Macroscopic and microvascular blood supply of the canine common calcaneal tendon

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Keywords
Canine Achilles tendon, canine common calcaneal tendon, microscopic, tendon blood supply, macroscopic

Summary
Chronic tendinopathy injuries to the canine common calcaneal tendon are relatively common in large breed dogs and typically affect the distal portion of the tendon. In humans, poor blood supply, biomechanical faults, poor training methods and fluoroquinolone administration have all been linked with the development of Achilles tendinopathy. The most common sites for Achilles tendinopathy in humans seem to correspond with areas of poor blood supply within the tendon.

The aim of this study was to evaluate the blood supply of the canine common calcaneal (Achilles) tendon to determine if variations occur along the tendon. The null hypothesis was that there would be no difference in the microvascular blood supply at varying points along the tendon.

Paired pelvic limbs were collected from 12 large breed dog cadavers. A 50% barium sulphate and 50% saline solution was infused along the tendon. Indian ink was infused into the musculotendinous junction had additional branches from the gastrocnemius muscles. Distally, vessels radiated proximally from the calcaneus 2 to 3 cm into the tendon. Mean total vessel counts at the insertion (138.54 ± SD 31.06) were significantly higher than all other sections (p <0.001). The mid-body had significantly lower total vessel counts. When the cross sectional area of the tendon was taken into account, only the insertion had a significantly higher mean vessel count/cm² than the mid-body of the tendon. There were no other significant differences in mean vessel count/cm².

Areas of poorer blood supply did not correspond with the most commonly reported chronic common calcaneal tendinopathies, suggesting that inherent poor blood supply at the site of injury may not play a role in the pathogenesis. Atraumatic handling and minimal manipulation should be used during the surgical approach and debridement to preserve the remaining blood supply in ruptured tendons.

Introduction
The canine Achilles mechanism comprises five muscles (the gastrocnemius, superficial digital flexor, biceps femoris, gracilis and semitendinosus), with three tendinous components forming the common calcaneal or Achilles tendon, which insert on the calcaneal tuberosity. The tendon of the gastrocnemius muscle forms the major portion of the common calcaneal tendon, with the tendon of the superficial digital flexor muscle and the combined tendon of the biceps femoris, gracilis and semitendinosus forming the remaining two components (1, 2).

Chronic injuries or tendinopathies of the canine common calcaneal tendon are common, especially in large breed dogs with Labrador Retrievers and Doberman Pinschers over-represented in multiple case series (2–4). These chronic injuries typically affect the insertion of the common calcaneal tendon onto the calcaneus or the distal portion of the tendon itself (2, 5). In a recent case series describing 14 chronic common calcaneal tendon injuries, none of which had a calcaneal avulsion injury, and all of the injuries were described as being mid-substance tears (6). The pathogenesis of chronic common calcaneal tendon injuries in canines is poorly understood. In people multiple factors including blood supply, biomechanics, overuse and drug administration have been implicated in the development of chronic Achilles tendinopathy (7–9). Areas of poor blood supply have been found within the human Achilles tendon; these have been correlated with the most common areas of injury by many authors (10–12). However there is still debate about the relationship between vascularisation and tendon rupture. To the authors’ knowledge, only one study has been performed in dogs to evaluate the vascularity...
of the common calcaneal tendon (13). This study looked at the alignment of blood vessels within the tendon and at the tensile strength of the tendon, and found avascular areas of fibrocartilage within the tendon adjacent to the calcaneal cap. The distal portions of the tendon containing fibrocartilaginous regions had a lower tensile strength, but not a lower tensile load than the proximal regions. It was hypothesised that these avascular fibrocartilaginous areas may play a role in acute tendon rupture (13).

The aim of this study was to evaluate the blood supply of the canine common calcaneal tendon to determine if blood supply varies throughout the tendon. The common calcaneal tendon (gastrocnemius, and the combined and superficial digital flexor tendon) was examined as a single tendinous unit. The null hypothesis was that there would be no difference in the blood supply at varying points along the canine common calcaneal tendon.

