Physical activity: does long-term, high-intensity exercise in horses result in tendon degeneration?

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Birch HL, Wilson AM, Goodship AE. Physical activity: does long-term, high-intensity exercise in horses result in tendon degeneration? J Appl Physiol 105: 1927–1933, 2008. First published October 2, 2008; doi:10.1152/japplphysiol.00717.2007.—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 that 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 mo. The SDFT, suspensory ligament, deep digital flexor tendon, and CDET were harvested from the forelimb. Tissue from the mid-metacarpal region of the right limb tendons was analyzed for water, DNA, sulfated glycosaminoglycan and collagen content, collagen type III-to-I ratios, collagen cross-links, 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. There were no signs of degeneration or change in mechanical properties of the SDFT. The CDET had a lower water content associated with increasing age, although aging in equine tendons results in matrix changes that do not equate to degeneration (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. The findings to date suggest that it is particularly important to determine the effects of exercise in skeletally mature indi-
individuals undertaking a well-defined exercise regime and to study the effects on matrix biology of tendons that 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 postmortem, 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-, sex-, and breed-matched individuals can be carried out, and tissue harvested for 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.

**METHODS**

**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 was randomly assigned to the high-intensity exercise group, and the other to a low-intensity exercise group. All horses were 18 mo 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 × 3.05 m) throughout the study. The study was conducted with appropriate regulatory approvals.

**Training regime.** The horses in the high-intensity exercise group were trained on a high-speed equine treadmill for 18 mo at a level similar to that which would be experienced in race training. A typical week’s work was as follows: Monday, 3 km at 12 m/s, 3% slope; Wednesday, two times, 1.5 km at 12 and 14 m/s, 4% slope, 5-min recovery; and Friday, three times, 1 km at 12, 13, and 15 m/s, 3% slope, 5-min recovery. This was combined with 40-min walking on a mechanical horse walker 6 days/wk and 20-min trotting on days when no treadmill exercise was given. Horses in the low-intensity exercise group undertook only walking, and this was for 40 min 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 discoloration. 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 −80°C before analysis. An adjacent 1-cm section from the SDFT was fixed in formalin and processed for routine hematoxylin and eosin staining for qualitative assessment of fiber 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 were 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 −20°C for mechanical testing.

**Water content.** Tissue was semin thawed at room temperature, and outer loose connective tissue and epitenon were 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.** Before DNA and GAG measurements, the lyophilized tissue was solubilized by papain digestion, as described previously (3). DNA was assayed by the fluorimetric method of Kim et al. (19) using the bisbenzimide 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 were 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 to 0.5 μg/ml. DNA content in tendon samples is expressed as micrograms DNA per milligram dry weight tissue and tissue fluorescence as arbitrary units per milligram of collagen.

**GAG assay.** Total sulfated 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 sulfate (0–10 μg in 3 ml dye). Results are expressed as micrograms chondroitin sulfate equivalent sulfated GAG per milligram dry weight tissue.

**Collagen content.** Collagen content was determined by measuring the amino 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.** Lyophilized tissue samples (~5 mg) 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 sulfate, 10% glycerol, 0.01% bromophenol blue) and heated for 30 min at 60°C 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

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>n</th>
<th>Age, mo</th>
<th>Body Weight, kg</th>
<th>SDFT, mm²</th>
<th>DDFT, mm²</th>
<th>SL, mm²</th>
<th>CDET, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low intensity</td>
<td>6</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>
</tr>
<tr>
<td>High intensity</td>
<td>5</td>
<td>39.0±1.2</td>
<td>452±2.1*</td>
<td>98±8</td>
<td>165±12</td>
<td>189±28</td>
<td>31±3</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of horses. SDFT, superficial digital flexor tendon; DDFT, deep digital flexor tendon; SL, suspensory ligament; CDET, common digital extensor tendon. *Significant difference relative to the low-intensity-trained group, P ≤ 0.05.
The high-intensity-trained horses, however, had a significantly lower water content than the CDET from the low-intensity-trained horse \((P = 0.003)\), and SDFT \((P = 0.026)\) 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 of the other structures (Table 2). The SDFT had a significantly lower GAG content than the SL, but was not significantly different from the DDFT. The SL had a significantly higher GAG content than the CDET and SDFT, but was not significantly different from 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 with the low-intensity exercised group of horses (Table 2).

