Physical activity: Does long term high intensity exercise in horses result in tendon degeneration?

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Running head Tendon response to exercise

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Abstract

This study explores the hypothesis that high intensity exercise induces degenerative changes in the injury prone equine superficial digital flexor tendon (SDFT) but not in the rarely injured common digital extensor tendon (CDET). The horse represents a large animal model which is applicable to human tendon and ligament physiology and pathology. Twelve age matched, female horses undertook galloping exercise three times a week with trotting exercise on alternative days (high intensity group, n=6) or only walking exercise (low intensity group, n=6) for 18 months. The SDFT, suspensory ligament (SL), deep digital flexor tendon (DDFT) and CDET were harvested from the forelimb. Tissue from the mid-metacarpal region of the right limb tendons was analysed for water, DNA, sulphated glycosaminoglycan and collagen content, collagen type III/I ratios, collagen crosslinks and tissue fluorescence. Left limb tendons were mechanically tested to failure. The analyses showed matrix composition to have considerable diversity between the functionally different structures. In addition the specific structures responded differently to the imposed exercise. High intensity training resulted in a significant decrease in the GAG content in the SDFT but no change in collagen content despite a decrease in collagen fibril diameters (previously published). There were no signs of degeneration or change in mechanical properties of the SDFT. The CDET had a lower water content following high intensity training and a higher elastic modulus. Long term high intensity training in skeletally mature individuals results in changes that suggest accelerated ageing in the injury prone SDFT and adaptation in the CDET.

Keywords: Ligament, Physical training, Extracellular matrix
Introduction

Many people are relatively physically inactive due to technological advances in the work place and home; a type of lifestyle known to contribute to disease. People are increasingly encouraged to compensate for this lifestyle by undertaking exercise to improve their health. The value of exercise is now also recognized for improving the symptoms of joint disease and arthritis (12; 48) although excessive exercise is a known factor that can contribute to the disease process (31). Little is however known about the effects of exercise on the skeletal system in general and in particular on soft connective tissues such as tendons and ligaments.

Injuries to tendon and ligaments are relatively common. A study in 1997 reported that 30-50% of human sports injuries were tendon related (16) while other studies have shown that the incidence of Achilles tendon rupture is increasing (14; 30). Some structures, such as the Achilles tendon, are particularly prone to injury while other tendons such as the anterior tibialis are rarely affected. Tendon injuries are not restricted to humans but also occur frequently in the horse (10; 18; 36; 51). As in humans, some equine tendons and ligaments, such as the superficial digital flexor tendon (SDFT) and suspensory ligament (SL) in the distal part of the forelimb, are particularly susceptible to exercise related injuries (10; 18; 36) while the anatomically opposing common digital extensor tendon (CDET) is rarely injured. This suggests there may be a structure specific response of tendon to exercise in both human and equine subjects.

Previous studies investigating the effects of exercise on tendon properties have shown conflicting results. Some studies have shown tendon hypertrophy in response to training (6; 54) and increased collagen content (54) while others have shown no change (32; 53) or a decrease in mechanical and structural properties (29; 45). These apparent contradictions may be due to the
variety of tendon types studied or due to variations in species, animal ages, exercise history, training duration and intensities used in the studies.

In tendons prone to injury it is now generally accepted that degenerative changes to the matrix precede clinical injury in human (17; 40) and equine subjects. Previous studies in horses have identified a reddish discoloration in the central core of the SDFT which was deemed to represent degeneration (50). This central core discoloration has been shown to be associated with increased levels of type III collagen, sulphated GAG, cellularity and rate of matrix turnover (3). In human tendons similar changes have been observed. Degenerate supraspinatus and subscapularis tendons have been found to have an increased proportion of type III collagen relative to type I (40) and higher levels of sulphated glycosaminoglycans (39). In addition, degenerated supraspinatus tendons had higher levels of the collagen crosslinks, hydroxyllysylpyridinoline and lysylpyridinoline and lower levels of pentosidine (2). Histopathological changes have also been reported in spontaneously ruptured human tendons including loss of parallel order of collagen fibres, hypoxic damage and mucoid degeneration (17). In human tendons these degenerative changes were associated with increasing age although ageing in equine tendons results in matrix changes that do not equate to degeneration; as older horses tend to have lower sulphated GAG levels and fewer cells (4). Increasing age in the equine SDFT is also associated with a decrease in collagen fibril diameters (9; 44).

