Injury and Healing Effect on Fatigue Properties of Collagen V Haploinsufficient Female Murine Tendons

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Introduction

- Classic Ehlers-Danlos Syndrome (*c*EDS) is characterized by mutation in the *COL5* genes, most commonly *COL5a1*, resulting in:
  - Skin hyperextensibility
  - Tendon and ligament fragility
  - Abnormal wound healing[1,2]
- Detrimental changes present in *c*EDS tendons may be exacerbated by decreased collagen synthesis and fibroblast activity in females[3,4,5]
- Quasi-static loading of the mouse patellar tendon[7,8,9] demonstrates decreases in modulus, failure stress, failure load, and stiffness due to reduced collagen V throughout healing
- Collagen V is essential in regulating collagen fibrillogenesis and the hierarchical structure of the tendon has been implicated in changes following cyclic loading[7,10]
- **Objective:** Define fatigue properties of female murine patellar tendons following injury, as well as the effect of a reduction in collagen V on these properties.
- **Hypothesis:** Reduction of collagen V following injury will delay improvements in the fatigue properties compared to wild-type (WT) tendons.

Materials and Methods

- Adult (day 120) female WT C57BL/6 and heterozygous ***Col5a1***$^{+/-}$ mice, a model for *c*EDS, were used (IACUC approved).
- Injured groups underwent bilateral patellar tendon injury surgery[11]
  - Sacrificed early in the remodeling healing phase (3 weeks) or later in remodeling (6 weeks) post-injury (PI)
- Uninjured age-matched mice were sacrificed
- *Mechanics*. The patella-patellar tendon-tibia complexes of all mice were dissected and tendons were subjected to a fatigue testing protocol[12] (Fig. 1) consisting of pre-conditioning and 1 Hz cyclic loading until failure
  - Cyclic loads corresponded to 20% and 55% maximum stress
  - Fatigue parameters were analyzed at breakpoints (BP), marking the ends of the primary phase (BP1) and secondary phase (BP2) (Fig. 2) of the fatigue life curve

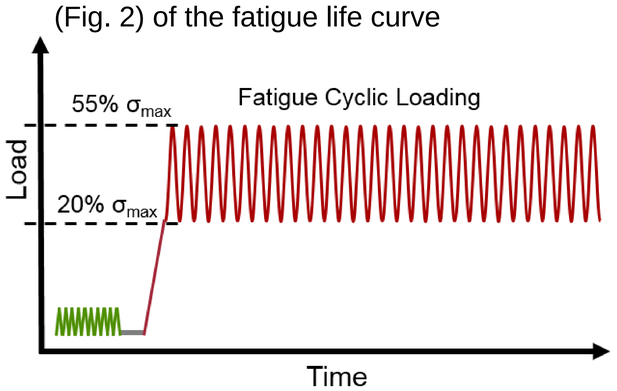

Figure 1. Fatigue mechanical testing protocol, including preconditioning followed by cyclic loading between 20 and 55% of max stress.

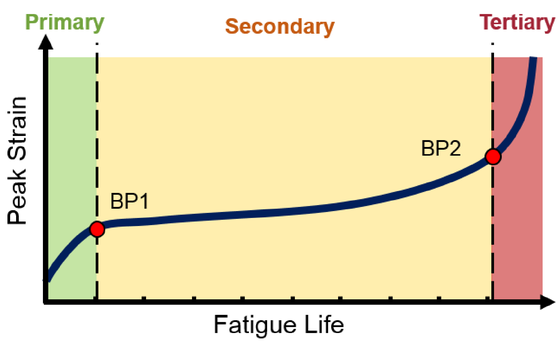

Figure 2. Triphasic fatigue life curve, including BP1 at the end of the primary phase and BP2 at the end of the secondary phase.

- *Statistics*. Two-way ANOVAs with post-hoc Bonferroni tests were used to assess the effects of genotype, injury time-point, and interaction. Significance was set at p≤0.05 and trends at p≤0.1.

