Collagen V Knockdown Alters Collagen Fibril Size, but Not Mechanics, in Mature Female Murine Tendons

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Introduction

Collagen V is a critical tendon matrix regulator that controls collagen I fibril size[1], and collagen V knockdown during tendon homeostasis increased the viscoelasticity of male murine tendons[2]. However, tendons display sex-dependent gene expression changes in response to collagen V knockdown[3] and the effect of this knockdown and differential gene expression on female murine tendon properties remains unknown. Therefore, the objective of this study was to define the effect of collagen V knockdown on mature female murine patellar tendon mechanical properties and collagen fibril size. Based on observed increases in matrix expression following collagen V knockdown[3], we hypothesized that collagen V knockdown would increase the mechanical properties and collagen fibril size of female murine patellar tendons.

Methods

Animals—Female wild-type (WT) and bitransgenic *Col5a1*$^{flox/+}$ and *Col5a1*$^{flox/flox}$ mice with *ROSA26-CreER*^{T2} were used in this study (IACUC approved). At 120 days old, mice received 3 consecutive daily tamoxifen (TM) injections (4mg/40g body weight) for Cre-mediated excision of floxed *Col5a1* alleles, resulting in I-*Col5a1*$^{+/-}$ and I-*Col5a1*$^{-/-}$ genotypes. Mice were sacrificed 30 days post-TM injections. Hindlimbs were harvested, and patellar tendons were isolated and prepared for mechanical testing (n = 15/genotype) or transmission electron microscopy (TEM, n = 4/genotype) as described[4]. *Mechanical Testing*—Tendons were immersed in a 37°C 1x PBS bath and loaded into an Instron 5848. Tendons underwent the following viscoelastic testing protocol: preconditioning, 10 min stress relaxations at 3, 4, and 5% strain, each followed by 10 cycle frequency sweeps at 0.1, 1, 5, and 10Hz, and a ramp-to-failure. Percent relaxation, dynamic modulus, and phase shift were computed from each stress relaxation and frequency sweep. Stiffness, max load, modulus, and max stress were measured from ramp-to-failure tests. *Collagen Fibril Imaging*—Following fixation and processing, tendons were sectioned at ~90nm and imaged with a JEOL 1400 TEM. 10 regions were analyzed per tendon. Collagen fibril diameter was measured across the fibril minor axis with BIOQUANT. *Statistics*—One-way ANOVAs with Tukey post-hoc tests were used to compare mechanical properties across genotypes. Collagen fibril diameter distributions from each genotype were compared against those of the other genotypes using Kolmogorov-Smirnov tests. Significance was set at $p \leq 0.05$ and trends at $p \leq 0.1$.

Results

No differences in any measured mechanical properties were observed across WT and knockdown genotypes; this included stiffness (Figure 1A), max load (Figure 1B), modulus

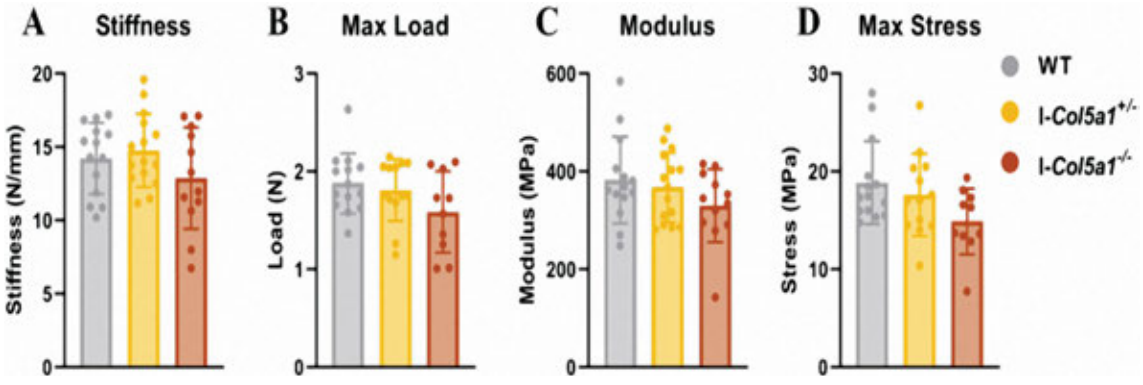

Figure 1. Collagen V knockdown does not impact female tendon mechanical properties. No differences in stiffness **(A)**, max load **(B)**, modulus **(C)**, or max stress **(D)** were observed across genotypes.