The findings to date suggest that it is particularly important to determine the effects of exercise in skeletally mature individuals, undertaking a well defined exercise regime and to study the effects on matrix biology of tendons which are prone to degeneration and injury. This type of study however is very difficult to carry out in human subjects as tissue is required for *in vitro* analysis. Although human tissue can be collected at post mortem this material does not have
detailed information on exercise history which precludes the study of exercise related changes to tendon. The horse represents an excellent ‘natural model’ for exercise induced degenerative tendon disease in humans. The advantage of using equine subjects is that controlled exercise studies on age, gender and breed matched individuals can be carried out and tissue harvested for \textit{in vitro} matrix analysis.

In this study we investigate the effects of long term high intensity exercise on the distal limb tendons in skeletally mature Thoroughbred horses. We explore the hypothesis that high intensity exercise induces degenerative changes in the SDFT and SL but not in the rarely injured CDET.

\textbf{Methods}

\textit{Animals}  Twelve age-matched female Thoroughbred horses that had previously received no physical training were used for the study. Horses were paired based on size and one of the pair randomly assigned to the high intensity exercise group and the other to a low intensity exercise group. All horses were 18 months old at the start of the study and age did not differ significantly between exercise groups (Table 1). Horses were kept in loose boxes (3.66 m x 3.05 m) throughout the study. The study was conducted with appropriate regulatory approvals.

\textit{Training Regime}  The horses in the high intensity exercise group were trained on a high speed equine treadmill for 18 months at a level similar to that which would be experienced in race training. A typical week’s work was: Monday, 3 km at 12 m/s, 3\% slope; Wednesday, two times 1.5 km at 12 m/s and 14 m/s, 4\% slope, 5 minutes recovery; Friday three times 1 km at 12 m/s, 13 m/s and 15 m/s, 3\% slope, 5 minutes recovery. This was combined with 40 minutes walking
on a mechanical horse walker 6 days/week and 20 minutes trotting on days when no treadmill 
exercise was given. Horses in the low intensity exercise group undertook only walking and this 
was for 40 minutes each day on a mechanical horse walker for 6 days of the week. The low 
intensity trained group allows comparison of high intensity exercise with a ‘normal’ level of 
activity rather than immobilization which is well known to result in deterioration of tendon and 
ligament properties (1).

_Tissue Collection_  The SDFT, SL, CDET and deep digital flexor tendon (DDFT) were harvested 
from the right and left forelimb of each horse immediately following death. One of the horses in 
the high intensity exercise group was not able to complete the training due to ill health and was 
therefore precluded from the analysis. Tendons from the right limb were used for matrix analysis 
and histological examination. The gross appearance of the whole tendon and transverse cross 
section were examined for any signs of damage or discoluration. A 1.5 cm section was taken 
from the mid-metacarpal region of each tendon, snap frozen in liquid nitrogen, wrapped in cling 
film to prevent dehydration and stored at -80ºC prior to analysis. An adjacent 1cm section from 
the SDFT was fixed in formalin and processed for routine Haematoxylin and Eosin (H & E) 
staining for qualitative assessment of fibre alignment. In addition, the cross sectional area (CSA) 
of each tendon was measured (results reported previously (5)) and a sample of tissue processed 
for determination of collagen fibril diameters using electron microscopy and the mass average 
fibril diameter (MAFD) calculated (results reported previously (9; 34; 35)). The left forelimb 
SDFT and CDET were dissected free from the limb, wrapped in cling film and stored frozen at 
-20ºC for mechanical testing.
Water content  Tissue was semi-thawed at room temperature and outer loose connective tissue and epitenon removed. The remaining tissue from the SDFT only was divided into central zone and peripheral zone tissue as described previously (3). Tissue was weighed and freeze dried until a constant weight was reached. Water content is expressed as a percentage of the wet tissue weight.

