Morphological and biomechanical studies on the common calcaneal tendon in dogs

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Summary
Spontaneous rupture at the distal part of the gastrocnemius tendon (GT) is the second most common non-traumatic tendon injury in dogs, whereas the other strands of the common calcaneal tendon do not seem to have a predisposition to rupture. In order to discover why we investigated the common calcaneal tendons of 63 dogs microscopically and biomechanically. Both the gastrocnemius and superficial digital flexor tendon (SFT) had multiple low vascularized fibrocartilaginous areas within their distal course as opposed to regular parallel fibered areas in the proximal tendon areas. Biomechanical testing revealed that the distal sections in both tendons show a 50% and 70% lower tensile strength ($F_{\text{max}}$/kg BW) than the proximal sections ($p<0.01$), respectively. On the contrary, tensile load ($F_{\text{max}}$/mm²) only differed minimally between proximal and distal sections in both tendons (8% and 9%, respectively), whereas the tensile load of the distal gastrocnemius tendon is 35% lower than of the distal superficial flexor tendon ($p<0.01$). To the authors’ knowledge, this is the first study to experimentally show that there are different biomechanical properties within the same tendon. The maximum load to failure is lower in the GT compared to the SFT within the same dog which explains its higher incidence of rupture in the field. The avascular fibrocartilaginous structure in the distal gastrocnemius tendon seems to play a further role in the pathogenesis of spontaneous rupture.

Keywords
Tendon, biomechanics, fibrocartilage, rupture, dog

Introduction
The common calcaneal tendon is composed of three separate strands: the gastrocnemius tendon, the superficial flexor tendon and the accessory tendon deriving from the long ischial muscles (1, 2). Rupture of the common calcaneal tendon is the second most frequent tendon rupture after the proximal biceps tendon in dogs (3). There is a lack of specific literature on the microscopic anatomy of the common calcaneal tendon in dogs, but it has been shown in humans and other species that there are local variations in microscopical structure along certain tendons, such as fibrocartilaginous areas, responding to different compressive and tensile forces (4–9). It has been hypothesized that fibrocartilaginous areas exhibit less tensile strength than parallel fibered areas, however, this has not yet been proven experimentally. It is indisputable though that these fibrocartilaginous inclusions play an important role in the pathogenesis of spontaneous tendon ruptures (5, 10). So far little is known about the microscopical and biomechanical properties of the separate strands of the common calcaneal tendon in dogs. Further understanding of these properties is required for the continued investigation of the potential etiology of spontaneous gastrocnemius tendon rupture.

Therefore, the aim of this study was to gather information about the macro- and microscopical structure of the individual tendons and to compare them with the results of biomechanical studies at the same locations.

Material and methods

Gross anatomy
Sixty-three dogs were used that had either died or been euthanized due to causes unrelated to this study. Only those dogs without any pathologic changes in the common calcaneal tendon were included. Their mean body weight was 27.9 kg (standard deviation [SD]: 15.7) with a mean age of 13.0 years (SD: 4.2). Their common calcaneal tendons were dissected soon after death or euthanasia at their musculotendinous junctions, sealed in a vacuum plastic bag and kept frozen at –20°C until further testing within the next two to seven days. The tendons used for microscopical examination were harvested within two hours after death or euthanasia and were immediately transferred into a formalin solution.

Vascularisation
The hind limbs of four middle sized dogs where recruited for ink injection of blood with a mean body weight of 16.3 kg (SD 5.7) and a mean age of 7.8 years (SD: 5.9). The femoral artery and vein were flushed and 10% ink stained gelatin emulsion was injected with the limbs heated up to 70°C. After filling was completed the limbs were cooled to 24°C, dissected and made transparent with Spalteholtz solution (11).

Microscopical examination
The mean body weight of the 11 dogs used for microscopical examination was 23.5 kg (SD: 10.8) and mean age was 9.6 years (SD 5.0). The tendons were dissected and the calcaneal tubercle was sawed off distally.
The specimen were fixed in formalin solution and cut in pieces. They were embedded in paraffin, cut into 5–8 µm slices, and Haematoxylin-Eosin (HE) stained. To differentiate the proteoglycans within the fibrous tissue we used Astra blue and Alcian blue staining with a nuclear counterstaining of aluminum red.

