Acute Reduction in Collagen V Expression Increases Viscoelasticity in Mature Tendons

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Introduction

Classic Ehlers-Danlos Syndrome (cEDS) is a disease caused by mutations in the gene encoding collagen V, a fibrillogenic protein present in tendon.[1] cEDS patients experience joint hypermobility, which is likely caused by connective tissue dysregulation in the absence of collagen V.[2] Tendon-specific knockout of collagen V decreases tendon mechanical properties due to an aberrant development of tissue fibrils.[3] However, the regulatory role of collagen V in tendon homeostasis has not been distinguished from its role in development. Understanding this homeostatic role is critical for establishing the baseline effect of collagen V knockdown in both healthy and injured mature tendons. Therefore, the objective of this study was to determine the effect of acute knockdown of collagen V on the mechanical properties of mature tendons. Since the tendon fibril network is well-established by tissue maturity, we hypothesized that acute knockdown of collagen V in mature tendons would result in minimal changes to tendon mechanical properties.

Methods

Animals

Male wild-type (WT) (n =15) and bitransgenic *Col5a1*$^{flox/+}$ (n = 15) and *Col5a1*$^{flox/flox}$ (n = 15) mice with a tamoxifen (TM)-inducible Cre were used in this study (IACUC approved). At 120 days old, mice received 3 consecutive daily TM doses (4mg/40g body weight) for Cre-mediated excision of the *Col5a1* gene. Mice were sacrificed 30 days later. Tibia-patellar tendon-patella complexes were harvested and prepared for mechanical testing as previously described.[4]

Mechanical Testing

Uniaxial, viscoelastic testing was performed with an Instron 5848. The testing protocol consisted of 10 cycles of preconditioning, followed by stress relaxations at 3%, 4%, and 5% strain. Following each stress relaxation, frequency sweeps of 10 cycles at 0.1, 1, 5, and 10Hz were performed.A ramp-to-failure followed the 5% stress relaxation. Percent relaxation, dynamic modulus (E*), and phase shift (δ) were quantified for each stress relaxation and frequency sweep. Stiffness, modulus, maximum load, and maximum stress were quantified from the ramp-to-failure data.

Statistics

For all mechanical properties, one-way ANOVAs with Bonferroni post-hoc tests were used to compare across genotypes. Significance was set at $p \leq 0.05$, and trends were set at $p \leq 0.10$.

Results

No differences in cross-sectional area (CSA) were observed between genotypes (data not shown).

Quasi-Static Mechanics

Col5a1$^{-/-}$ (NULL) tendons had a decreased modulus relative to WT tendons (Fig 1). No differences in stiffness, max load, or max stress were observed between genotypes (data not shown).

Stress Relaxation

Col5a1$^{+/-}$ (HET) tendons exhibited increased stress relaxation compared to WT tendons at 4% strain (Fig 2). NULL tendons trended towards increased stress relaxation compared to WT tendons at 4% strain. No differences were observed in stress relaxation between genotypes at 3% and 5% strain.

Dynamic Mechanics

At 3% strain, NULL tendons had increased tan(δ) values compared to WT tendons at 0.1Hz

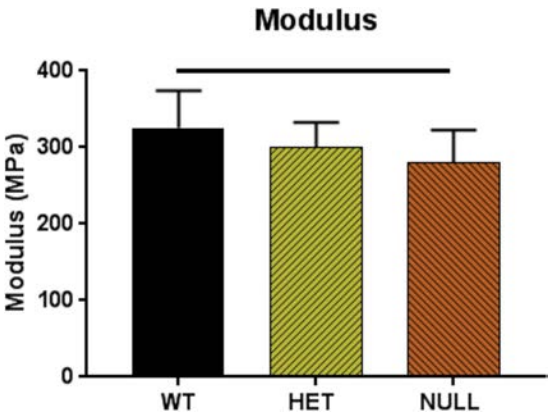

Fig 1. Elastic modulus. NULL tendons exhibited a decreased modulus relative to WT tendons. Solid bars indicate $p \leq 0.05$.

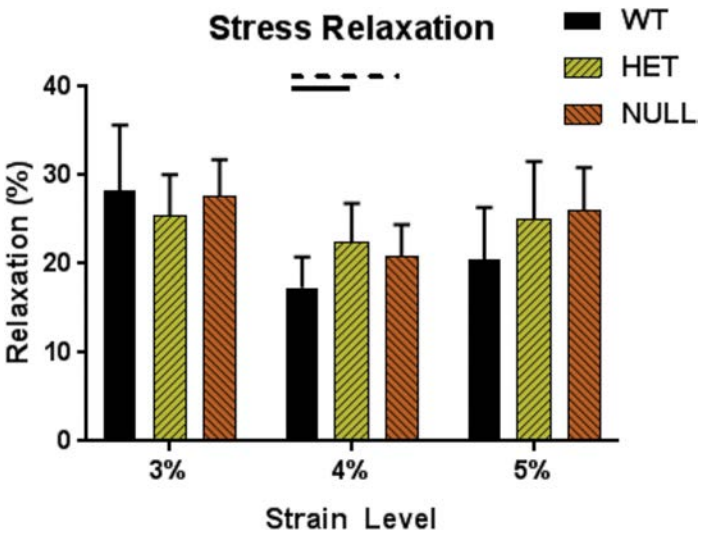

Figure 2. Stress relaxation. HET and NULL tendons displayed increased stress relaxation compared to WT tendons at 4% strain. Solid bars indicate $p \leq 0.05$, and dashed bars indicate $p \leq 0.1$.

