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

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Introduction

Patients with Classic Ehlers-Danlos Syndrome (*c*EDS), a disorder characterized by mutation in the *COL5* genes with *COL5a1* haploinsufficiency being the most common, suffer from articular hypermobility, skin hyperextensibility, tendon/ligament fragility and abnormal wound healing.[1,2] Furthermore, human studies have shown that females have decreased collagen synthesis and fibroblast activity[3-5] as well as altered gene expression during repair,[6] potentially exacerbating detrimental changes present in *c*EDS tendons. Quasi-static loading of the mouse patellar tendon[7-9] demonstrates decreases in modulus, failure stress, failure load, and stiffness due to reduced collagen V throughout healing. Although the hierarchical structure of the tendon has been implicated in changes following cyclic fatigue loading, and collagen V is essential in regulating collagen fibrillogenesis, fatigue properties have not been examined in *c*EDS tendons.[7,10] Therefore, the objective of this study was to define the fatigue properties of female patellar tendons following injury, as well as the effect of a reduction in collagen V on these properties. We hypothesized that reduction in collagen V following injury will delay improvements in the fatigue properties compared to wild-type tendons.

Methods

Adult female wild-type (WT) C57/BL6 and heterozygous *Col5a1*$^{+/-}$ mice, a model for *c*EDS, at 120 days of age (n = 60) were used (IACUC approved). Mice were randomly divided into uninjured and injured groups, with injured mice undergoing bilateral patellar tendon injury surgery as described.[11] Injured mice were sacrificed early in the remodeling healing phase (3w) or later in remodeling (6w) and uninjured age-matched mice were sacrificed.

Mechanics

The patella-patellar tendon-tibia complexes of all mice were dissected and prepared for mechanical testing.[12] Cross-sectional area was measured using a custom laser device.[13] Tendons underwent a fatigue protocol, consisting of pre-conditioning and 1 Hz cyclic loading until failure. Cyclic loads corresponded to 20% and 55% maximum stress (previously determined from quasi-static testing). Fatigue parameters were analyzed at the end of the primary phase (BP1) and secondary phase (BP2) of fatigue life, capturing changes in material parameters that occur with fatigue damage, including peak cyclic strain, tangent modulus, secant modulus, tangent stiffness, secant stiffness, hysteresis, and laxity. Secant modulus and stiffness are calculated in reference to the zero displacement point and tangent modulus and stiffness are calculated from a specific loading cycle.

Statistics

Two-way ANOVAs with post-hoc Bonferroni tests were used to assess the effects of genotype (collagen V expression), injury time-point, and their interaction on fatigue mechanical properties. Significance was set at $p \leq 0.05$ and trends at $p \leq 0.1$.

Results

WT patellar tendons 3w post-injury (PI) showed a significant decrease in tangent modulus (BP1 and BP2) (Fig.1A,B), tangent stiffness (BP1 and BP2 [trend]) (Fig.1C,D), and secant modulus (BP1 and BP2) (not shown) when compared to uninjured controls. The decrease in tangent modulus at BP2 persisted to 6w PI. However, no other parameters had differences at 6w PI. *Col5a1*$^{+/-}$ patellar tendons 3 and 6w PI exhibited reduced tangent modulus (Fig.1A,B), tangent stiffness (Fig.1C,D), and secant modulus at both BP1 and BP2 when compared to uninjured controls. There were no differences in *Col5a1*$^{+/-}$ tendons compared to uninjured tendons in peak strain (Fig.2C) or secant stiffness (not shown) at BP1. However, 3w PI at BP2, there was an increase in peak strain (Fig.2D) and a trending decrease in secant stiffness (not shown), with no differences 6w PI. Hysteresis was significantly higher in WT tendons 3w PI when compared to uninjured and 6w tendons at BP1. However only a trending difference was seen at BP2 between uninjured and 3w PI (Fig.2A,B). *Col5a1*$^{+/-}$ tendons showed no differences in hysteresis at BP1, but had significantly higher hysteresis 3 and 6w PI compared to uninjured tendons at BP2, and a trending increase between 3 and 6w PI (Fig.2A,B). Differences between genotypes, were primarily seen in the uninjured groups, with WT tendons having a significantly lower tangent

