Validation of magnetic resonance imaging for measurement of equine articular cartilage and subchondral bone thickness

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Objective—To validate use of magnetic resonance images (MRIs) for measurement of equine articular cartilage and subchondral bone thickness by comparison with measurements in histologic specimens.

Sample Population—32 cadaveric carpal joints from 16 horses.

Procedure—Magnetic resonance imaging was performed by use of 3-dimensional fast spoiled gradient echo (SPGR) and T2* 3-dimensional fast gradient echo (GRE) pulse sequences with and without fat saturation. Standard sites on the medial and lateral facets of the intermediate, radial, and third carpal bones were used for subchondral bone and articular cartilage thickness measurements. Digital image analysis software was used for MRI measurements 10 mm from the dorsal extent and perpendicularly to the articular surface. Histomorphometric measurements of hyaline, calcified cartilage, and subchondral bone thickness were obtained at selected sites. Comparisons between histomorphometric and MRI measurements and between magnetic resonance pulse sequences were evaluated.

Results—There were significant correlations between GRE and SPGR and SPGR and histologic measurements of articular cartilage, with no significant difference between measurements and good agreement. When calcified cartilage was excluded from the histologic measurement, MRI measurements were significantly greater than histologic measurements. For subchondral bone thickness, there was significant correlation between GRE and SPGR but GRE was significantly greater than SPGR measurements. Histomorphometric and MRI measurements were strongly correlated and not significantly different.


Magnetic resonance imaging is considered an important tool in the diagnosis and study of joint structure and pathologic lesions in humans and is increasingly being used for evaluation of lameness in horses. Joint disease is a major cause of lameness in horses and can cause changes to the morphology of articular cartilage and subchondral bone. To further understand osteochondral adaptation and injury, it is necessary to develop use of noninvasive imaging modalities suitable for in vivo use. Magnetic resonance imaging provides a potential noninvasive approach for visualizing and measuring articular cartilage and subchondral bone thickness. However, for magnetic resonance imaging to be used as a valid technique for studying joints, it is necessary that magnetic resonance imaging to be used as a valid technique for studying joints, it is necessary that magnetic resonance images (MRIs) of joint layers be validated against actual joint structure.

In humans, articular cartilage thickness on MRIs has been compared with histologic specimens and extracted cartilage plugs. However, there has been little evaluation of subchondral bone thickness measurements in human joints, and comparison of MRI and histologic measurements has not been reported in equine osteochondral tissues. There continues to be debate about whether the articular cartilage layer seen on MRIs represents only the hyaline layer or also includes the calcified layer, and no studies have measured calcified cartilage thickness to resolve this debate.

We hypothesized that MRI measurements of articular cartilage and subchondral bone thickness are a close representation of tissue measurements, as represented by histologic sections. The purpose of the study reported here was to validate the use of MRIs for measurement of equine articular cartilage and subchondral bone thickness by comparison with measurements in histologic specimens. Specifically, we wanted to compare histologic and MRI measurements of hyaline cartilage, calcified cartilage, and subchondral bone thickness at specific sites within the equine carpus by use of imaging parameters suitable for use in horses with lameness.

Materials and Methods

Sample population—Thirty-two cadaveric carpal joints were collected from 16 horses ranging in age from 2 to 30 years old. Horses were euthanatized for reasons unrelated to the study. Both carpi were collected immediately after death and preserved by freezing for 1 week at −20°C. Carpi were defrosted at 22°C for 24 hours prior to magnetic resonance imaging. Dissection was performed 4 hours after imaging; therefore, all carpi underwent only 1 freeze-thaw cycle. All carpi were allocated with unique identification numbers.

Magnetic resonance imaging—Magnetic resonance imaging was performed on thawed samples by use of a 1.5-T
magnetic resonance system. Carpi were placed on the right side in an extremity radiofrequency coil. Images were obtained in 3 planes (dorsal, transverse, and sagittal) by use of 3-dimensional fast spoiled gradient echo (SPGR) and T2* 3-dimensional fast gradient echo (GRE) pulse sequences.

