Ultrasonography has gained wide acceptance as an utmost practical and useful tool for the imaging of pathologic changes in equine tendons and ligaments. After introduction of the technique in the early 1980s by Rantanen, examination of tendinous and ligamentous structures in horses by use of ultrasonography has rapidly become a standard procedure. At present, the technique is routinely used in every-day equine practice for diagnostic purposes and for serial assessment of healing lesions or evaluation of treatments. 

A structure that is examined by use of ultrasonography is judged according to its echogenicity (ie, its capacity to generate echoes). Normal (injury-free) equine tendon tissue is highly echogenic because of its composition of longitudinally arranged, densely packed structures such as collagen fibrils. In the ultrasonographic image of a tendon, this echogenicity is reflected in the intensity (or brightness) and homogeneity (especially the spatial homogeneity over consecutive scans), which can be seen on the transverse image and in the axial alignment on the longitudinal scan. If a lesion develops in a tendon, part of the original structure is disrupted, which eventually, depending on the extent of the disintegration, becomes visible as loss of echogenicity. Initially, only semiquantitative grading methods have been developed for evaluation of the ultrasonographic images. Later, quantitative methods based on the quantification of the intensity of the echogenicity were introduced and expressed in terms of the gray level or image brightness of the transverse image. This technique has been used to quantify lesions in the tendons of racing Thoroughbreds, suggesting that the technique was sufficiently accurate to enable typing of pathologically changed tissues by analysis of the histogram of the corresponding ultrasonographic image. 

The value of so-called first-order statistics, such as gray level, gray sum, and mean gray level, as sole indicators of the integrity of tendons was questioned by Crass et al, who stated that ultrasonographic images remained abnormal until fibrillar realignment occurred with completion of the healing; this was further challenged by van Schie et al in a study on quantification of the effects of instrumental variables such as gain setting, transducer tilt, and transducer displacement. 

In the study reported here, correlation of the first-order gray level statistics of ultrasonographic images of normal equine superficial digital flexor (SDF) tendons and of equine SDF tendon tissues with various stages of lesion repair (and, therefore, various stages of tissue integrity) with the exact corresponding histologic specimens was investigated. The objective was to assess the discriminative power of the gray level statistics for accurate determination of the structural integrity of the tendon.

**Materials and Methods**

**Tendons**—The SDF tendons of 2 horses (a 9-year-old Dutch Warmblood gelding and a 3-year-old Thoroughbred...
mare) were studied. Both horses were known to have repeatedly developed lesions in 1 of their SDF tendons that had been documented over a prolonged period with respect to the moment of onset of each lesion and the exact location. The tendons were isolated within 2 hours after death. The tendons were proximally dissected from the limb at the junction of the superior check ligament. Distally, the insertion on the second phalanx was left intact as the first phalanx was cut at midshaft. The deep digital flexor tendon was severed at the site where it disappears into the hoof. A specimen consisting of hoof with the third phalanx, half of the first phalanx, and the SDF was obtained. Care was taken to keep the peritenon of the SDF intact. The contralateral tendons that were not pathologically changed were used as specimens of normal tendon tissue.

**Experiment setup**—Tendon handling, scanning procedure, and data collection have been described elsewhere. Briefly, the tendons were mounted in a water bath under standardized conditions. Various linear array transducers with frequencies of 7.5 MHz and 10 MHz could be moved with precise steps of 0.5 mm along and perpendicular to the tendon long axis. Approximately 200 steps/tendon were taken. At each step, transverse ultrasonographic images were captured. During these scan sessions, constant focus, brightness, and contrast settings were used, and the gain settings were fixed. The ultrasonographic information was collected in 2 ways: capture of the analogue video image (as presented on the monitor of the scanner) by means of a frame grabber; and direct collection, via a special digital output on the scanner or via storage on the internal hard disk of the scanner, of raw digital B-scan data.