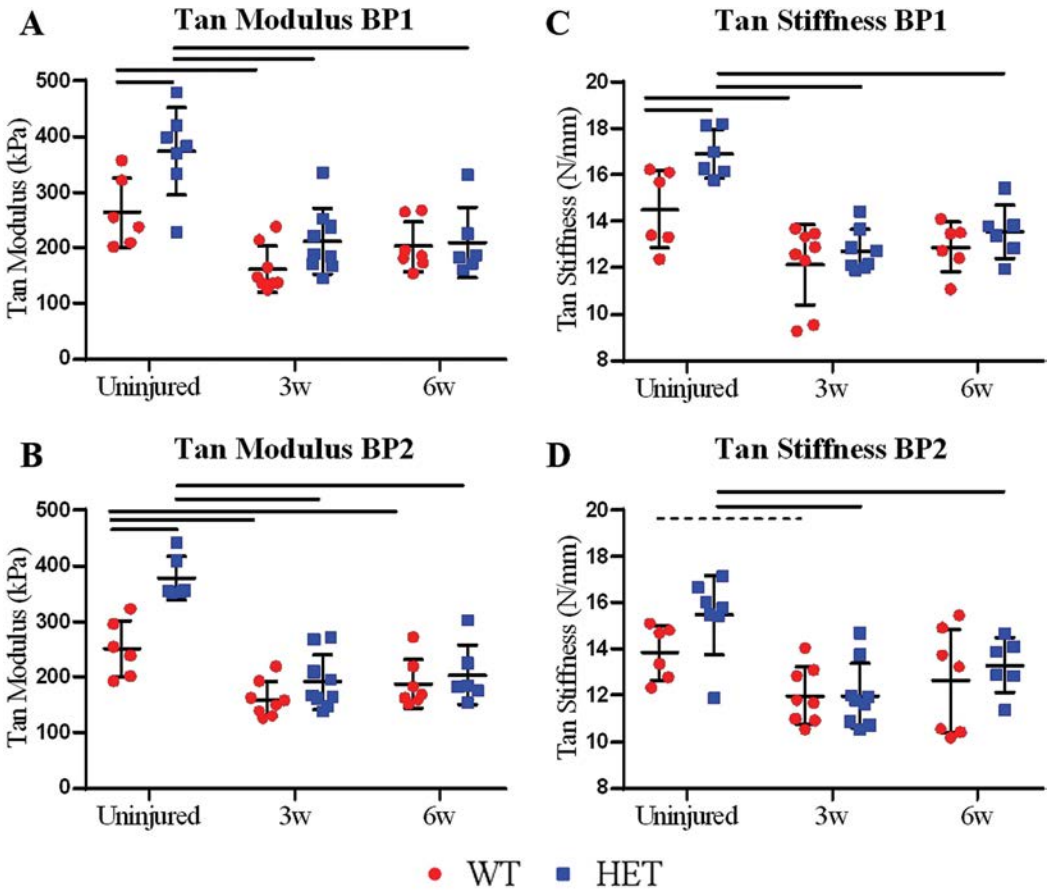

Figure 1. Tangent Modulus BP1 (A) and BP2 (B). Tangent Stiffness BP1 (C) and BP2 (D). *Col5a1*$^{+/-}$ tendons had persistent decreases in tangent modulus and tangent stiffness 3 and 6w PI at BP1 and BP2. WT tendon decreases in tangent modulus and tangent stiffness seen 3w PI were only persistent to 6w PI in tangent modulus at BP2. Solid lines denote significance and dashed lines denote trends.