DNA assay and tissue fluorescence  Prior to DNA and glycosaminoglycan (GAG) measurement, the lyophilized tissue was solubilised by papain digestion as described previously (3). DNA was assayed by the fluorometric method of Kim et al. (19) using the bisbenzimidazole dye, Hoechst 33258 to give an indication of tissue cellularity. Fluorescence was also measured in the absence of Hoechst dye and readings for DNA in the presence of Hoechst dye corrected to account for background tissue fluorescence. DNA concentrations were calculated by comparison to a standard curve prepared with calf thymus DNA diluted in dye solution to give a range of concentrations from 0 – 0.5 µg/ml. DNA content in tendon samples is expressed as µg DNA /mg dry weight tissue and tissue fluorescence as arbitrary units per mg of collagen.

Glycosaminoglycan assay  Total sulphated GAG content was quantified in aliquots of the papain digest by the method of Farndale et al. (11) using dimethylmethylene blue dye. Concentrations were calculated by comparison with a standard curve prepared with purified bovine trachea chondroitin sulphate (0-10 µg in 3 ml dye). Results are expressed as µg chondroitin sulphate equivalent sulphated GAG/mg dry weight tissue.
**Collagen content**  Collagen content was determined by measuring the imino acid hydroxyproline in an aliquot of the papain digest as described previously (3). Hydroxyproline concentrations were calculated by comparison with a standard curve prepared with standards (0 - 10 µg hydroxyproline /ml) and collagen content calculated assuming hydroxyproline to be present at 14%. Collagen content is expressed as a percentage of the dry weight of tendon tissue.

**Collagen type**  Lyophilised tissue samples (approx. 5mg) from the central zone of the SDFT were digested with CNBr by the method of Light and Bailey (26). The resulting peptides were dissolved in 200 µl sample buffer (125 mM Tris, 2% sodium dodecyl sulphate, 10% glycerol, 0.01% bromophenol blue) and heated for 30 minutes at 60ºC to ensure dissociation of the polypeptide chains. CNBr peptides were separated by SDS-PAGE on a 12.5% gel by the method of Laemmli (23) and stained with coomassie brilliant blue. Standards of purified types I and III collagen prepared from equine fetal skin and digested with CNBr as above, were also separated by electrophoresis along with the samples. The bands α1(I)CB8 and α1(III)CB5 were used to quantify types I and III collagen respectively. Quantification was carried out using a transilluminator and LabWorks™ (version 3.0.02.00) software (UVP Ltd, Cambridge).

**Collagen crosslink analysis**  The method used for the preparation and measurement of collagen crosslinks was that of Sims and Bailey (42). Approximately 15 mg of dry tissue was suspended in phosphate buffered saline, reduced with potassium borohydride and hydrolysed. Crosslinked amino acids were separated from non-crosslinked amino acids by fractionation on a CF-1-cellulose column. Crosslinked compounds were separated on a LKB 4400 amino acid analyzer.
and identified by comparison with a standard crosslink preparation. Results are expressed as moles of crosslink per mole of collagen.

**Mechanical properties**

The left SDFT and CDET from each horse were thawed at room temperature and mounted in a servohydraulic materials testing machine using cryoclamps. Tendons were preloaded with 25 N (CDET) or 100 N (SDFT) before precondition using 20 cycles from the preload to 1.5 kN (CDET) or 4 kN (SDFT). Tendons were then loaded to failure at 200% s\(^{-1}\) (SDFT) or 50% s\(^{-1}\) (CDET). Force and deformation data were collected and the ultimate force, ultimate stress, Stiffness and elastic modulus calculated.

**Statistical analysis**  Statistical significance was evaluated using a general linear model in SPSS (Version 13, Microsoft). Tendon type and exercise group were used as fixed factors for each variable measured. If tendons were found to be significantly different to each other for a variable they were considered separately to assess the influence of the exercise group for that variable. Differences between central and peripheral zone tissue in the SDFT were assessed using zone and exercise group as fixed factors. The level of significance was taken as \( p \leq 0.05 \). Data are presented as mean ± S.D.