**Experimental protocol**—Normal and pathologically changed tendons were scanned over their entire length. At the end of the scan session, the tendons were fixed in neutral-buffered formaldehyde and left for 48 hours in the same position and under the same tension to allow as accurate as possible comparison of histologic specimens and corresponding ultrasonographic images. After an extended period of fixation (minimum, 1 month), the tendon was cut by hand as precisely as possible in transverse sections (each with a mean thickness of 2.3 mm), which resulted in 60 to 75 cuts/tendon. High-resolution photographs were taken of all these tendon sections and were used for macroscopic evaluation, with the aid of low magnification, resulting in a first-instance estimation of the degree of structural integrity. Subsequently, the histologic specimens were embedded in plastic to preserve the shape of the tendon. Transverse microscopic cuts (4 μm thick) were made. Microscopic evaluation of the already macroscopically mapped areas of interest was made on the basis of histopathologic characteristics as formulated by Stromberg et al and Williams et al. Use of histologic criteria, such as hemorrhage, necrosis, edema, vascular proliferation, iron deposition, cellularity, mitosis, fibrosis, and endotenon-septation, and their combination with macroscopic evaluation and clinical history, led to a subdivision of tissue types. Various tissue types were discerned. Normal young tendon tissue was obtained from a young horse; a 3-year-old Thoroughbred mare (trained, but not raced) was used as a reference. Microscopically, it contained regular distribution of small fibroblasts, thin endotenon-septation with few small vessels, almost exclusively in the fine end-septa surrounding the primary tendon bundles. Normal old ten-

![Figure 1](image-url)

Figure 1—Findings in an area of equine superficial digital flexor (SDF) tendon classified as normal young tissue. Photograph (top, left) of SDF tendon specimen; photomicrograph (top, right) of a section of SDF tendon (H&E stain; bar = 80 μm); ultrasonographic image (left, bottom) of SDF tendon; and, histogram representation (right, bottom) of the distribution of gray levels/pixel on ultrasonographic evaluation.
don tissue was obtained from an old horse; a 9-year-old Dutch Warmblood gelding (high-performance showjumper) was used as a reference. Microscopically, it contained regular distribution of small fibroblasts and less cellularity (compared with normal young tendon tissue), somewhat thick endotendon-septation (compared with normal young tendon tissue), small vessels in septa, and localized fields with chondroid metaplasia. Necrotic tendon consisted microscopically of focal areas with acellularity, amorphous eosinophilic material and coagulation of cells, and areas with partially intact and partially ischemic endotendon. Early granulation tissue was obtained from an acute lesion. Microscopically, large numbers of swollen fibroblasts, some mitoses, enormous vascular proliferation, scattered small areas with ischemic changes, and loss of endotendon-septation with some mononuclear inflammatory cells were seen. Late granulation tissue was obtained from a subacute lesion. Microscopically, large numbers of swollen fibroblasts, and increased vascularity (especially in the endotendon) with perivascular iron deposits were seen. In the border, at the transition of normal tissue and lesion, moderate cellularity and vascular ingrowth were evident. Early fibrotic tissue (subacute lesion) was characterized microscopically by irregular distribution of moderate numbers of large fibroblasts and thick endotendon with increased numbers of vessels around it. Late fibrotic tissue (chronic lesion) was characterized microscopically by irregular distribution of moderate numbers of medium-large fibroblasts, thick endotendon, slight increase of vessels, and almost complete lack of normal endotendon-septation. Scar tissue (chronic lesion) was characterized microscopically by irregular distribution of small fibroblasts, thick endotendon, and complete lack of restoration of normal endotendon-septation.

