# MAGNETIC RESONANCE SIGNAL CHANGES DURING TIME IN EQUINE LIMBS REFRIGERATED AT 4°C

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**When *ex vivo* magnetic resonance (MR) imaging studies are undertaken, specimen conservation should be taken into account when interpreting MR imaging results. The purpose of this study was to assess MR changes during time in the anatomic structures of the equine digit on eight cadaver limbs stored at 4°C. The digits were imaged within 12 h after death and then after 1, 2, 7, and 14 days of refrigeration. After the last examination, four feet were warmed at room temperature for 24 h and reimaged. Sequences used were turbo spin echo (TSE) T1, TSE T2, short tau inversion recovery (STIR), and double-echo steady state (DESS). Images obtained were compared subjectively side by side for image quality and signal changes. Signal-to-noise ratio (SNR) was measured and compared between examinations. There were no subjective changes in image quality. A mild size reduction of the synovial recesses was detected subjectively. No signal change was seen subjectively except for bone marrow that appeared slightly hyperintense in STIR and slightly hypointense in TSE T2 sequence after refrigeration compared with day 0. Using quantitative analysis, significant SNR changes in bone marrow of refrigerated limbs compared with day 0 were detected in STIR and TSE T2 sequences. Warming at room temperature for 24 h produced a reverse effect on SNR compared with refrigeration with a significant increase in SNR in TSE T2 images. After 14 days of refrigeration a statistically significant decrease of SNR was found in bone marrow in TSE T2 and DESS sequences. The SNR in the deep digital flexor tendon was not characterized by significant change in SNR.** *Veterinary Radiology & Ultrasound, Vol. 51, No. 1, 2010, pp 19–24.*

**Key words: foot, horse, magnetic resonance imaging, preservation, temperature.**

## Introduction

THE USE OF magnetic resonance (MR) imaging to evaluate the equine digit is increasing.[1–7] Imaging of equine cadaver limbs has been performed to provide needed baseline anatomic information.[3,5,8–12] Some of these studies were realized on thawed limbs after freezing.[5,8,11,12] Because frozen tissue does not contain sufficient mobile protons to generate a radiofrequency signal, specimens must be thawed before imaging.[13] No differences have been detected between ante- and post-mortem examinations after the freezing/thawing process on the same feet when it was possible to compare.[5] When an *ex vivo* study is prolonged due to techniques being applied at different sites or times, preservation of the specimen is critical.[14] Equine cadaver specimens can be preserved by sealing the limb with a paraffin–polymer combination.[13] Although this allows multiple freeze–defrost cycles to be performed on the same specimen without degradation of the image quality, the method is relatively time consuming.[13]

Because the equine distal limb contains no muscles and the distal extremity is embedded in the hoof, we hypothesized that simple refrigeration at 4°C could be used to preserve equine limbs without major changes in MR signal. This would permit a prolonged examination without freeze–thaw cycles and without using paraffin. The purpose of this study was to assess MR signal changes during time in the equine digit during storage at 4°C.

## Materials and Methods

Eight fresh equine cadaver forelimbs were collected. These forelimbs were normal radiographically and were sectioned at the carpometacarpal joint to prevent air introduction around tendons and in the digital tendon sheath. The proximal end of each specimen was covered by an absorbing material and a latex glove to prevent blood loss during handling. Any shoe was removed. The feet were cleaned and excess frog trimmed.

All feet were imaged at room temperature between 8 and 12 h after limb acquisition (day 0) and then stored at 4°C between MR imaging examinations. Four feet were then imaged at 1, 2, 7, and 14 days after collection. Four other feet were imaged at 1 day and 14 days and then warmed to room temperature for 24 h before a last MR examination on day 15. Refrigerated feet were imaged immediately upon removal from the cold room to decrease the degra-

