The equine forelimb suspensory ligament exhibits a heterogeneous strain pattern under tensile load

M. Lavagnino; J. Madison; M. R. Williams; S. P. Arnoczky

Laboratory for Comparative Orthopaedic Research, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA

Keywords

Equine, suspensory ligament, strain

Summary

Objectives: To determine if regional variations in strain patterns occur within the suspensory ligament under tensile load. Local increases in strain may put certain regions of the suspensory ligament at risk and may explain the poor healing and high recurrence rates associated with suspensory branch injuries.
Methods: The suspensory ligament and its bone attachments were isolated from each of 10 adult equine cadaveric forelimbs and radiodense reference beads were inserted throughout the length of the ligament. Specimens were attached to a custom fixture secured to a materials testing system. Radiographs were acquired at 50, 445, 1112, and 2224 N of applied tensile load. Changes in distances between the beads in each region of the suspensory ligament were measured and the regional strain was calculated. Significant differences were determined using a repeated-measures analysis of variance.
Results: The suspensory ligament exhibited significant differences in regional strain ($p < 0.001$). The distal branches of the suspensory ligament had significantly greater strains than the proximal ($p = 0.025$) and mid-body ($p = 0.002$) regions. The mid-body of the suspensory ligament also exhibited local strain variation, with the distal mid-body having significantly higher strains than the proximal mid-body ($p = 0.038$).
Clinical significance: The equine suspensory ligament demonstrates a heterogeneous strain pattern during tensile loading, with the distal regions exhibiting significantly more strain than the proximal region. The non-homogenous strain pattern could explain the regional difference in injury and re-injury rates.

Correspondence to:
Steven Paul Arnoczky, DVM
College of Veterinary Medicine
784 Wilson Road, G-387
Michigan State University
East Lansing, MI 48824
United States
Phone: +1 517 353 8964
Fax: +1 517 353 8980
E-mail: arnoczky@cvm.msu.edu

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Introduction

The equine forelimb suspensory ligament is a commonly injured structure in many breeds and disciplines, with resultant impact on performance and long-term athletic career potential (1–5). Previous reports in the literature indicate a difference in healing and re-injury rates depending on the region of the suspensory ligament injured, with mid-body and branch injuries having higher recurrence rates as compared to the proximal region (2, 6). A clinical study has reported that, with stall rest alone, up to 90% of horses with acute proximal suspensory lesions returned to their previous activity level (6). In contrast, another study noted that mid-body suspensory desmitis in event horses was associated with a high recurrence rate, even after being treated with extended periods of rest (2). Despite anecdotal reports of a fair prognosis with suspensory ligament branch injuries in sport horses, consistent reports of high rates of recurrence have been noted (2, 6-8). However, the physiological basis for these variations in healing and re-injury remain unclear.

Previous reports from the human and veterinary literature have demonstrated a direct correlation between areas of decreased vascular supply and the common sites of tendon injury and compromised healing (9–18). However, a recent study found a consistently abundant intra-ligamentous microvascular supply throughout all regions of the equine suspensory ligament (19). In addition, while regional differences in biochemical characteristics of the normal equine suspensory ligament have been described, these are thought to be due to the various tissue origins of the different regions during foetal development and they do not appear to be related to the reported regional differences in healing and re-injury patterns (20, 21).

One possible explanation for the reported regional differences in healing and re-injury rate may be related to the biomechanical characteristics of the equine suspensory ligament. Previous experimental analyses have demonstrated a heterogeneous strain pattern in both the patellar and supraspinatus tendons of humans under simulated loading conditions (22–24). A localized increase in tendon strain was found to occur at the common sites of injury in these tendons and is thought to contribute to the impaired heal-

Figure 1 Image of the test set-up used to capture radiographic images of the equine forelimb suspensory ligament under varying applied tensile loads to determine regional ligament strains. The image shows the position of the x-ray unit, imaging plate, calibration marker, and the equine forelimb suspensory ligament specimen pinned into the fixture and attached to the material testing system.

ing and high re-injury rate observed in these structures (22–24). A similar situation may exist in the equine forelimb suspensory ligament.