Materials and methods

Paired pelvic limbs were collected from 12 large breed dogs (body weight >20kg) that were euthanatized for reasons unrelated to this study. Limbs were sealed in plastic bags, frozen and stored at –20°Celsius until the study was performed. Prior to use, the limbs were thawed at room temperature, and were covered in saline soaked gauze sponges throughout catheterisation, injection and sample collection.

Limbs were prepared in similar manner to that described in previously published reports (10, 14). The femoral artery of each limb was catheterized with a 3.3 mm polyethylene feeding tube secured with ligatures of 2–0 silk. Heparinised saline was then flushed through the femoral artery until there were not any visible clots seen in the effluent from the femoral vein.

One limb from each dog was infused with a 12% barium sulphate suspension (50% commercial barium sulphate suspension in saline) until filling of the nail beds occurred (14). The barium suspension was well agitated for a minimum of 30 seconds immediately prior to infusion. The infusion was performed with a 20 ml syringe using constant manual pressure over 60–90 seconds; the average volume of suspension used was 13–15 ml per limb. Filling of the nail beds with the barium solution was taken as the endpoint as this should outline the afferent blood supply (14).

The contralateral hindlimb was infused with Indian ink (Spalteholtz technique [15]) via the femoral catheter until filling of the nail bed occurred (Fig. 1). Dissection of the lower limb was performed noting the ink filled gross blood supply to the common calcaneal tendon. The common calcanean tendon was removed and placed in 10% formalin solution for at least five days. All tendons were between 8 cm and 9 cm in length. Each tendon was sectioned at 1 cm intervals, labelled 1 to 8 from proximal to the calcaneal insertion to the musculotendinous junction (Fig. 2). One 4 micron section was taken from each tendon section, stained with haematoxylin and eosin, and the number of ink filled arteries and arterioles counted (Fig. 3). The vessels in each individual section were counted three times by a single person (PJG). Numbered adhesive labels were used to obscure the label on the slide for each count, and slides were examined in a random order. The mean of the three counts was recorded as the count for the section, a four percent variance was evident between counts. Sections were assessed for overall degree of

Fig. 1
Indian ink filling nail beds (arrows), at the end-point of injection,

Fig. 2
Angiogram with numbers corresponding to points of tendon sectioning.

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vascular filling by looking for unfilled arterioles in the sections.

Mean vessel counts from each section were compared to evaluate the distribution of microscopic vasculature throughout the tendon. A commercially available software program\textsuperscript{a} was used; the variance in mean vessel counts was analysed using a one-way analysis of variance (ANOVA) test with a 95% confidence interval.

Mean vessel counts per cm\textsuperscript{2} were calculated to take into account the differences in cross sectional area along the length of the tendon; this was done by dividing the vessel count for each section by the cross sectional area of the sectioned tissue. The cross sectional area was determined by using a calliper to measure the width and length of the section tissue, and then applying the formula for calculation of the area of an oval as the sectioned tissue was ovoid in shape. The variance in mean vessel count/cm\textsuperscript{2} was analysed using an ANOVA test with a 95% confidence interval.

### Results

Radiographs of the barium injected limbs revealed multiple fine branches from the caudal branch of the saphenous artery entering the cranial aspect of the mid-body of the tendon; each branch radiated on entering the paratenon and seemed to supply a well-defined segment of tendon (Fig. 4). The distal third of the tendon had additional vessels radiating in from the calcaneus. These vessels exited the calcaneus distal to the tendinous insertion and entered the paratenon at the level of the insertion, terminating 2–3 cm proximal to the calcaneus, and appeared to be branches from the caudal branch of the saphenous artery. There were not any vessels observed to run the entire length of the paratenon. Additional vessels entered the musculotendinous junction from the gastrocnemius muscles, which were supplied by branches of the distal caudal femoral artery. During dissection of the Indian ink filled tendons, a similar distribution of ink-filled vessels was evident.

