsistencies in 2D interpretations of this 3D structure. The use of ultrasound tissue characterisation (UTC) has been developed to address the limitations of using ultrasound alone and uses conventional ultrasonography to objectively calculate fibre alignment of the tendon in vivo via the construction of a 3D image. This technique has successfully detected subtle (but important) changes in the echo pattern dynamics of the SDFT after maximal exercise23,98 with data returning to baseline values on day 3 post-race. Studies have reported that the reproducibility of using this method is high, and risks for operator error is low,100 making this a potentially valuable screening tool for monitoring structural changes in the SDFT over time in addition to diagnosis. Greater application of this tool across various disciplines will be of particular benefit in revealing the structural changes that occur in the SDFT in response to different forms of training and disciplines. From a practical perspective, horses often need to be sedated and distal limbs clipped before ultrasound, which can have implications in the management and training of horses.

12.2 | Monitoring biothermal effects of strain on the SDFT in vivo

A limited number of dated studies have attempted to study the biothermal response to exercise in vivo. Wilson and Goodship35 established a linear relationship between speed and tendon temperature, with a mean increase in tendon temperature of 2.5°C min⁻¹ during gallop on a treadmill. With speeds reaching 9.3-10.5 m.s⁻¹, mean peak temperature of 43.3°C was measured in the tendon central core, findings that are supported by Yamasaki et al101 who estimated the peak temperature of 45°C at speeds of 11 m.s⁻¹ in field conditions without a rider. An elite racehorse is capable of running at ridden speeds averaging 16.76 m.s⁻¹ and 15.91 m.s⁻¹ at 1000 and 2000 m races.102 Therefore, it is likely that the SDFT core reaches higher temperatures during training/racing, highlighting a potentially crucial link between increasing tendon temperature and tendon rupture beyond a certain threshold. However, it is worth noting that Fourier’s law was used to predict the core tendon temperatures reached during the gallop exercise period, based on subsequent readings post-exercise once the horses could be anaesthetised (approximately 3 minutes after cessation of activity). Such mathematical predictions rely on a number of assumptions which subsequently limit its accuracy. Furthermore, the invasive methods used to ascertain tendon core temperature in these studies have obvious ethical implications that significantly restrict future use and application of this technique in larger studies. Nevertheless, these studies do provide researchers with an insight into the mechanisms in the SDFT core in vivo during exercise.

There are a growing number of publications on the use of infrared thermography (IRT) to establish the distribution of body surface temperature in both human103 and equine104 athletes. It can denote areas of inflammation that could account for compromised performance, establish a source of pain or discover musculoskeletal overloads.105,106 Interest is recently emerging for further application of IRT due to its noninvasive nature and ability to promptly assess skin temperature, both of which are particularly beneficial when monitoring athletes in training.107 While IRT cannot reveal specific pathologies, it facilitates the localisation of increased heat (inflammation),108 commonly referred to as ‘hot spots’. The lack of insulating tissues overlying the tendon in the equine distal limb also provides a particularly accurate representation of internal temperature. In the acute phase, tendonitis is identified as a focal ‘hot spot’, later decreasing in temperature, but remaining elevated during the healing process.102 This technique has already shown promise as an early screening tool in soccer players, whereby IRT detection protocols were implemented during preseason training.109 Early detection of subclinical degenerative changes resulted in reduced incidences of injuries reported, as well as days lost to training due to injury. Routine thermographic evaluation of performance horses may also assist in detecting/preventing injury during training and providing an opportunity for preventative action. ‘Hot spots’ have been detected thermographically up to 2 weeks prior to clinical evidence of any swelling or pain in the horse.108 Most recently, the use of IRT as an early screening tool revealed significant temperature differences in racehorses before clinical manifestation of metacarpal conditions associated with training occurred.110

However, to date, there is still very limited research conducted on the use of thermography in equine athletes and further research is required to establish reference baseline profiles. Thermographic evaluation of the distal limb is complicated by environmental factors, such as airflow111 and ambient temperatures,112,113 where surface temperature of distal limbs at rest correlates strongly with ambient temperature.114 Differences of up to 5°C between SDFT core and skin temperature measurements during cryotherapy treatments...
also suggest a delayed response in skin temperature to extreme temperature changes, which need to be accounted for when using IRT.\(^\text{115}\) In addition to the effects of ambient temperatures on the interpretation of results, individual factors, such as breed, coat colour, a moist/dirty coat and time spent alternating weight on limbs during rest, have previously affected interpretation of thermal images.\(^\text{106}\) The generally high degree of symmetry between contralateral parts of the body in the healthy horse nonetheless provides a valuable asset in diagnosis of unilateral pathological conditions associated with inflammatory responses. Any thermal asymmetry could indicate abnormalities, although this would be absent in cases with bilateral pathology—the prevalence of which is 10%-35% in the SDFT.\(^\text{99,116}\) A difference greater than 1°C over 25% of the compared distal parts of the limbs\(^\text{105}\) or a threshold temperature variation of 1.25°C between the dorsal and palmar aspects of the third metacarpal region between limbs is indicative of subclinical inflammation, later clinically confirmed by radiography and ultrasonography.\(^\text{104}\) Recommended procedures/protocols have recently been published for the use of thermographic imaging in both human\(^\text{103}\) and equine\(^\text{114}\) studies. Unfortunately, standardisation of environmental and between-subject factors is not always possible in equine research due to the constraints associated with field testing, and so IRT still needs to be combined with conventional diagnostic modalities, such as ultrasonography, until sufficient data are available to establish the reliability of thermography across the various equine clinical applications.\(^\text{105,117,118}\)

