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Feasibility and repeatability for *in vivo* measurements of stiffness gradients in the canine gastrocnemius tendon using an acoustoelastic strain gauge

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Abstract

B-mode ultrasound is an established imaging modality for evaluating canine tendon injury. However, full extent of tendon injury often remains difficult to estimate, as small changes in sonographic appearance are associated with large changes in biomechanical strength. The acoustoelastic strain gauge (ASG) is an ultrasound-based tissue evaluation technique that relates the change in echo intensity observed during relaxation or stretching of tendons to the tissue's mechanical properties. This technique deduces stiffness gradient (the rate of change of normalized stiffness as a function of tissue strain) by analyzing the ultrasound dynamic images captured from gradually deforming tissue. Acoustoelastic strain gauge has been proven to accurately model strain and stiffness within tendons *in vitro*. To determine the feasibility and repeatability for *in vivo* ASG measurements of canine tendon function, stiffness gradients for the gastrocnemius tendons of ten clinically normal dogs were recorded by two non-independent observers at three sites (musculotendinous junction, mid tendon, and insertion). Average stiffness gradient indices (0.0132, 0.0141, 0.0136) and dispersion values (0.0053, 0.0054, 0.0057) for each site, respectively, were consistent with published mechanical properties for normal canine tendon. Mean differences of the average stiffness gradient index and dispersion value between observers and between limbs for each site were less than 16%. Using interclass coefficients (ICC), intraobserver (ICC 0.79–0.98) and interobserver (ICC 0.77–0.95) reproducibility was good to excellent. Right and left limb values were symmetric (ICC 0.74–0.92). Findings from this study indicated that ASG is a feasible and repeatable technique for measuring stiffness gradients in canine tendons.

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Conflict of Interest Statement

Two authors (R.V. and H.K.) hold a patent associated with some aspects of this concept for ultrasound analysis. No other authors have any conflicts of interest with the present study.

Introduction

Traditional B-mode ultrasonography is an established imaging modality for diagnosing tendon injury in dogs.^{1–3} Sonographic characteristics of tendon injury include changes in thickness, shape, fiber pattern and echogenicity.³ Assessment of tendon function can only be determined by extrapolation of this anatomic information combined with physical exam findings. However, the full extent of tendon injury often remains difficult to estimate, as small changes in B-mode sonographic appearance are associated with large changes in biomechanical strength.⁴ Evaluation of tendon's mechanical properties could potentially allow earlier detection of injury, more accurate monitoring of healing, as well as, more specific prognoses for recovery.^{5,6}

Because of the importance in determining the effect of injury on the function of tendons and ligaments, multiple methods for measuring strain (change in length in relation to applied force) in these tissues have been examined.^{7–10} Techniques such as liquid metal strain gauges, buckle transducers, implantable force or pressure transducers, Hall effect strain gauges, differential variable reluctance transducers and fiber optic transducers that directly measure strain are highly invasive, expensive, and often limit normal range of motion.⁷ Noninvasive techniques developed for human medicine, such as magnetic resonance imaging (MRI) cine phase contrast and sonographic average end-to-end strain calculations, are poorly adaptable to veterinary medicine due to unrealistic levels of patient cooperation needed, expense, and the requirement for specialized equipment.^{8,9} Preliminary studies in horses and pigs describing non-invasive, sonographic methods for evaluation of tendon function have been based either on measurement of the axial speed of sound,^{11–14} sonoelastography,¹⁵ or acoustoelasticity.¹⁶ These techniques currently offer the greatest potential for practical clinical application in canine patients with tendon injury.

Elastography works best when strain increments are small (<1%) because the model incorrectly assumes that the mechanical properties of a material and the ultrasound wave velocity within that material do not change with deformation.¹⁶ During activity, tendons undergo a relatively large amount of deformation. For example, strains from 2.2–4.5% at the walk and up to 16.6% at the gallop have been measured within the equine superficial digital flexor tendon.¹⁷ In addition, elastography indirectly assesses tissue stiffness by examining compression in the transverse plane whereas tendons are physiologically loaded in a longitudinal plane. While both of these shortcomings could be addressed by measuring axial speed of sound, the specialized transducers required for this technique limit the potential for widespread clinical adoption in canine patients.^{12,13}

Acoustoelasticity is a mathematical theory that relates changes in innate acoustic characteristics, specifically the speed of sound, to deformation applied to a target material. The acoustoelastic strain gauge (ASG) is an ultrasound-based tissue evaluation technique derived from this theory. The acoustoelastic strain gauge relates the change in echo intensity observed during relaxation or stretching of tendons to the tissue's mechanical properties.^{16,18,19} The acoustoelastic strain gauge has been proven to accurately model strain and stiffness within porcine flexor tendon *in vitro*.¹⁶ At the time of this study, no descriptions of *in vivo* tests using the acoustoelastic strain gauge in any species could be found.