For comparison of magnetic resonance imaging and histomorphometry, measurements were obtained on the intermediate carpal bone and exact apposing site on the lateral facet of the third carpal bone. For comparison of pulse sequences, measurements were also obtained on the radial and intermediate carpal bone and exact apposing site on the midsagittal site of the intermediate carpal bone and articular surfaces. The midsagittal site of the intermediate carpal bone was determined by use of calipers and marked with India ink, along with the exact corresponding site on the lateral facet of the third carpal bone. Four-millimeter sagittal sections at these sites were cut with a bandsaw and a small bandsaw. These sections underwent routine histologic preparation, as previously used for osteochondral measurement, and were found to have good agreement with needle-probe measurements of hyaline cartilage in situ. Decalcified, paraffin-embedded 8-µm sections from the center of the block were stained by use of H&E stain and toluidine blue. Specimens were only included when the entire cartilage layer was present with no defects and the dorsal aspect of the articular surface was clearly defined.

**Histomorphometry**—Hyaline and calcified cartilage and subchondral bone thickness measurements were obtained at these sites by use of the selected sagittal images. Measurements were taken perpendicular to the articular surface at a specific site 10 mm from the dorsal extent of the articular surface. Digital image analysis software was used for measurement. Boundaries between articular cartilage, subchondral bone, and cancellous bone were located as sharp alterations in pixel intensity. Pixel intensity limits of 0 to 30 for subchondral bone and 30 to 55 for articular cartilage were used on the basis of data from a preliminary study and the authors’ experience with other joints. Repeatability at each site was assessed by use of 10 repeated measurements/site for 10 limbs. Final measurements were obtained when the coefficient of variance was < 2%.

**Histologic preparation**—The dorsal aspect of the intercarpal joint was opened by sharp dissection to expose the articular surface. The dorsal aspect of the intercarpal joint at the level of the middle portion of the intermediate carpal bone (top) and lateral portion of the third carpal bone (bottom) from a horse. The synovial fluid (S) is visualized with low signal intensity relative to the high signal intensity of the cartilage layer (C). The low signal intensity of the subchondral bone (SB) is clearly different from the high, more heterogeneous signal intensity of the cancellous bone (CB).
obtained by use of a histomorphometric technique adapted from a previously reported technique. Subchondral bone thickness was measured by use of calibrated digital images of each slide, acquired by use of an optical digital zoom camera with electronic calipers. For articular cartilage measurements, digital images were obtained by use of a microscope linked to a digital camera at $2\times$ magnification. All measurements were taken perpendicular to the articular surface at the standard sites by use of image analysis software as previously described.

Repeatability at each site was assessed by use of 10 repeated measurements/site for 10 limbs. Final measurements were obtained when the coefficient of variance was $< 2\%$.

Statistical analyses—Measurements were compared between pulse sequences and between magnetic resonance imaging and histomorphometry for each individual site and

Figure 2—Sagittal plane fat-saturated SPGR image of the intercarpal joint at the level of the middle portion of the radial carpal bone (top) and medial portion of the third carpal bone (bottom) from a horse. The high signal intensity of the articular cartilage (C) has clear boundaries of low signal intensity in the synovial fluid (S) and underlying bone (B). The subchondral and cancellous bone layers cannot be visualized separately.

Figure 3—Sagittal plane fast gradient echo (GRE) image of the intercarpal joint at the level of the middle portion of the intermediate carpal bone (top) and lateral portion of the third carpal bone (bottom) from a horse. Compared with the SPGR images, the difference between the high signal intensity of the synovial fluid (S) and cartilage (C) is not as clear. Additionally, the definition between the low signal intensity of the subchondral bone (SB) and high, heterogeneous signal of the cancellous bone (CB) layers is not as clear, compared with the SPGR images. A small defect (arrow) in the articular cartilage of the intermediate carpal bone is evident.