Biomechanical and morphometrical studies

For biomechanical and morphometrical studies, the hind limbs of 28 dogs with a mean body weight of 32.7 kg (SD: 13.8) and a mean age of 7.1 years (SD: 4.2) were used. The four areas of interest in the gastrocnemius and flexor tendon were the proximal tendon waists (I) with only parallel fibres and the distal enthesis (II) respectively calcaneal cap (III) with pronounced fibrocartilages (Fig. 1).

Self-made interlocking stainless steel inserts for the biomechanical testing machine Z 010 (Zwick, Ulm, Germany) were used with deep-freezing (−75°C) of the clamping jaws in order to prevent slipping and cutting of the tendons (12, 13). The superficial flexor tendon was clamped on both ends while the gastrocnemius tendon was fixed to the biomechanical testing machine distally with a pin drilled through the bone so the calcaneus and the tendon were positioned in a 120° angle. A loading speed of 10 mm/min was chosen and the force (N) to maintain that speed was measured and documented in a force-elongation-diagram. The slope of the linear part was used to calculate the elasticitity modulus (E in N/mm²). The maximal force applied (F max in N) corresponds to the peak of the curve. Loading was stopped after complete failure. To compare the results they were referenced to cross sectional area (tensile strength in N/mm²) and body weight (tensile load in N/kg). The cross sectional areas were measured sonographically with a 7.5 MHz probe (Ultrasound Scanner 350C, Pie Medical, Maastricht, The Netherlands).

Statistical analysis

The Pearson correlation coefficient was calculated in order to judge the grade of dependence of the cross sectional area on body weight. Differences in cross sectional areas were evaluated with the t-test for paired samples and in tensile strength, tensile load and elasticity module by the t-test for independent probes. p<0.01 was considered to be statistically significant. All analyses were performed using SPSS 15.01 (SPSS Inc., Chicago, IL, USA).

Results

Gross anatomy

The gross anatomy of the common calcaneal tendon is shown in Fig. 2. The superficial flexor tendon runs from medial to caudal while coiling and widens as it coils over the calcaneal tuber. Underneath lies the sub-tendinous calcaneal bursa. The gastrocnemius tendon originates from the medial and lateral belly of its corresponding muscle, then runs caudally deep to the superficial flexor tendon where it inserts crescent shaped on the calcaneal tuber. Its insertion is always padded by the calcaneal tendon bursa which is wedge shaped. Proximal to the bursa a fat pad, which extends cranially above the calcaneal rim with the hock flexed, protrudes into the lumen of the bursa without penetrating the bursal wall (Fig. 4B). The lateral origin of the accessory tendon derives from the biceps femoris muscle while the medial portion comes from the semitendinosus and gracilis muscle. The oval tendon inserts on the dorso-lateral aspect of the calcaneal tuber.

The cross sectional area of the distal sections of the gastrocnemius as well as the superficial flexor tendon is on average 33% resp. 35% larger than that of the proximal sections (Table 1). In addition, the cross sectional area of the gastrocnemius tendon is approximately 10% less than the corresponding areas in the flexor tendon. There is a highly significant correlation between the cross sectional areas and the body weight in all four of the measured areas.
Common calcaneal tendon in dogs

Vascularisation

The blood vessels within the proximal areas of the tendons run straightly along the fibres connected with one another (Fig. 3A). This pattern stops abruptly with the blood vessels arcading just before the insertion of the gastrocnemius tendon and the calcaneal cap in the flexor tendon. Another avascular area is found in the distal plantar aspect of the gastrocnemius tendon opposite the calcaneal cap in the flexor tendon. The fat pad in the calcaneal tendon bursa shows very intense vascularization (Fig. 3B).

Microscopic anatomy

Microscopical examination revealed two distinctively different morphologic entities visible in the gastrocnemius and flexor tendon:
- Proximal: regular dense parallel fibered areas (Fig. 4A);
- Distal: five different fibrocartilaginous areas in the distal tendons at following localizations (Fig. 4B): 1) within the digital flexor tendon at the calcaneal cap, and 2) proximal to it a sesamoidal fibrocartilage, 3) in the enthesis of the gastrocnemius tendon, 4) a superficial fibrocartilage at the distal aspect, and 5) just proximal to 4 a sesamoidal fibrocartilage.

