Gastrocnemius Tendon Strain in a Dog Treated With Autologous Mesenchymal Stem Cells and a Custom Orthosis

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Objective: To report clinical findings and outcome in a dog with gastrocnemius tendon strain treated with autologous mesenchymal stem cells and a custom orthosis.
Study Design: Clinical report.
Animal: A 4-year-old spayed female Border Collie.
Methods: Bone-marrow derived, autologous mesenchymal stem cells were transplanted into the tendon core lesion. A custom, progressive, dynamic orthosis was fit to the tarsus. Serial orthopedic examinations and ultrasonography as well as long-term force-plate gait analysis were utilized for follow up.
Results: Lameness subjectively resolved and peak vertical force increased from 43% to 92% of the contralateral pelvic limb. Serial ultrasonographic examinations revealed improved but incomplete restoration of normal linear fiber pattern of the gastrocnemius tendon.
Conclusions: Findings suggest that autologous mesenchymal stem cell transplantation with custom, progressive, dynamic orthosis may be a viable, minimally invasive technique for treatment of calcaneal tendon injuries in dogs.

CLINICAL REPORT

A 4-year-old spayed female Border Collie was admitted with a 4-month history of slowly progressive, right pelvic limb lameness. Traumatic right-coxofemoral luxation 3 years earlier had been treated by surgical reduction and stabilization without complications and lameness resolved until the onset of the current lameness. Daily supplements of glucosamine and chondroitin (Tripple Joint Max, Pet Nutrition Products, Apopka, FL) and omega three fatty acids (Missing Link Plus, Designing Health, Velencia, CA) were administered. Carprofen (2.2 mg/kg orally) was being administered intermittently to provide analgesia for the lameness.

The dog was in good body condition, had a lateral splint immobilizing the right talocrural joint, and was grade 3/5 lame (Table 1) on the right pelvic limb at a walk and had moderate atrophy of the thigh musculature. Standing with the stifle held in extension by a veterinarian, the tarsal extension angles were 129° and 136° for the right and left tarsi, respectively. The right common calcaneal tendon was severely thickened and firm at its insertion. Direct palpation and flexion of the talocrural joint elicited a painful response. On survey radiographs there was increased soft-tissue opacity of the common calcaneal tendon adjacent to the insertion on the calcaneus. Ultrasonography findings included thickening, mineralization, and severe loss of gastrocnemius tendon-fiber pattern, and a large central core lesion just proximal to the insertion on the tuber calcis. The superficial digital flexor tendon was unaffected (Fig 1).

Treatment options discussed with the owner included conservative management and primary surgical repair. Because of the risk of surgical complications and the possibility of a poor outcome, the owner inquired about alternative therapies and was offered autologous mesenchymal stem cell (MSC) transplantation and custom, tarsal-paw orthosis (OrthoPets, Denver, CO) coaptation. The owner was thoroughly informed about the experimental nature of this method of treatment. Four days later, the dog was returned for force-plate gait analysis and bone marrow aspiration.

Gait Analysis

For force plate gait analysis (Force plate, Model BP400600-1000, Advanced Mechanical Technology, Inc., Watertown, MA), trials were considered valid if a walking speed between 1.8 m/seconds and 2.4 m/seconds was achieved, and if distinct ipsilateral thoracic and pelvic limb strikes were seen. All valid trials were averaged to obtain the final peak vertical and propelling-peak force values. Continuous data were screened for normal distribution using the Shapiro–Wilk test and compared using the Student's *t*-test. Mean ± SD values are reported. P values $<.05$ were considered significant.

Table 1 Lameness Scoring System Used

Lameness score	Lameness definition
0	None
1	Subtle weight-bearing
2	Mild weight-bearing
3	Moderate weight-bearing
4	Severe, predominantly weight-bearing
5	Severe, predominantly nonweight bearing

Mean ± SD peak-vertical force for the right (48.1 ± 0.7 N) pelvic limb was significantly less than for the left (110.6 ± 14.5 N) pelvic limb ($P = .003$). Peak propulsive force in the right pelvic limb (3.7 ± 0.6 N) was also significantly lower than for the left pelvic limb (18.2 ± 2.6 N; $P = .042$).