Image analysis—From each tendon section representing 1 of the aforementioned specified tissue types, the exact corresponding ultrasonographic images were selected from all stored images, plus the first 8 ultrasonographic images of both sides. This method, using ultrasonographic images from +4 mm to –4 mm, was used to obtain more information about the reproducibility of the representative ultrasonographic image and the gray level statistics. In each of the 17 ultrasonographic images, the same area of interest was selected, cut, and stored in a data file. Subsequently, gray level statistics were calculated from each cut and analyzed using a statistical analysis program. The calculated first-order gray level statistics were number of pixels; mean gray level (ie, mean gray level of each pixel, with SD and variance); gray sum (ie, sum of the gray levels of all pixels in the area of interest of the transverse image); maximum, minimum, mode (most frequently occurring gray level); histogram (ie, graphic representation of the distribution of gray levels/pixel); and skewness (indicative of symmetry around the mean) and kurtosis (indicative of pointedness) of the histogram.

After considering the fact that the scan converters internally have 6 bits available for representing the gray levels in the raw B-scan data, a gray scale from 0 to 64 was selected. Raw digital B-scan data were selected for calculation of these statistics.

Results

All selected tissue types (ie, normal young, normal old, necrotic, early granulation, late granulation, early fibrotic, late fibrotic, and scar tissues) were observed (Figs 1–8, respectively). For each tissue type, a photograph, photomicrograph, and corresponding ultrasonographic image were obtained. Also, a histogram of the selected area of interest in
the corresponding ultrasonographic image is depicted. The selected area of interest was small enough so that it included only 1 tissue type, which ensured that the histogram was representative for this specific tissue type only.

The first-order gray level statistics for the selected area of interest for all 8 tissue types were calculated (Table 1). These are standard parameters of quantitative image analysis, such as mean gray level, SD, gray sum, minimum and maximum gray level value, and skewness and kurtosis of the histogram. The minimum and maximum gray level values indicate the range of gray levels in the image. The SD gives an indication of the spreading of the gray level values around the mean. Skewness and kurtosis are other parameters that characterize the shape of the histogram. Skewness is a measure of the asymmetry or symmetry of the distribution of gray level values around the mean. Typically, if the distribution is symmetric around the mean, its skewness is equal to 0. Kurtosis is an indication for the peakedness or flatness of the distribution around the mean; it also indicates a heavily or lightly tailed distribution. Both can be seen as a test for normality of the measured distribution of gray levels. The normal distribution is symmetric and has a skewness value of 0. Higher values for skewness, kurtosis, or both mean a greater departure of the measured distribution from normality.

The gray level statistics of the investigated areas are depicted by corresponding histograms (Fig 1 to 8). It is evident that some conditions can be readily diagnosed from the ultrasonogram. Early granulation tissue (Fig 4) is characterized by a substantially lower mean gray level than is normal tendon tissue, be it young or old (Figs 1 and 2, respectively). In the histogram, this is reflected by a shift to the left and lower maximum gray level. Necrotic tissue (Fig 3) has a higher mean gray level. However, maximal values are not different from those found in normal old tendon tissue. In the case of late granulation tissue (Fig 5) and early fibrotic tissue (Fig 6), the mean gray level cannot be discerned from normal tendon tissue, nor can this be done on any other of the first-order gray level statistics (Table 1). The same applies to a large extent for late fibrotic (Fig 7) and scar tissues (Fig 8), where mean gray levels were found to be only fractionally lower than those of normal tendon tissue.

The mean gray level for each tissue type at the site of principal interest was determined (Table 2). Furthermore, the mean value taken over all 17 slices, equally spaced by 0.5 mm steps over a range from +4 to –4 mm around the principal site, is given together with the SD and range.

In normal young and old tendons, the values calculated in both ways are similar, and SD and ranges calculated from the 17 slices are low, indicating that the tissues examined were homogenous over a prolonged distance. The same applies to late granulation, early and late fibrotic, and scar tissues. However, this is not the case with early granulation tissue and necrotic tissue. Small steps in transducer displacement cause large...
Figure 4—Findings in an area of equine SDF tendon classified as early granulation tissue. See Figure 1 for key.