Results

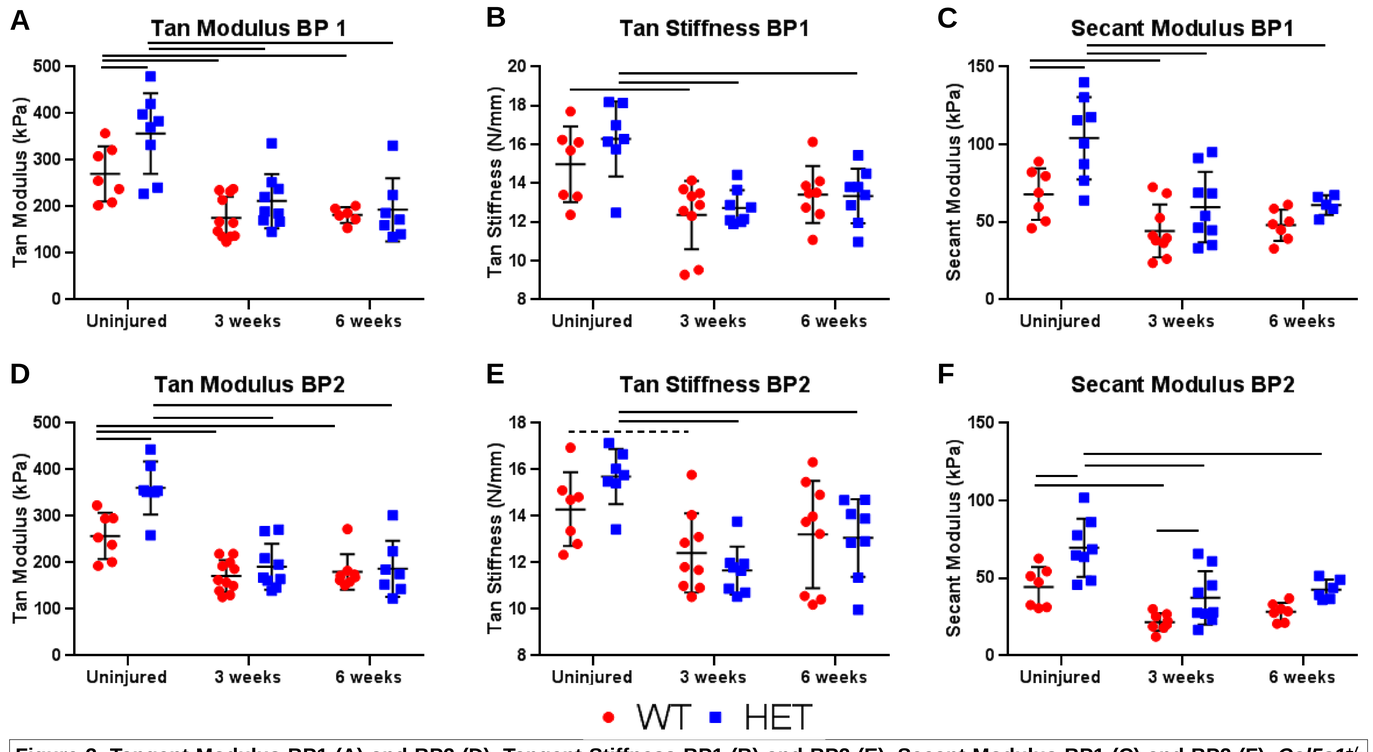

Figure 3. Tangent Modulus BP1 (A) and BP2 (D). Tangent Stiffness BP1 (B) and BP2 (E). Secant Modulus BP1 (C) and BP2 (F). ***Col5a1***$^{+/-}$ tendons had persistent decreases in tangent modulus, tangent stiffness and secant modulus 3 and 6 weeks PI at BP1 and BP2. WT tendon decreases seen 3 weeks PI were only persistent to 6 weeks PI in tangent modulus. Solid lines denote significance and dashed lines denote trends.

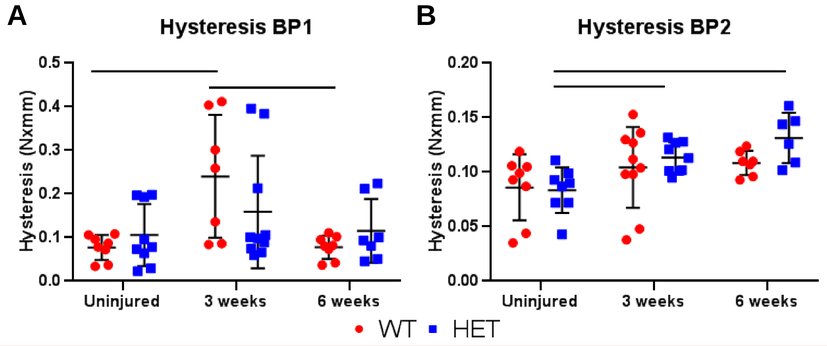

Figure 4. Hysteresis BP1 (A) and BP2 (B). WT tendons had increased hysteresis at BP1 3w PI, while ***Col5a1***$^{+/-}$ tendons had increased hysteresis 3 and 6 weeks PI at BP2. Solid lines denote significance and dashed lines denote trends.

Conclusions

- Properties of ***Col5a1***$^{+/-}$ tendons were persistently affected to a later time-point PI, while the fatigue properties of WT tendons showed minimal differences later in healing
  - Collagen V deficient mice have a delayed healing response, with changes persisting to 6 weeks PI, while WT tendon fatigue properties recover by 6 weeks PI
- Genotypic differences in uninjured tendons indicate that collagen V plays a role in the tendon response to cyclic loading
  - Differences are not consistently present PI, showing that WT and ***Col5a1***$^{+/-}$ tendon fatigue properties are affected to different degrees following injury, and the diminished healing of ***Col5a1***$^{+/-}$ tendons could be obscuring genotypic differences PI
- Hysteresis analysis indicates that energy loss is different throughout fatigue life between WT and ***Col5a1***$^{+/-}$ tendons following injury
  - WT tendons show increased hysteresis at the end of the primary phase, while ***Col5a1***$^{+/-}$ tendons show increased hysteresis at the end of the secondary phase
    - More energy is lost at the end of fatigue life in ***Col5a1***$^{+/-}$ tendons, while the opposite is true for WT tendons
    - Collagen V affects the ability of the tendon to heal in a manner that resists microstructural damage associated with cyclic use

SIGNIFICANCE: WT tendon fatigue properties recover following injury while a decrease in collagen V results in a delayed healing response.

References & Acknowledgments

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