The Effect of Freeze-Thawing on Magnetic Resonance Imaging T2* of Freshly Harvested Bovine Patellar Tendon

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Introduction: Magnetic resonance imaging (MRI) is commonly used to evaluate tendinopathy, however, tendons typically exhibit little signal on clinical fast-spin echo (FSE) sequences due to their highly ordered collagen composition, which causes rapid shortening of T2 relaxation and corresponding loss of signal. Only when the tendon is highly disrupted by edema or hemorrhage will it appear abnormal on FSE images [1]. Newer techniques, such as ultrashort echo time (UTE) imaging, can visualize tissue species with very short T2 relaxation times, and permits calculation of a reproducible decay constant (T2*), a biomarker of disruption of highly ordered tissues such as tendons, ligaments, and menisci [1-5]. Imaging of ex vivo tendon samples, particularly those from preclinical models, is best scanned shortly after death; however, it is often necessary to freeze samples for evaluation at a later date. The few studies which have addressed the effect of freezing on quantitative MRI properties of tendons, have had limited number of samples, samples only from humans, or samples from an older population [6]. The purpose of this study was to determine the effect of multiple freeze thaw cycles on T2* values for a sample of juvenile and young adult bovine patellar tendons. The patellar tendon was chosen for investigation since a large portion of tissue would be available for quantitative MRI analysis, and the patellar tendon is used as graft material in human anterior cruciate ligament reconstructive surgery.

Methods: Fourteen fresh bovine knees were acquired from a local abattoir within 8 hours of death. Four had closed physes (young adult) and ten had open physes (juvenile). The tendons were inspected visually by a veterinarian for gross evidence of disease or defects. The central patellar tendon was harvested from each knee and wrapped in saline-soaked gauze to maintain hydration. Specimens were refrigerated at 4°C for 12 hours prior to image acquisition. Initial (pre-frozen) MR imaging was performed within 24 hours of death. All scanning was performed on a clinical 3T scanner (GE Healthcare, Waukesha, WI) with an 8 channel phased-array wrist coil (Invivo, Gainesville, FL). Morphologic multi-planar fast-spin-echo (FSE) images were acquired: echo time (TE): 24 ms, repetition time (TR): 4000 ms, receiver bandwidth (RBW): ±50 kHz, acquisition matrix (AM): 512x256-384, number of excitations (NEX): 1-2, field-of-view (FOV): 16 cm, slice thickness (ST): 1.0-2.0 mm. Next, axial multi-slice multi-echo two-dimensional UTE images oriented along the length of each tendon were acquired for T2* calculations: TEs=0.05, 5, 10, 15 ms, TR=350 ms, RBW=±62.5 kHz, AM=512x701, NEX=2, flip angle = 45°, ST= 2mm, slice spacing = 1-2 mm. Following scanning, each tendon was placed in a sealed plastic bag to prevent dehydration, and was frozen at -20°C for at least 24 hours. Following freezing, each tendon was permitted to fully thaw at room temperature, requiring approximately 6 hours, and the MR imaging protocol was repeated. Each tendon underwent a total of four freeze-thaw cycles following scanning of
the fresh tendon sample. Image Analysis: Patellar tendon T2* values were calculated from the UTE images acquired following each freeze-thaw cycle by fitting the TE to the corresponding signal intensity: SI (TE)=S0*e(-TE/T2*)+C, where SI (TE) is the signal intensity at echo time TE, S0 is proportional to apparent proton density, T2* is the inherent transverse relaxation time constant, and C is a constant to account for image noise. Average bulk T2* values from all voxels comprising individual tendons, approximately 15,000 voxels, were generated for statistical analysis. Statistical Analysis: A two-way repeated measures analysis of variance (ANOVA) was performed to determine the effects of specimen age (juvenile or young adult) and freeze thaw cycles on tendon T2* values. A post hoc Student-Newman-Keuls (SNK) test was performed when statistical significance was found. Significance was set at p<0.05.

Results: The T2* values of all fresh bovine patellar tendons were significantly longer (1.66±0.35) than T2* values measured after 1, 2, 3, or 4 freeze-thaw cycles, p=0.0003 (1.47±0.24 ms, 1.45±0.20 ms, 1.46±0.18 ms, and 1.43±0.20 ms respectively). This represented approximately 12% reduction in tendon T2* values after having undergone a single freeze-thaw cycle. T2* values following any number of freeze-thaw cycles were similar to one another (Figure 1). Significant differences of T2* values were found between the young adult specimens, 1.38±0.31ms (mean ± std.), and those from juvenile specimens, 1.72±0.23ms, p=0.02. The interaction between the two factors of age and freeze thaw cycle was not significant, p=0.05. The juvenile specimens had a significant shortening of T2* after one freeze thaw cycle, p=0.001, but no subsequent changes of T2* were seen following the first freeze-thaw cycle. Fresh young adult specimens tended to have the longer T2* values as compared to T2* following individual the freeze-thaw cycles, but this was not significant, p=0.25. Seven samples demonstrated a slight reduction in T2* from the first to the last freeze-thaw cycle, three demonstrated no change, and four demonstrated a slight prolongation of T2* values. A representative T2* map of a fresh tendon sample and of the same tendon sample following 4 freeze-thaw cycles is shown in Figure 2.

Discussion: The current study evaluated the effect of numerous freeze-thaw cycles on the inherent MR parameter T2* of bovine patellar tendon samples from younger and older animals. Our study demonstrated a small but significant reduction of tendon T2* values after one initial freeze-thaw cycle, attributable primarily to the specimens from juvenile animals. The magnitude of the reduction was less than 1ms. Since the only significant difference was found between the fresh and the first freeze-thaw cycle, the difference seen may have been due to loss of free water from the cellular component, which is susceptible to freezing and the formation of ice crystals. Subsequent freeze-thaw cycles would not have a further effect on this lost cellular component explaining the lack of further reduction in T2* values. In this study, we found a significant 12% difference of T2* between the fresh and frozen samples. This corresponds well with previous studies which performed serial scanning of fresh and frozen specimens. In addition, since previous reports have demonstrated changes of 30-87% for T2* due to pathology [4], the small change in T2* found in the current study, while significant, is smaller than would be anticipated with pathologic changes in tendon. In conclusion, freezing of untreated tendons leads to a small but significant reduction in UTE T2* values. The results of our study demonstrate the importance of using uniform (fresh or frozen) tissue samples when assessing UTE T2* and noting that one freeze-thaw cycle may significantly reduce T2* values in young tendon samples.

Significance: MRI of untreated tendons following a single freeze-thaw cycle will result in shorter T2* values when performing ultrashort echo imaging. This change will be accentuated in tendon samples
from younger specimens. These data are relevant to translational application when using frozen specimens to assess response of T2* measurements to applied load or displacement.

Figure 1. Mean (± SD) of bovine patellar tendon T2* within 24 hours of death, and following 4 freeze-thaw cycles to -20°C. T2* values of the fresh specimens were significantly prolonged compared to T2* values obtained after any number of freeze-thaw cycles.
Figure 2: Representative slice matched axial T2* maps of a single bovine patellar tendon from a fresh sample (A) and after 4 freeze thaw cycles (B). The specimen displays a mild reduction of T2* through the tissue sample following the 4 freeze-thaw cycles. A total of 15 slices was evaluated for this specimen.

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