Collagen V-Deficient Tendons Exhibit Altered Dynamic Mechanical Behavior at Multiple Hierarchical Scales

Brianne K. Connizzo[1]
Lin Han[2]
David E. Birk[3]
Louis J. Soslowsky[1]

[1]McKay Orthopaedic Research Laboratory
University of Pennsylvania
Philadelphia, PA

[2]Department of Biomedical Engineering
Drexel University
Philadelphia, PA

[3]Department of Molecular Pharmacology & Physiology
University of South Florida College of Medicine
Tampa, FL

Introduction

Tendon's mechanical behavior is exhibited by a stress-strain curve with an initial, non-linear "toe-region", thought to be attributed largely to collagen I fiber and fibril re-alignment, uncrimping, sliding and deformation,[1-3] followed by the "linear-region". Recent studies with collagen V deficient mouse tendons have demonstrated altered fibril structure and reduced macroscale quasi-static and dynamic mechanical function.[4-6] However, the role of collagen V in developing the dynamic microstructural response has not yet been established. Therefore, the objective of this study was to investigate the microstructural response of collagen V deficient tendons to load at the fiber (re-alignment) and fibril (deformation, sliding) scale. We hypothesized that collagen V-deficient tendons would have impaired dynamic mechanical function at all length scales, with a stronger phenotype in the null than heterozygous mice. We also hypothesized that changes in dynamic responses at fibril and fiber levels would be more apparent than those we found at the tissue-level.

Methods

Male mice with three genotypes, *Col5a1*$^{+/+}$ (WT), *Col5a1*$^{+/-}$ (HET), and a tendon/ligament-targeted conditional knockout, *ScxCre+Col5a1*$^{-/-}$ (NULL) were sacrificed at P120 (IACUC approved). The supraspinatus tendon-bone complex was dissected from the shoulder and prepared for mechanical testing as described.[4] For collagen fiber re-alignment, tendons were subjected to a viscoelastic testing protocol as described and re-alignment was quantified using our established cross-polarizer technique.[7-8] Re-alignment during toe and linear region strain levels were analyzed by comparing the change in circular standard deviation of fiber angles between 0-4% strain (toe region) and 4-8% strain (linear region). In addition, the strain required to reach the plateau and the amount of re-alignment that occurred during that time was measured for each specimen using a bilinear curve fit. Statistical comparisons were made between these measures using one-way ANOVAs with post-hoc Bonferroni tests. To investigate collagen fibril deformation and sliding, samples were stretched to a randomly assigned grip-to-grip strain value (0, 1, 3, 5, or 7%) following preconditioning. Tendons were then immediately flash frozen, sectioned, and fixed. AFM imaging of 2μm × 2μm regions was performed in tapping mode using a modified protocol as described.[9] Fibril D-period was determined for ~20 fibrils for each image, across the width of each section, and across multiple sections.[9] An increase in the fibril D-period with increasing strain is indicative of fibril stretch, while a change in the variance of the distribution is indicative of strain heterogeneity between fibrils, or fibril sliding.[9] Statistical comparisons were made using non-parametric Kruskal-Wallis tests followed by post-hoc Dunn's tests. Comparisons of variance were performed using a Bartlett's test for unequal variances with post-hoc F tests. For all statistical comparisons in this study, $p < 0.05$ was considered significant.

Results

Re-Alignment: There were no differences in the amount of re-alignment during toe region strain levels between groups (Figure 1A). However, re-alignment during linear region strain levels was reduced in both groups (Figure 1B). All of the groups returned to a more disorganized state with the removal of strain and then re-aligned again during the ramp to failure. However, the null group re-aligned fully with less strain at the midsubstance (Figure 1C) and exhibited less re-alignment at the insertion site (Figure 1D). *Fibril Deformation*: At the insertion site of the wild type tendons, collagen fibril D-period showed a bimodal response, with an initial hold at 3% strain, followed by an increase at 5% strain (Figure 2A). At the midsubstance of the tissue, the fibril D-period increased monotonically, peaking at 3% strain. In the heterozygous group, the insertion site showed a similar trend to the wild type group, but with a large decrease at 3% strain (Figure 2B). At the midsubstance, the D-period in the heterozygous group peaked at 1% strain. At the insertion site in the null group, there was an initial decrease in D-period at 1% strain followed by an increase at 3% strain (Figure 2C). At the midsubstance in null tendons, the D-period initially decreased at 1% and increased thereafter. *Fibril Sliding:* At the insertion site of the wild type group, fibril sliding occurred at the insertion site between 1% and 3% applied strain (Figure 2A). Fibril sliding occurred in the heterozygous

