Use of quantitative analysis of sonographic brightness for detection of early healing of tendon injury in horses

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Objectives—To determine whether quantitative analysis of sonographic brightness could be used to detect healing of an induced injury of the superficial digital flexor tendon in horses and whether rate of healing was influenced by equine recombinant growth hormone.

Animals—8 clinically normal Standardbreds.

Procedures—A localized injury was created in the left and right superficial digital flexor tendons of each horse by injection of 2,000 units of collagenase. After injury, 4 horses received equine recombinant growth hormone, a possible promoter of tendon healing. Sonographic images (7.5 MHz) of the flexor tendons and ligaments of the metacarpal region were recorded on videotape prior to injury and weekly for 7 weeks after injury. Images were digitized, and sonographic brightness of tendons and ligaments was calculated.

Results—Collagenase-induced injury was sonographically similar to naturally occurring injury. After injury, sonographic brightness of the tendon decreased; after 3 weeks, brightness progressively increased, although by 7 weeks brightness had not returned to preinjury value. Equine recombinant growth hormone had no significant effect on the rate of tendon healing, as evaluated sonographically or at necropsy.

Conclusions and Clinical Relevance—As healing developed, alterations in sonographic brightness of injured tendons coincided with real changes in tendon structure. Quantitative sonographic brightness could be used to accurately monitor healing of equine tendon and ligament injuries and investigate the efficacy of various treatment regimens. (Am J Vet Res 2001; 62:1320–1327)

The usefulness of sonography for diagnosis of injuries of the flexor tendons and ligaments of the metacarpal region in horses continues to be confirmed. Characteristically, acute rupture and disruption of the collagen fiber bundles in tendons or ligaments are detected in initial sonographic images as an anechoic or hypoechogenic region within the structure. Reduction in echogenicity is associated with displacement of the collagen fiber bundles, which no longer act as specular reflectors of the sonographic beam, as well as by through-transmission of the beam, which occurs in areas of hemorrhage, fibrolysis, and early granulation tissue formation. The overall hypoechogenicity of the injured region is maintained as fibroplasia and further granulation tissue develop, but with healing there is a subjective increase in echogenicity. The progressive increase in echogenicity during the initial months of normal healing is thought to be associated with gradual reorientation of the collagen fiber bundles along the lines of stress in the limb (so that they again act as specular reflectors), a decrease in through-transmission of the sonographic beam (as areas of hemorrhage are resolved and the number of fibroblasts are reduced), and an increase in the scatter density (related to the formation of molecular linkages in the collagen fibers).

Observations of the mean gray scale (MGS) of sonographic images of tendons and ligaments have been used in an attempt to define the sonographic characteristics of the normal structures and study the effects of aging, injury, and race training. Repeatability of such observations is dependent on anisotropy (related to the angle of the sonographic beam) and the imaging variables used to produce the sonographic image. Although many imaging variables contribute to this variation, time gain compensation, which is usually controlled by the operator of the sonographic machine, has a major influence on the observed echogenicity of tendons and ligaments. Results of a previous study indicate that such variations can be normalized or accounted for by calibrating the gray scale images with a tissue-mimicking phantom.

Presently, tendon and ligament healing is largely assessed by subjective evaluation of changes of echogenicity in repeated sonographic images. To make interpretation of the images more accurate and reproducible, there is a need to develop a means of quantifying the changes in echogenicity that develop over time. Further, it is also known that the changes in MGS may be small and not easily detected with any level of confidence by a simple visual inspection of the sonographic image. Because quantitative techniques have the potential to detect small changes in echogenicity, we believe such analyses would improve the sensitivity of diagno-
sis. Also, there is interest in the role of growth hormone as a promoter of postsurgical healing of tissues. The purpose of the study reported here was to determine whether quantitative analysis of sonographic brightness could be used to detect healing of an induced injury of the superficial digital flexor tendon (SDFT) in horses and whether rate of healing was influenced by equine recombinant growth hormone.

