Magnetic resonance imaging of plantar soft tissue structures of the tarsus and proximal metatarsus in foals and adult horses

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Summary
Objectives: The object of this study was to describe previously defined soft tissue structures by using spin and gradient sequences in a 0.5 Tesla magnetic resonance system in order to improve the characterisation of tendon and ligaments at the plantar region of the equine tarsus and metatarsus while considering possible age-related variations. Methods: Cadaveric hindlimbs from twenty-two Warm-blood horses with an age range from one month to twenty-five years were examined in spin and gradient echoes. The proximal suspensory ligament from six limbs was dissected to assign the signal intensities histologically. For statistical analysis, horses were divided into two groups (<3 years and >3 years) for evaluating signal intensity and homogeneity of the plantar tendons and ligaments. Results: Focal increase of the signal intensity within the deep digital flexor tendon was significantly more present in horses older than three years. Signal alterations of the long plantar ligament were seen without a significant dependency to age. The accessory ligament of the deep digital flexor tendon could not be visualized on all images within the region of interest. The morphology of the proximal suspensory ligament was not affected by age-related changes. Clinical relevance: Spin and gradient echoes in MRI were suitable to identify and assess soft tissue structures at the plantar aspect of the equine tarsus and proximal metatarsus. Age-related appearance must be considered when interpreting magnetic resonance images.

Introduction
The plantar region of the equine tarsus and proximal metatarsus is covered by the superficial and deep digital flexor tendon, long plantar ligament, suspensory ligament, and deep digital flexor tendon accessory ligament. These soft tissue tendinous and ligamentous structures are often damaged by external trauma or excessive force during exercise (1-3). High suspensory desmitis in the hindlimb, a disease with an unpredictable prognosis where horses often do not reach previous performance levels after injury is a good example of this type of damage (4, 5). Generally, ultrasonography is the method of choice for diagnosing soft tissue lesions in the plantar region of the equine tarsus and proximal metatarsus. However, artefacts, echo attenuation at the proximal splint bones, and the narrow contact surface for the ultrasound probe at the plantar aspect of the hindlimb make interpretation of sonograms problematic (6-8). Magnetic resonance imaging (MRI) is increasingly being used in equine orthopaedics and several studies have shown improved detail and soft tissue characterisation of regions with limited access ultrasonographically (9-11). The clinical value of MRI examinations for injuries of the proximal metatarsal region is documented in many reports, however the pathologies described differ and show a variety of possible diagnoses (12-16).

There are few MRI studies of the normal tarsal anatomy in adult horses and the MRI systems used were different (17, 18). Therefore there are no standard magnetic resonance images of the equine plantar tarsus. In addition, detailed anatomical MRI descriptions of age-related differences of soft tissue structures for the plantar tarsus are non-existent. There are a few recent studies which found wide variability in MRI appearance and tissue composition at the origin of the suspensory ligament of adult horses (19-21). For the hindlimbs, only two reports that included six Warm-bloods and eleven horses of various breeds have been published to date (21, 22). The
The purpose of this study was to use MRI spin- and gradient-weighted echoes to describe the defined soft tissue structures at the plantar aspect of the equine tarsus and proximal metatarsus, focusing on their signal behaviour and to determine possible age-related changes. Specifically, we further describe the histological appearance of the proximal aspect of the suspensory ligament and compare these findings with MR images.

Materials and methods

Twenty-two cadaveric hindlimbs from Warmblood horses, ranging in age from one month to twenty-five years (mean: 7.6 years), and without any history of a disease related to the plantar tarsal and metatarsal soft tissue were examined. The horses were euthanatized due to reasons unrelated to orthopaedic problems of the hindlimbs. Prior to MRI, tarsi were examined radiographically in four standard planes followed by thorough sonographic examination of the plantar tarsal and proximal metatarsal region by an experienced examiner (AL). Magnetic resonance imaging was performed in a 0.5 Tesla system\(^a\). Each tarsus was placed in extended position in a human knee coil and scanned in spin and gradient echoes, T1- and T2-weighted, as well as proton weighted sequences in the transverse, sagittal, and dorsal planes (\(\text{Table 1}\)).