Figure 5—Findings in an area of equine SDF tendon classified as late granulation tissue. See Figure 1 for key.
Figure 6—Findings in an area of equine SDF tendon classified as early fibrotic tissue. See Figure 1 for key.

Figure 7—Findings in an area of equine SDF tendon classified as late fibrotic tissue. See Figure 1 for key.
differences in gray level statistics, indicating the non-homogeneous character of the tissues examined.

Furthermore, for each tissue type (Table 2), the ranges for skewness and kurtosis overlap between the various tissue types. When comparing the sets of 17 slices taken for each tissue type, only the skewness range values for early granulation tissue are significantly different from the other skewness range values.

Table 1—Results of first-order gray level statistics for various tissue types of equine superficial digital flexor tendons

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>N</th>
<th>Mean</th>
<th>SEM</th>
<th>SD</th>
<th>Skewness</th>
<th>Kurtosis</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal young</td>
<td>2,295</td>
<td>36.3</td>
<td>0.2</td>
<td>9.3</td>
<td>−0.19</td>
<td>−0.29</td>
<td>3.0</td>
<td>59.0</td>
</tr>
<tr>
<td>Normal old</td>
<td>2,610</td>
<td>37.1</td>
<td>0.2</td>
<td>9.0</td>
<td>0.05</td>
<td>−0.51</td>
<td>4.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Necrotic</td>
<td>1,140</td>
<td>42.5</td>
<td>0.3</td>
<td>8.5</td>
<td>−0.29</td>
<td>−0.05</td>
<td>9.0</td>
<td>61.0</td>
</tr>
<tr>
<td>Early granulation</td>
<td>2,090</td>
<td>13.8</td>
<td>0.2</td>
<td>7.9</td>
<td>0.34</td>
<td>−0.17</td>
<td>0.0</td>
<td>41.0</td>
</tr>
<tr>
<td>Late granulation</td>
<td>2,419</td>
<td>38.1</td>
<td>0.2</td>
<td>8.5</td>
<td>−0.13</td>
<td>−0.31</td>
<td>7.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Early fibrotic</td>
<td>1,824</td>
<td>36.9</td>
<td>0.2</td>
<td>8.4</td>
<td>−0.03</td>
<td>−0.12</td>
<td>12.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Late fibrotic</td>
<td>2,340</td>
<td>31.2</td>
<td>0.2</td>
<td>8.4</td>
<td>−0.45</td>
<td>−0.33</td>
<td>0.0</td>
<td>59.0</td>
</tr>
</tbody>
</table>

N = No. of pixels. Mean = Mean gray level. SEM = Standard error of the mean. SD = Standard deviation. Min = Minimum gray level. Max = Maximum gray level.

Table 2—First-order gray level statistics for equine superficial digital flexor tendon tissue types, using 17 slices around the principal site representing a tissue type