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TABLE 1. MR Imaging Protocols

| Type | Plane | TR (ms) | TE (ms) | FOV (mm) | Matrix | Thickn (mm) | Gap (mm) | Flip (°) | TA (min) | NEX | TI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| TSE T1 | Transverse | 512 | 12 | 180–180 | 307 × 512 | 3 | 1 | | 3:14 | 3 | |
| TSE T2 | Sagittal | 3800 | 84 | 160–160 | 288 × 384 | 3 | 0.3 | | 3:31 | 1 | |
| STIR | Sagittal | 3140 | 46 | 160–160 | 256 × 320 | 3 | 0.3 | | 4 | 2 | 150 |
| DESS | Dorsal | 20.7 | 6.34 | 180–180 | 256 × 256 | 1.5 | 0.3 | 40 | 3:38 | 2 | |

TR, repetition time; TE, echo time; FOV, field of view; Thickn, thickness; TA, acquisition time; NEX, number of acquisitions; TI, inversion time; TSE, turbo spin echo; STIR, short tau inversion recovery; DESS, double echo steady state.

dation of tissues, except for the four feet that were warmed to room temperature before the last MR examination.

MR images of the specimens were acquired with a human knee radiofrequency coil in a 1.5 T field.* Sequences were: turbo spin echo (TSE) T1-weighted in a transverse plane, TSE T2-weighted in a sagittal plane, short tau inversion recovery (STIR) in a sagittal plane, and 3D double-echo steady state (DESS) in a dorsal plane (Table 1). The transverse plane was oriented perpendicular to the proximodistal axis of the navicular bone, the dorsal plane was oriented parallel to the proximodistal axis of the navicular bone. Imaging series were obtained by the same technologist together with the first author by manually selecting the section prescription on the basis of a three-plane localizer series. Anatomic features visible on the localizer images were used as reference points. The digits were positioned with the dorsal aspect on the table to avoid the magic angle effect.[15]

DICOM images from the group of four feet acquired at 0, 1, 2, 7, and 14 days were compared subjectively by one operator side by side using an interactive workstation† to assess visual differences in signal and image quality. The window width and level for viewing were chosen subjectively by the reader for each sequence. The same window width and level were used for each sequence to compare images acquired at 0, 1, 2, 7, and 14 days. The reader was asked to define the signal of each anatomic structure (trabecular bone, synovial recesses, digital cushion, deep digital flexor tendon) on days 1, 2, 7, and 14, as being isointense, hypointense, or hyperintense to the signal of the same structure at day 0 and to assess any change in size of the synovial recesses. A five-point scale grading system (0 = nonvisible or nondiagnostic, 1 = poor, 2 = fair, 3 = good, 4 = excellent) was used to evaluate the image quality of anatomic structures and a score was given to each anatomic structure for each sequence at 0, 1, 2, 7, and 14 days.

A quantitative analysis was used in the eight feet to compare the changes in signal-to-noise ratio (SNR) between examinations. The SNR was calculated as the ratio of the amplitude of the MR signal (SI) of the tissue to the standard deviation of the amplitude of the background noise (SD) according to the equation SNR = SI/SD. Mean SI and mean SD were obtained by drawing three regions of interest (ROI) in each sequence and calculating the average. ROI were drawn in trabecular bone of the distal phalanx, the middle phalanx and the navicular bone, in the palmar proximal recesses of the distal interphalangeal joint, in the digital cushion, in the deep digital flexor tendon, and in the background region. The size of each ROI was determined subjectively in relation to the size of the structure to be evaluated. A ROI of 1 $cm^2$ was drawn in the distal half of the middle phalanx, just proximal to the distal subchondral bone plate, in the sagittal area. A ROI of 0.5 $cm^2$ was drawn in the proximal half of the distal phalanx, just distal to the proximal subchondral bone plate, in the sagittal area. For the navicular bone, a ROI of 0.2 $cm^2$ was drawn in the middle of the trabecular bone. A ROI of 0.1 $cm^2$ was used to assess the palmar proximal recesses of the distal interphalangeal joint in the sagittal area. The digital cushion was assessed palmar to the collateral sesamoidean ligaments in the sagittal area by drawing a ROI of 2 $cm^2$. A ROI of 0.1 $cm^2$ was drawn in the deep digital flexor tendon proximal to the collateral sesamoidean ligament in the sagittal area. A ROI of 3 $cm^2$ was positioned in the background noise in a consistent location for each sequence.