Stained tendon sections revealed a variation in the mean ink-filled vessel counts between sections; few non-filled vessels were evident (2 to 4 per section). One dog had abnormal chondroid metaplasia in the examined sections; the data from this dog was excluded from the study.

Sections taken from just proximal to the calcaneal insertion (section 1) consistently had the highest mean vessel count (138.54; SD 31.06, range 93 to 176). The number of vessels seen per section progressively decreased with each section from the insertion towards the tendon’s mid-substance (sections 4 and 5), and increased toward the musculotendinous junction (sections 6, 7, 8). (Fig. 5)

Analysis of variance revealed that mean vessel counts at section 1 (calcaneal insertion) were significantly higher than all other

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\textsuperscript{a} STATA 10\textsuperscript{TM}: Statacorp, College Station, Texas, USA
sections (p <0.001). The mean vessel counts in section 2 were significantly higher than section 4 (p = 0.05). Mean vessel counts at section 8 (musculotendinous junction) were significantly higher than sections 3, 4, 5, and 6 (p <0.05). No significant difference in mean vessel counts were found between sections 3, 4, 5, 6, and 7.

The mean vessel counts/cm² were highest at the insertion (section 1), decreased with each section towards the mid body (sections 4 and 5), and then increased toward the musculotendinous junction (section 8) (Fig. 6). Analysis of variance revealed the mean vessel count/cm² at the insertion (section 1) was significantly higher than the mid body (section 4) (p = 0.038). There were no other significant differences in mean vessel counts/cm² along the tendon.

In summary, the total number of vessels was highest at the insertion of the common calcaneal tendon, vessel counts gradually decreased through the tendon mid body, and then increased towards the musculotendinous junction. Less variance was seen in vessel counts per cm² with only the insertion having a significantly higher count than the mid-body.

**Discussion**

A tendon’s blood supply is derived from surrounding arteries, muscle branches and osseous vessels at its insertion. The term intrinsic system is used to refer to vessels from the myotendinous junction and osteotendinous junction. The extrinsic system refers to blood supply from the paratenon or synovial sheath if the tendon has one (16). The results of this study suggest the macroscopic blood supply of the canine common calcaneal tendon may be segmental, with little or no interconnection observed between regions. However microscopic angiography would be required to determine whether microscopic interconnections exist.

The results of this study proved our null hypothesis to be false; significant differences in blood supply do exist along the canine common calcaneal tendon.

The mid-body of the tendon had the poorest blood supply, which is similar to the findings of human studies (10–12, 17). In contrast to many human studies, we found that the insertion of the canine common calcaneal tendon had the best blood supply (12). Additional methods of evaluating the blood supply of the Achilles tendon including radioisotope tracking of tendon blood flow, laser Doppler flowmetry, immunohistochemical quantitative vascular density evaluation and power Doppler flow have been reported in the human literature (10–12, 21). In general most of these studies have agreed that the mid-section of the human Achilles tendon has the least vascularisation (10–12, 17, 18), and is the site most likely to rupture. However, there is debate as to the vascularity of the insertion of the human Achilles tendon with some studies finding a good blood supply and others poor vascularity (10–12, 17, 18). The application of these methods to both normal and abnormal canine calcaneal tendons is likely to enhance our understanding of its blood supply and
the pathogenesis of canine common calcaneal tendinopathies.

A recent study found areas of avascular fibrocartilage within the canine common calcaneal tendon at the level of the calcaneal cap, however no counts of total vessels were made in this study (13). These avascular fibrocartilages were not seen in our study, most likely due to differences in section placement and angle, with our sections being taken perpendicular to the long axis of the tendon just proximal to the calcaneal cap. In humans, the most common sites of chronic tendinopathy lesions or rupture are the mid-body followed by the insertion, with one study reporting a series of 698 cases for which a 66% incidence of mid-substance tendinopathy and a 23% incidence of insertional tendinopathy were found (9). However canine case series have tended to report chronic common calcaneal tendinopathy injuries as occurring close to or at the calcaneal insertion more commonly (2-3, 5). The results of this study suggest that unlike humans, dogs appear to have a good inherent blood supply at the common site of chronic injury, which would suggest that poor inherent local vascularity is less likely to be a predisposing factor in the development of chronic common calcaneal tendinopathy in dogs. However, the relatively poor vascularity mid-tendon may play a role in the development of the chronic mid substance tears reported in dogs (6). The progression of canine tendinopathy lesions may be due to tendon degeneration and secondary damage to the blood supply by the inciting cause as in humans (7-9).