Previous studies have recorded strains in the equine suspensory ligament as a single unit using surgically implanted strain gauges or ultrasonic kinematic markers (25-29). In addition, the mechanical properties of tissue segments removed from the mid-body and branch regions of the suspensory ligament have also been examined (20). However, to our knowledge, an analysis of the differential strains for each region of the entire, intact suspensory ligament has not been performed. Therefore, the purpose of the current study was to compare regional strains in the proximal, mid-body, and distal regions of the equine forelimb suspensory ligament *in vitro.* It was hypothesized that strain percentage would significantly increase progressively from the proximal to distal regions of the suspensory ligament. Significant differences in regional strains could provide a mechanical explanation for the poor healing and high rate of recurrence observed for injuries to the mid-body and distal branch regions of the suspensory ligament.

Materials and methods

Equine forelimbs (n = 10) were obtained from adult (8 to 16 years of age) mares (n = 6) and geldings (n = 4) of either Thoroughbred (n = 6) or American Quarter Horse (n = 4) breed. The horses were euthanatized for unrelated reasons and the forelimbs were dissected to isolate the suspensory ligament and its bone attachments (3rd metacarpal, proximal sesamoid bones, proximal and middle phalanges). The specimens were secured to a custom designed testing fixture with 6.35 mm diameter stainless steel pins drilled in the medial to lateral direction through the proximal aspect of the third metacarpal bone (2 holes), the distal aspect of the third metacarpal bone, the proximal phalanx, and the middle phalanx. The mid portion of the third metacarpal bone was then removed using a band saw. Radiodense beads were inserted at approximately one centimetre intervals along the palmar midline of the ligament and along its branches. Small stab incisions were made into the palmar aspect of the ligament with a number 11 scalpel blade and individual 2.6 mm diameter beads were inserted. Each stab incision was then closed with a single, simple, interrupted suture or cruciate suture of 2 metric or 3 metric polyglycolic acid or polyglactin 910. The fixture and specimen were aligned vertically along the length of the ligament in a materials testing system[a] with an 8896 N load cell (▶Figure 1). Initially, a 50 N preload was applied and

