Tendon-Targeted Collagen III Knockdown Minimally Alters Mouse Supraspinatus Tendon Insertion Mechanical Properties

Emma E. Kroll,[1] Jeremy D. Eekhoff,[1] Margaret K. Tamburro,[1] Michael S. DiStefano,[1] Stephanie N. Weiss,[1] Rebecca L. Betts,[1] Susan W. Volk,[2] Louis J. Soslowsky[1]

[1]McKay Orthopaedic Research Laboratory, University of Pennsylvania, Philadelphia, PA

[2]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA

krolle@seas.upenn.edu

Disclosures: Emma E. Kroll (N), Jeremy D. Eekhoff (N), Margaret K. Tamburro (N), Michael S. DiStefano (N), Stephanie N. Weiss (N), Rebecca L. Betts (N), Susan W. Volk (N), Louis J. Soslowsky (N)

INTRODUCTION: Tendons transmit force from muscle to bone, inserting at the enthesis—a frequent site of musculoskeletal injury and damage.[1] Tendons are a hierarchical structure composed of multiple collagens, with the most predominant being types I (COL1) and III collagen (COL3).[2] COL3 plays a distinct role in development[3] and healing[4] of many tissues, where it is utilized as a provisional building block that is remodeled into highly aligned and mechanically strong matrices. Despite COL3's presence in tendon,[2] its functional role in the adult tendon insertion to the enthesis remains undefined. Addressing this critical gap will influence our understanding of COL3's role in pathologies with decreased *Col3a1* expression and inform strategies to improve tendon-to-bone repair, a common treatment for supraspinatus tendon tears.[5] Therefore, the objective of this study was to define the role of COL3 in tendon-to-bone insertion mechanical properties using a novel, tendon-targeted *Col3a1* knockdown (KD) mouse (ScxCre; *Col3a1$^{f/f}$*) and biomechanical testing. We hypothesized that COL3 is critical to tendon load bearing capability, and its loss would impair the mechanical properties of the tendon's highly stressed insertion to the enthesis.

METHODS: Mixed sex mice with tendon-targeted *Col3a1* knockdown (ScxCre; *Col3a1$^{f/f}$*), and Cre-negative littermate controls (WT) were euthanized at 90 days old and upper limbs were harvested for KD validation and uniaxial tensile mechanical testing of the supraspinatus tendon insertion (IACUC approved). The insertion region was defined as the 1 mm of tendon extending proximally from the enthesis into the tendon midsubstance. Knockdown Validation: KD was confirmed via qPCR ($n \geq 3$/group) and sRNA *in situ* hybridization ($n = 1$/group, male only preliminary data). For qPCR, supraspinatus tendon insertions were isolated using a custom 3D printed device and pestled with a phenol lysis reagent. RNA was isolated (Direct-zol RNA Microprep, Zymo) and processed according to manufacturer's protocols (Standard BioTools). ΔCt values were calculated as follows: ΔCt = average Ct of housekeeping genes (*Abl1* and *Rps17*) – Ct gene of interest. For sRNA *in situ* hybridization, upper limbs were fixed in Prefer for 24-hours, optically cleared in 30% sucrose (w/v) for 24-hours, then embedded in optimal cutting temperature medium and sectioned at 8 µm. Sections were adhered to a glass slide and manufacturer's protocols were followed for RNA detection (ACD Biotech). Mechanical Testing: Supraspinatus tendons ($n \geq 10$/group) and humeri were isolated from the limb, tendon cross-sectional area was measured with a laser-based system, and stain lines were applied for optical tracking. The testing protocol was: preloading to 0.05 N, preconditioning, and a quasi-static ramp-to-failure (0.1 %/s, imaging at 2 Hz). Mechanical properties were analyzed using a custom MATLAB code. Statistical Analysis: After outlier removal (2.2 IQR), KD and WT comparisons were performed using two-tailed Student's t-tests ($\alpha = 0.05$, trends reported for $p < 0.1$).

