INTRODUCTION:
Anterior cruciate ligament (ACL) injury places the knee at risk for early osteoarthritis (OA) [1]. While ACL reconstruction (ACLR) improves knee joint stability, the procedure does not eliminate OA risk associated with ACL injury.

ACLR transection (ACLT) mimics many, although not all, aspects of a traumatically ACL-injured knee, and is often used to induce OA in animal models. In large animal models, which are crucial for studying disease-modifying interventions, data regarding the length of time between ACLT and OA development are unclear.

While advanced OA is marked by a decrease in articular cartilage (AC) thickness, a focal increase in AC thickness is thought to be one of the earliest detectable indications of OA [2-4]. These studies suggest that the breakdown of proteoglycan, a structural molecule, attracts water into AC [5]. The ability to detect these early changes in AC morphometry may allow researchers to investigate early-stage OA.

Our group recently validated a quantitative MRI (qMRI) approach for evaluating AC thickness both ex vivo and in vivo [5-7]. The validation of qMRI in a large animal model may further the understanding of disease progression in these animals. Therefore, the objective of this study was to apply an MR-based segmentation technique to evaluate porcine stifte joints treated with ACLT vs. ACLR. Our specific aim was to determine whether 15 weeks of healing would be sufficient for our qMRI method to detect changes in the AC thickness of the ACLT- and ACLR-treated knees, when compared to contralateral control limbs. We hypothesized that there would be significant differences in AC thickness between the surgical and control limbs 15 weeks after surgery.

METHODS:
Porcine Model of ACL Reconstruction: Ten skeletally mature Yucatan mini-pigs with a mean weight of 50kg (range 31-58kg) were included in the study. 5 pigs underwent unilateral ACLR using a bone-patellar tendon-bone allograft. The other 5 animals underwent unilateral ACLT. After surgery, the animals were allowed unrestricted weightbearing. Postoperative pain was managed with transcutaneous narcotics. The animals were euthanized after 15 weeks of healing. Knees were immediately harvested and imaged.

MR Imaging: Each knee was imaged on a 3T MRI (Siemens Trio, Germany), using a commercially available circular polarized knee coil. AC was imaged using the T1-WE-3D-FLASH sequence (0.3x0.3x0.8mm).

Manual Segmentation: The tibiofemoral AC of each scan was manually segmented in the sagittal plane by an experienced examiner (MEB). Once segmented, the AC was reconstructed, and a 3D voxel model of each structure was created. Each voxel model was wrapped with a triangular mesh to create a virtual solid model.

AC Thickness: Cartilage thickness measurements were performed on load-bearing regions of interest (ROIs) [5-7]. A cylinder was fit to the bone-cartilage interface of the 3D femoral cartilage model. A line was drawn from a distinctive notch on the condyle to the center of the cylinder. Each femoral condyle was divided from the notch point toward the posterior aspect of the femur to create 6 femoral ROIs (3 medial, 3 lateral). Two ROIs (1 medial, 1 lateral) were defined on the cartilage regions of the tibial 3D model. The inertial axes of the medial compartment and the centroid of each compartment were calculated using MATLAB (The Mathworks, Inc., MA). The calculated inertial axes were projected onto the centroid of each compartment to determine ROI orientation. The mean thickness of each cartilage patch was calculated with a closest point algorithm using MATLAB [5-7].

Statistical Analyses: Data were analyzed using a mixed linear model (SAS version 9.2, SAS Institute Inc., NC and MATLAB). Fixed effects were included in the model for group (ACLT vs. ACLR), limb (surgical vs. control), bone (tibia vs. femur), and two effects describing the ROIs within each bone: 1) compartment (lateral vs. medial), and 2) region (anterior vs. middle vs. posterior). A covariate for animal weight was included. The model allowed for different variances for each measurement location in surgical and control limbs, as well as a different covariance between the surgical and control limb for each ROI. Twenty-four a priori planned comparisons were made using orthogonal linear estimates between surgical and control limbs for each ROI within each group, as well as a comparison of these differences between groups. Alpha was maintained at 0.05 across these comparisons using the Holm test.

RESULTS:
There were significant increases in AC thickness in surgical limbs compared to control limbs in virtually all ROIs in both ACLT and ACLR groups (adj. p<0.05; Fig. 1). One exception was in the ACLT group at the anterior medial femur (adj. p=0.06047); a second exception was in the ACLR group at the posterior lateral femur (adj. p=0.4812). There was no significant difference in cartilage thickness increase between the ACLT and ACLR groups.

Fig. 1: Differences in mean articular cartilage thickness values (mm) between the surgical and control limbs in each group, divided by ROI. Error bars indicate 95% confidence intervals. * denotes adj. p<0.05.

DISCUSSION:
Although ACL injury is associated with early OA, the mechanisms involved in this relationship remain poorly understood. A reliable method that can quantify in vivo changes in a large animal model of knee injury would allow researchers to study disease progression and evaluate disease-modifying interventions.

The present study demonstrates that qMRI can detect a difference between the AC thickness of ACLT-transected, ACLR-reconstructed, and their respective contralateral control limbs in mini-pigs after 15 weeks of healing. The limbs that underwent ACLT and ACLR had significant increases in AC thickness from their respective control limbs. It is likely that, after 15 weeks of healing, the animals used in this study were euthanized while their limbs were undergoing the initial cartilage thickness changes characteristic of early OA. While additional animals must be added to each treatment group before firm conclusions can be drawn, the results of this study may indicate that 15 weeks are not sufficient to observe the cartilage loss one may expect during OA disease progression.

REFERENCES:

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