The coefficient of variation was calculated for each ROI value to assess the repeatability of the value obtained by manual drawings of the ROI. With these coefficients of variation, a threshold value was determined by calculating a unilateral confidence interval of 95% using a *t*-distribution with 479° of freedom.

A linear model with a mixed procedure was performed using SAS software ‡ to test statistical significance of image quality scores changes ($P < 0.05$) and of SNR changes ($P < 0.05$).

## Results

Statistically significant results are reported in Table 2. There was a minimal difference in section planes between examinations of the same limb at different times. Visibility and margination of the anatomic structures of the digits and overall image quality were unchanged subjectively

*Siemens Symphony 1.5 T, Siemens S.A., Bruxelles, Belgium.
†e-Film Medical, e-Film Medical Inc., Toronto, Canada.
‡SAS Institute Inc., Cary, NC.

TABLE 2. Summary of Statistically Significant Changes in SNR

| | T0/T1d | T14d/TRT | T0/T14d | T1d/T14d | T0/TRT |
|---|---|---|---|---|---|
| T1-W | | | | | |
| P2 | | | | | |
| P3 | | | | | |
| DSB | | | | | |
| Synovial rec. | ↑ | | ↓ | | ↓ |
| Digital cush. | | | ↑ | ↑ | |
| DDFT | | | | | |
| T2-W | | | | | |
| P2 | ↓ | ↑ | ↓ | | ↓ |
| P3 | ↓ | ↑ | ↓ | ↓ | |
| DSB | ↓ | ↑ | ↓ | ↓ | |
| Synovial rec. | ↑ | ↓ | | ↓ | |
| Digital cush. | ↓ | | ↑ | | |
| DDFT | | | | | |
| DESS | | | | | |
| P2 | | | ↓ | | ↓ |
| P3 | | | ↓ | ↓ | |
| DSB | | | ↓ | ↓ | ↓ |
| Synovial rec. | ↑ | | ↑ | ↓ | |
| Digital cush. | ↑ | | ↑ | ↑ | |
| DDFT | | | | | |
| STIR | | | | | |
| P2 | ↑ | | ↓ | ↓ | |
| P3 | ↑ | | ↓ | | |
| DSB | ↑ | | ↓ | | |
| Synovial rec. | | ↓ | ↓ | ↓ | ↓ |
| Digital cush. | | | ↓ | | |
| DDFT | | | | | |

P2, middle phalanx; P3, distal phalanx; DSB, distal sesamoid bone; Synovial rec., synovial recess; Digital cush., digital cushion; DDFT, deep digital flexor tendon; T1-W, T1-weighted; T2-W, T2-weighted; STIR, short tau inversion recovery; DESS, double echo steady state; T0, within 12 h after the horse death; T1d, 1 day after death; T14d, 14 days after death; TRT, warmed at room temperature for 24 h before the last MR examination; ↑, increase of signal-to-noise ratio (SNR) between the two examination times; ↓, decrease of SNR between the two examination times.

in all feet. No significant change in image quality score was observed over time in the feet imaged at day 0, 1, 2, 7, and 14 days.

No subjective change in signal was seen, except for bone marrow in STIR and TSE T2 sequences. Subjectively, at day 1, trabecular bone appeared slightly hypointense compared with day 0 in the TSE T2 images and slightly hyperintense compared with day 0 in the STIR images (Figs. 1 and 2). No subjective change in bone marrow signal was seen between 1, 2, 7, and 14 days (Figs. 1 and 2). For all synovial recesses assessed, a mild reduction in size of the synovial recesses was found subjectively and this was mainly visible at 14 days.

The repeatability of ROI manual drawing was good with 93.33% of signal values being under the threshold in the four feet examined at 0, 1, 2, 7, and 14 days, and 95.5% of signal values being under the threshold in the four feet examined at 0, 1, and 14 days—24 h. The structures with the greater number of coefficients of variation above the threshold value were the deep digital flexor tendon and the synovial recess. No significant change was observed in the background noise between examinations.

