Acute Knockdown of Collagen V Alters Mature Supraspinatus Tendon Regional Structure and Function

Michael S. DiStefano, Stephanie N. Weiss, Andrew F. Kuntz, Louis J. Soslowsky
McKay Orthopaedic Research Laboratory, University of Pennsylvania, Philadelphia, PA
micdis@seas.upenn.edu

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INTRODUCTION: Collagen V is a critical fibril-forming collagen expressed during development and in mature tendons that is essential in regulating initial fibril assembly and fiber organization [1]. Clinically, classic Ehlers-Danlos syndrome (EDS) is a connective tissue disorder with greater than 50% of patients being haploinsufficient for *COL5A1* and is characterized by hyperextensible skin, joint hypermobility and instability, and abnormal wound healing [2]. Recent data from mature mouse supraspinatus tendon, which experiences a complex, region-dependent (insertion and midsubstance) loading environment within the rotator cuff of the shoulder, demonstrated that deficiency of collagen V from development resulted in severely altered collagen fibril structure, biomechanical properties, and dynamic responses to load [3]. However, the region-specific regulatory roles of collagen V during tendon homeostasis have not been differentiated from its role in development. Determining the role of collagen V during tendon homeostasis is critical for establishing the baseline effect of collagen V knockdown in both mature and aging tendons. Therefore, the objective of this study is to elucidate the effect of acute knockdown of collagen V on region-specific structure and function of mature supraspinatus tendons. Since tendon hierarchical structure is well-established by maturity, we hypothesized that acute knockdown of collagen V would result in minimal changes to regional mechanical properties, collagen fiber realignment and fibril diameter distribution.

METHODS: Animals: Male wild-type (WT; n=10) and bitransgenic *Col5a1*$^{flox/+}$ (I-HET; n=10) and *Col5a1*$^{flox/flox}$ (I-NULL; n=10) with a tamoxifen-inducible *ROSA-CreER*^{T2} were used. At 120 days old, Cre excision was induced via two consecutive IP injections of tamoxifen. Mechanics and Collagen Fiber Realignment: All mice were sacrificed at 150 days old (IACUC) and were subjected to our mechanical testing and collagen fiber realignment protocol [3]: stress relaxations at 3%, 5%, and 7% strain each with subsequent frequency sweeps at 0.1, 1, 5, and 10 Hz, followed by a quasistatic ramp-to-failure. Throughout the ramp-to-failure, dynamic collagen fiber realignment was quantified using cross-polarization imaging, and regional fiber alignment data was interpolated with a polynomial fit as a function of strain from the load-displacement data. Elastic parameters failure load and linear stiffness were quantified. Viscoelastic parameters percent relaxation, dynamic modulus and phase shift (tan δ) were also quantified for each stress relaxation and frequency sweep. Images were acquired during the ramp-to-failure for optical strain tracking of stain lines demarcating the insertion and midsubstance regions of the tendon to calculate the modulus of each region. Collagen Fibril Diameter: Tendons were fixed, processed, sectioned, stained, and imaged via transmission electron microscopy (TEM) as described [4]. Collagen fibril diameter was measured across the fibril minor axis using a custom MATLAB script. Statistics: Comparisons between genotypes were conducted using one-way ANOVAs followed by Bonferroni post-hoc tests for mechanical properties and collagen fiber realignment. Collagen fibril diameter distributions from each genotype were compared to those of the other genotypes using Kolmogorov-Smirnov tests. Significance was set at $p \leq 0.05$ and trends at $p \leq 0.1$.

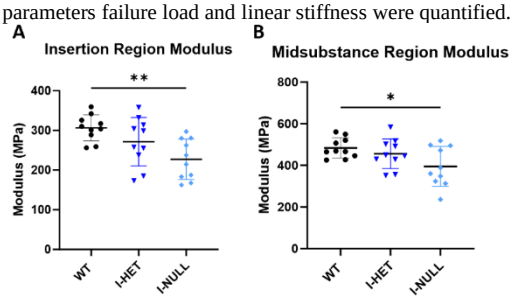

Figure 1. Acute collagen V knockdown (I-NULL) resulted in tendons with reduced insertion (A) and midsubstance (B) moduli relative to wild type (WT) controls. Data as mean ± standard deviation (--p≤0.1, *p≤0.05, **p≤0.01, ***p≤0.001).