For signal assignment of the origin of the suspensory ligament, full cross-sectional tissue samples, 3 cm distal from the tarsometatarsal joint, from six horses were taken for further histological examination. Histological staining included haematoxylin-eosin for qualitative criteria and trichrome Galloycanine 2R Anilin (GRA) for distinguishing the muscle (red) from connective tissue (blue). The histological analysis of the cross sections used qualitative criteria when describing the occurrence and arrangement of different tissue types.

The region of interest (ROI) was set between the calcaneal tuber to 3 cm distal of the tarsometatarsal joint to evaluate the following structures: the superficial and deep digital flexor tendons, the long plantar ligament, the accessory ligament of the deep digital flexor tendon, and the suspensory ligament. The signal intensities of these structures were characterised by using a grey scale with five levels (hyperintense, high-intermediate, intermediate, low-intermediate, hypointense) in relation to the trabecular bone due to the absence of muscle tissue on most images. The appearance of the proximal suspensory ligament on the MRI at the distal level of the ROI was analysed morphometrically with the help of an image processing computer programme\(^b\).

For statistical analysis, the horses were divided into two groups. Group A comprised nine limbs from horses three years and younger, whereas group B included thirteen limbs from horses older than three years. This allocation was made on the basis of physeal fusion to ensure that full skeletal maturity had been reached by the horses in group B (23). The Shapiro-Wilk test was performed to test for normal distribution; this was followed by the Pearson test to evaluate MRI findings for each defined structure between group A and B. To assess correlation between the age of the examined population and the amount of signal intense tissue to the cross sectional area (CSA) at the origin of the suspensory ligament, the Students t-test was used. The significance level was set at \(p < 0.05\).

Results

None of the limbs showed abnormal findings in the ROI on radiographic and ultrasound examination and none were excluded prior to MRI examination. Transverse and sagittal planes were used for anatomical evaluation because they provided the best access to the defined structures. Dorsal images were used in cases of unclear assignment, but were considered inappropriate for assessing the plantar tarsal region in general. Spin-weighted sequences provided the best overall soft tissue contrast and resolution, whereas gradient-weighted sequences could demonstrate the best signal intensity differences within signal intensity regions and improved resolution in detail.

Superficial digital flexor tendon

The superficial flexor tendon was visible in all sequences used as a homogeneous hypointense structure, clearly demarcated along all transverse image sections by the surrounding paratenon of intermediate signal intensity. Proximally the crescent-shaped superficial digital flexor tendon covered the caudal surface of the proximal calcaneal tuber completely changing into a more oval-shaped structure further distally.

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\(\text{Table 1}\) Magnetic resonance imaging parameters. For optimal signal-to-noise-ratio, slice thickness was set at 3.5 mm or 4.5 mm considering the different tarsal sizes. Imaging time ranged from two to 10 minutes.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T1-spin echo (T1W)</th>
<th>T2-turbo spin echo (T2W/TSE)</th>
<th>T1-gradient echo (T1W/3D/FFE)</th>
<th>T2-gradient echo (T1W/3D/FFE)</th>
<th>Proton density weighted echo (PDW/TSE)</th>
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<td>Repetition time (mms)</td>
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<td>24</td>
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<td>Echo time (mms)</td>
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<td>27</td>
<td>17</td>
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<td>90(^\circ)</td>
<td>55(^\circ)</td>
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<td>Field of view (mm)</td>
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<td>300</td>
<td>180</td>
<td>180</td>
<td>200</td>
</tr>
</tbody>
</table>

\(\text{a}\) GYROSCAN T5-NT, Philips Healthcare, Hamburg, Germany

\(\text{b}\) Adobe Photoshop CS2 9.0.: Adobe, San José, CA, USA
Figure 1 Transverse T1-weighted gradient echo, left hindlimb of a 12-year-old gelding. Increased signal intensity in the core of the deep digital flexor tendon is visible. A) Deep digital flexor tendon (a), tarsal tendon sheath (b), and superficial digital flexor tendon (c). Part B is the enlarged detail from part A. The deep digital flexor tendon is marked with an (a).