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Mean_{slice 0}</th>
<th>Mean_{slice-8/+8}</th>
<th>SD_{slice-8/+8}</th>
<th>Range_{slice-8/+8}</th>
<th>Skewness_{Min-Max}</th>
<th>Kurtosis_{Min-Max}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal young</td>
<td>36.3</td>
<td>36.6</td>
<td>0.5</td>
<td>35.8 to 37.3</td>
<td>−0.33 to −0.03</td>
<td>−0.52 to −0.09</td>
</tr>
<tr>
<td>Normal old</td>
<td>37.1</td>
<td>36.9</td>
<td>0.6</td>
<td>36.0 to 37.7</td>
<td>−0.09 to 0.12</td>
<td>−0.52 to −0.01</td>
</tr>
<tr>
<td>Necrotic</td>
<td>42.5</td>
<td>43.7</td>
<td>3.1</td>
<td>37.2 to 48.3</td>
<td>−0.53 to −0.07</td>
<td>−0.60 to 0.08</td>
</tr>
<tr>
<td>Early granulation</td>
<td>13.8</td>
<td>14.2</td>
<td>2.9</td>
<td>10.2 to 18.7</td>
<td>0.29 to 0.63</td>
<td>−0.42 to 0.61</td>
</tr>
<tr>
<td>Late granulation</td>
<td>38.1</td>
<td>38.1</td>
<td>0.4</td>
<td>37.3 to 38.7</td>
<td>−0.31 to −0.06</td>
<td>−0.38 to 0.31</td>
</tr>
<tr>
<td>Early fibrotic</td>
<td>36.8</td>
<td>36.4</td>
<td>0.6</td>
<td>35.4 to 37.0</td>
<td>−0.14 to 0.17</td>
<td>−0.41 to 0.21</td>
</tr>
<tr>
<td>Late fibrotic</td>
<td>31.2</td>
<td>32.1</td>
<td>0.6</td>
<td>31.4 to 33.0</td>
<td>−0.38 to 0.10</td>
<td>−0.30 to 0.33</td>
</tr>
<tr>
<td>Scar</td>
<td>35.0</td>
<td>34.7</td>
<td>0.5</td>
<td>33.6 to 35.5</td>
<td>−0.33 to 0.19</td>
<td>−0.30 to 0.31</td>
</tr>
</tbody>
</table>

Mean_{slice 0} = Mean gray levels for the area of principal interest. Mean_{slice-8/+8} = Mean over all 17 slices in the range (+4 mm to −4 mm) around the principal site. SD_{slice-8/+8} and Range_{slice-8/+8} = The SD and range over the 17 slices around the principal site. Skewness_{Min-Max} and Kurtosis_{Min-Max} = The range of skewness and kurtosis over the 17 slices around the principal site, respectively.
Discussion

Data collection—Ultrasonographic data were collected in 2 ways. Capture of the analogue ultrasonographic image, as presented on the monitor of the scanner, by use of the frame grabber, is the most practical method, but data are influenced by the post- and video-processing of the scanner and by the frame grabber. Digital B-scan data are collected either by use of a digital output on the scanner or via storage on the internal hard disk. These data were raw, which allowed the resampling and interpolation procedure to be done using a more surveyable routine. However, because gray level statistics provide only first-order information, in contrast to higher order analysis of spatial (eg, echo-textural) information, interpolation of the raw data from this study was not necessary. Similar to that in another study, the effect of the frame grabber did not exceed 1 gray level on the 64-level scale, so this effect was negligible. The effects of post- and video-processing on the gray level statistics were determined by comparing the statistics of the grabbed analogue information with those of the raw digital B-scan data. Results of this comparison indicated that the post- and video-processing procedures did not interfere with the gray level statistics. Nevertheless, the choice was made to use the raw digital B-scan data for all the statistics reported here to provide uniformity of data for future research on higher order analysis of echo-textural information.

Matching of histologic specimens with corresponding ultrasonographic images—To facilitate matching of histologic specimens with the corresponding ultrasonographic images, special attention was given to the careful correction for crimp of the tendon during fixation, as well as for the variation in thickness of the hand-cut slices. This exact matching is essential for the technique described here and became more important with decreasing homogeneity of the tissues, as is the case with various stages of lesions.

Characterization of tissue types—Echogenicity is related to differences in acoustic impedance at local interfaces in the insonated tissues. In tendons and ligaments, acoustic impedance is mainly related to the content and especially the density of collagen, which is the most important structural protein. In normal tendons, the intact collagen fibers are densely packed and arranged in a strict hierarchic architecture along the tendon axis. These tissue characteristics lead to a high acoustic impedance and to a specific axial arrangement of acoustic interfaces and, thus, to high echogenicity, which is viewed on ultrasonographic images as intensity and homogeneity on transverse scans, and as an intact axial alignment on longitudinal scans.