(trend), 1Hz, and 5Hz and had increased tan(δ) values compared to HET tendons at 0.1Hz (data not shown). HET tendons had increased tan(δ) values compared to WT tendons at 1Hz and 5Hz (trend). At 4% strain, NULL tendons had increased tan(δ) values compared to WT tendons at all frequencies and trended towards higher tan(δ) values relative to HET tendons at 0.1Hz and 1Hz (Fig 3A). HET tendons had increased tan(δ) values compared to WT tendons at all frequencies (trend at 0.1Hz). At 5% strain, NULL tendons had increased tan(δ) values compared to WT tendons at all frequencies (Fig 3B). NULL tendons had increased tan(δ) values compared to HET tendons at 1Hz and 5Hz, with trending increases at 0.1Hz and 10Hz. No differences in dynamic moduli were observed between genotypes across strain levels and frequencies (data not shown).

Discussion

Surprisingly, acute reduction in collagen V expression in mature tendons led to numerous changes in tendon viscoelastic properties. NULL tendons exhibited increased stress relaxation at 4% strain and increased tan(δ) values at nearly every strain and frequency. HET tendons exhibited increased stress relaxation at 4% strain and displayed intermediate tan(δ) values between those of WT and NULL tendons. These results are in direct contrast to our hypothesis, as knockdown of collagen V increased tendon viscoelasticity in an allele dosage-dependent manner. While mature tendons were generally believed to be quiescent tissues, there is growing evidence that tendon fibril networks are dynamic and remodel on shorter time scales than previously thought.[5] Results of this study strongly support the notion of these dynamic networks, with collagen V playing a large role in regulating fibril properties beyond the developmental time frame. While this study is limited by global knockout models and potential confounding effects on neighboring tissue, the induced and short period of knockdown minimizes these effects. Future studies will analyze the composition and gene expression of these collagen V-knockdown tendons to further elucidate the surprising regulatory role of collagen V in mature tendons. Overall, this study demonstrates that acute reduction of collagen V expression in mature tendons leads to an increase in their viscoelastic properties.

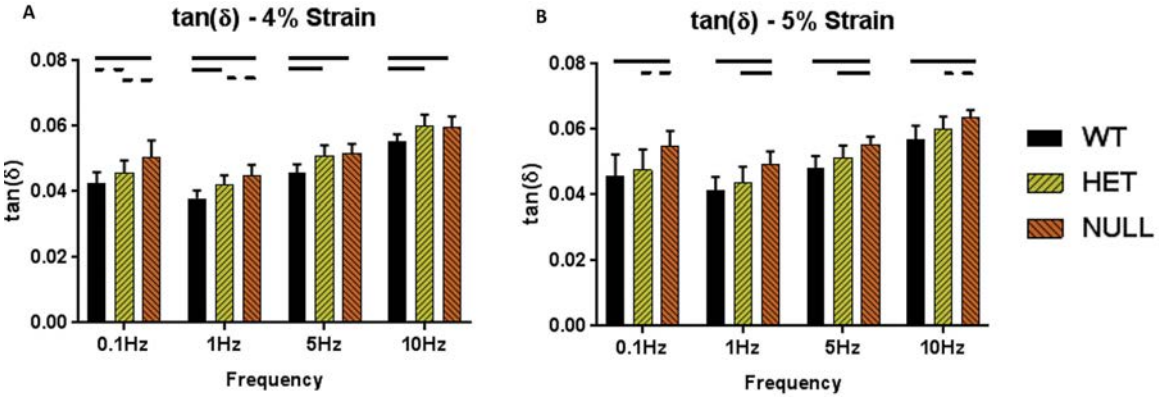

Figure 3. Phase shift. (A,B) NULL tendons had higher tan(δ) values than WT tendons at every frequency of both strains. HET tendons exhibited intermediate tan(δ) values between those of WT and NULL tendons. Solid bars indicate $p < 0.05$, and dashed bars indicate $p < 0.1$.

Significance

This study reveals that acute reduction in collagen V expression increases viscoelasticity in mature tendons. These results provide further insight into the surprising role of collagen V in regulating mechanical properties during tendon homeostasis.

Acknowledgements

This work was supported by the NIH (R01AR065995, P30AR069619) and the NSF GRFP.

References

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