

Meniscus Remodeling after ACL Injury is Region-Specific in a Skeletally Immature Porcine Model

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INTRODUCTION: The number of anterior cruciate ligament (ACL) injuries are increasing in pediatric patients [1]. These ACL injuries are commonly associated with meniscal tears or injuries. The lateral meniscus (LMEN) is more commonly torn at the time of injury, whereas the medial meniscus (MMEN) is more commonly injured over time in an ACL-deficient knee [2,3]. Some clinicians favor non-surgical treatment of pediatric ACL injuries, though the risk of degeneration in the joint is increased [2,3,4]. Studies have suggested that meniscal injuries may be isolated to the posterior region after ACL tears, suggesting that regional changes to the meniscus after ACL injury should be investigated [5]. Our previous studies showed menisci volume increased after ACL transection (ACLT), however, regional volumes of the meniscus were not investigated [6]. Therefore, the objective of this study is to determine regional changes in MRI-based size and signal parameters, histology, and biochemistry of the menisci after an ACLT using a juvenile porcine model.

METHODS: All animal protocols were approved by IACUC. Seven female juvenile (3 month) Yorkshire cross-breed pigs underwent a unilateral ACL transection arthroscopically and a sham incision was made on the contralateral joint. After 12 weeks, both hind limbs were imaged using the 3.0-T Siemens MAGNETOM Skyra MRI system (T2 SWI sequence, voxel size 0.5x0.5x0.8mm) (Fig 1A). Meniscal measurements were done for both the medial and lateral menisci. These measurements included middle height and width. The medial and lateral menisci were then segmented manually from the MRI scans. The segmented menisci were also separated by the posterior, middle and anterior regions respectively. All volumes (total, posterior, middle, and anterior) were recorded. Using the meniscal segmentations, T2* relaxation maps were created by fitting a monoexponential decay function on a voxel-by-voxel based on 6 echo times using MATLAB. Biochemical analysis was performed on the inner and outer areas of the middle region of both the medial and lateral menisci. A dimethylmethylene blue (DMMB) assay was performed to investigate the glycosaminoglycan (GAG) content of the meniscus before and after injury. Furthermore, compositional changes were histologically evaluated using a safranin O and fast green stain, where safranin O stains GAGs. Meniscus volume, height, meniscus T2* values and meniscus GAG content were all compared between the ACLT and sham operated joints using paired t-tests with Bonferroni-Dunn method as correction for multiple comparisons. Overall significance was set at $\alpha=0.05$.

RESULTS: At 12 weeks post-ACLT, total volume of both medial and lateral menisci increased by 27% ($P=0.090$) and 22% ($P=0.111$) relative to contralateral sham-operated controls (Fig 1B), respectively. Specific meniscal regions also increased in volume. The middle region of both the MMEN ($P=0.048$) and LMEN ($P=0.035$) significantly increased along with the posterior region of the MMEN ($P=0.014$) (Fig 1D-F). Similarly, the MMEN posterior horn height ($P=0.024$) significantly increased along with the MMEN middle width ($P<0.001$) and LMEN middle height ($P<0.01$) which both significantly increased (Fig 1G-I). T2* values significantly decreased compared to the sham operated controls in the entire lateral meniscus ($P=0.05$) and regionally in the posterior lateral meniscus ($P=0.017$) (Fig 2A-B). However, the T2* values in the medial posterior meniscus remained unchanged (Fig 2C). Via histology, GAG content is stained by the Saf-O (red). In normal tissue, the inner two thirds mainly consist of GAGs and the outer third is primarily fibrous tissue. Visually, the GAG content remains similar in LMEN, but decreases in MMEN after ACLT (Fig 3A). Overall, similar mean changes were observed via biochemistry, but with substantial variability (LMEN ($P=0.122$); MMEN ($P=0.525$)) (Fig 3B).

DISCUSSION: ACLT caused significant changes to the meniscus within 12 weeks of injury, specifically in the posterior and middle regions of the meniscus. Although meniscus remodeling has not been a primary metric for degeneration, studies have shown hypertrophy to be indicative of osteoarthritis [7]. Our findings align with this. After an ACLT, the meniscus size increased. Furthermore, our study determined specific regions to better understand how the meniscus is impacted and where degeneration begins after an ACLT. Higher T2* values show greater disorganization in collagen fibers [8]. Our results show a decrease in T2*, which could be a result of tissue remodeling in response to altered loading. Our results show greater differences when looking at size versus T2* values, which may be valuable since more complex imaging sequences would not be required. In future studies, T1rho mapping via MRI mapping can be used to associate changes in regional T1rho values with changes in GAGs due to degeneration. This could be an additional metric with histology to better quantify the compositional makeup of the meniscus after injury. Better understanding of where meniscal changes occur after ACL injury can lead to appropriate metrics for early detection of degeneration, which may aid in prevention of long-term changes, such as osteoarthritis.

SIGNIFICANCE/CLINICAL RELEVANCE: This study helps to determine where early meniscal changes occur to better understand degeneration after ACL injury.

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ACKNOWLEDGEMENTS: We would like to acknowledge the NCSU College of Veterinary Medicine and Laboratory Animal Resource for their help.

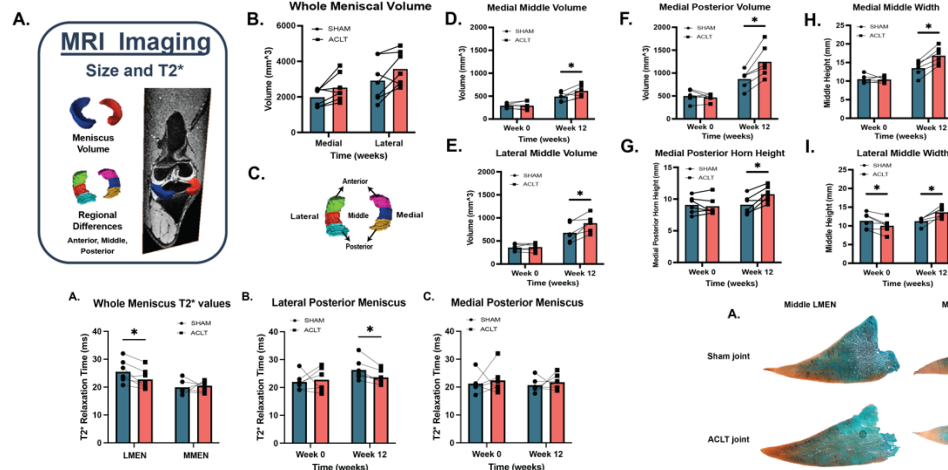


Figure 2: Graphs show T2* Relaxation times (ms) in (A) lateral and medial menisci (B) lateral posterior meniscus (C) medial posterior meniscus * <0.05 between sham and injured

Figure 1: (A) Shows segmented meniscus, (B) medial and lateral volume at 12 weeks, (C) meniscus regions, (D) medial middle volume, (E) lateral middle volume, (F) medial posterior volume, (G) medial posterior horn height, (H) medial middle width and (I) lateral middle width. * <0.05 between sham and injured

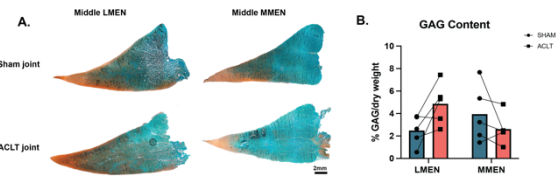


Figure 3: (A) Typical histological images of the lateral and medial meniscus of the sham and ACLT groups and (B) DMMB for GAG content * <0.05 between sham and injured