Using quantitative analysis, significant SNR changes in bone marrow of refrigerated limbs compared with day 0 were detected in STIR and TSE T2 sequences. Warming at room temperature for 24 h produced a reverse effect on SNR compared with refrigeration with a significant increase in SNR in TSE T2 images. The SNR in the deep digital flexor tendon was not characterized by significant change in SNR (Table 2). After 14 days of refrigeration a statistically significant decrease of SNR was found in bone marrow in TSE T2 and DESS sequences.

## Discussion

In this study the initial images were acquired between 8 and 12 h after death but not immediately after death. Therefore early post-mortem changes and changes due to differences between physiologic temperature and room temperature have not been assessed. Changes in T1 and T2 relaxation times of tissues between ante- and post mortem have been found.[16] *In vivo* MR images of the digits used for this study were not available and a comparison between ante- and post-mortem MR images was not made.

The effect of the temperature on T1 and T2 relaxation times has been evaluated.[16–18] In this study, except for the last examination on four feet at room temperature, the feet were not warmed before imaging to avoid bacterial proliferation and to limit deterioration. This necessarily resulted in a lower specimen temperature compared with the initial MR examination completed before refrigeration.

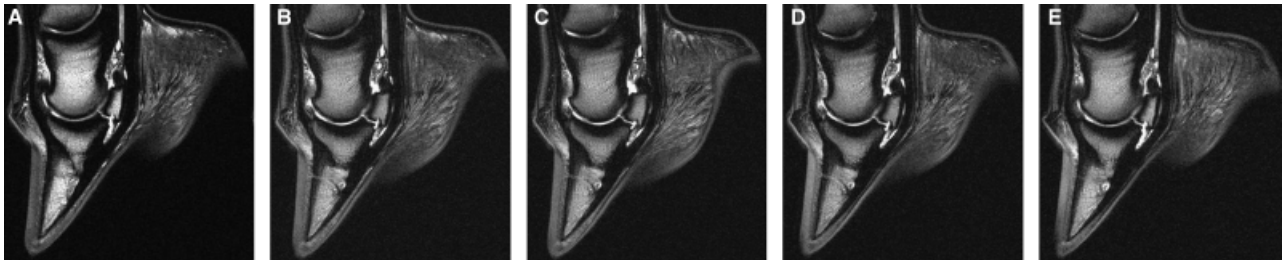


FIG. 1. Sagittal T2 turbo spin echo images acquired on day 0 (A), day 1 (B), day 2 (C), day 7 (D), and day 14 (E). At day 1 (B), bones appeared slightly hypointense compared with day 0 (A). No changes were seen subjectively in bones between day 1 (B), day 2 (C), day 7 (D), and day 14 (E).

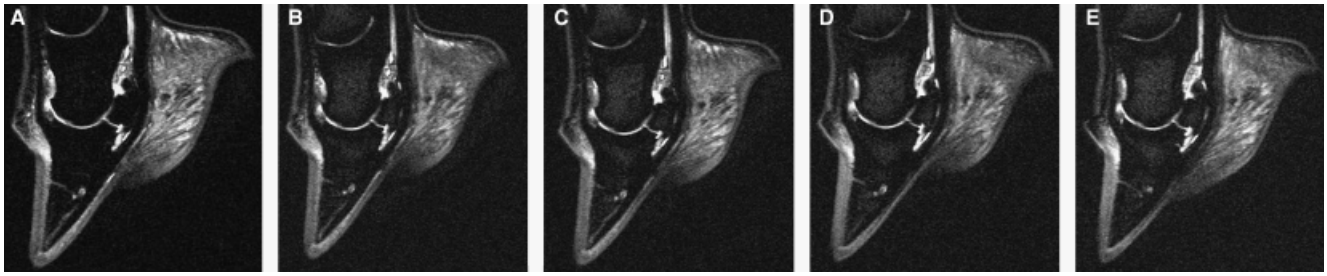


FIG. 2. Sagittal short tau inversion recovery images acquired at day 0 (A), day 1 (B), day 2 (C), day 7 (D), and day 14 (E). Subjectively, at day 1 (B), bones appeared slightly hyperintense compared with day 0 (A). No changes were seen subjectively in bones between day 1 (B), day 2 (C), day 7 (D), and day 14 (E).