RESULTS: Successful *Col3a1* KD was achieved in the insertion via qPCR (Fig.1A) and sRNA *in situ* hybridization (Fig.1B). When analyzing mechanical material properties, tendon insertion cross-sectional area (CSA) was significantly higher in KD mice compared to WT (Fig.2A), KD mice had trending decreased insertion moduli (p=0.0849, Fig.2B). Tendons failed at the insertion during ramp-to-failure tests in both groups. Max force (Fig.2C) and max stress (Fig.2D) showed no differences in KD mice.

DISCUSSION: COL3 is important in development and healing in multiple tissues. In this study, we evaluated the role of COL3 in young adult mouse supraspinatus tendon mechanical properties with a focus on the insertion site, given the preponderance of injuries and tissue damage at this location. *Col3a1* KD increased insertion CSA, indicating there may be compensatory matrix production to account for COL3 loss which may, in turn, relate to the trending reduced insertion modulus seen in KD mice. No significant differences were observed in failure properties, indicating that KD tendons are capable of withstanding the same loads as WT tendons. Future studies will include mechanistic structural and biological investigation of the effect of COL3 loss during development and injury of the supraspinatus tendon insertion and its adjacent enthesis. Overall, these results partially support our hypothesis, indicating that while COL3 may not be essential for maximum load bearing capacity, it does contribute to the mechanical properties of the tendon insertion.

SIGNIFICANCE: Defining COL3's role in tendon mechanics at the insertion lays a foundation for the improvement, development, and implementation of site-specific therapeutic interventions for tendon healing.

REFERENCES: [1] Derwin. *The Journal of Bone & Joint Surgery*. 2018. [2] Tresoldi. *Muscles Ligaments Tendons*. 2013. [3] Liu. *Proceedings of the National Academy of Sciences of the USA*. 1997. [4] Volk. *Cells Tissues Organs*. 2011. [5] Sugaya. *The Journal of Bone & Joint Surgery*. 2007.

ACKNOWLEDGEMENTS: This study was funded by NIH R01AR080029 and the Penn Center for Musculoskeletal Disorders (P30AR069619).

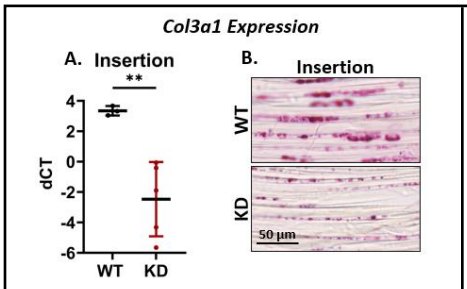

Figure 1. (A) KD tendon insertion had significantly decreased expression shown by qPCR. **(B)** Representative images of sRNA *in situ* hybridization (red fast = *Col3a1*) demonstrating KD. Numerical data displayed as mean ± SD, * = p<0.05, and ** p<0.01.

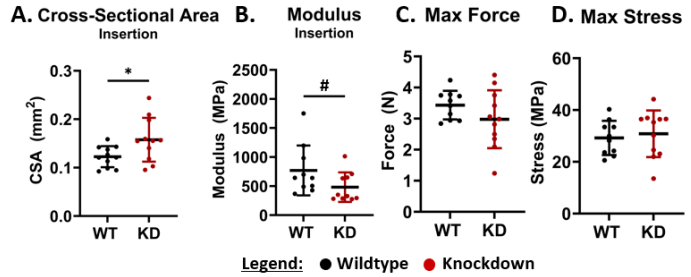

Figure 2. KD tendons had **(A)** increased insertion CSA (p=0.0312) **(B)** and trending (p=0.0849) decreased insertion modulus. **(C)** Max force and **(D)** max stress remain unaffected by KD. Data displayed as mean ± SD, * = $p < 0.05$, and # = $p < 0.10$.