Biomechanical and morphometrical studies

Biomechanical testing revealed highly significant differences in tensile strength (Fig. 5) between the proximal parallel fibered areas and the distal fibrocartilaginous areas in both the gastrocnemius and superficial flexor tendon. With regard to tensile load (Fig. 6) there was no statistically significant difference between these areas in either tendon. There was a lack of statistically significant difference when comparing tensile strength within the same regions in both tendons. On the contrary, tensile load was significantly less in both the parallel fibred and the fibrocartilage areas in the gastrocnemius tendon (Table 2).

The elasticity module of the parallel fibered area of the gastrocnemius and the flexor tendon was 449.17 ± 214.75 and 488.51 ± 215.55 N/mm², respectively, and therewith similar. The fibrocartilaginous areas of both tendons only measured 94.63 ± 38.84 and 94.12 ± 20.66 N/mm², respectively, which is almost five-fold less than in the parallel fibred areas.

Discussion

The common calcaneal tendon is a complex structure composed of three separate strands with currently limited data available on its individual microscopical and biomechanical properties.

Our microscopical results clearly show zones with very different structural properties along the tendon length: parallel fibred areas in the proximal parts of the tendons and fibrocartilaginous areas in the distal parts. We were able to purposefully select specific tendon sections for biomechanical testing in order to further investigate the influence of morphology on biomechanical properties in tendons.

The presence of fibrocartilaginous areas in tendons has been known for a long time (14, 15). There are different fibrocartilages based on their structure, localization and embryology. The terms insertion fibrocartilage at the entheses, sesamoid fibrocartilage for fibrocartilage deep within tendons, and periosteal fibrocartilage as superficial layer of boney pulleys characterize both their function and localization. Their structural difference is due to their embryologic origin (16). In fibrocartilages, as in the twisted cranial cruciate ligament (4) or the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Cross sectional area (CSA) in the proximal and distal sections of the superficial flexor tendon (SFT) and the gastrocnemius tendon (GT).</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFT prox.</td>
<td>13.6 ± 7.9</td>
</tr>
<tr>
<td>SFT dist.</td>
<td>19.9 ± 11.2</td>
</tr>
<tr>
<td>GT prox.</td>
<td>11.3 ± 5.4</td>
</tr>
<tr>
<td>GT dist.</td>
<td>17.6 ± 8.9</td>
</tr>
</tbody>
</table>

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canine gastrocnemius tendon, proteoglycan sheaths develop around their fibres as an adaption to impacting pressure and shearing forces while the development of gliding tendons over a boney pulley is different. They express collagen fibres long before proteoglycans are formed (17). While the development and the maintenance of fibrocartilage is genetically determined, their expression is influenced by environmental factors. This is evident in the pronounced periosteal fibrocartilage in the calcaneal cap of the flexor tendon only experiencing pressure and shearing forces compared to the one in the gastrocnemius tendon also experiencing tensile forces. So far, the reason for and clinical effect of fibrocartilage is not fully understood. It could be pathological, but the general consensus today is not that it is a pathologic entity, rather an adaption in areas of pressure (5, 7). We did not find any evidence of degeneration in the tendons and since fibrocartilage was only found in areas of pressure we assume that their existence is physiologic and functional.

The zonal structure of the enthesis in the gastrocnemius tendon demonstrates another mode of attenuation in addition to the calcaneal tendon bursa and its fat pad. It allows the gradual transfer of load due to the various tensile and elastic properties of the different zones. It also prevents thinning of the tendon while loaded and kinking of the fibres while bent (18).

The fibrocartilaginous structure of the calcaneal cap in the flexor tendon is explained by the pressure exerted on the tendon by the boney pulley. The different zones derive from varying pressure of the pulley according to their position.

The layers of the periosteal fibrocartilage in the gastrocnemius tendon opposite the calcaneal cap in the flexor tendon is mirror-inverted and less pronounced. It represents a modification of the fibrocartilaginous sliding face of the calcaneal tuber which continues as this fibrocartilage. Fibrocartilage embedded in the surface of a tendon with this structure and function is rare and has not yet been reported.

