Tendon-Targeted Collagen XII Knockout Attenuates Male and Female Achilles Tendon Function Similarly

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Introduction

Collagen XII is a Fibril-Associated Collagen with Interrupted Triple Helices (FACIT) that regulates collagen fibril assembly and is primarily expressed throughout tendon growth and development.[1] Mutations in the *Col12a1* gene result in myopathic Ehlers-Danlos syndrome, a connective tissue disorder in which patients exhibit weakness at birth, absence of deep tendon reflexes and distal joint hypermobility and contracture.[2] Our novel tendon-targeted collagen XII mouse model demonstrated that flexor digitorum longus (FDL) tendons exhibited reduced elastic mechanical properties in both male and female mice.[1] However, the role of collagen XII on the elastic, viscoelastic, and dynamic response to load in the Achilles tendon, which experiences a complex and distinct mechanical loading environment, remains unknown. Therefore, the objectives of this study were to (1) elucidate the role of collagen XII on Achilles tendon mechanics and dynamic response to load using tendon-targeted (Scleraxis-Cre) collagen XII knockout mice and (2) understand whether the role of collagen XII on Achilles tendon biomechanical function and dynamic response to load is comparable between sexes. We hypothesized that tendon-targeted knockout of collagen XII would result in consistent reductions in elastic and viscoelastic function and collagen fiber realignment in both sexes.

Methods

Achilles tendons from male and female, day 30 tendon-targeted collagen XII knockout (KO) mice (ScxCre;*Col12a1*$^{f/f}$, n = 4/group) and wild-type (WT) control mice (Cre-littermates, n = 4/group) (IACUC approved) were subjected to our mechanical testing protocol[3]: stress relaxation testing at 3, 5, and 7% strain each with subsequent dynamic frequency sweeps at 0.1, 1, 5, and 10 Hz, followed by a quasistatic ramp-to-failure. During the ramp-to-failure, dynamic collagen fiber realignment was quantified using cross-polarization imaging,[3] and alignment data was interpolated with a polynomial fit as a function of strain from the load-displacement data. For each sex, comparisons between genotypes were conducted using two-tailed, t-tests with significance set at $p \leq 0.05$ and trends at $p \leq 0.1$.

Results

Cross-sectional area (CSA) was not different across genotypes for both male and female tendons (data not shown). Consistent with our hypothesis, structural properties, maximum load and linear stiffness (Figures 1A, B), and material properties, maximum stress and linear modulus (Figures 1C, D), were significantly decreased in both male and female KO tendons. Further, genotypic differences were observed in viscoelastic properties in both sexes. Percent relaxation was significantly increased in KO tendons at all strain levels (7% strain shown in Figure 2A). Additionally, dynamic modulus was significantly decreased in male and female KO tendons, while phase shift was significantly

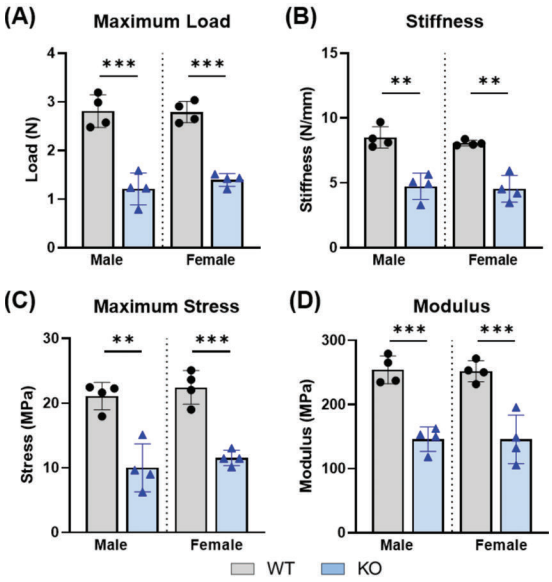

Figure 1. Male and female KO Achilles tendons demonstrated significant reductions in structural (A-B) and material (C-D) properties relative to WT controls. Data as mean ± standard deviation (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$).