**Results**

**Gross morphology and histological examination**  All tendons appeared macroscopically normal on examination during the harvesting procedure and showed no signs of central core discolouration. H & E staining of longitudinal sections of the SDFT showed parallel collagen
fibre alignment and rows of elongated nuclei with no apparent differences between sections from high and low intensity trained tendons (Fig. 1a & b). The CSA did not differ significantly between high and low intensity exercised groups for any of the structures (Table 1) as reported previously (5).

Water Content  Water content, which can have a significant influence on tissue stiffness, ranged from 56.3% to 67.6% and all structures were significantly (p < 0.001) different to each other (Table 2). The water content did not differ between the central and peripheral zone tissue for the SDFT. Water content was not significantly different between low and high intensity trained groups for the SDFT, DDFT and SL. The CDET from the high intensity trained horses however had a significantly (p = 0.007) lower water content than the CDET from the low intensity trained horses (Table 2).

DNA content  DNA content gives an indication of cellularity and did not differ between the central and peripheral zone tissue of the SDFT but was significantly different between the structures (p < 0.001). The SL had the highest levels followed by the SDFT then the DDFT while the CDET had the lowest levels (Table 2). DNA content did not differ significantly between the low and high intensity exercised groups for any of the structures.

Glycosaminoglycan content  Glycosaminoglycans influence tissue hydration and have also been implicated in the control of collagen fibril diameters. The high intensity trained group of horses had a significantly lower GAG content in the SDFT (p = 0.003), SL (p = 0.021) and CDET (p = 0.026) than the low intensity trained group (Table 2). This difference was greatest in the central
zone of the SDFT (Fig. 2). The GAG content was significantly different between structures (p < 0.001). The CDET had a significantly lower GAG content than all the other structures (Table 2). The SDFT had a significantly lower GAG content than the SL but was not significantly different to the DDFT. The SL had a significantly higher GAG content than the CDET and SDFT but was not significantly different to the DDFT. There was no significant difference between the central and peripheral zone tissue of the SDFT.

Collagen content  The collagen component provides high tensile strength and content varied between 70.7% and 75.2% of the dry weight of tendon tissue and this was significantly (p ≤ 0.018) higher than the collagen content of the SL (Table 2). Collagen content did not differ significantly between the central and peripheral zone tissue of the SDFT. The SL had a significantly (p = 0.012) higher collagen content in the high intensity exercised group compared to the low intensity exercised group of horses (Table 2).

Collagen crosslinks  The ability of collagen to resist high tensile forces depends on the formation of strong covalent crosslinks between collagen molecules. The predominant mature crosslink detected in the SDFT, DDFT and SL samples was hydroxylysylpyridinoline (HP). The levels did not differ significantly between the high and low intensity trained horses for any of the structures (Table 2). There was no difference between the central and peripheral zone tissue of SDFT, however the SDFT, DDFT and CDET had significantly different levels (p < 0.001) to each other with the SDFT having the highest levels followed by the DDFT and the CDET having the lowest levels. Histidinohydroxylysinoonorleucine (HHL), a major crosslink found in mature skin, was detected in all the CDET samples (0.069 ± 0.005 moles/mole collagen) and some of
the DDFT samples at trace levels but did not differ significantly between high and low intensity trained groups. A further peak of histidinohydroxymesodesmosine (HHMD) was detected in high amounts in the CDET samples (1.24 ± 0.15 moles/mole collagen) and at trace levels in the DDFT samples but did not differ significantly between high and low intensity trained groups. The divalent immature aldimine crosslink dehydro-hydroxylysinonorleucine (dehydro-HLNL) was detected in the CDET samples (0.04 ± 0.01 moles/mole collagen) but none of the other structures.

Type III collagen The ratio of type III collagen to type I collagen has been shown to increase in degenerated tendons (3). The central zone tissue of the SDFT contained 9.7 ± 2.2% type III (high intensity group) and 7.5 ± 4.0% (low intensity group) and this was not significantly different between the two exercise groups (95% confidence interval for difference between means = -2.3 to +6.8).