Materials and Methods

Horses—This study used 8 clinically normal Standardbreds (7 mares, 1 gelding; age, 3 to 7 years; mean ± SD body weight, 381 ± 32 kg) that were part of a larger cohort of horses involved in an investigation of the effects of equine somatotropin on healing of an induced tendon injury. The project was approved by the Animal Care and Ethics Committee of the University of Sydney.

In each horse, a localized injury was induced in the SDFT of both forelimbs. Horses were sedated by IV administration of detomide hydrochloride (0.01 mg/kg of body weight) and given phenylbutazone IV (4.4 mg/kg, IV), and the skin of the palmar aspect of each metacarpal region was clipped and aseptically prepared with providone-iodine and 70% alcohol. A 27-gauge 1.25-cm needle was inserted into each tendon via its medial border at each of 3 sites (6, 7, and 8 cm distal to the midpoint of the palmar border of the accessory carpal bone), and 0.15 ml (807 units) of collagenase was injected. Using sonography, care was taken to ensure that the needle tip was located within the center of the tendon. After collagenase injection, each horse was placed in a 10 × 10-m yard, clinically examined daily for evidence of lameness or discomfort, and administered phenylbutazone (2.2 mg/kg, PO, q 24 h) for 5 days. In addition, 4 of the horses received equine recombinant growth hormone (equine somatotropin; 10 µg/kg, IM, Q 24 h, for 1 week, then 20 µg/kg, q 24 h, for 5 weeks), commencing 1 week after injection of collagenase. At the completion of the sonographic observations, each horse was euthanized by IV administration of pentobarbital, and a necropsy was performed.

Sonography—Sonographic images (7.5-MHz mechanical sector probe) were made in dorsal and sagittal anatomic planes of the palmar aspect of the left and right metacarpal regions of each forelimb 4, 8, 10, 12, 14, 16, and 20 cm distal to the midpoint of the accessory carpal bone. Images were recorded on videotape prior to induction of tendon injury and at weekly intervals up to 7 weeks (except in horse 1, in which observations were made at 1, 2, 4, 6, and 7 weeks). On each day that the observations were made, sonographic images were also recorded (using identical imaging variables) of 3 sonographic soft-tissue equivalent phantoms, as described.

Videotaped equine and phantom images were analyzed offline by use of a personal computer and digitized (640 × 480 × 8) at the rate of 1 image/10 s; no a priori decision was made in selecting images, and 75 images (mean value) for each week of observation were digitized. Each image made at each level in the dorsal or sagittal anatomic plane was arranged with the gray scale images of the 3 phantoms.

Mean gray scale measurements were made in each horse image, using a dedicated software package. Regions of interest were traced in the SDFT, the deep digital flexor tendon (DDFT), the accessory ligament (AL) of the deep digital flexor muscle, and the interosseous medius muscle (suspensory ligament [SL]) as displayed in the image (Appendix). Each tendon and ligament was present in each image, except images made 20 cm distal to the accessory carpal bone, where only the SDFT and DDFT were present (the branches of the SL were seen in the 20-cm dorsal-plane images, but regions of interest were not drawn). In the sagittal plane, a consistently sized region of interest (2,730 pixels) was used, and in the dorsal plane, the region of interest (2,000 to 7,000 pixels) was drawn to encompass as much of the respective tendon or ligament as possible. Using the software package, each region of interest was copied to each of the 3 phantom images. The size, shape, and location of each region of interest in the phantom image were identical to that used in the images.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Dorsal plane</th>
<th>Sagittal plane</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDFT</td>
<td>1.22 (0.19)</td>
<td>1.28 (0.17)</td>
</tr>
<tr>
<td>DDFT</td>
<td>1.46 (0.13)</td>
<td>1.55 (0.15)</td>
</tr>
<tr>
<td>AL</td>
<td>1.67 (0.23)</td>
<td>1.64 (0.23)</td>
</tr>
<tr>
<td>SL</td>
<td>2.18 (0.20)</td>
<td>1.87 (0.20)</td>
</tr>
</tbody>
</table>

Within a column, mean values with different superscripts are significantly (P < 0.05) different.


