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Tendon-Targeted Collagen V Knockout Influences Mechanical Properties of Aged Supraspinatus Tendon

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Introduction

Collagen V is one of the minor collagens in tendon, yet it plays a critical role in collagen fibrillogenesis by influencing the hierarchal assembly of collagen I into fibrils, fibers, and fascicles.[1] Clinical manifestations of reduced collagen V expression are present in patients with classic Ehlers-Danlos syndrome (EDS), a heritable connective tissue disorder with generalized connective tissue fragility as well as joint hypermobility and instability.[2] In addition to the altered tissue properties caused by reduced collagen V expression in EDS, the supraspinatus tendon is at high risk for injury with increased age and exhibits region-specific properties due to its complex leading environment.[3-4] Previous work has demonstrated that collagen V deficiency during development resulted in severely altered collagen fibril structure, biomechanical properties, and dynamic responses to load in the mouse supraspinatus tendon.[5] However, little remains known regarding the region-specific roles of collagen V in mouse supraspinatus tendon with more advanced age. Therefore, the objective of this study was to elucidate the region-specific role of collagen V in supraspinatus tendon mechanical properties of aged mice. We hypothesized that reduction in collagen V would result in inferior mechanical properties of the supraspinatus tendon, and that the mechanical changes would be greater in the insertion region than in the midsubstance region.

Methods

Supraspinatus tendons (n = 10/group) from male, 300 day old tendon-targeted collagen V heterozygous (Ten-Het) (ScxCre;Col5a1$^{f/wt}$), knockout (Ten-Null) (ScxCre;Col5a1f/f), and Cre-littermate controls (control) were used in this IACUC approved study. Supraspinatus tendon-humerus complexes were finely dissected, and the cross-sectional area was measured using a laser displacement sensor. Lines were applied to the tendon using Verhoeff's stain at 0, 1, 2, and 2.5mm from the humeral insertion to demarcate the insertion region (0-1mm) and midsubstance region (1-2mm) for optical strain tracking and to establish the gauge length (2.5mm). After potting the humerus and securing the free end of the tendon between sandpaper using cyanoacrylate glue, the samples were subjected to mechanical testing: after preloading to 0.05N and performing 10 cycles of preconditioning, stress relaxations were conducted 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 at a rate of 0.1% strain/second. Failure load and linear stiffness were quantified from the ramp to failure. Percent relaxation was calculated for each stress relaxation, and dynamic modulus and phase shift (tan δ) were quantified for each frequency sweep. Images were acquired during the ramp-to-failure for optical strain tracking of stain lines to calculate the modulus of the insertion and midsubstance regions. Comparisons between genotypes were conducted using one-way ANOVAs followed by Bonferroni post-hoc tests.

Results

Whole tendon cross-sectional area did not differ between groups (Figure 1A). Significant differences were seen in tendon elastic and viscoelastic mechanical properties. Ten-Null tendons failed at a significantly lower loads compared to both control and Ten-Het tendons and demonstrated a lower stiffness than controls (Figure 1B-C). Additionally, Ten-Null tendons exhibited increased percent relaxation at 7% strain compared to control tendons (Figure 1D). There were no differences in percent relaxation between groups at 3% or 5% strain (data not shown). Ten-Null tendons demonstrated a decreased dynamic modulus and increased phase shift across all strain levels and frequencies (7% strain at 1 Hz shown in Figure 1E-F). Ten-Null tendons demonstrated a decreased elastic modulus in the insertion region compared to control and Ten-Het tendons, while the Ten-Null tendons had a lower modulus only compared to controls in the midsubstance region Figure 2A-B).