a Instron 8501: Instron Corp., Canton, MA, USA

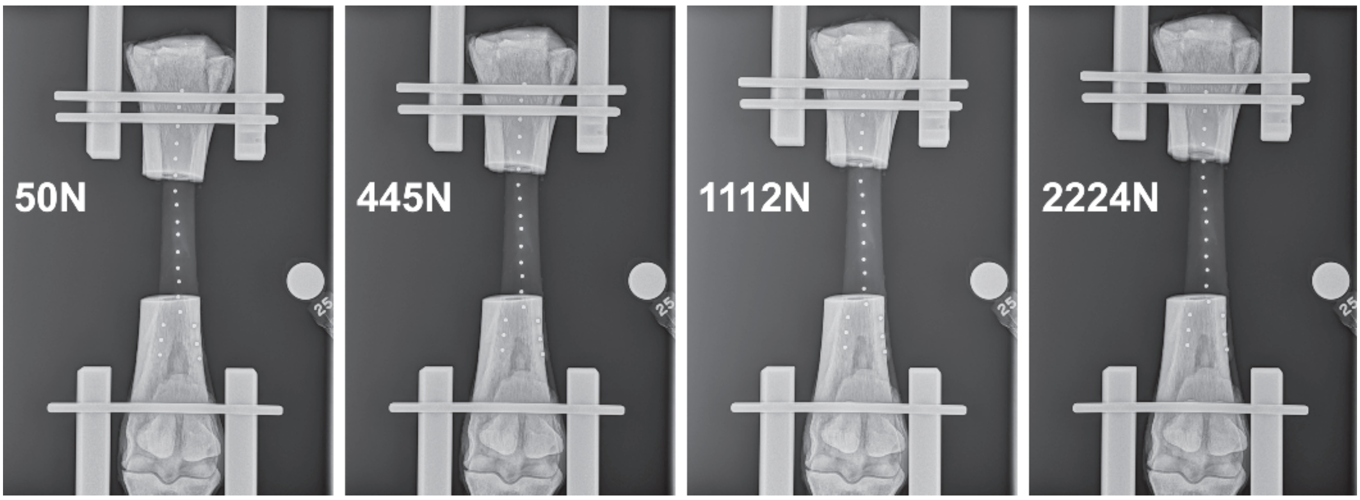

Figure 2 Radiographic image series of a representative equine forelimb suspensory ligament under 50, 445, 1112, and 2224 N of applied tensile load with radiodense beads spaced throughout the ligament to help determine regional strains.

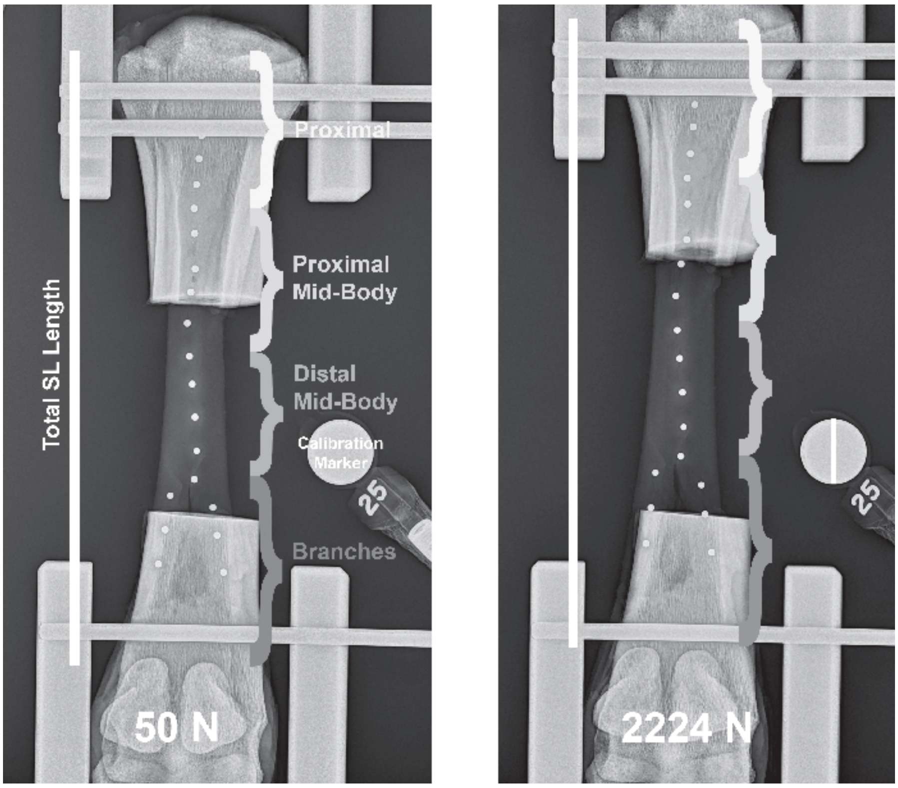

Figure 3 Representative radiographic images of an equine forelimb suspensory ligament under 50 and 2224 N of load. Defined regions of the suspensory ligament were based on previous clinical definitions and determined using osseous landmarks and radiodense beads. A 25 mm radiodense calibration marker aligned in the plane of the suspensory ligament was used to address any magnification disparities between specimens.

maintained on the specimen while a palmarodorsal radiograph was acquired to both ensure a proper alignment and as an initial point to measure changes in length of the suspensory ligament with increased loading. The material testing system was then programmed, in a load control manner, to proceed in a stepwise fashion with a linear increase in load to the next predetermined load level over a 10 second period. Loading was momentarily paused for 100 seconds, as per the programming protocol, at predetermined load levels of 445 N, 1112 N, and 2224 N while radiographs were acquired. (►Figure 2). These predetermined loads were within the lower levels of physiological loads on the suspensory ligament that have been reported to range from 3000–4000 N at a walk to 6000-13000 N at a trot (30-32). Force and displacement data were recorded at 100 Hz. Digital radiographs were acquired using an x-ray unit[b] and a wireless plate detector[c].