Figure 1—Sonographic images of the flexor tendons and ligaments of the metacarpal region, made in the dorsal plane of the left forelimb (a and b; medial is toward the left side of the figure) and sagittal plane of the right forelimb (c and d; proximal is toward the left side of the figure), of a horse with an induced injury of the superficial digital flexor tendon (SDFT). X = Stand-off attachment on face of the sonographic probe. Y = Reverberation artifact related to stand-off attachment. S = Superficial digital flexor tendon. D = Deep digital flexor tendon. A = Accessory ligament. L = Suspensory ligament.
sonographic image of the tendon or ligament of each horse. The MGS in each region of interest was measured on a scale of 0 to 255, where 0 is black, and 255 is white. At each level in each limb of each horse, the brightness of each structure in the sonographic image was calculated by dividing its MGS value by the mean value for the MGS of the 3 phantoms. Individual digitized sonographic images were selected from the screen of the computer workstation and printed.

Analysis of data—Sonographic brightness (gray scale units on a scale of 0 to 255) was calculated as a mean ± SD value for each level of each tendon or ligament. A multivariate repeated-measures ANOVA was used for overall comparisons, and paired t-tests were performed to compare pairs of means; Values of P ≤ 0.05 were considered significant. Because complete data were necessary for performance of multivariate repeated-measures analysis, the values of any missing data points were considered to be values obtained during the preceding week. Analyses were performed to determine differences between sonographic brightness values of each normal tendon and ligament. Further analyses tested whether there were variations in the sonographic brightness of each tendon and ligament between horses and between left and right forelimbs. After injury to the SDFT, the sonographi...
ic brightness value of a tendon or ligament (obtained at a particular time and level) was compared with each of the other 7 values collected at the same level but at differing times.

Results

Acquisition of data—Sonographic images were unavailable from horse 1 for the third and fifth weeks after injury. Of 6,400 possible observations, sonographic brightness of the individual flexor tendons and ligaments was measured on all but 18 occasions when, for technical reasons, an individual structure could not be analyzed.

Normal tendons and ligaments—There were significant \( P \leq 0.001 \) differences among sonographic brightness values obtained for the SDFT, DDFT, AL, and SL. The main effect of the anatomic plane of observation was not significant \( P = 0.11 \); however, interaction between structure and anatomic plane was significant \( P \leq 0.001 \), indicating that there were differences in sonographic brightness between the 2 planes but not for all structures. There were no significant differences between observations made of each structure in the left and right forelimbs of the same horse. Thus, data

Figure 3—Changes in sonographic brightness (gray scale units ± SD [Y axis]; pooled data from sagittal images of the left and right forelimbs) of the SDFT of 8 horses, made at 7 distances (cm [schematic diagram]) distal to the midpoint of the palmar border of the accessory carpal bone. Sonographic brightness was measured weekly (X axis) after induction of an injury to the SDFT at the 8-cm level at time 0. See Fig 2 for key.
obtained from sonographic observations of the 4 structures of both limbs (at the 7 levels, prior to injury of the SDFT) were pooled for each of the left and right forelimbs. In each anatomic plane, statistical analyses revealed that the SL was the most echogenic and had the highest sonographic brightness, followed by the AL, DDFT, and SDFT (Table 1).

Effect of somatotropin—During the 6 weeks of somatotropin administration to 4 horses, somatotropin had no effect on the sonographic brightness of the injured SDFT or the other tendons and ligaments. Thus, data collected from the 4 somatotropin-treated and 4 untreated horses were pooled.