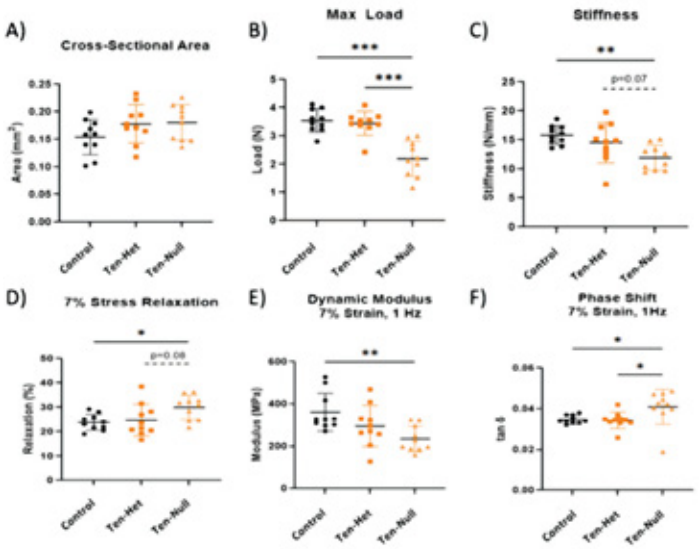

Figure 1. (A) No differences were seen in whole-tendon cross-sectional area. Ten-Null tendons demonstrated decreased in **(B)** max load and **(C)** stiffness. Within viscoelastic properties, Ten-Null tendons had **(D)** increased percent relaxation, **(E)** decreased dynamic modulus, and **(F)** increased phase shift. Data shown as mean ± standard deviation. (--p≤0.1, *p≤0.05, **p≤0.01, ***p≤0.001).

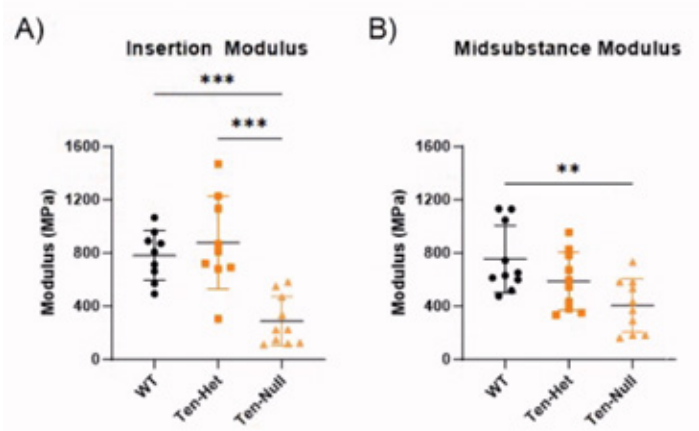

Figure 2. Ten-Null tendons demonstrated reduced modulus in the **(A)** insertion region, compared to control and Ten-Het samples, and in the **(B)** midsubstance, compared to controls. Data shown as mean ± standard deviation. (**p≤0.01, ***p≤0.001).

Discussion

This study investigated the role of collagen V on aged supraspinatus tendon elastic and viscoelastic mechanical properties. Results demonstrate that collagen V plays a critical role in regulating the extracellular matrix of supraspinatus tendon that has lasting effects into advanced age. Both the insertion and midsubstance regions were affected by collagen V knockout, yet its influence caused a greater decrease in modulus in the insertion region where the supraspinatus tendon is the least organized and experiences the highest strains.[6] Previous work investigating the regional influence of collagen V on fibril morphology in the supraspinatus tendon has shown that collagen V knockout mice demonstrate a significant disruption of fibril assembly with an increase in structurally aberrant fibrils at the insertion region compared to controls.[7] This regional variation in how collagen V influences collagen fibril structure could contribute to the respective mechanical responses of the insertion and midsubstance regions. This is the first study to evaluate the role of collagen V in tendons from mice aged 300 days as previous work evaluated tendons from younger mice (60-120 days).[5-7] Evaluating tendons at this age allows us to gain an understanding of the influence of collagen V on tendon properties after maturation into more advanced age. Future studies will investigate the underlying structural and compositional properties of the tendon extracellular matrix caused by knockout of collagen V that give rise to these mechanical findings. Moreover, continued work will investigate the varied effect of collagen V knockout at different ages to gain a better understanding of the distinct roles of collagen V in tendon properties during development, maturation, and aging.

Clinical Relevance

EDS is a clinical syndrome with limited treatment options. Furthering our understanding of collagen V in tendon under different conditions will aid the development of therapeutic targets for EDS.

Acknowledgement:

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