Since chronic tendinopathy injuries tend to occur at the insertion, the progression of these lesions into complete or partial ruptures may be due to damage to the blood supply, thus impairing the ability of the tendon to heal (2, 5). It has been suggested in the human literature that along with repeated trauma, a reduction in the normal blood supply or insufficient blood supply is an important factor in the failure of tendon injuries to heal (17). Our results would suggest that given the good blood supply in the region of the insertion of the common calcaneal tendon, repetitive overuse leading to tendon microtrauma is the likely cause of insertional tendinopathy. This theory is supported by the human literature with histological examination of affected tendons revealing mucoid degeneration, fibrinoid necrosis along with microtearing, proliferation of fibroblasts and thin walled vessels, all of which are suggestive of repetitive trauma (19). Histological examination of chronic common calcaneal tendinopathy would be useful to determine if similar changes are present in dogs. Only one paper, to our knowledge, has reported on the histological changes in dogs, which found fibrovascular proliferation adjacent to the tendinosseous junction (2).

In humans, Achilles tendinopathy has also been associated with poor training and abnormal biomechanics or conformation (excessive foot pronation and ankle instability) leading to excessive loading during physical activity and resulting in a chronic overuse injury (7,8,9). Gait analysis of predisposed breeds would be of benefit in evaluating the potential influence of bio-

Fig. 6
Mean vessel counts/cm² with standard deviation per section.
mechanics as a contributing factor in the development of chronic common calcaneal tendonopathy.

Achilles tendinopathy and complete rupture have been recorded in people following administration of fluoroquinolone antibiotics (8, 20). Fluoroquinolones have been shown to cause a decrease in collagen synthesis by canine fibroblasts in an in vitro study (21). Administration of ciprofloxacin was found to decrease protein and collagen levels in the common calcaneal tendon of juvenile dogs (22). Given that fluoroquinolone antibiotics are widely available in veterinary medicine their potential role in the development of common calcaneal tendinopathy in dogs may warrant further investigation.

Potential sources for error in this paper include the use of frozen cadaveric specimens, and inability to inject all of the microvessels of the tendon. However the trend in vessel counts was similar in each tendon examined, with very few unfilled arterioles visible and similar methods have been reported in both the human and veterinary literature (10, 14). A way to overcome this would have been to use live dogs, which would then be anasthetized, heparinized and lastly euthanatized directly prior to performing the study. The lack of a controlled injection pressure may be a source of error; it has been suggested that inadequate pressure may fail to fill all the microvessels of interest, while excessive pressure may lead to a false positive result with leakage giving the appearance of vessels where none are present (12). However controlled injection pressures are not consistently used in veterinary and human studies (10, 11, 14, 18, 23, 24).

In clinical cases, an atraumatic approach with minimal manipulation of the tendon should be employed to avoid damage to the extrinsic blood supply arising from the caudal saphenous artery. Also, the inherent distribution of blood vessels throughout the tendon should be considered when determining the amount of tendon debrided, as debriding to a relatively avascular area may impair healing. At this time it is not known whether the differences in vessel counts affect healing of the canine Achilles tendon.

Further evaluation is necessary to determine the significance of these findings in clinical cases, and in tendon healing after surgical debridement and tenorrhaphy. Additional studies are needed to evaluate the effect of tendon debridement and tenorrhaphy on blood supply and healing of the canine Achilles tendon.

References