Tissue fluorescence Tendon tissue fluorescence has been shown previously to correlate to the age of the horse (4) and shows a significant decrease in the core of degenerated SDFT relative to the peripheral zone tissue (3). The tissue fluorescence was not significantly different between central and peripheral zone tissue of the SDFT. The high intensity trained group had lower levels of tissue fluorescence in all the structures than the low intensity trained group (Table 2) and this was significant (p < 0.001) when all structures were grouped together. The CDET had significantly (p ≤ 0.011) lower levels of fluorescence than the SDFT, DDFT and SL however the other structures were not significantly different to each other.
Mechanical properties  The force and stress withstood by the tendons before gross failure was not significantly different between the high intensity and low intensity trained groups for the SDFT or CDET (Table 3). The SDFT had a similar stiffness and elastic modulus in the linear region of the loading curve for both exercise groups. The elastic modulus of the CDET however was significantly (p=0.004) higher following high intensity training (Table 3).

Discussion

The results of this study do not support our hypothesis that high intensity exercise results in tendon degeneration in the injury prone SDFT. We were unable to show any of the matrix changes such as increased type III/I collagen ratios, increased sulphated GAG levels, high cellularity and low tissue fluorescence associated with macroscopically degenerated equine tendons; a condition we consider to be analogous to long standing painless tendinopathy in humans (27; 37). It may be that a more intense training programme would have resulted in overt tendon damage however the level of activity in this study was similar to that experienced by horses in race training where tendinopathies are common. Furthermore, if our high intensity trained group of horses had been compared to a group of horses confined to box rest we may have seen significant differences but this would not be of particular relevance to high intensity exercise versus moderate levels of activity.

Although the tendons studied showed remarkably few changes in response to the high intensity training regime, tendons which have evolved for different functional roles varied in their response to the increased exercise. This may, in part, explain the variation in results obtained in other training studies where a variety of tendons such as the Achilles (13), anterior tibialis (32), flexor digitorum longus (32), digital extensor (54) and patella (22; 38) tendons have
been assessed. This difference in response is likely to relate to the different physiological function of individual tendons and also differences in their composition and rates of maturation. Indeed, the data from this study have demonstrated marked differences in the matrix composition including collagen crosslinks, sulphated GAG and cellularity between the anatomically and functionally distinct structures. Such variation is likely to result in specific mechanical properties which relate to the different role of these tendons and ligament during locomotion.

In response to high intensity training, the positional low-strain and rarely injured tendon (CDET) showed a decrease in water content while the proportion of collagen in the dry weight of tissue remained the same signifying an over all increase in collagen. This change would be expected to increase the stiffness of the tendon tissue, a response which seems appropriate for a positional tendon such as the CDET which is required to be relatively inextensible for efficient function. This finding is supported by the mechanical data showing a significantly higher elastic modulus thus demonstrating a stiffer material in the high intensity trained CDETs even though fibrils diameters and interfibrillar spacing were not significantly different as reported previously (9).

In contrast to the positional tendon, in response to high intensity training the high-strain SDFT did not show a decrease in water content or an increase in collagen content. When considering the function of the tendon this is not surprising given that the force the SDFT experiences is due, to a large extent, the gravitational and inertial forces of locomotion rather than muscle contraction (52) and this would not be expected to increase with high intensity training. An increase in collagen content or an increase in the CSA, which would both increase the stiffness of the structure, would reduce the efficiency of the SDFT. Indeed the mechanical data demonstrate no changes in material stiffness or ultimate properties in the SDFT following
high intensity training. Furthermore, we have previously reported no difference in the CSA of the SDFT from the high and low intensity trained horses (5).

Interestingly, the SL which is also a high-strain energy storing structure showed a significantly higher collagen content in the high intensity trained group of horses. This apparent contradiction is likely to result from a difference in the maturation time of the different structures studied. The SL is a vestige of the interosseus muscle and at birth has a considerable muscle component. As the ligament matures the muscular tissue is replaced by collagenous tissue. The results of the present study suggest that this maturation process is accelerated by the imposition of high intensity exercise or alternatively may be slowed down in the low intensity trained group by withholding high speed exercise. In the high intensity trained group of horses the collagen content of the SL was not significantly different to that of the SDFT.