Injured SDFT—One week after injection of collagenase, the injured area was clearly detected in sonographic images made in the dorsal and sagittal anatomic planes as a mottled hypoechoic region within the tendon (Fig 1). The tendon was thickened, and the typical echogenic spots in the dorsal anatomic plane and the echogenic lines in the sagittal plane, corresponding to transverse and longitudinal sections of bundles of collagen fibers, respectively, were not evident. The qualitative

Figure 4—Changes in sonographic brightness (gray scale units ± SD [Y axis]; pooled data from dorsal images of the left and right forelimbs) of the SDFT of 8 horses, made at 7 distances (cm [schematic diagram]) distal to the midpoint of the palmar border of the accessory carpal bone. Sonographic brightness was measured weekly (X axis) after induction of an injury to the SDFT at the 8-cm level at time 0. See Fig 2 for key.
reduction in echogenicity of the injured site was accompanied by a significant progressive decrease, typically during a 3-week period, in sonographic brightness of the injured region. Significant reductions in sonographic brightness at the other levels of the tendon were also evident (Fig 2–4). During the period from 3 to 7 weeks after injury, there was a subjective increase in echogenicity of the injured region of the tendon (Fig 1), although this region still retained a mottled granular appearance. There was an accompanying significant progressive increase in sonographic brightness of the injured tendon (at the site of injury and at the other levels), but at 7 weeks after injury brightness of the SDFT still remained significantly lower than before injury.

DDFT, AL, and SL—In sonographic images of the DDFT recorded after injury to the SDFT, architecture of the tendon was unchanged. There were, however, sub-

Figure 5—Changes in sonographic brightness (gray scale units ± SD [Y axis]; pooled data from dorsal and sagittal sonographic images of left and right forelimbs) of the deep digital flexor tendon of 8 horses, made at 2 distances (cm [schematic diagram]) distal to the midpoint of the palmar border of the accessory carpal bone. Sonographic brightness was measured weekly (X axis) after induction of an injury to the SDFT at the 8-cm level at time 0. *Significant (P ≤ 0.05) increase at week 4, compared with time 0. †Significant (P ≤ 0.05) increase at week 7, compared with time 0.
jective increases in the echogenicity of the tendon, which were accompanied by significant progressive increases in sonographic brightness of all but the most proximal and distal levels of the DDFT (Fig 5). Echogenicity usually peaked at 4 weeks after injury to the SDFT and seemed subjectively to decrease during the ensuing 3-week period, but not significantly, either qualitatively or quantitatively. After injury to the SDFT, there were no subjective alterations in overall sonographic appearance or echogenicity of the AL or SL and no significant changes in sonographic brightness of either ligament.

Necropsy findings—Major thoracic and abdominal organs of each horse were grossly normal. Gross appearance of the injured SDFT of the somatotropin-treated and untreated horses were similar, and each tendon was thickened, especially at the site of collagenase injection. The DDFT, AL, and SL of each horse were grossly normal.

Discussion

After collagenase injection, sonographic images of the injured regions of the SDFT appeared similar to those found after naturally occurring trauma.1-5 There were clearly defined hypoechoic regions, corresponding to sites of disruption of the collagen fiber bundles in the tendon (possibly accompanied by localized hemorrhage and edema). At the site of induced injury, there was also an increase in thickness of the tendon and loss of its normal architecture, especially in the sagittal anatomic plane, where the tendon had a granular appearance. These sonographic findings were consistent with results of a histologic study17 of the healing of collagenase-induced equine tendon injuries; at 8 weeks after injection of collagenase, the SDFT had a firm fibrous tissue scar. The authors concluded that repair of such injuries mimicked that of a naturally occurring tendon sprain. The dose of collagenase in that study was not given, but it may have been higher than in our study, because in that study, the cross-sectional area of the SDFT of 1 horse that was necropsied 8 weeks after induction of the injury was 6 times greater than before injury.21 The dose of collagenase (2,000 units) in our study was at the upper range (1,200 to 2,000 units) of that used in a recent investigation of the effects of SDFT tendinitis on limb kinematics and ground reaction forces.22

