INTRODUCTION: Contrast enhanced imaging of articular cartilage is predicated on the use of anionic contrast agents that function as mobile ionic probes that partition themselves in inverse proportion to the glycosaminoglycan (GAG) content of the cartilage matrix. GdDTPA₂⁻ (charge -2, 548 g/mol) has been used for delayed gadolinium enhanced magnetic resonance imaging of cartilage (dGEMRIC) and Ioxaglate²⁻ (charge -1, 1269 g/mol) or Iothalamate¹⁻ (charge -1, 809.17 g/mol) has been used for contrast enhanced computed tomographic imaging of articular cartilage (CECT). dGEMRIC is able to differentiate between healthy and arthritic cartilage both in vivo and in vitro. Changes in T1 relaxation time measured by MRI in the presence of GdDTPA²⁻ reflect variations in the structure and composition of the cartilage extracellular matrix (ECM) including GAG¹⁻. Similarly, changes in the x-ray attenuation of cartilage measured by CT in the presence of Ioxaglate or Iothalamate have been used to quantify changes in the GAG content and structure of normal and degraded articular cartilage¹⁻. According to Donnan equilibrium, partitioning of these anionic contrast agents are affected both by the GAG content and permeability of the cartilage ECM, making it difficult to ascertain what changes are specifically measured by contrast enhanced imaging of cartilage. Assuming that water content reflects the porosity (and permeability) of articular cartilage, the aim of this study was to evaluate the effect of naturally occurring changes in the water and GAG content of bovine articular cartilage on the variation of the x-ray attenuation of cartilage measured by microCT in the presence of the anionic contrast agents GdDTPA²⁻ and Ioxaglate.

METHODS: Specimen Preparation: Sixty-six osteochondral plugs (7mm diameter) were extracted from the knee joints of freshly slaughtered, skeletally mature cows. The osteochondral plugs were harvested from the tibial, femoral, and patellar surfaces. Contrast Enhanced Computed Tomography Imaging of Cartilage: Anionic contrast agents GdDTPA²⁻ (Magnevist) and Ioxaglate (Hexabrix) were used in this study. The samples were first immersed in Hexabrix (64 mg/mL) for 24hrs. Sequential transaxial images of the cartilage and underlying subchondral bone were acquired using µCT imaging system (Scanco Biomedical, USA) at an isolectric voxel resolution of 36 μm³, 70 kVP tube voltage, 113 μAmp current and 300 ms integration time. The sequential transaxial µCT images were converted into DICOM image format. The 3D µCT data sets were then analyzed using commercial image processing software (Analyze™, Mayo Clinic, Minnesota, USA). Cartilage was segmented from subchondral bone using a semi-automatic threshold based algorithm. The mean cartilage x-ray attenuation values for each sample were obtained by averaging attenuation values in the cartilage tissue over all transaxial µCT images. The same bovine samples were immersed in saline for 24 hrs to clear the Hexabrix, before being immersed in Magnevist (0.2M) for 24 hrs and being re-scanned using µCT. The osmolarities of both contrast agents were maintained at 400 mOsm/kg. Biochemical assessment of GAG content: The articular cartilage was separated from the subchondral bone using a razor blade and the wet mass of the cartilage was obtained. The cartilage was lyophilized for 24 hours and its dry weight was measured, following which the water content was obtained as a difference between the wet and dry weights. The total GAG weight per mg wet weight of cartilage for each sample was calculated using the 1,9-dimethylmethylene blue (DMMB) colorimetric assay. Statistical Analysis: Linear regression analysis (SPSS 17.0, Chicago) was used to express the x-ray attenuation measured by CECT as a function of the GAG content and water content for all the osteochondral plugs. The coefficient of determination was used to test the strength of each association. Further, to assess the extent that the variation in x-ray attenuation was affected by the variation in water content in addition to variation in GAG content measured by CECT in the presence of either Hexabrix or Magnevist, both GAG content and water content were used as predictors in a hierarchical multiple linear regression model. The change in R² when the water content was included in the model in addition to the variation in GAG content alone was used to assess the additional affect of water content (permeability) on the x-ray attenuation measured by CECT.

RESULTS: The x-ray attenuation measured using CECT was linearly and inversely related to the GAG content of the articular cartilage for both Hexabrix (R² = 0.44, P < 0.001) and Magnevist (R² = 0.36, P < 0.001) (Fig.1). The x-ray attenuation measured using CECT was linearly and directly related to the water content of the articular cartilage for both Hexabrix (R² = 0.25, P < 0.001) and Magnevist (R² = 0.44, P < 0.001) (Fig.2). Accounting for the variation in both water and GAG content explained 51.5% of the variation in CECT when Hexabrix was used and an additional 7.4% (P = 0.03) of the variation in x-ray attenuation measured by CECT when Magnevist was used and an additional 22.6% (P = 0.001) of the variation in x-ray attenuation measured by CECT when Hexabrix was used compared to the variation in GAG content alone. Assuming that water content reflects the porosity of articular cartilage, the statistically significant improvement in the relationship between x-ray attenuation measured by CECT and water & GAG content of articular cartilage suggests that partitioning of anionic contrast agents in cartilage is significantly affected by tissue porosity in addition to GAG content. The concurrent loss of collagen with GAG depletion that occurs in osteoarthritis affects the water content of the ECM. The resulting biochemical and structural changes influence the partitioning of anionic contrast agents at equilibrium. The work presented here demonstrates that when using anionic contrast agents, water content contributes significantly to the x-ray attenuation of cartilage measured by CECT beyond that of GAG content alone which may also reflect changes in the porosity of the ECM. Further parametric characterization of the effects of structural and biochemical factors on the partitioning of ionic contrast agents according to Donnan equilibrium will be needed before CECT imaging of cartilage can be developed into a viable clinical tool.