In the energy storing structures following long term high intensity exercise, the levels of sulphated GAG were significantly lower compared to low intensity trained horses. Sulphated GAG chains are a component of proteoglycan molecules which in the tensional region of tendon are represented predominantly by the family of small leucine-rich proteoglycans namely decorin, fibromodulin, biglycan (49) and lumican (15). These molecules bind to collagen fibrils and play a role in fibrillogenesis and regulation of collagen fibril diameters (7; 8; 15; 47).

Correspondingly, we have previously reported that collagen fibril diameters were significantly smaller in the core of the SDFT from high intensity trained horses than those in the low intensity trained horses (35) although the area covered by collagen was not significantly different between the two groups (high intensity group – 66.2%, low intensity group – 71.7%). However, reduction in collagen fibril diameters did not occur in the SL or the positional tendon following high intensity training despite a lower sulphated GAG content (9; 34). Other non-collagenous proteins
have been implicated in the process of collagen fibril formation including the glycoprotein, cartilage oligomeric matrix protein (COMP). The level of COMP was also found to be lower in the central zone tissue of the SDFT in the high intensity trained horses as reported previously (43).

The analyses of the SDFT extracellular matrix suggested that accelerated collagen formation was not taking place. For example, we were unable to detect any immature crosslinks and the levels of tissue fluorescence, which we have previously found to be a good indicator of matrix age (3; 4) did not change. Our results therefore suggest that small diameter collagen fibrils result from breakdown of larger fibrils. Small diameter collagen fibrils are associated with tissues with a less stiff matrix and this has been suggested to be due to an increase in interfibrillar interactions relative to crosslinks within the fibrils (33). This change in the matrix of the SDFT may be an adaptive response to increase the elasticity of the tendon or may represent micro-damage although we were not able to detect either with mechanical testing, whichever way; the mechanism for this is unclear but may involve loss of the proteoglycan molecule. The measurement of sulphated GAG content does not differentiate between loss of the whole proteoglycan molecule from the matrix, removal or partial removal of the GAG chain from the protein core or synthesis of new proteoglycan molecules with shorter GAG chains. Interestingly, smaller fibril diameters and reduced levels of sulphated GAG have been reported in old tendons suggesting reduction occurs as part of the natural ageing process (39; 46).

Studies into the effects of exercise on human tendons are restricted to measurements that can be made non-destructively in vivo. Although this precludes matrix composition and tissue morphology analysis, measurement of gross tendon CSA and stiffness have been made using imaging techniques. Several studies comparing athletes and non-athletes found that the CSA of
the energy storing Achilles tendon was larger in the athletes suggesting that there might be an adaptive hypertrophy in response to training (28; 41; 55). However a subsequent study which measured Achilles tendon CSA and stiffness before and after 9 months of training found no change (13). In contrast, strength training of the quadriceps muscle group in young and old human subjects was found to increase the stiffness and elastic modulus of the patella tendon (22; 38); a positional tendon having a similar function to the equine CDET. Studies in other species also suggest a tendon specific response to an imposed training regime. Following 12 months running exercise of miniature swine the digital extensor tendons underwent significant hypertrophy while the flexors tendons were not significantly larger than those of the sedentary animals (53; 54).

Exercise studies in humans have not been able to assess collagen and proteoglycan content and organization within tendon. Measurements have however been made on peritendinous tissue around the human Achilles tendon and suggest an increase in blood flow (24), collagen turnover (25) and change in protease activity (21) with exercise which may represent an adaptive response (20).