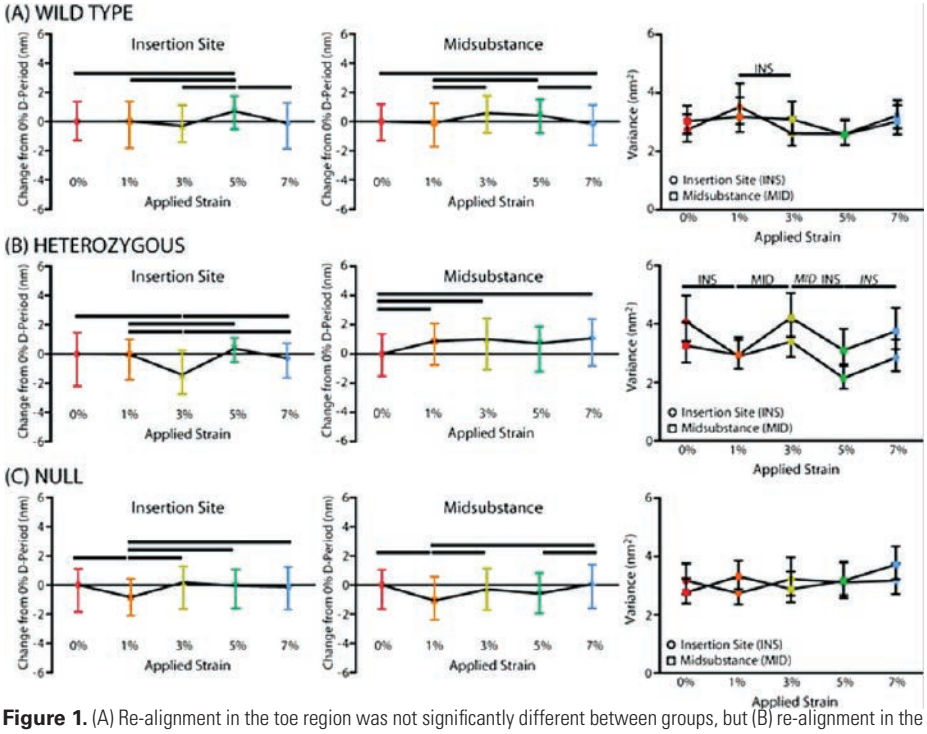

Figure 1. (A) Re-alignment in the toe region was not significantly different between groups, but (B) re-alignment in the linear region was reduced in the experimental groups. (C) The null group required less strain overall to full re-align and (D) re-aligned less overall. Data is reported at mean ± standard deviation.

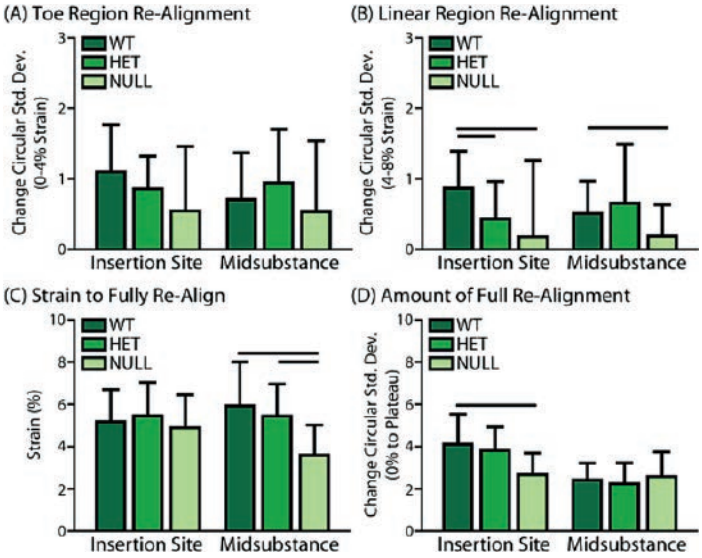

Figure 2. Results from fibril stretch at the insertion site (left) and midsubstance (middle) and fibril sliding at both location (right) are shown for each group. Data is reported at median ± interquartile range.

group continuously throughout the test, while the null group did not exhibit significant fibril sliding (Figure 2B,C).

Discussion:

Collagen V has a major role in regulating tendon fibrillogenesis. As hypothesized, a severely diminished response at the microscale and nanoscale was revealed as collagen V expression was decreased and therefore regulation of fibril assembly was further disrupted (in the heterozygous and null groups compared to the wild type group). With all of the multi-scale results taken together, these results suggest different mechanisms whereby tendons with reduced collagen V (50 to100%) expression attempt to ameliorate their altered fibril assembly at maturity. The wild type group is able to reduce stress at the lower hierarchical scales (fibers, fibrils) through a series of coordinated dynamic responses, specifically collagen re-alignment and sliding. The heterozygous group compensates for the lack of fibril strength via earlier re-alignment and a large amount of fibril sliding. The sliding reduces strain on the collagen fibrils initially, and thus, prevents early fibril failure, but with a large amount of repeated fibril sliding, the fibrils eventually pull away from each other and fail in shear. This allows the heterozygous group to respond elastically, but only at low strain levels, which is consistent with clinical observations. In contrast, the null group also responds early to load, but is incapable of producing significant fibril sliding, and therefore, the tendons fail earlier and with lower maximum loads. These studies highlight the hierarchical relationships that exist in tendon and suggest that this unique set of dynamic processes provide normal tendons with a series of protective measures to prevent early failure.

Significance

These studies deepen our understanding of the multi-scale response of tendons to loading and define a key role for collagen V in developing the structure responsible for this response.

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References

1. Rigozzi S, *et al.* *J Struct Biol.* 176(1):9-15. (2011).
2. Connizzo BK, *et al.* *Clin Orthop Relat Res.* 472(8):2433-9. (2014).
3. Miller KS, *et al.* *J Biomech.* 45(12):2061-5. (2012).
4. Connizzo BK, *et al.* *J Orthop Res.* (2015).
5. Connizzo BK, *et al.* Summer Biomechanics, Bioengineering, and Biotransport Conference; (2015) Snowbird, Utah.
6. Sun M, *et al.* *Am J Pathol.* 185(5):1436-47. (2015).
7. Dunkman AA, *et al.* *Matrix Biol.* 32(1):3-13. (2013).
8. Miller KS, *et al.* *J Biomech Eng.* 134(3):031007. (2012).
9. Connizzo BK, *et al.* *J Biomech.* 47(16):3794-8. (2014)