When a lesion develops, the original structural arrangement of the tendon is damaged to a certain extent. Collagen fibrils rupture; hemorrhage, edema, necrosis, and lysis of fibrils occurs, and an inflammatory response with cellular infiltration ensues. In the process of healing, granulation tissue appears; gradually, it is replaced by more fibrous structures that will progressively reorient along the lines of stress. During genesis and the ensuing healing of a lesion, changes in the microanatomy of the tendon take place, and echogenicity changes substantially. This loss and gradual recovery of echogenicity has been reported by several authors who used a semiquantitative scale for judging echogenicity. However, it would be more interesting if it were possible to detect impending subclinical lesions to prevent a possibly disastrous breakout or to be able to monitor accurately the later stages of healing to aid in the decision making when horses should resume training or be allowed to race again. This can only be achieved when a correct and reliable assessment of the structural integrity of the tendon can be made on the basis of the ultrasonographic image (ie, when some sort of tissue typing is possible). A report by Tsukiyama et al on SDF tendonitis of Thoroughbreds claims that evaluation of the ultrasonographic image of this tendon is possible, on the basis of quantitative analysis of the histogram representing mean gray level, described earlier by Nicoll et al.

Results of this study indicate that some stages of the lesions can indeed easily be detected by either qualitative or quantitative analysis of the ultrasonographic images. Early granulation tissue is hardly echogenic and, hence, has a significantly lower gray level than normal tendon tissue. Acute or hyperacute lesions in the acute tear or the cell inflammatory stage were not detected, but there is no doubt that, in such instances, differences between the lesion and normal tendon tissue is more evident and, therefore, easy to diagnose. In the event of necrosis (which can be seen in an acute lesion), the acoustic impedance of the tissue increases, resulting in a higher mean gray level. This can be detected on the image itself by subjective assessment and from the first-order gray level statistics. The shape of the histogram, however, is hardly discernable from that of normal tendon tissue. This is in contrast to the histogram of early granulation tissue, which is clearly different from the normal histogram.

Conversely, late granulation tissue and early fibrotic tissue cannot be distinguished from normal tendon tissue by use of the gray level statistics, because there is no difference in mean gray level; neither are there any other differences in the first-order gray level statistics nor in the shape of the histograms. On the other hand, for late fibrotic tissue or scar tissue, the mean gray level was fractionally subnormal, although without any other abnormalities in the first-order statistics or in the shape of the histogram. The previous observations led to the conclusion that the mean gray level cannot be used as an implicit and reliable indication for the age of the lesion.

Another important observation was that when examining pathologically changed tissues characterized by some loss of structural integrity, sequential scanning over a limited distance could lead to large differences in first-order gray level statistics. This means that use of first-order gray level statistics for tissue typing may lead to different conclusions with only minor transducer displace-
ments. It also means that the results of Tsukiyama et al. should be interpreted with caution, because they did not exactly match ultrasound images with the corresponding histologic sections, and they compared the images obtained by ultrasonography with a tendon section length of not less than 6 cm. This observation emphasizes how critical it is to evaluate the structural integrity of tendon tissue with three-dimensional architecture by only quantifying 1 two-dimensional representation, either transverse or longitudinal, of the structures.

It may be concluded that although quantification of the transverse ultrasonographic image by use of first-order gray level statistics may be helpful, the method is not sufficiently sensitive to accurately and unequivocally determine the type of tendon tissue under investigation. This is mainly because in quantitative gray level analysis, only 1 aspect of echogenicity (namely intensity) is quantified, whereas the other main aspects of tendon echogenicity (homogeneity and axial alignment) are not taken into account. For accurate assessment of tendon integrity, some additional longitudinal information is essential. To achieve this goal, not only should the intensity of the transverse image be quantified, but also some degree of homogeneity of sequential transverse images should be added as well, which would introduce a longitudinal component. Further, it is of vital importance for the final assessment of tendon integrity to combine longitudinal and transverse information.

References