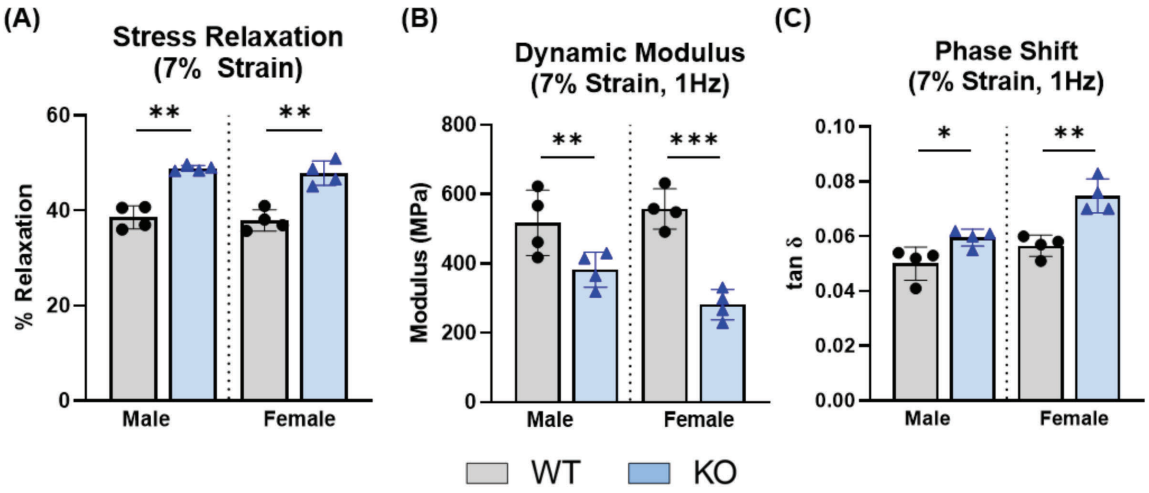

Figure 2. Male and female KO Achilles tendons had increased stress relaxation **(A)** and significantly decreased dynamic modulus **(B)** and increased phase shift **(C)** relative to WT controls. Data as mean ± standard deviation (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001).

increased in male and female KO tendons across at all strain levels and frequencies (7% strain at 1Hz shown in Figures. 2B and 2C, respectively). These results are supported by reductions in collagen fiber realignment in both male and female KO tendons, as demonstrated by significantly greater normalized circular variance values from 3-9% strain (Figures. 3A, B), encompassing the toe and linear elastic regions of these tendons.

Discussion

This study investigated the role of collagen XII on Achilles tendon elastic and viscoelastic mechanics and dynamic collagen fiber realignment using tendon-targeted male and female ScxCre;*Col12a1*$^{f/f}$ mice. Consistent with previous data from collagen XII knockout FDL tendons [1], we showed that tendon-targeted collagen XII knockout resulted in striking reductions in Achilles tendon elastic and viscoelastic mechanical properties and collagen fiber realignment in both sexes. These mechanical deficits could be attributed to the improper hierarchical assemblies of KO tendons resulting in disorganized tendon matrices with an inferior ability to quasi-statically and dynamically respond to load. This was evidenced by marked reductions in the KO tendons' responses to realign resulting in inferior mechanical properties, especially maximum load, modulus, and dynamic modulus. Although similar differences in elastic and viscoelastic mechanical properties were present in both male and female tendons in response to collagen XII knockout, more genotypic differences were present in female mice. Genetic variations in the *Col12a1* gene have been associated with an increased risk of ACL ruptures in women,[4] implicating potential sex-specific effects of collagen XII knockout. Our results demonstrate that decreased collagen XII expression detrimentally affects male and female Achilles tendon elastic and viscoelastic properties and dynamic collagen fiber realignment in response to load.

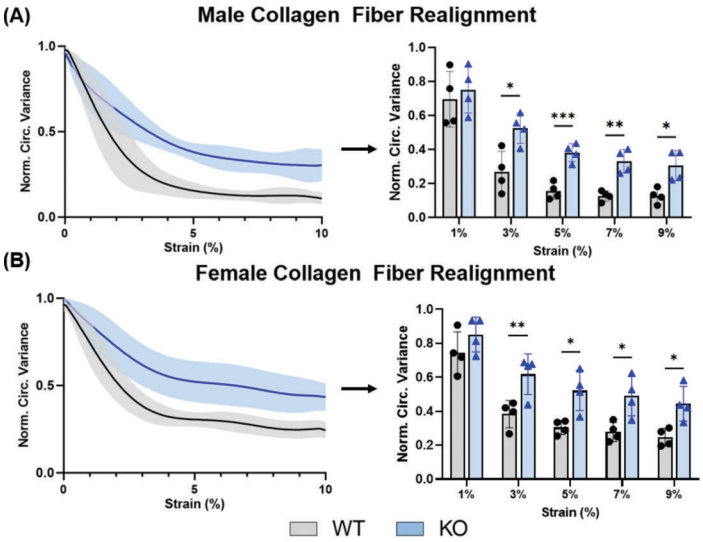

Figure 3. Male **(A)** and female **(B)** KO Achilles tendons exhibited significantly reduced collagen fiber realignment with increasing strain. Decreased normalized circular variance is indicative of increased collagen fiber realignment. Data as mean ± standard deviation (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001).

Significance

This study further elucidates the role of collagen XII in regulating tendon function. Future studies will evaluate the mechanisms that contribute to these results. Understanding the effects of collagen XII in both male and female tendons can be used to develop potential treatments modalities for myopathic Ehlers-Danlos syndrome.

Acknowledgements

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References

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