In conclusion, the results of this study show that specific tendons respond differently to an imposed training regime. Long term high intensity exercise resulted in an adaptive change in the low-strain rarely injured CDET whereas changes observed in the high-strain energy storing SDFT suggest accelerated ageing as a result of training although macroscopic pathology was not evident. We were not able to show any differences in the mechanical properties of the SDFT and therefore it is difficult to say whether these ageing associated changes represent a beneficial response or very early signs of micro-damage. This study is not able to determine whether these changes occurred in the first few months of training or in the longer term hence further work is
needed to determine whether a rest period between episodes of high intensity training would
reverse the changes seen. In addition, the analyses in this study showed substantial differences
between functionally distinct tendons. The precise relationship between composition, structure
and function is an important area with regards to tendon and ligament physiology.
Acknowledgement

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Figure Legend

**Figure 1**  H & E stained longitudinal section of the SDFT (x400) from a high intensity trained horse (a) and low intensity trained horse (b).

**Figure 2** Total sulphated glycosaminoglycan content in the central and peripheral zone tissue of the SDFT following high and low intensity training. Data are presented as mean ± S.D.
Table 1  Horse age, body weight and tendon cross sectional area at the end of the training period.

<table>
<thead>
<tr>
<th>Exercise group</th>
<th>Age (months)</th>
<th>Body wt (kg)</th>
<th>SDFT</th>
<th>DDFT</th>
<th>SL</th>
<th>CDET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low intensity</td>
<td>38.4 ± 1.1</td>
<td>504 ± 32</td>
<td>105 ± 25</td>
<td>159 ± 5</td>
<td>178 ± 37</td>
<td>32 ± 2</td>
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<tr>
<td>(n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High intensity</td>
<td>39.0 ± 1.2</td>
<td>452 ± 21*</td>
<td>98 ± 8</td>
<td>165 ± 12</td>
<td>189 ± 28</td>
<td>31 ± 3</td>
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<tr>
<td>(n=5)</td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

Data are presented as mean ± S.D. Areas are in mm². * denotes a significant difference relative to the low intensity trained group.
Table 2  Matrix composition of the SDFT, DDFT, SL and CDET following low and high intensity training.

<table>
<thead>
<tr>
<th></th>
<th>SDFT</th>
<th>DDFT</th>
<th>SL</th>
<th>CDET</th>
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<tr>
<td>% Water Content</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High intensity</td>
<td>64.3 ± 0.8</td>
<td>61.8 ± 1.2</td>
<td>67.6 ± 1.5</td>
<td>56.3 ± 0.8 *</td>
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<tr>
<td>Low intensity</td>
<td>64.1 ± 0.9</td>
<td>61.4 ± 1.3</td>
<td>67.4 ± 1.1</td>
<td>57.7 ± 0.5</td>
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<td>CI</td>
<td>-1.0 to +1.5</td>
<td>-1.3 to +2.2</td>
<td>-1.6 to +2.0</td>
<td>-2.2 to -0.5</td>
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<tr>
<td>DNA (µg/mg)</td>
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<td></td>
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</tr>
<tr>
<td>High intensity</td>
<td>1.49 ± 0.13</td>
<td>0.73 ± 0.16</td>
<td>2.32 ± 0.29</td>
<td>0.39 ± 0.09</td>
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<td>Low intensity</td>
<td>1.53 ± 0.19</td>
<td>0.85 ± 0.12</td>
<td>2.23 ± 0.31</td>
<td>0.54 ± 0.22</td>
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<td>CI</td>
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<td>-0.39 to +0.09</td>
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<tr>
<td>GAG (µg/mg)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>High intensity</td>
<td>8.1 ± 1.1 *</td>
<td>9.6 ± 3.5</td>
<td>12.1 ± 1.2 *</td>
<td>2.2 ± 0.4 *</td>
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<tr>
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<td>9.9 ± 1.3</td>
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<td>Collagen (mg/mg)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High intensity</td>
<td>0.72 ± 0.05</td>
<td>0.75 ± 0.05</td>
<td>0.68 ± 0.07 *</td>
<td>0.72 ± 0.03</td>
</tr>
<tr>
<td>Low intensity</td>
<td>0.72 ± 0.04</td>
<td>0.73 ± 0.04</td>
<td>0.57 ± 0.04</td>
<td>0.71 ± 0.02</td>
</tr>
<tr>
<td>CI</td>
<td>-0.07 to +0.06</td>
<td>-0.04 to +0.08</td>
<td>+0.03 to +0.19</td>
<td>-0.02 to +0.04</td>
</tr>
<tr>
<td>Pyridinoline (mol/mol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High intensity</td>
<td>0.48 ± 0.07</td>
<td>0.35 ± 0.06</td>
<td>0.45 ± 0.05</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Low intensity</td>
<td>0.47 ± 0.05</td>
<td>0.36 ± 0.04</td>
<td>0.48 ± 0.04</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>CI</td>
<td>-0.08 to +0.08</td>
<td>-0.08 to +0.05</td>
<td>-0.10 to +0.04</td>
<td>-0.02 to +0.03</td>
</tr>
</tbody>
</table>
Tissue fluorescence (units/mg)