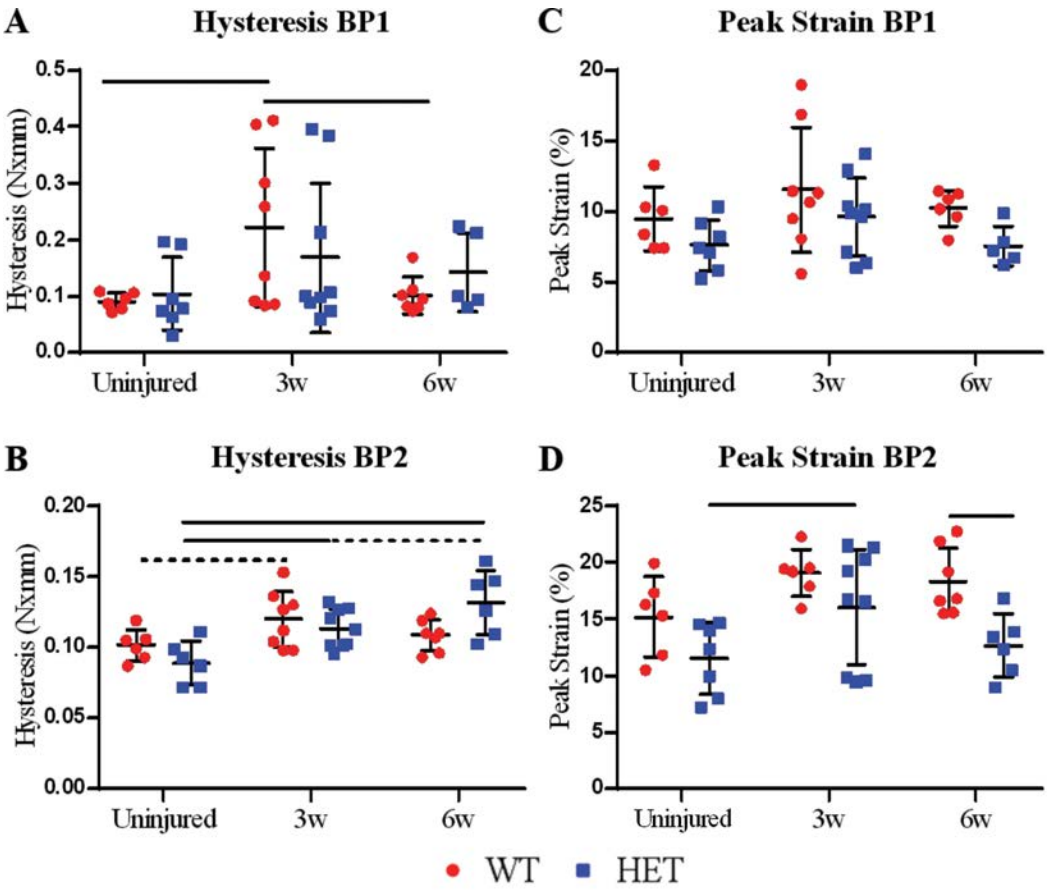

Figure 2. Hysteresis BP1 (A) and BP2 (B). WT tendons had increased hysteresis at BP1 3w PI, while *Col5a1*$^{+/-}$ tendons had increased hysteresis 3 and 6w PI at BP2. **Peak Strain BP1 (C) and BP2 (D).** Peak strain was increased 3w PI in *Col5a1*$^{+/-}$ tendons at BP2. Solid lines denote significance and dashed lines denote trends.

modulus (BP1 and BP2) (Fig.1A,B), tangent stiffness (BP1) (Fig.1C), and secant modulus (BP1 and BP2) (not shown), and a significantly higher laxity (BP1) (not shown). When compared to WT tendons, *Col5a1*$^{+/-}$ tendons at 6w PI had a decreased peak strain (BP2) (Fig.2D) and increased secant stiffness (BP1 [trend] and BP2) (not shown).

Discussion

This study evaluated the fatigue properties of the patellar tendon in uninjured and injured mice as well as the role of collagen V. Cyclic fatigue loading mimics the *in vivo* loading pattern of the patellar tendon, and therefore is a relevant approach to study mechanic properties. Overall, fatigue properties of *Col5a1*$^{+/-}$ tendons were persistently affected to a later time-point post-injury, while the fatigue properties of WT tendons showed minimal differences later in healing. Therefore, as hypothesized, collagen V deficient mice have a delayed healing response, with changes persisting to 6w PI, while WT tendon fatigue properties recover by 6w PI. Additionally, genotypic differences in uninjured tendons indicate that collagen V plays a role in the tendon response to cyclic loading. However, these differences are not consistently present PI. This shows 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 post-injury. Lastly, hysteresis analysis indicates that energy loss is different throughout fatigue life between WT and *Col5a1*$^{+/-}$ tendons following injury, as 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. This indicates that collagen V affects the ability of the tendon to heal in a manner that resists microstructural damage associated with cyclic use. Therefore, this study demonstrates that collagen V plays a role in the tendon's ability to respond to fatigue loading, and following injury, collagen V plays a crucial role in the tendon healing process.

Significance

This study demonstrates that WT tendon fatigue properties recover following injury while a decrease in collagen V results in a delayed healing response, highlighting the importance of evaluating the effect of collagen V in the tendon healing process.

Acknowledgements

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