Figure 4—Bland-Altman plots of subchondral bone thickness measurements of a standard site $10$ mm from the dorsal extent of the articular surface on the radial, intermediate, and third carpal bones from horses obtained by use of GRE and SPGR images. A—On the comparison plot, the identity line illustrates that GRE measurement of cartilage thickness is equal to SPGR measurement, indicating moderate agreement between the methods. B—Difference plot indicating positive bias in GRE measurements, compared with SPGR measurements. SB = Subchondral bone. Gray line = Zero bias. Dotted line = Level of bias. Dashed line = $95\%$ limits of agreement.
for all sites. Spearman correlation was used to test for an association between measurements obtained by use of magnetic resonance imaging and histomorphometry and between magnetic resonance pulse sequences. A Wilcoxon signed rank test was used to test for differences between pulse sequences and between the histomorphometric and MRI measurements. A Bland-Altman bias plot was used to test for agreement and limits of agreement between measurements for each pulse sequence, between articular cartilage histomorphometric and MRI measurements, and between subchondral bone histomorphometric and MRI measurements. A value of $P < 0.05$ was considered significant. All statistical analyses were performed with a statistical software package.

**Results**

**Comparison of pulse sequences**—There were no significant differences in measurements between sites.

The SPGR and SPGR fat saturation images delineated subchondral bone and cartilage more clearly than GRE images, leading to better repeatability and lower SD values at 70% of cartilage sites and 67% of subchondral bone sites (Figures 1–3). The SPGR and SPGR fat saturation measurements for the same site were identical. There was a significant correlation between fat-suppressed GRE and SPGR measurements of articular cartilage ($P = 0.019$) and subchondral bone thickness ($P < 0.001$). There was no significant difference between articular cartilage measurements obtained by use of GRE (mean ± SD, $0.578 ± 0.077$ mm) and SPGR ($0.587 ± 0.085$ mm) images, but GRE measurements of subchondral bone thickness ($5.37 ± 8.0$ mm) were significantly greater than SPGR measurements.
(4.71 ± 7.72 mm; \(P < 0.001\)). When agreement was evaluated, significant positive bias (bias, 0.662; 95% confidence intervals [CI], 0.17 to 1.153 mm) was detected for subchondral bone GRE measurements (Figure 4). There was no significant bias observed in articular cartilage measurements (bias, –0.009; 95% CI, –0.026 to 0.009 mm) and reasonable agreement between fat-suppressed SPGR and GRE measurements (Figure 5).

Comparison of MRI and histomorphometric measurements—There was no significant difference in measurements between sites, and comparisons between MRI and histomorphometric measurements gave the same results for individual sites and pooled data.

There was no significant difference between MRI (1.436 ± 0.303 mm) and histomorphometric (1.446 ± 0.311 mm) measurements of articular cartilage, and there was a strong correlation \((r = 0.96; P < 0.001)\) between MRI and histomorphometric measurements. There was excellent agreement between histomorphometric and MRI measurements with no significant bias detected (bias, –0.006; 95% CI, –0.030 to 0.017; Figure 6). When calcified cartilage thickness was excluded from the articular cartilage measurements and only hyaline cartilage histomorphometric thickness measurements were compared with MRI measurements for articular cartilage, there was an association between MRI and histomorphometric measurements \((r = 0.69; P < 0.001; \text{Figure 7})\). However, MRI (1.430 ± 0.302 mm) measurements were significantly \((P < 0.001)\) greater than histomorphometric (0.921 ± 0.267 mm) measurements, and there was significant positive bias (bias, 0.509; 95% CI, 0.439 to 0.579; Figure 8).

There was no significant difference between MRI (2.357 ± 1.2 mm) and histomorphometric (2.374 ± 1.242 mm) measurements for subchondral bone, and there was a strong correlation \((r = 0.98; P < 0.001)\) between MRI and histomorphometric measurements (Figure 9). There was excellent agreement between histologic and MRI measurements with no significant bias detected (bias, –0.016; 95% CI, –0.098 to 0.065; Figure 10).
Discussion

Results of the study reported here support use of magnetic resonance imaging as a representation of osteochondral thickness. The SPGR measurements were in agreement with histomorphometric measurements for cartilage and subchondral bone; however, measurement of subchondral bone by use of GRE images introduced significant bias.