MSC Preparation

Bone-marrow was aspirated (11 g Jamshidi needle, 12 mL syringe) from the proximal aspect of the right humerus under anesthesia (premedication with hydromorphone 0.07 mg/kg subcutaneously and anesthetized with propofol 3 mg/kg titrated to effect intravenously [IV] and supplemented with 100% oxygen). The sample was immediately transferred to the laboratory (Advanced Regenerative Therapies, Fort Collins, CO) for stem cell isolation, culture, and expansion; initial total nucleated cell count was 264×10^6. The sample was centrifuged at 200*g* for 5 minutes then resuspended in 10 mL phosphate buffered saline (PBS). This process was repeated twice. The sample was incubated at 37°C in a 95% oxygen and 5% CO_2 environment for 5 days. After cell expansion, the final MSC count was $>20 \times 10^6$. The sample was cryogenically preserved in a 95% serum and 5% dimethylsulfoxide (DMSO) solution at −86°C until the day of transplantation. At injection, cells were thawed in a 37°C water bath and diluted 1:1 with lactated Ringers solution immediately before injection. No cell viability testing was performed before injection. A final volume of 1 mL purified, autologous MSC was injected percutaneously using a 22 g needle under light sedation (butorphanol 0.2 mg/kg IV) and ultrasound guidance into the core lesion of the right gastrocnemious tendon. No complications or difficulties were experienced during injection.

Custom Orthosis

At this appointment, a cast-molding was made for a custom, progressive, dynamic orthosis (CPDO) with the tibiotarsal and metatarsophalangeal joints positioned in 165° and 75° of extension, respectively. The previously molded lateral splint was re-applied and continued for 2 weeks until the custom orthosis was available.

Posttreatment Care and Follow-Up

Follow-up examinations were planned for 30, 60, 90, 180, and 365 days after initial treatment. We recommended limited activity and 3–5 five minute leash walks each day for the first 2–3 months after treatment. The owner was also informed that controlled, passive range of motion (PROM) exercises would be encouraged during the healing process.

Outcome

At the 28-day recheck examination, the owner reported no additional outside activities other than the prescribed 5-minute leash walks. The orthosis was being worn at all times with the exception of daily tendon massage which was performed by the owner. A lameness grade was not assigned at this visit, but the dog was reported to be using the right pelvic limb well. A small, focal area of alopecia was noted on the proximal aspect of the tibia beneath a Velcro strap associated with the orthosis. Recheck ultrasonographic examination revealed no change compared with day 1, with continuous thickening of the gastrocnemius tendon with loss of fiber pattern and a heterogeneous echogenicity. The small, mineralization was also evident in the core lesion. Continued activity restriction and 60-day recheck examination was recommended.

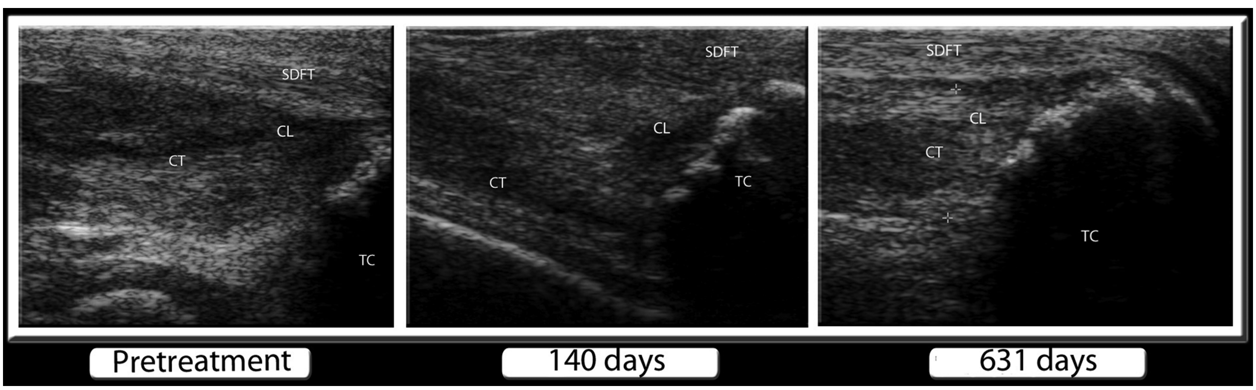

Figure 1 Ultrasound images of the common calcaneal tendon: pretreatment, 140 days posttreatment, and 631 days posttreatment. CL, core lesion; TC, tuber calcis; SDFT, superficial digital flexor tendon; CT, conjoined tendon of the semitendinosus, biceps femoris, and gracilis muscles.