However, because the exact temperature of the feet during each MR examination of this study was not available, a quantitative correlation between SNR changes and temperature was not possible.

A decrease in the transverse (T2) relaxation time as temperature decreases has been described in several studies on human tissues and in the rat brain.[16] T1 and T2 relaxation times of bone marrow decrease slightly to moderately with temperature until about $-5^{\circ}C$, and then decrease rapidly thereafter.[18] In our study, a statistically significant decrease of the SNR was seen in bone marrow in T2-weighted images after 24 h of refrigeration. As the feet were not warmed at room temperature, except for images acquired on day 15, these initial signal changes most likely reflect the higher temperature of the limbs at time 0 compared with the lower temperature at subsequent examinations. A reverse effect toward a statistically significant increased SNR of bone marrow in T2-weighted images between the day 14 (refrigerated) and day 15 (ambient) images supports this hypothesis. In spite of this, SNR changes were only statistically significantly different between 0 and 2 days in the TSE T1 sequence and no hyperintensity was observed subjectively in T1-weighted images after refrigeration. In studies on human tissues, the change in T2 relaxation time with temperature decreases was more pronounced than the change in T1 relaxation time[18] and the slope of the regression line of the signal against temperature depended on the repetition time (TR) with a longer TR leading to a higher slope.[19] Signal changes in the TSE T2 sequences (long TR) may therefore have been more evident than changes in the TSE T1 sequence (short TR).

Changes in signal in STIR images as a function of temperature have not been described to our knowledge. An increase in bone marrow SNR was seen in STIR images acquired after refrigeration and was visible subjectively as a hyperintense bone marrow. The optimal inversion time (TI) may vary between individuals or with body part.[20,21] This may be due to variations in the T1 relaxation time of fat between patients or between different parts of the body.[20,21] Because the T1 relaxation time of bone marrow decreases slightly to moderately with decreasing temperature[18], incomplete suppression of fat in STIR sequences of refrigerated limbs may have occurred because of temperature changes and incomplete fat saturation should probably also be expected between ante- and post-mortem images. A technique is described to determine the TI that produces the lowest fat peak for the best suppression of fat signal in subsequent STIR MR imaging.[21] Even though only slight hyperintensity was subjectively visible after refrigeration, it would be interesting to vary the TI in cadaver limbs examined after refrigeration to produce the lowest fat signal intensity and improve lesion detection. Although changes were less consistently statistically significant, the digital cushion was characterized by similar behavior to bones in relation to temperature changes between 0 and 1 day, except for the STIR sequence. The mixed composition of fat and connective tissue of the digital cushion may explain these results.

Changes between 1, 2, 7, and 14 days, and also changes between day 0 and the day 15 images more likely represent changes related to post-mortem interval and degradation of tissues; a decrease of SNR was seen in most instances in bone marrow and the synovial recess. Post-mortem changes inducing changes in water mobility and structure loss may influence relaxation times.[16] A decrease in T2 relaxation time, which may explain the decrease in SNR in T2-weighted images after 14 days of refrigeration, has been demonstrated in rats[16] and in excised porcine brain tissue.[22] Changes in the T1 relaxation times have, on the contrary, been less constant in relation to post-mortem intervals[16,22] and less time dependent.[23] T1 relaxation time decreases with cell shrinkage and increases with cell swelling in studies on apoptosis.[24] Post-mortem changes in T1 relaxation times are different between tissues and T1 relaxation time rapidly decreases after death and then increases to a plateau.[25] Nonlinear changes in the T1 relaxation time in relation to post-mortem interval as well as differences between tissues may be responsible of the less constant results in the present study with regard to T1-weighted images obtained 14 days after refrigeration.

The synovial recess was characterized by a statistically significant increase in SNR in all sequences except the STIR sequence between 0 and 1 day, and this differs from the other tissues. Because a statistically significant reverse effect is seen between the days 14 and 15 images, this

difference may reflect a difference in relation to temperature changes of the synovial recess, which includes a large fluid component, in comparison with other solid tissues. However, a difference related to very early changes after death in the synovial tissue leading to increased membrane permeability, water diffusion and molecular changes may not be excluded.[26,27] Further studies on synovial fluid will be needed to better elucidate changes in MR signal in relation to temperature and post-mortem interval.