### 13 | Initial Detection of Subclinical SDFT Pathology: Early Intervention Strategies

Similar to human studies,\(^\text{109}\) early detection of subclinical changes may provide trainers with the opportunity for preventative action during the training programme to minimise incidences of clinical injury and reduce recovery periods. Standardising training regimes by following the scientific principles of training\(^\text{2}\) should ensure progressive loading of the various systems and provide sufficient recovery times. However, we discuss specific intervention strategies that can be used to temporarily minimise biomechanical and biothermal stresses to the SDFT if subclinical degenerative changes have been detected using appropriate screening protocols.

#### 13.1 | Early intervention strategies: Reducing biomechanical stresses

Guidance has been published on the effect of artificial surfaces on the musculoskeletal system,\(^\text{4}\) possibly due to the rise in reported musculoskeletal injuries since the increased use of artificial surfaces. Research on the effects of surface type on performance have been investigated in flat racing,\(^\text{119,120}\) over hurdles,\(^\text{121}\) high-speed trotting,\(^\text{122}\) and showjumping.\(^\text{26}\) However, epidemiological data specifically related to injuries associated with various surfaces used across the equestrian disciplines still require further investigation. Studying hoof-surface interactions, in particular, can elucidate biomechanical stresses experienced by the limb that contribute to current knowledge to aetiology of musculoskeletal injuries in field conditions.\(^\text{4,123}\) Arena surface assessment made between professional showjumping riders also demonstrated a substantial level of inter-rider variation,\(^\text{124}\) which further suggests a lack of objective guidance available to inform riders and trainers of appropriate surface quality.

In an attempt to minimise biomechanical strains during exercise, a recent study showed that 85.6% of racehorse trainers in Australia use water-based exercise\(^\text{125}\) and swimming, in particular, is emerging as an accepted part of training programmes for racehorses.\(^\text{126}\) Other semi-flotation forms of water-based exercise include water walkers, ridden ‘surge’ training\(^\text{125}\) and water treadmills.\(^\text{127}\) With previous studies demonstrating that submerging quadrupeds (horses and dogs) in water at various depths reduces bodyweight by between 10% and 60%\(^\text{128,129}\), water-based exercise is likely to significantly reduce biomechanical strain placed on the tendons in the distal limb during exercise due to hydrostatic buoyancy in the water. However, the specific effects of different water-based exercise on SDFT stresses and strains are yet to be determined. Previous studies suggest that water treadmills and water walkers are classed as low-intensity exercise,\(^\text{127,130}\) while swimming and ridden ‘surge’ training may be a form of relatively high-intensity exercise.\(^\text{126,131}\) Therefore, water treadmills and water walkers may be suitable at an earlier stage of the fittening programme before ridden work commences. The use of water treadmills specifically for the rehabilitation of musculoskeletal injuries in sport horses has been reviewed and includes recommendations and contraindications for use.\(^\text{132}\)

#### 13.2 | Early intervention strategies: Reducing biothermal stresses

Understanding the factors that initiate inflammatory responses and managing the frequency of hyperthermic insults during training in preparation for elite levels of performance may assist in the prevention of SDFT injury. Adaptive processes in vivo after intense exercise involve complex and intricate interactions between tendon tissue and vascular, nervous and immune systems,\(^\text{76,77}\) and involve inflammatory agents which play a key role in the early stages of healing. However, prolonged or repetitive exposure to hyperthermic conditions has shown to damage tendon cells which play an important role in maintaining the extracellular matrix. The onset of tendonitis manifests as acute inflammation in the distal limb, often accompanied by swelling, inflammation and tenderness/pain at the affected site.\(^\text{101}\) This hyperthermic response may be exacerbated by the use of boots or bandages during training, as skin temperatures of 40.3 ± 1.9°C have been recorded in booted limbs after 14-18 minutes of intense exercise, a difference of 7.11 ± 1.9°C in comparison to the unbooted limb.\(^\text{3}\) While core tendon temperatures have not been recorded in response to the application of boots during training, insulation of the distal limb is not
recommended due to the hyperthermic conditions already observed in the tendon core during high-speed galloping without insulation.