The question of whether sonographic characterization of tissues can provide clinically useful basic quantitative information about the normal structure and function of tendons and ligaments continues to be addressed. In in vitro studies of the SDFT and DDFT, measurements of ultrasound velocity, attenuation, and backscattering were made in the transverse and dorsal anatomic planes, as well as along the length of the tendon.23,24 It was found that these variables varied with the tendon; the SDFT had lower velocities and attenuations than the DDFT. The echotexture of the human Achilles tendon has an ellipsoidal spatial spectrum that was quantified.25 In vivo sonographic observations, a tissue elliptical ratio was measured, which successfully differentiated between normal tendons and those with cholesterol deposits and appeared to have potential for characterization of diffuse and focal tendon abnormalities.26 In horses, simple in vivo measurements of MGS, often with associated histograms, have also been used to provide quantitative information about tendons and ligaments.6,7,12,26,27 Such data was often not standardized by concurrent use of a tissue-equivalent phantom or corrected for any changes in imaging variables, especially time-gain-compensation; thus, usefulness of the technique was reduced. It is important to understand that the structures in a sonographic image may have variable MGS and distribution of gray levels (as illustrated in a histogram), depending on the selected imaging variables and the orientation of the sonographic beam. Furthermore, in in vitro studies of SDFT, there is not always a direct correlation between such uncorrected MGS and the histologic appearance of the tendon.20

Results of our study provide further evidence of the practical value of sonographic brightness measurements for characterizing certain aspects of tendon and ligament structure. In clinically normal horses, the sonographic brightness of the SL was significantly greater than that of the other flexor tendons and ligaments in the metacarpal region.14 This difference may be related to the fetal structure of the SL as the interosseous medius muscle; it has been reported in mature horses that muscle fibers were scattered through the SL.25,28 After the induced injury, there were consistent significant decreases in the sonographic brightness of the SDFT, followed by significant progressive increases in brightness as tendon healing commenced. These patterns of change in sonographic brightness, although most pronounced at the site of injury, also developed throughout the length of the SDFT; this suggests that the collagen fiber bundles in the uninjured region of the tendon were no longer perpendicular to the sonographic beam and, therefore, did not act as specular reflectors. These observations suggest that the entire SDFT was affected by the localized injury and that the tendon was no longer under its normal weight-bearing tension. Results of another study indicate reduction in MGS of the normal SDFT when it is non-weight-bearing; the MGS is higher during weight-bearing. Concurrent with changes in brightness of the SDFT were consistent significant opposite changes in the brightness of the DDFT. The initial increase in brightness of the DDFT and the decrease in brightness as the SDFT healed were probably related to increased transmission of the sonographic beam through the abnormal SDFT. The increased through-transmission did not appear to affect the sonographic brightness of the AL or SL, suggesting that the DDFT had effectively attenuated the increased through-transmission of the sonographic beam.

In laboratory animals, human growth hormone increases collagen deposition and breaking strength of colonic anastomoses.16,19 We found, however, that an equine recombinant growth hormone (somatotropin) had no effect on the initial rate of healing of the collagenase-induced tendon injury, as indicated by measurements of quantitative sonographic brightness and gross pathologic findings at necropsy. The reliable in vivo techniques we applied in this study used standard sonographic imaging equipment to quantify changes in tendon brightness that developed
after an injury. Sonography was performed in an identical manner to that used clinically, and as the sonographic beam was swept through the flexor tendons, it was perpendicular to the structures in the dorsal and sagittal anatomic planes. The repeatable significant changes in sonographic brightness coincided with real changes in tendon structure. Quantitative information provided by measurements of sonographic brightness may be used by equine clinicians to more accurately monitor the healing of tendon and ligament injuries and investigate the efficacy of various treatment regimens.

References