The ideal tendon for acoustoelastic strain gauge evaluation is straight, superficial, with a large difference between relaxed and stretched positions. The canine gastrocnemius tendon was selected for this study based on these characteristics. The gastrocnemius tendon is one of the three major structures forming the common calcaneal tendon.²⁰ Although common calcaneal tendon injury in the dog is rare, the gastrocnemius tendon is the most commonly

injured portion.²¹ The injury is usually caused by an acute trauma; however, it has been suggested that degeneration due to repetitive stress may also play a role.²⁰ Both full and partial ruptures of the musculotendinous junction, mid tendon, and insertion have been described in the dog.^{2, 20, 21} Diagnosis is usually obtained by a combination of physical exam findings, radiography, and ultrasound. Surgical management is recommended if a full or partial thickness tear is identified as conservative management often leads to unsatisfactory results.²⁰ While sonographic assessment of the healing of common calcaneal injury has been documented, investigators could not determine the point at which the healing process ceased.² The purpose of this study was to determine the feasibility and repeatability of the acoustoelastic strain gauge for *in vivo* measurements of gastrocnemius tendon stiffness gradients in a cohort of normal dogs.

Materials and Methods

All animal protocols were approved by and in accordance with the University of Wisconsin Research Animal Resources Center. With informed client consent, five female and five male, clinically normal, medium to large breed dogs with no history of lameness were recruited from the population of client-owned animals presenting to the University of Wisconsin Veterinary Medical Teaching Hospital for elective spay/neuter procedures. Dogs were defined as clinically normal based on a complete orthopedic and lameness examination performed by a surgical resident (K.D.).

Dogs were sedated by the anesthesia service, using a premedication protocol deemed most appropriate for their scheduled elective surgery. Dogs were then positioned and maintained in right lateral recumbency with a soft towel placed between the hind limbs in order to maintain consistent medial to lateral support. Longitudinal and transverse images of the musculotendinous junction, the subjective mid portion, and insertion of the right and left gastrocnemius tendons were obtained with a 6–18 Mhz linear electronic transducer (MyLab 70, Esaote, Indianapolis, IN) in order to establish that no B-mode sonographic abnormalities were evident. Cine loops optimized for the gastrocnemius tendon in longitudinal orientation at each of these sections were obtained as the tarsus was moved by an assistant from a maximally flexed to extended position. At each site, the cine loop acquisition was performed three times by one sonographer (M.E.) and a single time by a second sonographer (F.D.). Sonographers were aware of each other findings at the time of the exam as they acted as the assistant when not scanning.

Uncompressed audio/video interleaved (AVI) format files for each of the cine loops were then used for acoustoelastic strain gauge analyses by a single observer (H.K.) who was unaware of patient demographics and sonographer identity. A region of interest (ROI) consisting of approximately the middle half of the tendon section was selected. In order to maintain this same ROI throughout the cine loop, a region-based optical flow tracking technique was used to estimate the movement of speckles between consecutive frames.¹⁶ The change in relative pixel intensity over time was analyzed to allow selection of the segment of the cine loop containing the most consistent movement of the tendon from a stretched to relaxed state. This segment was evaluated with the acoustoelastic strain gauge mathematical model allowing calculation of a stiffness gradient (the rate of change in stiffness in relation to the change in strain) from the change in relative intensity of each pixel within the ROI.^{16, 18, 19} The stiffness gradients were depicted visually as a continuous color-coded scale overlaid on the B-mode ultrasound image of the tendon in order to allow subjective assessment (Figure 1B). Blue indicated a region of low stiffness gradient (i.e. firm tissue that exhibits little change in stiffness in relation to deformation) and red indicated a region of high stiffness gradient (i.e. soft tissue that exhibits large changes in stiffness in relation to deformation).

In order to quantify stiffness gradients, a standardized 50 pixel $\times$ 300 pixel area located centrally within the original region of interest was selected. A histogram relating stiffness gradient to number of pixels was obtained from this standardized area (Figure 1A). The stiffness gradient index for each cine loop was defined as the average stiffness gradient for the histogram. The distribution of stiffness gradients (dispersion value) was defined as the standard deviation of the histogram. Mean, mean difference between observers, and mean difference between limbs were calculated for each site for both the stiffness gradient index and dispersion values.