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At 56 days, the dog continued to do well with no changes in treatment at home. A lameness grade of 1 to 2/5 was present at a walk. Recheck ultrasonographic examination revealed a 10 mm to 7 mm decrease in thickness of the core lesion in the dorsoplantar plane compared with day 1, but no apparent changes in fiber-pattern loss or heterogenous echogenicity. The progressive, dynamic, motion-control limiter on the orthosis was set to allow an additional 10° of flexion at the talocrural joint. This created 10° of motion at the talocrural joint. The paw segment of the orthosis (Fig 2) was not removed at this time. The owner was directed to start daily PROM exercises. Continued activity restriction and 90-day recheck examination was also recommended.

At 98 days, the dog continued to do well according to the owner. A lameness grade of 1/5 was assigned at a walk. Ultrasonographic examination revealed similar thickness as on the previous examination, but a subjectively mild return of a linear fiber pattern in some small sections of the gastrocnemius tendon. The previously observed mineralized structure was unchanged. Continued activity restriction was advised and a recheck examination was recommended at 120 days.

At 140 days, the dog continued to do well with no changes in treatment at home. Repeat ultrasonographic examination revealed subjective mild continued improvement in the linear fiber pattern of the gastrocnemius tendon (Fig 1). The thickness of the tendon and the presence of the mineralized structure were unchanged. The paw segment of the orthosis (Fig 2) was removed. Progressively increased amounts of controlled activity during leash walking (i.e., 15 minutes, 2–3 times a day for 2 weeks, then 30 minutes, 2–3 times a day for 2 weeks, followed by 60 minutes, 2–3 times a day) were recommended. Additionally, weekly underwater treadmill exercises were advised until recheck examination at 180 days.

Over the next month, once weekly underwater treadmill exercise was performed without the CPDO. The 1st treatment consisted of 15 minutes, at 2.2 mph, with a water level of 14.5″. The last treatment consisted of 22 minutes, at 2.8 mph, with a water level of 15″. Tendon massage, swimming, and periods of off-leash activity were performed regularly with the owner for the next 6 months. The orthosis was used intermittently at the owner's discretion, but was effectively discontinued 6 months after MSC treatment.

Figure 2 Custom, progressive, dynamic, orthosis used in the reported case. Initially, the talocrural and metatarsophalangeal joints were fixed at 165° and 75°, respectively. At 56 days posttreatment, 10° of extension was allowed in the talocrural joint. At 140 days posttreatment, the paw segment was removed.

At 343 days, after MSC treatment, the dog was rechecked. Current activities reported included extensive walks, stair climbing, and running through the snow while the owner was cross-country skiing. These activities were performed without the orthosis. The owner reported no lameness associated with any of these activities. On physical examination, the dog had a body condition score (BCS) of 4/9 and was not lame at a walk. Standing with the stifle held in extension by the veterinarian, the tarsal extension angles were 130° and 139° for the right and left tarsi, respectively. Mild joint effusion and a subtle cranial drawer sign with no pain on extension of the right stifle were noted. Lateromedial and dorsoplantar radiographs of the right stifle showed moderate stifle effusion evident by a soft tissue opacity in the cranial and caudal aspects of the right stifle joint with partial obliteration of the infra-patellar fat pad, and caudal displacement of the fascial plane between the gastrocnemius muscle and deep digital flexor muscle group, an irregular tibial-intercondylar eminence and periarticular osteophytes on the proximal aspect of the patella and lateral aspect of the tibial plateau representing mild stifle osteoarthritis. Lateromedial and craniocaudal radiographs of the right tarsus demonstrated an irregular calcaneal tuberosity with evidence of chronic new bone formation and surrounding soft tissue thickening at the site of insertion of the common calcaneal tendon representing enthesopathy of the previously diagnosed common calcaneal tendinopathy.

Ultrasonographic examination demonstrated no changes from the previously described gastrocnemius tendinopathy findings. The owner was informed of the possibility of an early onset, partial cranial cruciate ligament injury and was instructed that continued healing of the gastrocnemius tendon was unlikely. She was cautioned that continued activity without use of the orthosis might result in recurrence of right pelvic limb lameness; however, there was no indication to continue orthosis support with the intention of encouraging further healing of the gastrocnemius tendon. The owner was instructed to monitor for signs of lameness in the right pelvic limb and to return for recheck if seen. No further activity recommendations were made at this time and the owner declined further evaluation of the suspected early-partial CCL tear.