<table>
<thead>
<tr>
<th></th>
<th>High intensity</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>152.7 ± 9.9</td>
<td>149.0 ± 7.8</td>
<td>160.2 ± 7.9</td>
</tr>
<tr>
<td>Low intensity</td>
<td>156.8 ± 15.7</td>
<td>160.7 ± 13.0</td>
<td>162.0 ± 19.0</td>
<td>135.3 ± 24.2</td>
</tr>
<tr>
<td>CI</td>
<td>-22.6 to +14.3</td>
<td>-26.8 to +3.4</td>
<td>-22.5 to +18.9</td>
<td>-47.6 to +10.1</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.D. * denotes a significant difference relative to the low intensity trained group. CI = 95% confidence interval for difference between means.
Table 3  Mechanical properties of the SDFT and CDET following low and high intensity training.

<table>
<thead>
<tr>
<th></th>
<th>SDFT</th>
<th>CDET</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ultimate force (N)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>High intensity</em></td>
<td>$13355 \pm 2923$</td>
<td>$5307 \pm 789$</td>
</tr>
<tr>
<td><em>Low intensity</em></td>
<td>$13520 \pm 4188$</td>
<td>$5420 \pm 1474$</td>
</tr>
<tr>
<td><em>CI</em></td>
<td>$-5739$ to $+5369$</td>
<td>$-1782$ to $+1555$</td>
</tr>
<tr>
<td><strong>Ultimate stress (MPa)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>High intensity</em></td>
<td>$123 \pm 24$</td>
<td>$203 \pm 34$</td>
</tr>
<tr>
<td><em>Low intensity</em></td>
<td>$128 \pm 33$</td>
<td>$187 \pm 43$</td>
</tr>
<tr>
<td><em>CI</em></td>
<td>$-50$ to $+39$</td>
<td>$-37$ to $+70$</td>
</tr>
<tr>
<td><strong>Stiffness index (kN/strain)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>High intensity</em></td>
<td>$135 \pm 20$</td>
<td>$39.7 \pm 1.6$</td>
</tr>
<tr>
<td><em>Low intensity</em></td>
<td>$136 \pm 17$</td>
<td>$37.6 \pm 4.9$</td>
</tr>
<tr>
<td><em>CI</em></td>
<td>$-28$ to $+27$</td>
<td>$-3.1$ to $+7.3$</td>
</tr>
<tr>
<td><strong>Elastic modulus (MPa)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>High intensity</em></td>
<td>$1245 \pm 121$</td>
<td>$1514 \pm 55$ *</td>
</tr>
<tr>
<td><em>Low intensity</em></td>
<td>$1310 \pm 95$</td>
<td>$1303 \pm 110$</td>
</tr>
<tr>
<td><em>CI</em></td>
<td>$-228$ to $+99$</td>
<td>$+88$ to $+333$</td>
</tr>
</tbody>
</table>

Data are presented as mean $\pm$ S.D. * denotes a significant difference relative to the low intensity trained group. CI = 95% confidence interval for difference between means.
H & E stained longitudinal section of the SDFT (x400) from a high intensity trained horse (a) and low intensity trained horse (b).
35x22mm (320 x 320 DPI)
H & E stained longitudinal section of the SDFT (x400) from a high intensity trained horse (a) and low intensity trained horse (b).
35x23mm (320 x 320 DPI)