All statistical tests were selected and performed by or under the supervision of a statistician. Regional differences in mean stiffness gradient and dispersion values were compared across all sites for each limb using a repeated measure analysis of variance (ANOVA). Effects of age and gender on mean stiffness gradient were tested using a Pearson's correlation and Student's t-test, respectively. Variation in the rate of returned error from the acoustoelastic strain gauge was analyzed using Fisher's exact test. For all comparison tests, a statistically significant difference was defined as $p < 0.05$. Intraobserver repeatability, interobserver repeatability, and symmetry between right and left limbs at each of the three sites for both the stiffness gradient and dispersion values were measured using intraclass correlation coefficients (ICC). Intraclass correlation coefficient cutoffs were defined as follows: < 0.4 poor agreement, $0.41\text{--}0.6$ moderate agreement, $0.61\text{--}0.79$ good agreement, > 0.8 excellent agreement.²² Any patient that had more than one error for a site was removed from the intraobserver repeatability analysis for that site. Any patient that had an error for the second observer was removed from the interobserver repeatability and right to left symmetry analysis for that site. Commercially available statistical software (R (version 2.14.0), R Foundation for Statistical Computing, Vienna, Austria) was used for all data analyses.²³

Results

Included dogs ranged in age from 5 to 30 months old (mean 12.3 months, median 7 months). Six different breeds were represented: Australian Shepherd, Border Collie, Labrador Retriever (3), Siberian Husky, Rhodesian Ridgeback, and Rottweiler (3). The mean stiffness gradient indices and dispersion values for the musculotendinous junction, the mid portion, and the insertion of a normal canine gastrocnemius tendon are summarized in Table 1. The mean stiffness gradient index for the insertion of the right gastrocnemius tendon was increased compared to the remainder of the sites ($p = 0.03$). There was no statistical difference among any of the remaining values. No statistically significant differences or trends for gender or age effects on stiffness gradient index or dispersion value were identified.

Data from 51/240 cine loops (21%) returned errors when the acoustoelastic strain gauge was applied. There was a statistically significant increased error rate at the insertion of the tendon (30%) compared to other sites, musculotendinous junction (22%) and mid portion (11%) ($p = 0.01$). There was no difference in error rate between observers.

The mean differences for the stiffness gradient and dispersion values between observers and between limbs for each site are summarized in Tables 2 and 3, respectively. All differences between observers and between limbs were less than 16% of the metric evaluated and the 95% confidence intervals contained zero. The intraclass correlation coefficients evaluating repeatability within the same observer, between observers and between right and left limbs are summarized in Tables 4, 5, and 6, respectively. Intraobserver (ICC $0.79\text{--}0.98$) and interobserver (ICC $0.77\text{--}0.95$) reproducibility was good to excellent. Right and left limb values were symmetric (ICC $0.74\text{--}0.92$).

Discussion

Acoustoelastic strain gauge findings for the normal canine gastrocnemius tendon were consistent with a tissue that was relatively stiff (small stiffness gradient indices). In addition, there was minimal internal variation in functional characteristics of the tendon as dispersion values were an order of magnitude smaller than the measured stiffness gradient indices. These results were expected, as the normal canine gastrocnemius tendon is composed of a dense, homogeneous, parallel arrangement of fibers with a high tensile strength and elastic modulus.²⁴ The mean stiffness gradient index for the insertion of the right gastrocnemius tendon was increased compared to the remainder of the sites. The fibrocartilaginous region of the insertion of the canine gastrocnemius tendon has a lower elastic modulus and therefore a higher stiffness gradient would be expected for this tissue.²⁴ Although the portion of the tendon measured at the insertion in this study was located immediately proximal to the expected location of the fibrocartilaginous region, the recumbent position of the right limb during evaluation may have caused unintended inclusion of this region, resulting in the reported increased stiffness gradient.