At 631 days, the dog was re-examined and the owner reported the dog was normal with no activity restrictions and no apparent lameness. The dog was in good body condition (BCS 4/9) and was not lame at a walk and trot. Standing with the stifle held in extension, tarsal-extension angles were 124 and 135 for the right and left tarsi, respectively. Mean peak vertical forces for the right (79.6 ± 3.3 N) and left (86.9 ± 5.3 N) pelvic limbs were not significantly different ($P = .03$). Likewise, peak propulsive forces were not significantly different between right (8.5 ± 5.9 N) and left (14.0 ± 6.4 N; $P = .07$) pelvic limbs. Recheck ultrasound examination showed subjective improvement of the linear fiber pattern of the gastrocnemius tendon (Fig

1). A persistent thickness of the gastrocnemius tendon with areas of heterogeneous echogenicity, a focus of mineralization, and an irregular calcaneal tuberosity were still evident.

DISCUSSION

In this report, the pet owner considered the outcome of a gastrocnemius tendon strain treated with MSC and CPDO as excellent. Although serial orthopedic and ultrasonographic examinations as well as force plat gait analysis were consistent with incomplete healing of the injured gastrocnemious tendon, functional outcome was considered good.

To our knowledge there are no other clinical reports of a common calcaneal tendon injury treated with MSC and CPDO. Historically, conservative treatment has been unrewarding because of high recurrence rates[1] and surgical repair has been the preferred treatment. Many variations in surgical technique have been advocated, but in general, primary tenorrhaphy and temporary immobilization of the tarsus are performed.[2–9] Most recent veterinary publications of common calcaneal tendon injuries have focused on surgical repair, augmentation with either myocutaneous autografts or biologic scaffolds, and immobilization techniques.[5,6,9–12] One of these studies was a biomechanical study examining the use of either polypropylene mesh in conjunction with a 3-loop pulley suture or mesh alone versus suture tenorrhaphy. Based on results from that study, the use of polypropylene mesh alone or in conjunction with suture could not be recommended because of unacceptable gap formation during loading.[5] Clinical outcome of dogs treated by suture tenorrhapy and augmentation with a semitendinosus muscle flap has been evaluated and short-term clinical outcome was considered good.[6] Norton et al.[9] reported clinical use of a transarticular ring fixator in conjunction with suture tenorrhaphy and although the complication rate was high, 80% dogs had no lameness at 3 months after surgery.

We offered surgical repair to the owner in the current report as the gold standard treatment for gastrocnemius tendon injury; however, because of the risk of complications and possibility of failure to return to full athletic ability, the owner was unwilling to pursue this treatment option.[13] Instead, a more conservative, albeit investigational, approach using autologous MSC and CPDO was chosen. Currently, there is no published research on the clinical use of MSC for common calcaneal tendon strains in dogs.

The primary motivation for use of MSC in tendon injury is to provide the healing tendon with precursor cells which have the ability to provide a more normal tendon matrix in place of scar formation.[14,15] Unfortunately, evidence to support the use of MSC in common calcaneal tendon injuries is inadequate in the veterinary literature; however, mixed results have been identified in multiple *in vitro* and *in vivo* canine flexor-tendon injury models when treated with MSC and different growth factors. One such *in vitro* study evaluated the structural effects of platelet-rich plasma (PRP) and/or bone marrow-derived stromal cells (BMSC) on canine flexor tendons. After cell culture, significant increases in maximum strength and stiffness were detected with tendons treated with both PRP and BMSC together when compared to negative controls and tendons treated only with BMSC.[16] Based on these results, this group is in the process of further testing using an *in vivo* canine model.[16]

Whereas there are no clinical reports of MSC treatment of common calcaneal tendon injuries in dogs, there are clinical publications on the use of MSC in the treatment of superficial digital flexor tendon (SDFT) strains in horses.[14,15] Reported clinical outcomes were compared to previous reports with significantly improved return to function and reduced recurrent injury rates being documented after MSC treatment.[15] Histopathology was available for some horses treated with MSC that were euthanized at various time points after treatment. Proliferation of collagen fibers and apparent healing was documented.[15] Whereas there are numerous limitations with extrapolating equine study results to the dog in this report, the treatment protocols used were very similar and the clinical outcomes appear to be positive. It is unknown whether or not MSC contributed to the healing process in our dog, but it is worth further investigation and controlled experimentation is indicated.