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when coming into contact with the long plantar ligament dorsolaterally and the deep digital flexor tendon dorsomedially (►Figure 1 to ►Figure 4). No clear delineation between the long plantar ligament and the superficial digital flexor tendon was identified in laterally positioned sagittal planes due to signal equality. Limbs of group A and group B showed no differences regarding homogeneity of the tissue signal and delineation to the surrounding structures.

Deep digital flexor tendon

Overall the deep digital flexor tendon was defined as a homogenous hypointense structure in all of the sequences used. A subtle area of increased signal intensity (low-intermediate and intermediate in all sequences used) was visible at the level of the sulcus of the sustentaculum tali in transverse planes in four out of nine limbs in (44%) group A; in group B significantly more limbs (12/13; 92%) showed these signal alterations (p = 0.013) (►Figure 1). Depending on the position of the plane, delineation of the deep digital flexor tendon ranged from excellent to insufficient. Transverse planes always provided definition of the oval-shaped deep digital flexor tendon because of the intermediate to hyperintense signal intensities of the paratenon and the synovia of the tarsal tendon sheath. In the sagittal planes, the border to the adjacent superficial digital flexor tendon, accessory ligament of the deep digital flexor tendon, and the suspensory ligament was ill-defined distal to the tarsometatarsal joint due to insufficient signal contrast.

Long plantar ligament

The long plantar ligament was assessed completely from its origin at the calcaneal tuber to the insertion at the plantar part of the distal joint capsule and fourth metatarsus. This ligament changed shape from flat proximally to round or trapezoid at the mid-calcaneal level to transverse-oval distally. The generally hypointense signal intensity showed alterations particularly at mid-level in five out of nine limbs in group A and seven out of 13 in group B without setting a significance between the groups. These increased signal patterns (low-intermediate in T2-spin weighted and proton weighted sequences, intermediate in T1- and T2-weighted sequences) were arranged longitudinally in sagittal images and had an irregular patchy appearance transversally (►Figure 2, ►Figure 3). The signal similarity to the adjacent cortical bone of the caudal calcaneous dorsally, the deep digital flexor tendon medially, and the superficial digital flexor tendon plantaromedially impeded clear delineation. Depending on the extent of the described patterns, differentiation of the border zones was notably improved.

Accessory ligament of the deep digital flexor tendon

The thin and flat accessory ligament of the deep digital flexor tendon is a homogenous, hypointense structure. Due to the
close anatomical relation dorsal to the hypointense deep digital flexor tendon and plantar to the suspensory ligament, there was limited contour signal difference for clear identification in all sagittal planes, whereas in the transverse planes the accessory ligament of the deep digital flexor tendon was well-defined, surrounded by soft tissue of low-intermediate to high intermediate signal intensity (Figure 4). However, in four out of nine limbs in group A, and one out of 13 limbs in group B, the accessory ligament of the deep digital flexor tendon was not identifiable. There was a significant difference in images between groups A and B (p = 0.043) for the accessory ligament of the deep digital flexor tendon.

**Suspensory ligament**

The transverse plane images allowed for the most detailed assessment of the suspensory ligament morphology. The origin of the suspensory ligament was visible as a flat to rectangular, hypointense structure, which could not be differentiated dorsally from the cortical bone of the metatarsus. The lateral part of the origin of the suspensory ligament proximal to its attachment to the metatarsal bone became an oval, hypointense band. This separate branch of origin was detectable in transverse plane images located laterally to the limb axis and adjacent to the deep digital flexor tendon, the long plantar ligament and the plantar tarsal joint capsule. This small structure, between the metatarsal joint and the distal intertarsal joint, is defined by higher signal intensity loose connective tissue (Figure 3). Proximal to the intertarsal joint, the suspensory ligament could not be clearly differentiated due to the direct contact to the hypointense cortical bone of the plantar calcaneus and the long plantar ligament. Distal to its metatarsal attachment, an increasing amount of signal intense (high-intermediate to intermediate in all sequences) connective tissue, surrounding the suspensory ligament from the position 2-3 cm distal to the tarsometatarsal joint, facilitated differentiation. Here, the suspensory ligament developed an oval, bilobed structure containing two semilunar tissue bundles of hyperintense (predominantly T2-weighted and proton density weighted sequences) to intermediate (predominantly T1-weighted sequences) signal intensity (Figure 4). Some small areas of those bundles showed signal densities one grade lower or higher then described above. Proximal to this level these bundles were highly irregular and poorly distinguishable from each other.