**Collagen cross-links.** The ability of collagen to resist high-tensile forces depends on the formation of strong covalent cross-links between collagen molecules. The predominant mature cross-link detected in the SDFT, DDFT, and SL samples was hydroxylysylpyridinoline. 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) from each other, with the SDFT having the highest levels followed by the DDFT, and the CDET having the lowest levels. Histidinohydroxylysinonorleucine, a major cross-link found in mature skin, was detected in all of the CDET samples (0.069 ± 0.005 mol/mol 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 was detected in high amounts in the CDET samples (1.24 ± 0.15 mol/mol collagen) and at trace levels in the DDFT samples, but it did not differ significantly between high- and low-intensity-trained groups. The divalent immature aldimine cross-link dehydrohydroxylsinonorleucine was detected in the CDET samples (0.04 ± 0.01 mol/mol 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 of 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 from each other.

**Mechanical properties.** The force and stress withstood by the tendons before gross failure were 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).
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>Ultimate force, N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High intensity</td>
<td>13,335±2,923</td>
<td>5,307±789</td>
</tr>
<tr>
<td>Low intensity</td>
<td>13,520±4,188</td>
<td>5,420±1,474</td>
</tr>
<tr>
<td>CI</td>
<td>−5,739 to +5,369</td>
<td>−1,782 to +1,555</td>
</tr>
<tr>
<td>Ultimate stress, MPa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High intensity</td>
<td>123±24</td>
<td>203±34</td>
</tr>
<tr>
<td>Low intensity</td>
<td>128±33</td>
<td>187±43</td>
</tr>
<tr>
<td>CI</td>
<td>−50 to +39</td>
<td>−37 to +70</td>
</tr>
<tr>
<td>Stiffness index, kN/strain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High intensity</td>
<td>135±20</td>
<td>39,7±1.6</td>
</tr>
<tr>
<td>Low intensity</td>
<td>136±17</td>
<td>37.6±4.9</td>
</tr>
<tr>
<td>CI</td>
<td>−28 to +27</td>
<td>−3.1 to +7.3</td>
</tr>
<tr>
<td>Elastic modulus, MPa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High intensity</td>
<td>1,245±121</td>
<td>1514±55*</td>
</tr>
<tr>
<td>Low intensity</td>
<td>1,310±95</td>
<td>1,303±110</td>
</tr>
<tr>
<td>CI</td>
<td>−228 to +99</td>
<td>+88 to +333</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Significant difference relative to the low-intensity-trained group, P ≤ 0.05.

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 sulfated 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 program 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 with 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 vs. moderate levels of activity.

Although the tendons studied showed remarkably few changes in response to the high-intensity training regime, tendons that 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 cross-links, sulfated 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 overall increase in collagen. This change would be expected to increase the stiffness of the tendon tissue, a response that 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 fibril 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, our laboratory has 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 sulfated GAG were significantly lower compared with low-intensity-trained horses. Sulfated 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, our laboratory has 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 sulfated GAG content (9, 34). Other noncollagenous proteins have been implicated in the process of collagen fibril formation, including the glycoprotein, cartilage oligomeric matrix protein. The level of cartilage oligomeric matrix protein 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 cross-links, and the levels of tissue fluorescence, which our laboratory has 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 cross-links 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 microdamage, 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 sulfated 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 sulfated GAG have been reported in old tendons, suggesting reduction occurs as part of the natural aging process (39, 46).

Studies into the effects of exercise on human tendons are restricted to measurements that can be made nondestructively 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 nonathletes 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 mo 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 into the effects of exercise on human tendons are limited to measurements that can be made nondestructively 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 nonathletes 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 mo 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-mo running exercise of miniature swine, the digital extensor tendons underwent significant hypertrophy, while the flexor 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 aging 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 aging-associated changes represent a beneficial response or very early signs of microdamage. 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 regard to tendon and ligament physiology.

GRANTS

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REFERENCES