No age or gender effects were identified for acoustoelastic strain gauge measurements in dogs in this study. This finding was not consistent with previous reports describing age-related differences in tendon biomechanics in humans and horses. However, it should be noted that none of the included dogs were older than 30 months. In the equine superficial digital flexor tendon, stiffness is increased in animals greater than 2 years of age.^{17, 25} In human quadriceps and Achilles tendons decreased stiffness has been correlated with increasing age.^{26, 27} If the effect of age on tendon mechanics is consistent across species, this may be explained by an initial increase in tendon stiffness during maturation followed by a decrease in tendon stiffness secondary to age-related degeneration. In horses, tendon mechanical properties can be further altered by exercise in juvenile animals, the effect of which can extend beyond maturation.¹⁷ In humans, gender specific differences have also been reported and may play a role determining tissue strength as tendons from females have lower stiffness and elasticity compared to male subjects.^{28, 29} The small number of subjects and narrow age range of the current study limited the likelihood of finding any effect of age or gender on the canine gastrocnemius tendon. It is considered unlikely that the magnitude of change in the mechanical properties from the potential effects of age, gender and/or exercise on canine tendon would be on a similar scale to that caused by acute tendon injury.³⁰ Additional research would be required to determine if these potential changes could affect the interpretation of the acoustoelastic strain gauge findings in terms of evaluating more chronic lesions, increased risk of tendon injury, or degree of tendon healing.

Approximately one-fifth of all cine loops returned errors when applying the acoustoelastic strain gauge. The error rate was greatest at the gastrocnemius tendon insertion and musculotendinous junction, which have a greater complexity of anatomy and movement than the mid-portion of the tendon. This is especially true at the tendon insertion where the calcaneal tuber could interfere with maintenance of contact between the transducer and tendon when the tarsus was extended. In future studies, at least three cine loop acquisitions should be obtained to guarantee that suitable data would be available for analysis.

The 16% mean difference between observers for both stiffness gradient and dispersion values indicated that there was good sonographer repeatability for measuring characteristics of normal canine gastrocnemius tendons *in vivo* using the acoustoelastic strain gauge. As the 95% confidence intervals for the differences between observers all contained zero, there actually may have been no true difference. Good (> 0.6 ICC) to excellent (> 0.8 ICC) intraobserver repeatability and interobserver repeatability also indicated that acoustoelastic

strain gauge measurements had high precision when performed by the same or different sonographers. Similar values were observed between right and left limbs, demonstrating the low homotypic variation that is expected in normal canine tendons.

A limitation of this study was the small sample size, which resulted in large 95% confidence intervals for some of the reproducibility statistics. This problem was amplified by data loss due to error. There was a much larger confidence interval when evaluating interobserver and intraobserver repeatability for data from the mid portion than the other sites along the tendon. It is possible that this was due to lost data from the musculotendinous junction and insertion sites when cine loops were less than perfectly acquired. The opposite effect was seen when evaluating limb symmetry as the musculotendinous junction and insertion sites had the largest confidence intervals. This was most likely caused by the greater complexity of anatomy at these locations. Small variations in proximal to distal location along the tendon between the right and left limb being evaluated would likely have greater effects at these sites than the mid portion of the tendon. For example, a more distal acquisition at the gastrocnemius insertion could have included a portion of the fibrocartilagenous region while a more proximal acquisition at the musculotendinous junction could have included a region with a different proportion of interspersed muscle and tendon fibers.²⁴ This variation may have been reduced if the cine loop acquisition at each site had been paired rather than evaluating all of left limb followed by all of the right as done in this project.

Another limitation to this study was that animals were presumed to have normal gastrocnemius tendons through a combination of owner history and physical exam findings, including a complete orthopedic evaluation. No histopathologic confirmation of normal tendon status was performed. Patients with any clinical abnormalities were excluded, as the sensitivity of the acoustoelastic strain gauge for subtle compensatory changes due to lameness is unknown. Patient age was restricted to younger animals to minimize the possibility of unknown degenerative process or previous injury. Despite these restrictions, chronic or subclinical tendon injury could have been present in some of the subjects.

Acutely injured tendon has been previously described as having decreased elastic modulus, stiffness, and echogenicity compared to normal tissue.^{30–32} Clinical pilot studies using sonoelastography in humans with tendon injury have most commonly detected decreased tissue stiffness and increased heterogeneity within the elastogram.^{33–35} However, some conflicting results have also been published.³⁶ During the healing process echogenicity of the tendon may return to normal; however, the mechanical properties may remain inferior to uninjured tissue.^{32, 37} Both axial speed of sound and kinematic analysis have shown a gradual return towards normal parameters during healing of equine superficial digital flexor tendons; however, only the initial course of the healing process was examined.^{12, 31} Additional research is needed to determine if evaluation of injured and healing canine tendons with the acoustoelastic strain gauge would yield similar results.