The radiographic images were analysed using imaging software[d] to calculate regional strain in the previously described proximal, proximal mid-body, distal mid-body, and branch portions of the suspensory ligament (►Figure 3) (33). The overall suspensory ligament length was measured from the proximal epiphyseal attachment to the top of the proximal sesamoid bones. The proximal region was defined as the proximal fourth of the total length of the suspensory ligament while the mid-body of the suspensory ligament was defined as being located between the proximal fourth and the point where the suspensory ligament separates into the lateral and medial branches (33). The mid-body region of the suspensory ligament was then further bisected into proximal and distal mid-body segments. The suspensory ligament branch length was determined by measuring the vertical length between the branching point of the suspensory ligament to the proximal aspect of one of the proximal sesamoid bones. For each specimen, the medial or the lateral proximal sesamoid bone was chosen and maintained as the end point of the branch length for all of the applied loads. The radiodense beads closest to these delineations were used as fiduciary markers to calculate the strain of the respective suspensory ligament regions.

The length of each defined portion of the suspensory ligament was measured at the preload (50 N) to obtain the original length (L_O) as well as at the applied loads (445, 1112, and 2224 N) to obtain the applied lengths (L_A). The regional strain values were calculated by dividing the change in length of the suspensory ligament region with each applied load by the original length [strain = $(L_A - L_O)/L_O$]. Each radiographic image had a 25 mm spherical calibration marker[e] that was aligned with the suspensory ligament and used to scale each radiographic image and account for radiographic magnification variations.

To determine if strain differences existed between regions at each of the applied loads (445, 1112, or 2224 N), a repeated measures analysis of variance (ANOVA) was performed at each applied load with a Bonferroni post-hoc analysis[f]. Significance was set at $p \leq 0.05$.

Cross-sectional area measurements

Additional suspensory ligament specimens were dissected from their proximal and distal attachments, frozen, and sequential 5 mm thick cross-sections were cut along the full length (proximal, mid-body, branches) of the specimens using a band saw. The individual specimens were photographed and the area of each section determined

b HF100/30+ Ultra Light portable x-ray unit: Minray, Northbrook, IL, USA
c Wireless plate detector: Sound-Eklin, Carlsbad, CA, USA
d Image J software: U.S. National Institutes of Health, Bethesda, Maryland, USA. Available from: http://imagej.nih.gov/ij/
e Akucal- Image Scaling Device: J2 Medical LP, Pittsburgh, PA, USA
f Systat 13: Systat Software Inc., San Jose, CA, USA

using imaging software[d], with the individual branch cross-sectional areas combined for a total cross-sectional value at these sections. Cross-sectional area measurements were grouped by region and compared using an ANOVA[f]. Significance was set at $p \leq 0.05$.

Results

The equine suspensory ligament demonstrated a heterogeneous strain pattern under increasing tensile load. While the overall strain of the suspensory ligament reached 4.4% at 2224 N of tensile load, there were significant differences in regional strain magnitude at 445 N ($p = 0.024$), 1112 N ($p = 0.021$), and 2224 N ($p = 0.031$).

At 445 N of tensile load, the branch region of the suspensory ligament exhibited significantly more strain than the proximal ($p = 0.010$) and proximal mid-body ($p = 0.047$) regions of the suspensory ligament (►Table 1).

At 1112 N of tensile load, the branch region of the suspensory ligament exhibited significantly more strain than the proximal ($p = 0.03$), proximal mid-body ($p = 0.02$), and distal mid-body ($p = 0.023$) regions of the suspensory ligament (►Table 1).

At 2224 N of tensile load, the branch region of the suspensory ligament exhibited significantly more strain than the proximal ($p = 0.025$), proximal mid-body ($p = 0.008$), and distal mid-body ($p = 0.05$) regions of the suspensory ligament. In addition, the distal mid-body portion of the suspensory ligament demonstrated significantly more strain ($p = 0.038$) that the proximal mid-body portion of the suspensory ligament (►Table 1).