The transversally recognisable areas of higher signal intensities within the suspensory ligament were visible as linear patterns in sagittal planes. The proportion of these patterns to the total SL varied with the position of the sagittal plane, causing unique images in each plane. Differentiation between the hypointense part of the suspensory ligament to the cortical bone of the metatarsus dorsally, and the plantar located deep digital flexor tendon and the accessory ligament of the deep digital flexor tendon, was impeded when border zones lacked surrounding soft tissue of increased signal intensity.

Morphometric analysis revealed an age-independent amount of embedded, signal intense tissue bundles at the level 3 cm distal to the tarsometatarsal joint. The cross-sectional area occupied by these bundles of high signal intensity was 32 ± 5% (mean 30%) in group A and 30 ± 2% (mean 30%) in group B, respectively. Due to a poor signal-to-noise ratio causing misrepresentation of the border zone between hyperintense areas, one limb (three-year-old-filly) had to be excluded from statistical data. Histologically, the proximal suspensory ligament was composed of muscle fibres and adipose cells embedded in collagen tissue. Muscle and adipose tis-

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**Figure 4** Transverse T2-weighted gradient echo, left hindlimb of a 10-year-old mare. The embedded tissue bundles within the bilobed proximal suspensory ligament appear in hyperintense and high-intermediate signal intensities. The accessory ligament is only visible as a thin, poorly developed hypointense structure. (a) Accessory ligament of the deep digital flexor tendon, (b) deep digital flexor tendon, (c) medial embedded tissue bundle of the suspensory ligament, (c') medial lobe of the suspensory ligament, and (d) superficial digital flexor tendon.

**Figure 5** Gallocyanin 2R Anilin (GRA) stained histological feature of the centre of the suspensory ligament origin 3 cm distal from the tarsometatarsal joint; adipose tissue detectable near muscle cells. (a) Collagen-tendinous tissue, (b) muscle tissue, (c) fat tissue, and (d) vessels; green bar = 10 µm.
sue were in close relation to each other, implying several layers of fat cells surrounded the muscle fibres to separate them from the collagen tissue. Vessels and nerves were predominantly found at the border region of collagen to muscle tissue (Figure 5). No age-dependant differences regarding the tissue characterization or composition were detected. None of the examined samples showed any visible signs of an acute or chronic inflammatory disease.

Discussion

This study presents new findings regarding the magnetic resonance images of tendons and ligaments at the plantar tarsus and proximal metatarsus. As this study focused on the anatomical description of soft tissue structures, established magnetic resonance sequences with already verified soft tissue resolution were used (9, 13, 17, 18). It is in agreement to those studies, that spin-weighted sequences were useful to achieve a generally satisfying tissue contrast and resolution for anatomic overview. Gradient-weighted sequence magnetic resonance images had an improved resolution of detail by showing that intermediate to hyperintense signals of embedded bundles within in the suspensory ligament were classified histologically as not only muscle but also adipose tissue, underscoring the sensitivity of gradient-weighted sequences for these tissues (24). While fat-suppressed sequences were not used for anatomical descriptions in this study, it is clear that they are of beneficial value in diagnosis of injuries with suspected bone involvement, soft tissue injuries at bone attachments or identification of fluid in tissues with adipose cell content (9, 12, 13, 24, 25).