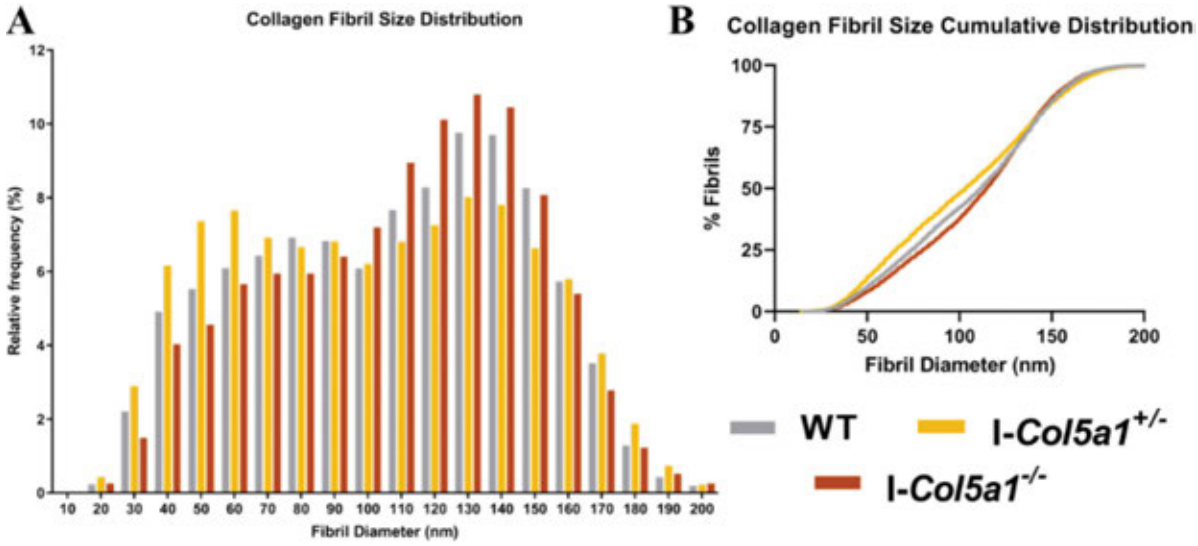

Figure 2. Collagen V knockdown alters collagen fibril size in an allele-dependent manner. WT tendon fibrils demonstrate a characteristic bimodal size distribution. I-Col5a1$^{+/-}$ tendons had a higher proportion of small (< 70nm) and large (> 160nm) diameter fibrils. I-Col5a1$^{-/-}$ tendons displayed a larger proportion of intermediately sized fibrils (100-140nm). All distributions were significantly different from each other ($p < 0.0001$).

(Figure 1C), max stress (Figure 1D), percent relaxation, dynamic modulus, and phase shift (data not shown). Conversely, collagen fibril size distributions were significantly different across all genotypes (Figure 2, $p < 0.0001$). WT tendons displayed a characteristic bimodal fibril size distribution (Q1: 74.3nm, Q2: 111.6nm, Q3: 139.0nm). I-*Col5a1*$^{+/-}$ tendons exhibited a larger spread in fibril size, with increased proportion of small (< 70nm) and large (> 160nm) diameter fibrils (Q1: 65.7nm, Q2: 103.1nm, Q3: 137.2nm). I-*Col5a1*$^{-/-}$ tendons contained an increased proportion of fibrils between 100-140nm in diameter (Q1: 80.1nm, Q2: 114.3nm, Q3: 138.2nm).

Discussion

Contrary to our hypothesis, acute knockdown of collagen V in mature female mice did not significantly alter patellar tendon mechanical properties. Despite the lack of mechanical changes, collagen V knockdown resulted in allele-dependent changes to collagen fibril size distribution. Taken together, these results provide key insights into the sex-linked role of collagen V in homeostatic tendon function. While collagen V knockdown in mature female murine tendons did not impact mechanical properties as shown here, collagen V knockdown did lead to increased viscoelasticity in mature male tendons[2]. Both sexes experienced changes in collagen fibril size in response to collagen V knockdown. This suggests that homeostatic female tendon function is less sensitive to collagen V presence than male tendon function. The decreased sensitivity to collagen V presence in female tendons may be due to observed increases in matrix synthesis expression in response to collagen V knockdown[3]. A limitation of this study is the global nature of the *Col5a1* knockdown model used. While this may lead to confounding effects in other tissues, the short knockdown window employed here likely minimized these effects. Future studies will assess the histological properties of knockdown tendons to further delineate the sex-dependent response to collagen V knockdown.

Significance

This work demonstrates a sex-linked role of collagen V in dictating homeostatic tendon function. Understanding this sex-dependent role can inform therapeutics that treat collagen V-associated clinical disorders.

References

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