Cross-sectional area measurements

There was no significant difference in cross sectional area between regions ($p = 0.628$) when the branch areas were combined. The mean and standard deviations for the cross sectional area for the proximal region was 2.72 ± 0.17 cm^2, the main body region was 2.66 ± 0.15 cm^2, and the combined branches region was 2.73 ± 0.11 cm^2.

Table 1 Mean and standard deviation of strains in each region of the suspensory ligament at **A)** 445 N, **B)** 1112 N, and **C)** 2224 N. Values are expressed as percentage of change in length. Regional strain comparisons with statistical significance (p <0.05) are indicated in bold.

A)				
445 N	**Proximal (P)**	**Proximal mid-body (PB)**	**Distal mid-body (DB)**	**Branches (B)**
Mean strain (%) ± SD	1.0 ± 0.9	1.2 ± 1.4	2.0 ± 1.2	3.3 ± 0.9
Bonferroni corrected p-values	PB: 1.000 DB: 0.880 B: **0.010**	P: 1.000 DB: 1.000 B: **0.047**	P: 0.880 PB: 1.000 B: 0.055	P: **0.010** PB: **0.047** DB: 0.055

B)				
1112 N	**Proximal (P)**	**Proximal mid-body (PB)**	**Distal mid-body (DB)**	**Branches (B)**
Mean strain (%) ± SD	2.4 ± 1.2	2.4 ± 1.9	2.2 ± 1.4	5.5 ± 1.7
Bonferroni corrected p-values	PB: 1.000 DB: 1.000 B: **0.031**	P: 1.000 DB: 1.000 B: **0.020**	P: 1.000 PB: 1.000 B: **0.023**	P: **0.031** PB: **0.020** DB: **0.023**

C)				
2224 N	**Proximal (P)**	**Proximal mid-body (PB)**	**Distal mid-body (DB)**	**Branches (B)**
Mean strain (%) ± SD	4.1 ± 1.6	2.7 ± 2.0	5.2 ± 2.1	7.6 ± 2.6
Bonferroni corrected p-values	PB: 0.254 DB: 0.924 B: **0.025**	P: 0.254 DB: **0.038** B: **0.008**	P: 0.924 PB: **0.038** B: **0.050**	P: **0.025** PB: **0.008** DB: **0.050**

Discussion

The suspensory ligament is a primary component of the suspensory apparatus of the horse that functions to support the fetlock and prevent excessive extension of that joint during the weight-bearing or stance phase of the stride (33-35). Injury to the suspensory ligament most often occurs as a result of overextension (dorsiflexion) of the fetlock during the maximal weight-bearing that occurs at the middle of the stance phase of the stride (33). The injury may result from either an acute traumatic episode or as a result of a more chronic repetitive strain injury (2, 36). While lesions can occur in the proximal, mid-body, or branches of the suspensory ligament, mid-body and branch injuries have demonstrated a poorer prognosis and higher re-injury rates than lesions in the proximal region (2, 6). However, the reason for this anatomical variance in healing and re-injury of suspensory ligament lesions has not been elucidated.

The results of the current study demonstrate that under tensile loading, a heterogeneous strain pattern exists within the equine suspensory ligament with the distal branches exhibiting significantly more strain than the proximal and mid-body areas. This difference was noted at all levels of applied tensile force. While strains in the mid-body were not significantly different from those experienced in the proximal portion of the suspensory ligament, at the highest force (2224 N), the distal portion of the mid-body experienced significantly more strain than the proximal portion of the mid-body. A previous study that examined the mechanical properties of isolated tissue segments harvested from the mid-body and branches of the equine suspensory ligament found similar results to the current study in that the mid-body specimens experienced significantly less tensile strain and were significantly stiffer than the branch regions (37).