The sesamoid fibrocartilage in the gastrocnemius tendon and the flexor tendon are not as prominent as the aforementioned and cannot be found at the same level. There has to be some pressure impact. In the flexor tendon this might be the fascia-like reinforcement running perpendicular to the tendon just next to the fibrocartilage. In the gastrocnemius tendon flexion of the hock exerts pressure on the tendon at the cranial border of the calcaneal tuber. Although there is the bursa between tendon and bone, not only tensile forces affect the tendon here. Another possible explanation is the pressure arising from the separate strands twisting around each other. This causes development of fibrocartilage in the human Achilles tendon (19).

The subtendineous calcaneal bursa protects the tendon from pressure of the calcaneal tuber. The calcaneal tendon bursa is filled with a fat pad in every dog that was

![Fig. 4 Longitudinal histologic sections of GT and SFT. a) parallel fibred tendon tissue in the proximal GT (HE stain) b) fibrocartilaginous areas in the distal GT and SFT (Astrablue stain). 1 – SFT calcaneal cap, 2 – SFT sesamoidal fibrocartilage, 3 – GT enthesis, 4 – GT distal superficial fibrocartilage, 5 – GT sesamoidal fibrocartilage, FP – fat pad, CT – calcaneal tuber.](image)

![Fig. 5 Mean tensile strength of two different localizations in the GT and SFT. Various superscripts mark statistical significant differences (p<0.01).](image)
examined but has not been described yet. The structure and probably the function are similar to the retromalleolar fat pad in humans where it prevents excessive pressure on the tendon with ankle flexion. We also suggest a nutritive function to the avascular area of the gastrocnemius tendon underneath the fat pad according to the infrapatellar fat pad in the knee.

We selected tensile strength (\(F_{\text{max}}/A\)) and tensile load (\(F_{\text{max}}/BW\)) as the most important parameter to characterize the biomechanical properties of tendons. For rheological properties we selected the elasticity module. These parameters guarantee high reproducibility and a good comparison.

The tensile strength of tendons has been tested extensively with results varying from 40 to 170 N/mm\(^2\). The large range can be explained by different microscopical structures in the studied tendons or tendon sections. It has been hypothesized that fibrocartilaginous areas possess less tensile strength than parallel fibered areas (5). None of the previously performed studies report on the precise section and microscopical structure of the tested tendon nor its area.

Tensile strength for fibrocartilaginous areas is explicitly below the values of parallel fibered areas. For the first time it was possible to prove experimentally that there are different biomechanical properties along the length of the same tendon. This also explains why so far determined values in literature vary so much between different authors. It was also possible to prove for the first time that fibrocartilaginous areas possess less tensile strength than parallel fibered areas which was shown experimentally in both tested tendons. The difference in tensile strength demonstrates an adaption to the impacting different loads. Although tensile strength is a good parameter to describe biomechanical properties in a tendon, it does not tell anything about the loading capacity of a tendon in the body which is influenced by the body weight. A good parameter therefore represents the tensile load where the maximum tensile force is correlated with the body weight.

The values of tensile load compared to the values of tensile strength seem to show inconsistent results. Both sections of the gastrocnemius tendon and the superficial flexor tendon respectively have significantly different values of tensile strength (Fig. 5) whereas the values of tensile load (Fig. 6) do not vary significantly. The explanation lays within different cross sectional areas in both tendons and their sections. As discussed earlier the reason for the decreased tensile load in the gastrocnemius tendon is the smaller cross sectional area compared with the flexor tendon. The significantly smaller tensile strength of fibrocartilaginous sections does not lead to a decreased tensile load, but is compensated by a greater cross sectional area at these sections. Decreased tensile strength of specific tendon sections does not generally mean an area of least resistance and disposition to rupture. Thus a greater incidence of rupture in fibrocartilaginous areas in tendons generally does not represent clinical experience. However if less tensile strength is not compensated by a greater cross sectional area the section is prone to rupture. Examples herefor are the fibrocartilaginous areas in the canine cranial cruciate ligament and in the Achilles tendon in humans (4, 19).