The reduction of the synovial content, probably due to fluid loss, may be responsible for subjective impression of size reduction of the synovial recesses, which was mainly seen at 14 days and for the change in signal in the distal interphalangeal joint recess especially between 14 days and other times. However, as the synovial recesses are small amorphous structures, drawing a ROI of 0.1 $cm^2$ is difficult without including adjacent tissues; this error could explain the higher coefficient of variation for the synovial recess compared with other structures. The subjective impression of a size reduction of the synovial recesses could not be confirmed quantitatively mainly because of the variable shape of the recesses.

A technique for preserving equine cadaver specimens using a freezing/thawing process has been described.[13] However, the thawing process is time consuming and the MR examination has to be scheduled to permit the foot to be completely thawed. The simple method presented in this study has the advantage of being less time consuming than the method presented previously[13] and to allow the MR examination to be done at any time. Freezing and thawing can be responsible for cell membrane damage that may consequently reduce the quality of histopathologic samples.[28] Refrigeration at 4°C induces less cellular damage than cryopreservation in studies on human semen storage.[29]

There was a minimal difference in the imaging planes between some examinations. Although attention was paid to orient the imaging planes consistently, positioning the foot in the magnet may have been slightly different between examinations and may have indirectly produced slightly different imaging planes. Images were obtained by the same technologist by manually selecting the imaging plane on the basis of a three-plane localizer series. Anatomic features visible on the localizer images were used as reference points. Methods for automatic section prescription may give more reliable image plane selection.[30] Although differences in imaging plane were minimal, they may have produced different values in the selected ROI.

Because limbs were removed from the MR gantry between examinations, signal intensity between examinations may have changed due to differences such as limb placement with relation to the magnet isocenter, and radiofrequency coil placement with respect to the foot. Therefore the measure of SNR was chosen to assess changes between examinations instead of using the absolute signal value of the tissues.

Overall image quality was considered good and there was no significant difference in the quality score between examinations in the 4 feet on which the scoring system was applied. This suggests that refrigeration is sufficient to preserve equine cadaver limbs before MR examination and to prevent putrefaction. Most bacterial contamination at slaughter arises from hide and intestinal tract.[31] The foot is not in contact with the gastrointestinal tract at the abattoir which reduces contamination by bacteria. Also, the distal extremity has few hydrated tissues. These characteristics are probably a major reason why refrigeration at 4°C is adequate to preserve the distal limbs for a relatively long time without major changes in image quality. Long-term storage at 4°C has been used in several studies to preserve allografts.[32–37] In these studies, the graft was stored in different preservation solutions.[32–37] A preservation solution or preservation by wrapping in moistened tissue was not used in our study. Preservation by soaking in a solution or by wrapping may reduce dehydration. Because the skin was preserved and because of the hoof capsule, we presume that dehydration was relatively slow. We decided to avoid external humidification to limit deterioration and to decrease bacterial proliferation on the skin.

There were no significant changes in the SNR in the deep digital flexor tendon. However, the deep digital flexor tendon was the structure with the greater number of coefficients of variation above the threshold value and this may have influenced the results. The extremely low signal value of the deep digital flexor tendon could have induced these greater values of the coefficient of variation. Even if the feet were positioned on the dorsal aspect to avoid magic angle effect, this artifact may have been present in the suprasesamoidean area when the tendon was not perfectly straight and this may have influenced SNR measurements in the deep digital flexor tendon.

In conclusion, mild subjective changes in signal can be seen in trabecular bone in STIR and TSE T2 images between the first examination performed between 8 and 12 h after death and the examination after 1 day of refrigeration at 4°C. Although no significant changes in image quality occur in refrigerated limbs between 24 h and 2 weeks, changes in temperature after refrigeration and changes related to a post-mortem interval of 14 days of refrigeration result in variations of the SNR of tissues of equine cadaver digits. Our results suggest that MR sequence parameters (such as TI) for *in vivo* imaging should be optimized for *ex vivo* studies and that results of post-mortem studies should be interpreted considering temperature changes, storage conditions, and length of the post-mortem interval.

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