It is well-known that soft tissue structures such as ligaments and tendons normally undergo non-uniform deformation (strain) under functional loading conditions (38, 39). It has been postulated that regions of ligaments and tendons exposed to increased levels of strain may be more prone to micro tears within the extracellular matrix following excessive loading (40). In the suspensory ligament, this increased regional strain could cause localized collagen fibre disruption, ultimately leading to tissue degeneration and the pathological changes associated with desmitis (33). Indeed, previous finite element analysis models of human rotator cuff and patellar tendons have demonstrated localized regions of increased tendon strain at the common sites of tendinopathy lesions in these structures (22–24). The increased strain experienced by the branches of the suspensory ligament in the current study suggests that they may be at more risk for damage during extremes of loading. This concept is supported by a recent epidemiological study involving Thoroughbred racehorses that examined the prevalence of suspensory apparatus lesions in 322 equine cadaveric forelimbs (41). The study found that 80% of the limbs had an injury to some aspect of the suspensory apparatus (41). In the suspensory ligament, lesions were more common in the branch region (22%) compared to the mid-body (7%) and proximal (3%) regions (41).

The increase in regional strain demonstrated in the branches of the suspensory ligament may also explain the higher rate of poor healing and re-injury associated with lesions in this region (2, 6). The histological changes found in suspensory ligament desmitis (fibrosis, hyaline degeneration, chondroid metaplasia, neovascularization) have also been associated with a reduction in the material properties of the tissue, making it more susceptible to further injury (6, 42, 43). This would be especially critical in areas of normal high strain such as the branches of the suspensory ligament.

The reason for the significant increase in strain experienced by the branch region of the suspensory ligament is unclear. A recent study could not find any significant differences in matrix composition between different regions of the suspensory ligament (37). It has also been suggested that the branches of the suspensory ligament may be more vulnerable to damage due to their smaller cross-sectional area (33). However, in the current study, the combined cross-sectional area of the suspensory ligament branches was not significantly different from that of the proximal or mid-body regions. Thus, if the tensile load was shared equally by both branches, the stresses should be the same as those experienced by other regions of the suspensory ligament. However, if the position of the distal limb deviated from the vertical axis (valgus or varus) of the distal aspect of the suspensory ligament, it is possible that the branches could experience asymmetrical loading, with one branch experiencing a significant increase in load. This could indeed place the medial or lateral branch at greater risk for injury. A conformational change in the hindlimbs may place abnormal stress on the suspensory ligament and may influence the prognosis (8). Conformational changes in forelimb may also place abnormal stress on the forelimb, but this requires further investigation.

A potential limitation of the current study is the fact that a maximum of 2224 N of tensile force was placed on the suspensory ligament. While a previous theoretical model has suggested that the suspensory apparatus of the equine forelimb experiences significantly higher forces at the walk and trot, a previous *in vivo* study that utilized implantable strain gauges placed on one of the branches of the suspensory ligament found peak strains of 5.4 ± 0.9% at the walk and 9.1 ± 1.3% at the trot (43, 44). The peak strain values within the branches of the suspensory ligament in the current study were 7.6 ± 2.6% suggesting that the tensile loads used in our *in vitro* testing system appeared to reflect clinically relevant forces.

Another potential limitation is the fact that the *in vitro* test apparatus used in the current study applied a pure, axial tensile force to the suspensory ligament specimens. While this is likely to be an accurate representation for the majority of the length of the suspensory ligament, a theoretical model of the ligament based on *in vitro* strain data suggested that the line of action of the distal branches of the ligament may be more similar to a rope curving around a pulley at the level of the fetlock (45). However, this same study found that the strain in the suspensory ligament at 3000 N of applied force was not significantly different between the axial (straight) and theoretical curved loading scenarios (45). Therefore the axial loading applied in the current study would indeed appear to have clinical relevance.

The results of the current study demonstrate a heterogeneous strain pattern within the equine suspensory ligament. The significant increase in regional strain demonstrated within the branches of the suspensory ligament, compared to the proximal and mid-body regions, could explain the higher rates of injury and recurrence in these structures. Additional epidemiological and mechanistic studies will be needed to confirm this theory.

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Conflict of interest

No conflicts of interest have been declared.

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