A number of magnetic resonance imaging studies in human joints have underestimated articular cartilage thickness. It has been speculated that differences detected between MRI and histologic measurements for cartilage are attributable to visualization of the calcified layer within the subchondral bone plate. However, the calcified layer has not previously been measured and compared with MRIs. In our study, there was excellent agreement between histomorphometric measurements of cartilage thickness when histomorphometric measurements of full-thickness cartilage were compared with MRI results. However, when histologic measurements of hyaline cartilage thickness were obtained without including the calcified layer, MRI measurements were significantly greater than the hyaline cartilage thickness, indicating that the thickness measured by use of magnetic resonance imaging was a better representation of hyaline and calcified layers together. Therefore, results of our study suggested that for the pulse sequences used, the calcified layer may be visualized as part of the articular cartilage on MRIs. As alterations in osteoarthritis may relate to changes in cartilage total thickness and an alteration in the relative thickness of the calcified and hyaline layers, it is important that this is understood as a potential limitation for quantitative magnetic resonance imaging.

Results of previous studies in human joints indicate that SPGR sequences are reliable and accurate for measurement of cartilage and subchondral bone thickness. However, there has been limited comparison between GRE and SPGR sequences for osteochondral measurement. Our results indicated that articular cartilage thickness measurements may be obtained by use of GRE fat saturation, SPGR fat saturation, or SPGR images, but subchondral bone measurements may be overestimated if GRE images are used with the imaging parameters used in this study.

Visual assessment of images is considerably easier by use of SPGR sequences, and the layers are more clearly delineated with higher contrast between tissues, so inaccuracy in defining the subchondral-cancellous bone interface is potentially more of a problem when GRE images are used; repeatability of measurement was slightly better for SPGR than GRE images.

There have been few comparisons of MRI and histologic measurements of subchondral bone thickness. Validation of absolute measurements against histologic results has not previously been reported, although patterns of thickness on MRIs have reflected histologic patterns and there was good inter-rater and intrarater reliability for MRI measurements of subchondral bone by use of an SPGR sequence. Chemical shift artifacts
lead to potential problems for subchondral bone measurement and are a particular issue in gradient echo sequences. However, results of our study indicated that measurement of subchondral bone thickness by use of SPGR images provided good representation of the thickness as detected histologically at a specific site.

The magnetic resonance imaging parameters were based on clinical protocols, with the plan that these could potentially be used for in vivo assessment of the osteochondral unit. Therefore, increased or decreased accuracy may be obtained by use of different imaging parameters or a different imaging system.

There were limitations in the study reported here. Three-millimeter contiguous slices with a 1.5-mm overlap were used, and ZIP was used for reconstruction. Therefore, some inaccuracy was possible in the location of standard sites and measurement. However, we have previously obtained images with and without ZIP by use of a phantom and found measurements to be comparable and margins better defined with ZIP; therefore, we consider our results valid within the confines of practical clinical scanning. Volume averaging would be a potential problem in areas of variability in cartilage thickness. There is little variability in the thickness of cartilage across the dorsal aspect of the carpal bones in the regions assessed in our study; therefore, volume averaging was unlikely to have provided substantial error in the sagittal images used. However, at sites with considerable curve in the articular surface or variability in cartilage thickness, potential error associated with volume averaging should be considered.

In the study reported here, defined pixel intensity limits were used for determining the interfaces between tissues on the basis of results of a preliminary study and experience with other joints. An image standard was not used within the field of view for each measurement, which was a limitation of the study because variation in signal intensity between images could have altered the absolute limits used for measurement, and it is possible that alternative limits could have altered accuracy. In a preliminary study with equine tarsal joints, we used an imaging standard within the field of view, compared the subchondral bone pixel intensity ratio with the standard between the same tarsal joint acquired by different observers and between tarsal joints imaged on different days, and found only a small range of variation between subchondral bone pixel intensity ratio and the standard between images. Therefore, although the lack of imaging standard in the study reported here was a limitation, we do not believe that it is likely to have altered the overall findings. The osteochondral boundary was visibly detectable with a sharp alteration in pixel intensity in all cases, and the subchondral-cancellous bone boundary was visually detectable as a clear change in pixel intensity for nearly all cases; therefore, a slight alteration in the pixel intensity limits would have been unlikely to alter the overall results of this study.

Results of our study indicated that magnetic resonance imaging with parameters applied in horses with lameness can provide a good representation of cartilage and subchondral bone thickness. Results support the use of magnetic resonance imaging in the study of osteochondral structure and alteration, with potential for clinical application in the diagnosis of osteochondral abnormalities.

References