Therefore, temperature manipulation for injury prevention purposes may be of clinical benefit in the distal limb of horses post-exercise. Cryotherapy and cold-water immersion have become popular forms of therapy in human athletes where athletes are exposed to extremely cold air/water for short periods of time. Cryotherapy has been documented to cause analgesia, hypometabolism and vasoconstriction, thereby functioning as an anti-inflammatory mechanism. Some studies have produced conflicting results on the proposed physiological mechanisms by which whole-body cryotherapy is postulated to improve recovery rates in comparison to passive recovery in trained athletes. However, in the distal limb of horses, Petrov et al demonstrated that the topical application of cryotherapy reduced core SDFT temperature, and that soft tissue temperatures did not elevate to initial temperatures for 2 hours after 60 minutes of application. Within the first 5 minutes alone of topical application, central SDFT temperature had also decreased by nearly 10°C. This notably rapid temperature response is most likely as a result of the lack of subcutaneous fat/other insulating tissues overlying the flexor tendons in the distal limb, as well as poor vascularity of the tissues. Metabolic enzymatic activity has shown to reduce by half for every 10°C decrease in tissue temperature and temperatures between 10°C and 11°C have previously been recommended for effective inhibition of metabolism, as well as minimisation of secondary tissue damage. Tissues cooled to between 10°C and 15°C and skin temperatures below 13.6°C are also believed to have local analgesic benefits. Furthermore, a study in which researchers examined the defensive role of surface cooling on traumatised tissue revealed the inhibition of apoptosis in tissues when cooled to a surface temperature of 10°C. Therefore, the application of cryotherapy is likely to be of clinical benefit for inflamed tissues; however, a standardised method of application that has been verified in the research is still yet to be established.

14 | CONCLUSION

The SDFT is an energy-storing structure essential for efficient locomotion and thus performance, yet has very narrow mechanical margins for error, making it susceptible to injury. Our understanding of the pathophysiology of SDFT injuries has developed in recent years. However, this has not translated to a reported reduction in injury rates in the performance industry. Regardless of the varying levels of knowledge and experience among industry stakeholders, the literature has strongly suggested that trainers are considerably more interested in measures to prevent SDFT injury rather than treatment due to the destructive nature of this injury. Therefore, implementing evidence-based training practices may help to standardise protocols and decrease the risk of musculoskeletal injuries during training programmes. This review recommends considering some key areas that require further investigation by researchers, so that recommendations can be made for training programmes (in line with the principles of training) that specifically aim to prevent incidences of SDFT injury. (a) An understanding of the physiological demands of the different types of high-intensity training sessions is imperative for trainers to be aware of. (b) Extraneous factors, such as surface type, gradient, ambient temperatures, fence height and bandaging, should all be considered before commencing training sessions, as these have a direct influence on SDFT strain and temperature values during training. (c) The frequency of any training sessions that subject the SDFT to extreme stresses which mimic the competition environment (galloping, jumping, etc.) should be monitored closely and repeated a minimum of 72 hours apart in order to allow the SDFT sufficient time to repair and adapt. Individual factors, such as the horse’s age, previous competition experience and history of injuries, play an important role in deciding the frequency of intense sessions, and so require further investigation. Greater variation of exercise sessions (such as water-based exercise) may also aid in allowing sufficient recovery time for the musculoskeletal system while maintaining effective stimulus to other systems. However, there is still a very limited amount of research on these relatively novel forms of training and further investigation is required. (d) Objective measures (to ascertain physiological responses by individual animals to training sessions) should contribute to decisions on when to increase training intensity, and this should be increased incrementally over an appropriate length of time. Routine veterinary consultation and screening/protocol protocols should be employed to detect any pathological changes as early as possible. The modalities mentioned in this review that monitor structural changes in the SDFT have shown high reproducibility and low user error, making them a potentially valuable screening tool. However, monitoring the inflammatory response of the distal limb by means of identifying ‘hot spots’ using IRT requires further investigation and should not currently be used in isolation until reference baseline profiles are established. (e) Temperature manipulation may play an important role in modulating the inflammatory response associated with high-intensity work, but again standardised protocols in equine athletes have not been established and so still have scope for further investigation.

CONFLICT OF INTERESTS

No competing interests have been declared.

ETHICAL ANIMAL RESEARCH

Not applicable.

OWNER INFORMED CONSENT

Not applicable.

FUNDING INFORMATION

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. C. Thorpe is funded by a Versus Arthritis Career Development Fellowship.

AUTHOR CONTRIBUTIONS

C. O’Brien was responsible for the concept, overall preparation and final editing of the manuscript. C. Thorpe and N. Marr had major
input into Tendon Specialisation and Effect of Ageing sections of the manuscript final editing and N. Marr created Figure 1.

DATA ACCESSIBILITY STATEMENT

Not applicable.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/evj.13331.

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How to cite this article: O’Brien C, Marr N, Thorpe C. Microdamage in the equine superficial digital flexor tendon. Equine Vet J. 2021;53:417-430. https://doi.org/10.1111/evj.13331