Hinged splints are frequently used in people after Achilles tendon repair to encourage early, controlled use of the limb.[17,18] The reported benefits include early return to function and less disuse atrophy.[17,18] In veterinary medicine, typical immobilization techniques include: coaptation splinting or transarticular external skeletal fixation.[2–4,6,7,9] It is interesting that in contrast to human surgery where early return to controlled motion is recommended, in veterinary surgery rigid immobilization has been the cornerstone of postoperative management. This aspect of our case management deserves special attention, especially given the results of a recent study that demonstrated no difference in gastrocnemius tendon strain in tarsi of dogs immobilized with an external fixator versus no immobilization.[10] However, external fixators were placed at a standing angle in that study. Immobilization of the joints in an extended position may have lead to different results. To our knowledge this is the first report of CPDO stabilization in the treatment of calcaneal tendon injuries in dogs. Use of CPDO after primary tenorrhaphy may also be useful and should be considered as a possible subject of further investigation.

The use of ultrasound in evaluating the canine calcaneal tendon has been reported.[19] Ultrasound was also used in a previous study to evaluate healing after primary tenorrhaphy and semitendinosus augmentation.[6] In that study, ultrasound imaging was effective in assessing catastrophic complications as well as subjective healing. This is in agreement with our case in which subjective linearization of tendon fibers and objective diminishing lesion size was seen during the healing process. Ultrasound appears to be an effective method of diagnosing and monitoring calcaneal tendon injuries in dogs.

We evaluated tarsal posture as a gross method of assessing the potential regenerative healing abilities of the MSC. The lack of improvement in overall tarsal extension angle by the end of the study period was consistent with our other results, namely, persistent weight-bearing deficits and incomplete healing of the gastrocnemius tendon fiber pattern.

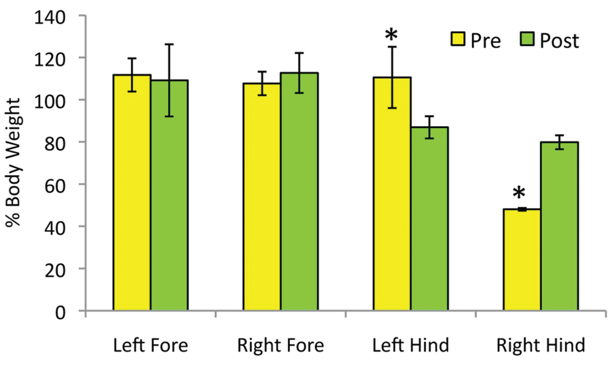

Figure 3 Bar graphs demonstrating pretreatment and 631days posttreatment peak vertical force for both forelimbs and pelvic limbs. Asterisks represent significant differences between pre and posttreatments.

The subjective lameness grade of 0/5 achieved by this dog 1.5 years after treatment was considered good and consistent with the best reported surgical outcomes in dogs.[6,7,9] It is also interesting in that it demonstrates the need for more objective methods of outcome measure in dogs given the persistent weakness in the surgical limb demonstrated by our force plate results.

We used force plate gait analysis as an objective outcome measure. Of all ground reaction forces measured, we examined peak vertical and peak propulsive forces as they represent the largest loads applied to the limb during ambulation in the vertical and horizontal directions, respectively. This dog ultimately regained 92% of the peak vertical force and 61% of the propulsive force of the contralateral limb. To our knowledge there are no clinical studies evaluating force plate analysis after calcaneal tendon injury in dogs; however, a recent *in vivo* study looking at peak vertical force measurements in dogs after calcaneal surgery and immobilization of the tarsus, found preoperative peak vertical forces similar to the outcome values of the dog we report.[10] Significant differences in posttreatment kinetic variables between the contralateral pelvic limbs were noted in peak vertical force, and peak propulsive force which indicates a residual lameness or altered gait within the right pelvic limb (Fig 3). The development of mild cranial-caudal instability in the right stifle is of interest in that it may have played a role in the lameness seen in our dog. While there was never any discomfort associated with hyperextension of the stifle, it may have affected the dog's recovery. The owner was offered arthroscopic exploration of the dog's stifle joint for diagnosis, but this was declined because of the dog's perceived excellent outcome.

