EFFECT OF CYCLIC LOADING AND TRYPsin TREATMENT ON THE MR PROPERTIES OF THE NUCLEUS PULPOsUS IN RELATION TO ITS COMPRESSIVE PROPERTIES AND BIOCHEMICAL CONTENT

INTRODUCTION
Diseases of the spine that result from disc degeneration afflict a large proportion of the elderly population. Early and non-invasive detection of disc problems prior to the appearance of symptoms would open the door for the development of preventive therapies. Quantitative magnetic resonance imaging (MRI) is currently being tested for such purposes. Recent work has shown that the physiological state of the disc, including loading, must be considered when studying in vitro the relationships between the quantitative MR parameters, the biochemical state of the disc, and its mechanical properties [1].

Since the human intervertebral disc is loaded in compression for approximately 16h per day [2], we tested the hypothesis that different loading conditions for 16h while in saline solution and enzyme treatments induce changes in MR parameters, mechanical properties, and biochemical contents of intervertebral nucleus pulposus (NP).

METHODS
Experimental Groups: Thirty-six bovine caudal discs (2-3 years-old; non-degenerated) as 3-disc segments were injected in the NP with either 5 mg of trypsin (Sigma, ON, Canada) in 40 μl Tris buffer or with Tris buffer only. The 3-disc segments were placed in bags containing saline solution and antibiotics and were kept at 37°C throughout the experiment. The segments were subjected to either 16h of cyclic compression loading (50N–300N–50N at 1Hz) or were left unloaded for 16h. The segments were then paraffin embedded for MRI.

MRI Procedure: The MR examinations were carried out in a 1.5T whole-body Siemens' Avanto system using the standard circularly polarized head coil. The T1 and T2 relaxation times, magnetization transfer ratio (MTR), and trace of the apparent diffusion coefficient (TrD) were measured as described previously [1].

Biochemical Composition: The intervertebral discs were dissected and the NP was separated for biochemical and mechanical analyses. The NP tissue was analyzed for contents of water, glycosaminoglycan (GAG), total collagen, and denatured collagen [1].

Mechanical Testing Procedure: The portion of NP tissue for mechanical analysis was immediately frozen on dry ice and kept at –80°C until analysis. NP tissue plugs of 5 mm-diameters were prepared using a cryostat and biopsy punch and tested under confined compression as previously described [1]. Briefly, swelling pressure, compressive modulus H_{A}, and hydraulic permeability k were obtained using a ramp stress-relaxation experiment (10% strain increments and relaxation times and TrD (Fig. 1A) decreased while MTR increased approximately 16h per day [2], we tested the hypothesis that different loading conditions for 16h while in saline solution and enzyme treatments induce changes in MR parameters, mechanical properties, and biochemical contents of intervertebral nucleus pulposus (NP).

RESULTS
Loading of the disc motion segments for 16h in a saline solution significantly affected the measured MR properties of the NP. The relaxation times and TrD (Fig. 1A) decreased while MTR increased following loading. Neither the biochemical components of the NP, except the water content (Fig. 1B), nor the mechanical properties were significantly influenced by loading.

Enzymatic treatment of the NP had no effect on its MR properties, but increased the percent of denatured collagen (Fig. 2A) and decreased the compressive modulus of the NP (Fig. 2B).

** REFERENCES **
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![Figure 1](image-url)

**Figure 1.** Effect of 16h of cyclic loading on (A) the trace of the apparent diffusion coefficient (TrD) and (B) the water content of NPs injected with trypsin or buffer. * \( P \leq 0.05 \).

![Figure 2](image-url)

**Figure 2.** Effect of enzymatic treatment (trypsin vs. buffer) on (A) the percent of denatured collagen and (B) the compressive modulus (H_{A}) of NPs subjected to 16h of cyclic loading or left unloaded.

* \( P \leq 0.05 \), ** \( P = 0.0688 \).