Widespread adoption of functional ultrasound in veterinary clinical practice requires an inexpensive, uncomplicated, easily repeatable, non-invasive method of analysis. As software-based techniques that use equipment present within most veterinary practices, the acoustoelastic strain gauge and sonoelastography currently meet these requirements. While acoustoelastic strain gauge provides more accurate modeling and longitudinal plane assessment of tendon mechanics, its application is limited to those tendons for which a sonographic window can be maintained while moving from a stressed to relaxed state.¹⁶ Additional research comparing the two methods for a variety of tendons, injuries, and species is needed to determine if there are clinically significant differences in the functional analysis.

In conclusion, the stiffness gradients measured with the acoustoelastic strain gauge in clinically normal canine gastrocnemius tendons *in vivo* were consistent with expected mechanical properties based on previous reports for normal canine gastrocnemius tendon. The technique was found to have high repeatability and precision for measuring stiffness gradients in normal canine gastrocnemius tendons. Additional research is needed to determine the ability of the acoustoelastic strain gauge to detect tendon injury and monitor tendon healing in clinically affected dogs.

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Figure 1.

(A) Histogram depicting the stiffness gradients for the musculotendinous junction (MTJ, red), mid-portion (blue) and insertion (green) in a clinically normal gastrocnemius tendon. (B) Corresponding B-mode ultrasound images with color-coded overlay. Blue indicates a region of low stiffness gradient (firm tissue) and red indicates a region of high stiffness gradient (soft tissue).

Table 1

Mean Stiffness Gradient Index and Dispersion Values

Site	SGI*	95% CI†	DV‡	95% CI
Musculotendinous Junction	0.0132	0.0118–0.0147	0.0053	0.0046–0.0060
Mid Portion	0.0141	0.0125–0.0157	0.0054	0.0048–0.0061
Insertion	0.0136	0.0118–0.0153	0.0057	0.0049–0.0066

* Stiffness Gradient Index

† Confidence Interval

‡ Dispersion Value

Table 2

Mean Difference between Observers

Site	SGI*	95% CI†	DV‡	95% CI
Musculotendinous Junction	0.0014	-6×10^{-5} –0.0035	0.0004	–0.0004–0.0012
Mid Portion	–0.0022	–0.0048–0.0004	-7×10^{-8}	–0.0013–0.0013
Insertion	0.0007	–0.0008–0.0023	0.0006	-9×10^{-5} –0.0014

* Stiffness Gradient Index

† Confidence Interval

‡ Dispersion Value

Table 3

Mean Difference between Limbs

Site	SGI*	95% CI†	DV‡	95% CI
Musculotendinous Junction	-0.0019	-0.0046–0.0008	-0.0011	-0.0023–0.0001
Mid Portion	-0.0014	-0.0033–0.0006	-0.0002	-0.0010–0.0005
Insertion	0.0017	-0.0017–0.0050	0.0008	-0.0008–0.0024

* Stiffness Gradient Index

† Confidence Interval

‡ Dispersion Value

Table 4

Intraclass Correlation Coefficients for Intraobserver Repeatability

Site	S _{GI} [*]	95% CI [†]	DV [‡]	95% CI
Musculotendinous Junction	0.97	0.93–0.99	0.84	0.63–0.95
Mid Portion	0.79	0.59–0.92	0.78	0.58–0.91
Insertion	0.98	0.90–1.0	0.81	0.45–0.97

* Stiffness Gradient Index

† Confidence Interval

‡ Dispersion Value

Table 5

Intraclass Correlation Coefficients for Interobserver Repeatability

Site	SIG [*]	95% CI [†]	DV [‡]	95% CI
Musculotendinous Junction	0.92	0.74–0.98	0.90	0.69–0.97
Mid Portion	0.77	0.32–0.92	0.71	0.20–0.90
Insertion	0.95	0.87–0.98	0.93	0.78–0.98

* Stiffness Gradient Index

† Confidence Interval

‡ Dispersion Value

Table 6

Intraclass Correlation Coefficients for Right to Left Limb Symmetry

Site	S _{GI} [*]	95% CI [†]	DV [‡]	95% CI
Musculotendinous Junction	0.74	−0.09–0.94	0.75	0.02–0.94
Mid Portion	0.92	0.69–0.98	0.92	0.67–0.98
Insertion	0.77	0.07–0.95	0.70	0–0.93

* Stiffness Gradient Index

† Confidence Interval

‡ Dispersion Value