RESULTS: As expected, no differences were observed in elastic mechanical properties, failure load and linear stiffness, and viscoelastic properties, percent relaxation, dynamic modulus, and phase shift, with acute collagen V knockdown (I-HET and I-NULL) (data not shown). Surprisingly, acute collagen V knockdown resulted in reductions in both the insertion (**Fig. 1A**) and midsubstance (**Fig. 1B**) moduli relative to wild type controls. These results are further supported by reductions in collagen fiber realignment in I-HET and I-NULL tendons across region, as demonstrated by greater normalized circular variance values for insertion (**Fig. 2A**) and midsubstance (**Fig. 2B**) regions from 3-9% strain, encompassing the toe and linear elastic regions of these tendons. Collagen fibril diameter distributions were different across genotype (**Fig. 3**, $p<0.0001$). I-NULL tendons exhibited more heterogeneous fibril diameter distributions with a greater percentage of larger diameter fibrils compared to WT and I-HET tendons in the insertion (**Fig. 3A**) and midsubstance (**Fig. 3B**) regions.

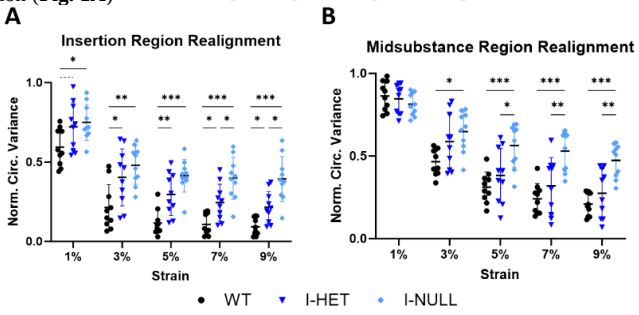

Figure 2. I-HET and I-NULL tendons demonstrated reduced collagen fiber realignment in the insertion (A) and midsubstance (B) regions. Decreased normalized circular variance is indicative of increased collagen fiber realignment. Data as mean ± standard deviation (--p≤0.1, *p≤0.05, **p≤0.01, ***p≤0.001).

DISCUSSION: In direct contrast to our hypothesis, acute reduction of collagen V expression in mature supraspinatus tendons resulted in regional structural and functional differences with reduced regional moduli and collagen fiber realignment and altered collagen fibril diameter distributions. Although mature tendons were generally believed to be quiescent tissues, there is increasing evidence that tendon collagen fibril networks are dynamic and remodel on shorter time scales than previously presumed [5]. Results of this study strongly support this notion, with collagen V playing a large role in regulating fibril properties beyond the postnatal developmental timeframe. While this study is limited by global knockdown models and potential confounding effects on neighboring tissue, the induced and short period of knockdown minimizes these effects. Future studies will evaluate the composition and gene expression of these acute collagen V-knockdown tendons to further elucidate the surprising regulatory role of collagen V in mature supraspinatus tendons.

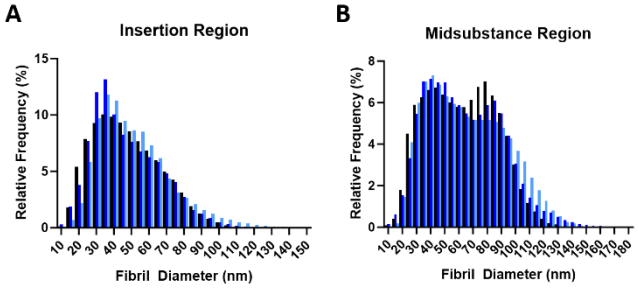

Figure 3. Fibril diameter distributions demonstrate a shift towards larger diameter fibrils in I-NULL tendons relative to the distributions of I-HET and WT tendons in the insertion (A) and midsubstance (B) regions. All distributions were significantly different from each other ($p<0.0001$).

SIGNIFICANCE/CLINICAL RELEVANCE: This study reveals that acute reduction in collagen V expression alters supraspinatus tendon regional mechanical properties, collagen fiber realignment and collagen fibril diameter distributions. These results provide further insight into the surprising role of collagen V in regulating tendon function during homeostasis and establishes a baseline for elucidating the role of collagen V in both mature and aging tendons.

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