As expected the absolute cross sectional area increases with increasing bodyweight whereas the relative cross sectional area decreases with higher weights. Therefore we expect less loading capacity in tendons of heavy dogs. This hypothesis was proved in parallel fibered tendon areas with our biomechanical investigations as already verified in the cranial cruciate ligament (4).

![Fig. 6](image)

**Mean tensile load of two different localizations in the GT and SFT. Various superscripts mark statistical significant differences (p<0.01).**

**Table 2** Maximum load, tensile strength, and tensile load in the proximal and distal sections of the superficial flexor tendon (SFT) and the gastrocnemius tendon (GT).

<table>
<thead>
<tr>
<th>Section</th>
<th>Max. load mean(N) ± SD</th>
<th>Max. load range (N)</th>
<th>Tensile strength mean (N/mm(^2)) ± SD</th>
<th>Tensile strength range (N/mm(^2))</th>
<th>Tensile load mean (N/kg) ± SD</th>
<th>Tensile load range (N/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFT prox.</td>
<td>1488.3 ± 543.4</td>
<td>699.8–3000.5</td>
<td>99.9 ± 29.2</td>
<td>38.4–160.1</td>
<td>32.8 ± 16.0</td>
<td>23.9–97.5</td>
</tr>
<tr>
<td>SFT dist.</td>
<td>1692.9 ± 861.9</td>
<td>507.3–3441.3</td>
<td>31.4 ± 10.8</td>
<td>11.8–49.9</td>
<td>44.5 ± 11.4</td>
<td>18.9–60.4</td>
</tr>
<tr>
<td>GT prox.</td>
<td>1031.3 ± 317.6</td>
<td>408.4–1446.5</td>
<td>82.3 ± 36.3</td>
<td>37.1–147.2</td>
<td>32.2 ± 8.5</td>
<td>17.0–47.1</td>
</tr>
<tr>
<td>GT dist.</td>
<td>1107.1 ± 352.7</td>
<td>489.1–1494.8</td>
<td>43.6 ± 11.7</td>
<td>22.2–65.1</td>
<td>29.2 ± 7.6</td>
<td>15.8–37.0</td>
</tr>
</tbody>
</table>
The elasticity modulus tells about the rheologic properties of a tendon and gives evidence about the attenuation ability of a tendon. The results show that fibrocartilaginous sections possess a considerably greater elasticity – shown by a lower elasticity modulus – than parallel fibered sections. The difference in elasticity expresses an adaptation to different functional demands. Parallel fibered sections are to transmit tensile forces efficiently so they show little elasticity. On the other hand fibrocartilaginous sections have to absorb pressure and shearing forces not meant to be transmitted and hence need more elasticity as we have been able to show.

Spontaneous ruptures of the canine gastrocnemius tendon only occur just proximal the insertion on the calcaneal tuber (20, 21, 22) where we were able to find fibrocartilaginous areas. As revealed with the biomechanical studies the gastrocnemius tendon in fact shows lower tensile strength at this section but the same tensile load as in parallel fibered areas. For this reason the disposition to rupture at this specific localization is not explained by a lower tensile strength but other factors must apply.

Scarse vascularization is a typical structural feature of fibrocartilaginous sections in tendons and has been linked to tendon rupture in people (23, 24). In our studies we have been able to show avascular areas in both the flexor and the gastrocnemius tendon. The results show that fibrocartilaginous sections possess a considerably greater elasticity – shown by a lower elasticity modulus – than parallel fibered sections. The difference in elasticity expresses an adaptation to different functional demands. Parallel fibered sections are to transmit tensile forces efficiently so they show little elasticity. On the other hand fibrocartilaginous sections have to absorb pressure and shearing forces not meant to be transmitted and hence need more elasticity as we have been able to show.

Another predisposing factor in dogs could be a small standing angle in the hock (22) or a breed specific very prominent form of the calcaneal tuber like in humans suffering from “Haglund’s disease” (29, 30). Radiologic or anatomical studies about the form of the calcaneal tuber in predisposed breeds like the Doberman (22) have to be carried out yet.

References