INTRODUCTION

Osteoarthritis (OA) is a crippling degenerative joint disease that commonly develops in the knee, severely affecting quality of life. Since OA is a cartilage disorder, most studies focus on the effects of OA on the cartilage itself. However, since OA is a multifactorial disease, there is evidence that the meniscus is also very much involved in its progression. It has previously been shown that increased degradation is associated with a decrease in collagen content and an increase in hydration in the meniscus (effect on proteoglycan content was mixed). Quantitative MRI has proven to be a powerful diagnostic tool for OA in cartilage. However, while a few studies have begun reporting quantitative imaging data for the meniscus, the relationship between the biochemical composition of the meniscus and imaging parameters have not been established. Therefore the purpose of this study was to evaluate changes in T1rho and T2 relaxation times and tissue biochemistry after enzymatic digestion of collagen in bovine menisci.

METHODS

Ten fresh bovine knees were acquired locally from a veal slaughter house. The knees were opened to reveal the meniscus, and the tibia was disarticulated from the tibia; the menisci were left attached to the body. The knees were opened to reveal the meniscus, and the femur established cartilage sequences were modified specifically for shorter relaxation times. Sagittal 3D T1rho-weighted images were acquired with the following imaging parameters: TR/TE = 9.0/3.7 ms; FOV = 10 cm, matrix=256 x 128, slice thickness=3 mm, BW=31.25 kHz, VPS=64, Trec=1.5 s, TSL=0, 2, 4, 10, 20, 40, 80 ms, FSL = 500 Hz. T2 mapping was performed immediately after the T1rho sequence by adding a nonselective T2 imaging sequence with TR/TE=2000/2.8, 7.3, 11.7, 20.5, 29.5, 38.5, 73.9 ms. T1rho and T2 maps were quantified on a pixel-by-pixel basis (Figure 1). In-plane spatial resolution for both map sequences was 0.4 x 0.4 mm. During pre-digestion MRI acquisition, specimens were placed in plastic Ziploc bags and fully immersed in phosphate-buffered saline.

Immediately after scanning, the specimens were immersed in a Collagenase digestion solution with penicillin and streptomycin. Collagenase VII was chosen as a digestion enzyme for its ability to specifically target collagen, with minimal trypsin activity to leave proteoglycans intact, and for its precedence in the literature. Digestion was carried out at 37°C, for 40 hours; enzymes were refreshed at 20 hours by replacing the digestion solution. The digestion was stopped immediately before the post-digestion scan, and the specimens were prepared and scanned using the same methodology described above.

Following digestion, punches were taken from 3 regions of both the lateral and medial menisci for biochemical assay: anterior horn, body, and posterior horn. Both collagen concentration and GAG concentration were acquired post-digestion using previously established methodology. Due to the destructive nature of biochemical assay, pre-digestion assays were not taken. For comparison of biochemistry data, three punches from each of the six regions of a non-digested control sample were analyzed. Segmented masks for each meniscus were overlaid on the T1rho and T2 relaxation time maps for quantification. Paired samples t-tests were used for pre-post digestion comparisons. For biochemistry data, percentage differences from the control sample is reported for data description but no statistical analyses were performed. All statistics were performed using SPSS software with significance set at p<0.05.

RESULTS

No significant difference was observed in T1rho relaxation time when comparing post-digested values (22.3 ± 4.8 ms) to pre-digested values (23.3 ± 4.1 ms, p=0.05). In contrast, a significant 22% increase in T2 relaxation time was observed between pre- (20.8 ± 3.9 ms) and post-digested (25.1 ± 4.6 ms, p=0.001) digestion (Figure 2). Average GAG concentration post-digestion was 0.75 ± 0.23 % wet mass, compared with 0.54 ± 0.18 % wet mass in the control sample. Collagen content post-digestion displayed a 19% decrease (17.1 ± 3.3 % wet mass) when compared to the control sample (21.2 ± 1.6 % wet mass).

DISCUSSION

These data reveal that T1rho remains constant and T2 increases substantially with Collagenase IV digestion (Figure 1). As our digestion was designed to be specific to collagen, this is consistent with the theoretical understanding of the physical basis of T1rho and T2, with T1rho being more strongly affected by the macromolecules of the tissue and T2 being more strongly influenced by collagen and water. Interestingly, the magnitude of T2 increase following digestion (22%) is similar to the decrease is collagen content post-digestion when compared to the control sample (19%). GAG content following enzymatic digestion was very slightly higher when compared to the control sample. However, GAG content accounted for less than 1% of tissue wet weight in both digested and control samples.

REFERENCES


ACKNOWLEDGEMENTS

This study was funded by R01 AG17762. Thank you to Eric Han from GE Healthcare for his help with sequence development.