INTRODUCTION
Gross morphologic, biochemical and histologic grading schemes have been used to characterize degenerative disc disease. However, to better understand and treat disc degeneration, an accurate and non-invasive diagnostic tool is required to objectively detect changes in the disc matrix with aging and degeneration. Quantitative Magnetic Resonance Imaging (MRI) allows for an objective and reproducible way of assessing MR-sensitive protons, such as those of water in the intervertebral disc (IVD), and their interaction with matrix macromolecules (proteoglycans, collagens). In previous studies, we showed that T1, T2 and the magnetization transfer (MT) correlate to the disc biochemical content and grade of disc degeneration [1]. Furthermore, the apparent diffusion coefficient (ADC) is not a reflection of the disc’s biochemical subunit content but a direct reflection of matrix integrity [2]. Using directed controlled enzymatic techniques, we have recently found that enzymatic matrix degradation can be quantified by MR techniques in an enzyme-specific manner [3]. In this study, we tested the effect of hyaluronidase, which cleaves glycosidic bonds in hyaluronate, chondroitin and chondroitin sulfate, on the MR parameters and the matrix content of the treated NP. Results are compared to those obtained with collagenase-treated NP.

METHODS
Bovine caudal IVDs were harvested within 1-2 hours after slaughter, and kept as part of single motion segments. Nine nucleus pulposus (NP) of the IVDs were injected with 5 mg hyaluronidase type V from sheep testis (Sigma) in 50 mM sodium phosphate, pH 7.1, or with 50 mM sodium phosphate buffer (control). Another nine NPs were injected with 5 mg bacterial collagenase type A (Sigma) in 50 mM Tris with calcium chloride, or with 50 mM Tris with calcium chloride buffer (control). After injections, the single motion segments were embedded in paraffin blocks, and incubated at 37°C for 13-14 hours. Standard SE sequences were used for T1, T2, and MT determination [1] and the ADCs were determined along the anterior/posterior axis, as previously described [2,3]. After MRI, the NP was isolated from each segment, and 60 mg sections of NP were treated separately to quantitate the water, glycosaminoglycan (GAG), total collagen and denatured collagen contents [3]. Statistically significant differences between enzymatically-treated NPs and the control NPs were determined by ANOVA (P< 0.05).

RESULTS
Analysis revealed that treatment of NPs with hyaluronidase did not have any significant effect on either water or GAG (Fig 1A) contents when compared to the control. Not surprisingly, total collagen content and percent denatured collagen content (Fig 1B) of the NP were not affected by hyaluronidase treatment. The GAG (Fig 2A), water and collagen contents of collagenase-treated NPs did not differ from the control. As expected, collagen denaturation in NP increased significantly with collagenase treatment (P < 0.0001) (Fig 2B).

The MRI parameters, T1, T2 (Fig 1C) and MT (Fig 1D) of hyaluronidase-treated NPs did not differ from those of the control. On the other hand, T1 (P < 0.002) and T2 (P < 0.0001) (Fig 2C) decreased while MT (P < 0.03) (Fig 2D) increased in collagenase-treated NPs when compared to the control NPs. The ADCs of the NPs measured in this experiment were neither affected by hyaluronidase treatment or by collagenase treatment.

Stepwise, unforced regression analysis revealed the following statistically significant relationships between matrix content and integrity, and quantitative MR:

1. H2O (%) = 173.9-77.5*MT (r = 0.511; P < 0.004),
2. Denatured Collagen (%) = -167.3+158.9*MT (r = 0.244; P < 0.02).

DISCUSSION
Given constant water, GAG and collagen contents throughout all aged-matched bovine caudal discs, any changes in quantitative MRI signal would be a result of the change in matrix integrity induced by these enzymes and not a reflection of content. Treatment of NP with hyaluronidase was expected to cleave the GAG chains, leading to a loss of GAG chains, as well as a decrease in water retention. This was not observed with our experimental setup, probably due to a lack of fluid flow. Furthermore, the observed lack of effect of hyaluronidase on the T1, T2, MT and ADCs of the NP was also observed when NPs were treated with trypsin, which cleaves proteoglycan core proteins [3]. In view of these results, it is hypothesized that introduction of physiologic mechanical loading after enzyme injection will allow better detection of changes in matrix integrity by MRI, especially those induced through proteoglycan denaturation or enzymatic cleavage. However, proof of concept is clearly shown through our observation that collagenase induced a significant alteration in the collagen matrix integrity, as reflected by an increase in denatured collagen and alteration in the MR parameters. Moreover, the formulas obtained by regression analysis show a relationship between matrix content and integrity, and MR parameters. Future work will be aimed at studying the relation between the enzyme-induced biochemical changes with the mechanical properties and the MR parameters.

REFERENCES

ACKNOWLEDGMENTS: This work was supported by grants from the AO-ASIF Foundation, the OREF, and the CIHR.

** CHUM, Universite de Montreal, Montreal, QC, Canada H2L 4M1.
*** Orthopaedic Research Laboratory, Royal Victoria Hospital, McGill University, Montreal, QC, Canada H3A 1A1.

5th Combined Meeting of the Orthopaedic Research Societies of Canada, USA, Japan and Europe
Podium No: 049