INTRODUCTION:
Recently, significant attention has been focused on better understanding the progression of knee osteoarthritis (OA). In addition to cartilage degeneration, damage to the menisci—the crescent-shaped, fibrocartilaginous tissues crucial for joint stability—can occur during the course of knee OA. Hence, minimally-invasively techniques that can monitor both cartilage and meniscus biochemical properties will be particularly useful for diagnosing OA and evaluating treatment response. Previous studies have explored the use of delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) to image the meniscus. Contrast Enhanced Computed Tomographic (CECT) imaging is a promising new technique that has been shown to predict glycosaminoglycan (GAG) content in articular cartilage. Previously, our group has shown that CECT using a novel cationic contrast agent (CA4+) can sensitively monitor changes in the GAG content of articular cartilage. Similar to articular cartilage, the GAG content of meniscus has been shown to deteriorate in the early stages of OA, hence we hypothesize that cationic CECT imaging can predict the GAG content of bovine meniscus as an additional indicator of the health of the meniscus besides its structural integrity. Specifically, the aims of this study were to evaluate the ability of CECT imaging of bovine meniscus to quantify the GAG content and its distribution within the meniscus and to determine if there is a predominant surface(s) through which the contrast agent diffuses.

METHODS:
Specimen Preparation and Study Design: Four medial menisci were carefully excised from the knees of skeletal mature cows. The menisci were sectioned into three regions: anterior, central and posterior; the exposed ends of each region were sealed with cyanoacrylate to prevent contrast agent from diffusing through the cut surfaces. This study consisted of two parts: (1) a diffusion-in study to determine the immersion time required for the cationic contrast agent (CA4+) to reach equilibrium within the different anatomic regions of the meniscus and (2) a fixed-time immersion study with additional samples to compare the CECT attenuation with the tissue GAG content at equilibrium.

Contrast Enhanced Computed Tomography Imaging: For the diffusion-in study, two posterior, one central, and one anterior sample were immersed in 30 ml solutions of CA4+ containing protease inhibitors and antibiotics and imaged using CECT at various time points up to 95h. The time required for the contrast agent to reach equilibrium within the different regions of the meniscus was determined as the time after which the change in CT attenuation was less than 1%. Six more samples (1 posterior, 3 central, 2 anterior) were each immersed to equilibrium in 30 ml of the same contrast agent solution. For both studies, following immersion, each sample was blotted, and the meniscus section positioned in a µCT imaging system (µCT40, Scanco Medical AG) using a custom airtight holder that maintained a humid environment to prevent drying of the tissue. Sequential transaxial µCT images of the center of each meniscus section were acquired in the coronal plane at an isotropic voxel resolution of 36 µm³, 70 kVp tube voltage, 113 µAmp current and 300 ms integration time. The µCT image data were converted into DICOM format and imported for post-processing (Analyze, BIR, Mayo Clinic). The meniscus was segmented from air and the holder using an automated threshold-based algorithm. The mean CECT attenuation (HU) for each sample was obtained by averaging the attenuation values over all pixels contained within the meniscal tissue. Additionally, one slice from the center of the scan region of each sample was examined qualitatively using a color map to examine if the diffusion of the contrast agent occurred predominately through a particular surface(s). Following CECT, the imaged region of each meniscus was excised from the surrounding tissue and immersed in saline at 4 °C for 24h to wash out the contrast agent.

Biochemical Assessment of GAG: Each excised meniscus section was cut into a three segments (inner, middle, outer), and the GAG content of each of these subregions was determined using the DMMB assay.

Statistical Analysis: Univariate linear regression analysis was applied to evaluate whether the CT attenuation for the samples correlated with their GAG content. The coefficient of determination (R²) was used to assess the strength of the correlation. Significance was set as a two-tailed p-value < 0.05.

RESULTS:
Figure 1A shows the diffusion-in curves which suggest that contrast agent diffusion begins to plateau around 90h. The color maps in Figure 1B illustrate that the increasing CECT attenuation in the meniscus over 95h of diffusion agrees with the natural GAG distribution in meniscus (Figure 1C), and occurs preferentially through the proximal and secondary through the distal surface, with little diffusion through the medial surface. The CECT attenuation accounts for 89% of the GAG content for the meniscus segments (anterior, central, or posterior, R²=0.89, p<0.001); Figure 1D.

DISCUSSION:
This study demonstrates that CECT imaging using a cationic agent can be used to evaluate the GAG content of isolated bovine menisci. The cationic contrast agent diffused into meniscus primarily through the proximal, and secondarily through the distal surface. Since the clinical administration of contrast for CECT of cartilage requires intra-articular injection, the proximal surface of the meniscus would be exposed to the injected contrast agent, allowing simultaneous evaluation of the articular cartilage and meniscus. However, the time required to reach passive diffusion equilibrium is not practical in a clinical scenario, but utilization of mechanical convection by way of repetitive joint loading using flexion-extension and axial compression can facilitate the mass transfer of the contrast agent into the meniscus. Nevertheless, the equilibrium CECT attenuation using CA4+ accounts for 89% of the GAG content, which suggests that this technique can be applied for determining the biochemical content of the meniscus. The color maps representing the CECT attenuation in the meniscus are in agreement with a previous study. Future studies using healthy and degenerated menisci samples from human patients are planned to further validate our findings.

SIGNIFICANCE:
Degeneration of meniscus tissue as well as meniscal tears contribute to compromised loading in the knee and hence are implicated in the initiation and progression of OA. A sensitive method for imaging the menisci as well as articular cartilage in the knee would improve upon current clinical practice for diagnosing and monitoring OA.

REFERENCES:

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