In the present study, MRI of the origin of the suspensory ligament agrees with descriptions from other authors (19-22). The suspensory ligament has a consistency in tendinous collagen tissue in which two bundles of muscle fibres and adipocytes are embedded, and there is no age-dependency as measured by the percentage of bundle-area compared to the cross sectional area (26-29). As other studies have already demonstrated, the amount of muscle fibres in the suspensory ligament varies individually, but as derived from literature, a certain influence by breed is also obvious. It has been shown that the suspensory ligament in Standardbred horses had 40% more muscle tissue than Thoroughbreds (27). In contrast, Dyson and colleagues, who did not indicate the age, breed or limb side, found a 2.1% to 11% muscle content in the suspensory ligament (28). In the forelimb of Warmbloods, means of 20.3% and 17.1% have been found for muscle and adipose tissue content (19, 21). Reports in the hindlimb of Warmblood horses and a mixed breed population reported an average of 19% and 25% of the cross sectional area on a comparative level with our examination (19, 22). The maximum percentage measured was 41% in the proximal segment of Standardbred horses in the hindlimb (27). Considering the different methods and their respective standard deviations, the high variety of embedded tissue in the proximal suspensory ligament is most likely due to the horse’s individual morphology. The relatively high percentage of muscle fibres within the suspensory ligament suggests their important role in limb stability and elastic storage of energy (29). The function of the adipose cells is still unknown. Although not quantitatively measured, we also found more adipose tissue than muscle fibres in some histological sections. This agrees with recent findings where adipose tissue represented approximately 60-80% in some bundles (22). In this study, because of the large age span of the examined horses and the significant size ranges of the limb and the suspensory ligament, we did not measure absolute values of the total cross sectional area and bundle size. Instead, with regard to clinical applications, percentage values allow comparison of bundle-to-cross sectional area values demonstrating the variety of the equine population. To consider the quantity of different tissue types in the suspensory ligament origin when interpreting sonograms is beneficial for clinical examinations. The earlier described proximal branch of the origin at the rear suspensory ligament could be seen in all limbs although a clear outline was dependent on image quality (30). Theoretically, lesions of this segment are possible but not yet confirmed in diagnostic imaging. It has been hypothesized that a positive high flexion test can also be related to pathologies in this branch.

Interpretation of the signal variations in the deep digital flexor tendon and long plantar ligament are only conjecture because histological examination of these structures was not part of this work. A previous history of a soft tissue injury cannot be fully ruled out, although the limbs were examined radiographically and sonographically by an experienced clinician. However, the high percentage of limbs with such variations and the repeatability of the signals are indicative of non-pathological findings. The locally increased signal intensity of the deep digital flexor tendon was more common in horses older than three years (group B). This may be a sign of a structural adaption or chondroid metaplasia; this is supported by the fact that this tendon runs closely around the underlying bone of the calcaneus within a tendon sheath and is therefore prone to higher stress and ischemia (31). Our study indicates that clinicians should use caution in interpreting an increased MRI signal intensity as a diagnosis for tendon lesions.

In both groups, there was an increase in signal intensity within the long plantar ligament; this could be due to trabecular morphology of connective tissue of different composition. When dissected, the long plantar ligament appeared trabecularly subdivided by connective tissue. Further work using histological sections would be needed to confirm this hypothesis.

Compared to the forelimbs, the hindlimb accessory ligament of the deep digital flexor tendon is poorly developed and may even be absent (32-34). Still, as pointed out in several clinical studies, it appears to play a role in hindlimb lameness (35, 36). In this study, eight percent of group B had no visible accessory ligament of the deep digital flexor tendon corresponding well with previous results (32). The significantly higher percentage of younger horses (group A) lacking an accessory ligament of the deep digital flexor tendon could be due to the low image resolution of small structures in the lower magnetic field strength MRI. Alternatively, the distal level of the region of interest may not have contained sufficient
connective tissue to enable clear demarcation. The signal intensity of the hindlimb accessory ligament of the deep digital flexor tendon was classified as homogeneously hypointense in all MRI, in contrast to earlier studies on the forelimb accessory ligament of the deep digital flexor tendon (11, 20, 37). This is most likely due to different tissue compositions in forelimbs and hindlimbs.

This study clearly shows that interpretation of MR images in spin- and gradient-weighted sequences should take into account the age of the patient. Especially when increased signal intensities are seen in the deep digital flexor tendon of horses older than three years and, for horses younger than three years, when the accessory ligament of the deep digital flexor tendon cannot be visualised until 3 cm distal to the tarsometatarsal joint. The superficial digital flexor tendon, the suspensory ligament and the long plantar ligament showed significant similarities among the groups.

Conflict of interest

No conflicts of interest have been declared.

References

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