Calcification in Intervertebral Disc Degeneration and Scoliosis
Hristova, GI; Mwale, F; Jarzem, P; Ouellet, J; Roughley, P; Antoniou, J
McGill University, Montréal, QC, Canada, Shriners Hospital for Children, Montreal, Quebec, Canada.
fmwale@ldi.jgh.mcgill.ca

INTRODUCTION
Calcification of connective tissues is a feature of tissue pathology, involving the deposition of inorganic calcium phosphates in crystalline forms. Alkaline phosphatase (ALP) initiates this process by hydrolyzing its phosphate substrates such as pyrophosphate. This reaction yields inorganic phosphate (Pi) which in the presence of calcium (Ca\(^{2+}\)) in the extracellular fluid forms apatite. The specific aim of this study was to assess the calcification potential in IVDs of patients with degenerative disc disease (DDD) or adolescent idiopathic scoliosis (AIS) by measuring ALP activity, Ca\(^{2+}\) and Pi concentrations in comparison with control non-diseased IVD.

MATERIALS AND METHODS
19 degenerative discs (Thompson grade 4 and 5) from between levels L1 and S1 were recovered from 14 patients aged between 23 and 55 years. Degenerative IVDs were analyzed in comparison with 15 control IVDs (Thompson grade 1 and 2) from 38 and 48 year-old donors. 21 scoliotic discs from between levels T11 and L5, with Cobb angles between 40 and 78 degrees, were recovered from patients aged between 11 and 21. Scoliotic IVDs were analyzed in comparison with 4 IVDs from a 15 year-old donor. Cartilage endplate (EP), annulus fibrosus (AF) and nucleus pulposus (NP) from each degenerative, scoliotic or control disc were analyzed separately, as well as the concave and convex parts of each scoliotic disc.
Discs were analyzed for ALP activity, Ca\(^{2+}\) and Pi concentrations, using commercially available kits from Bio Assay Systems. Specimens were homogenized mechanically in buffer and the liquid phases were analyzed for ALP activity and the concentrations of soluble Ca\(^{2+}\) and Pi. The IVD tissues were then digested completely using proteinase K. Insoluble calcium phosphate salts were solubilized using 0.1N hydrochloric acid. Subsequently, the assays for Ca\(^{2+}\) and Pi were performed and the results for the soluble and insoluble fractions of calcium and phosphate were combined. Results were normalized against the wet weight of the IVD tissues being analyzed.

RESULTS
ALP activity (Fig. 1), Ca\(^{2+}\) concentration (Fig. 2) and Pi concentration (Fig. 3) demonstrated consistently higher levels in EP, AF and NP of degenerative IVDs compared to the control discs statistically significant (p<0.0001). However, the variability between different degenerative discs was large, with no clear association either with the age of the patient or the disc level. ALP activity (Fig. 4), Ca\(^{2+}\) concentration (Fig. 5) and Pi concentration (Fig. 6) also show a tendency to be higher in scoliotic IVDs compared to control discs.

DISCUSSION AND CONCLUSION
The aim of this study was to assess the potential for calcification in IVD tissue (EP, AF and NP) in degenerative and scoliotic discs. The results of the study clearly demonstrate that disc degeneration in adults involves a significant increase in calcification potential. A similar magnitude of calcification potential is present in both the degenerative and scoliotic IVDs. ALP activity in both degenerative and scoliotic discs is higher in NP, whereas the Ca\(^{2+}\) and Pi concentrations are higher in the EP. The results suggest that IVD mineralization in AIS might reflect an ongoing premature degenerative process.

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Figure 3: Pi Concentration (degenerative versus control IVDs)
However, the differences between scoliotic and control discs were found to be statistically significant (p<0.05) regarding the ALP activity and Pi concentration only. Considerable individual variations in the levels of calcification markers in scoliotic discs were also observed. The variability did not show obvious correlation with the disc level or the Cobb angle of the scoliotic deformity. Although the average levels of calcification markers were predominantly higher in the concave aspect (except for Ca\(^{2+}\) and Pi concentrations in AF), the differences were not statistically significant.

Figure 4: ALP Activity (scoliotic versus control IVDs)

Figure 5: Ca\(^{2+}\) Concentration (scoliotic versus control IVDs)

Figure 6: Pi Concentration (scoliotic versus control IVDs)