In conclusion, the outcome after treatment of a gastrocnemius tendon strain in a dog using MSC and CPDO was considered good. Although incomplete healing was seen with serial orthopedic and ultrasound examinations, functional outcome was equivalent to what is reported with successful surgical treatments.[6,7,9] However, further studies are indicated to evaluate the efficacy of this treatment before it can be recommended in clinical dogs.

REFERENCES

1. King M, Jerram R: Achilles tendon rupture in dogs. *Compend Contin Educ Pract Vet* 2003;25:613–620.
2. Reinke JD: Treatment of avulsion of the gastrocnemius tendon, in Bojrab MJ, Ellison G, Slocum B, (eds): *Current Techniques in Veterinary Surgery*. Baltimore, MD, Williams & Wilkins, 1998, pp 1257–1260.
3. Montgomery R, Fitch R: Muscle and tendon disorders, in Slatter D, (ed): *Textbook of Small Animal Surgery*. Philadelphia, PA, Saunders, 2003, pp 2264–2272.
4. Piermattei DL, Flo GL, DeCamp CE: *Handbook of Small Animal Orthopedics and Fracture Repair*. St Louis, MO, Saunders Elsevier, 2005, pp 674–678.
5. Gall TT, Santoni BG, Egger EL, et al: In vitro biomechanical comparison of polypropylene mesh, modified three-loop pulley suture pattern, and a combination for repair of distal canine achilles' tendon injuries. *Vet Surg* 2009;38:845–851.
6. Baltzer WI, Rist P: Achilles tendon repair in dogs using the semitendinosus muscle: surgical technique and short-term outcome in five dogs. *Vet Surg* 2009;38:770–779.
7. Guerin S, Burbidge HM, Firth E, et al: Achilles tenorrhaphy in five dogs: a modified surgical technique and evaluation of a cranial half cast. *Vet Comp Orthop Traumatol* 1998;11:205–210.
8. Moores AP, Comerford EJ, Tarlton JF, et al: Biomechanical and clinical evaluation of a modified 3-loop pulley suture pattern for reattachment of canine tendons to bone. *Vet Surg* 2004;33: 391–397.
9. Norton JC, DeCamp CE, Yu J, et al: Use of a single-ring transarticular fixator construct for immobilization of the talocrural joint following common calcaneal tenorrhaphy. *Vet Comp Orthop Traumatol* 2009;22:430–435.
10. Lister SA, Renberg WC, Roush JK: Efficacy of immobilization of the tarsal joint to alleviate strain on the common calcaneal tendon in dogs. *Am J Vet Res* 2009;70:134–140.
11. Shani J, Shahar R: Repair of chronic complete traumatic rupture of the common calcaneal tendon in a dog using a fascia lata graft. *Vet Comp Orthop Traumatol* 2000;13:104–108.
12. Gilbert TW, Stewart-Akers AM, Simmons-Byrd A, et al: Degradation and remodeling of small intestinal submucosa in canine Achilles tendon repair. *J Bone Joint Surg [Am]* 2007; 89-A:621–630.
13. Worth AJ, Danielsson F, Bray JP, et al: Ability to work and owner satisfaction following surgical repair of common calcanean tendon injuries in working dogs in New Zealand. *NZ Vet J* 2004;52:109–116.
14. Smith RW: Mesenchymal stem cell therapy for equine tendinopathy. *Disabil Rehabil* 2008;30:1752–1758.
15. Godwin EE, Young NJ, Dudhia J, et al: Implantation of bone marrow-derived mesenchymal stem cells demonstrates improved outcome in horses with overstrain injury of the superficial digital flexor tendon. *Equine Vet J* 2011;44:25–32.
16.

Morizaki Y, Zhao C, An KN, et al: The effects of platelet-rich plasma on bone marrow stromal cell transplants for tendon healing in vitro. *J Hand Surg Am* 2010;35:1833–1841.

17. Maffulli N, Tallon C, Wong J, et al: Early weightbearing and ankle mobilization after open repair of acute midsubstance tears of the Achilles tendon. *Am J Sports Med* 2003;31:692–700.
18. Mortensen NHM, Skov O, Jenson PE: Early motion of the ankle after operative treatment of a rupture of the Achilles tendon. *J Bone Joint Surg Am* 1999;81:983–990.
19. Lamb CR, Duvernois A: Ultrasonographic anatomy of the normal canine calcaneal tendon. *Vet Radiol Ultrasound* 2005;46:326–330.