Gender Dependent Alterations in the Mechanical Response of Injured Collagen V Haploinsufficient Murine Tendons

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Introduction

Classic Ehlers-Danlos syndrome (*c*EDS) is most commonly characterized by haploinsufficiency in *COL5α1* with patients suffering from hyperextensible skin, joint instability and laxity. Collagen V is significantly upregulated following injury[1,2] and abnormal wound healing is associated with a diminished expression of collagen V[1,3]. Furthermore, estrogen in females is suggested to be associated with decreased collagen synthesis[4,5] and altered gene expression during repair[6]. However, the way gender-specific differences influence the healing response after injury and in the presence of collagen V deficiency has not yet been investigated. Therefore, the objectives of this study were to evaluate the mechanical response of injured patellar tendons over time in normal and *c*EDS mice, as well as differences between genders. We hypothesized that gender related differences in collagen V expression would result in an abnormal wound matrix in the injury response, contributing to the abnormal wound phenotype resulting in reduced mechanical properties of injured EDS tendons compared to normal tendons. In addition, the injury response in the female *c*EDS tendons will be inferior to that of male tendons due to these gender influences, resulting in greater mechanical properties of male tendons when compared to females.

Methods

Adult male and female wild-type (WT) C57/BL6 and heterozygous (HET) ***Col5a1***$^{+/-}$ *c*EDS mice (n = 120) at 120 days of age were used (IACUC approved). All mice underwent bilateral patellar tendon injury surgery as described[7] and were sacrificed 3 weeks or 6 weeks post-injury.

Mechanics

The patella-patellar tendon-tibia complexes of all mice were dissected and prepared for mechanical testing[8]. Cross-sectional area was measured using a custom laser device[9]. Tendons were subjected to a viscoelastic testing protocol[8,10] consisting of: 1) preconditioning, 2) stress relaxation at strain levels of 2%, 3% and 4%, 3) a sinusoidal frequency sweep (10 cycles at 0.1, 1, 5, and 10 Hz) at each strain level, 4) return to gauge length, and 5) ramp to failure. Tendon length was measured at nominal load prior to test initiation.

Statistics

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

Results

Quasi-static

WT male tendons had higher failure stress at 3w and 6w post-injury (PI), tissue modulus at 6w, and a trending increase in tissue modulus at 3w when compared to WT female tendons (Figure 1A,B). WT male tendons also had higher failure loads and failure stiffness at 3w and 6w PI (Figure 1C,D). HET males had higher failure stress and failure loads at 3w and 6w PI, and higher stiffness at 3w PI when compared to HET females (Figure 1B,C,D).

Viscoelastic

WT male tendons, when compared to WT female tendons 3w PI, had reduced dynamic moduli at 2% strain (.1 Hz and 1 Hz), with a trend towards a decrease at 2% strain, 10 Hz and 3% strain, 0.1 Hz, however no differences in HET mice between genders were seen at 3w PI (Figure 2A,B). No WT differences in dynamic modulus were seen between genders at 6w PI (Figure 2C,D). Additionally, WT males had a higher tan(δ) at 2% strain, .1 Hz at 3w and 6w PI when compared to WT females (data not shown). HET male and female tendons showed trending differences in dynamic modulus at 6w PI at lower frequencies at 3% and 4% strain (Figure 2D, only 3% shown). The viscoelastic response of HET male tendons showed an increase in tan(δ) across all strains and frequencies (excluding 4%, 1 Hz and 4%, 10 Hz) 6w PI when compared to HET females (Figure 3B, only 3% shown). Additionally, no differences were seen between genders or genotypes in cross sectional area (data not shown).

Discussion

WT and HET male injured patellar tendons demonstrated higher material and structural

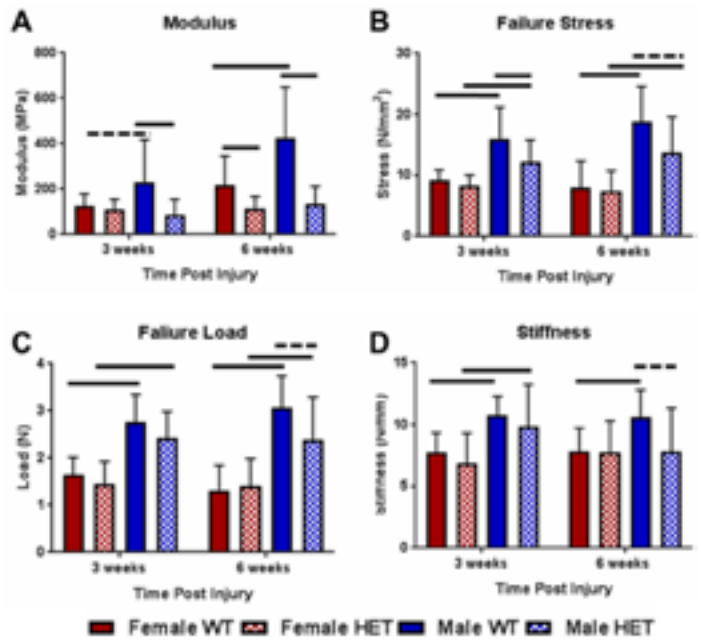

Figure 1. Material and structural and properties of female and male WT and HET patellar tendons. WT and HET male patellar tendons had increased moduli **(A)**, failure stress **(B)**, failure loads **(C)** and had increased stiffness **(D)** compared to female tendons.

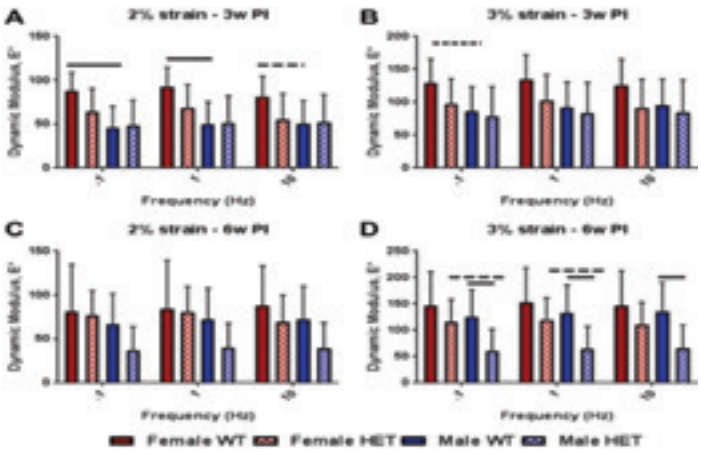

Figure 2. Dynamic moduli of female and male WT and HET patellar tendons. WT female patellar tendon dynamic moduli are increased across all frequencies at 2% strain, 3w PI **(A)**, with no differences seen at 6w PI **(C)**. Trending differences between genders were seen at lower frequencies at 3% strain, 6w PI **(D)**.

properties compared to WT and HET female injured tendons, respectively, at both time points following injury. Reduction in collagen V had a greater effect on male tendon material response than female tendon response, which is consistent with previous findings in uninjured tendons[11]. When examining the viscoelastic response, although dynamic modulus was decreased in WT male tendons compared to WT female tendons 3w PI, these differences did not persist to 6w PI. Additionally, there were no gender differences in viscoelastic properties of HET tendons 3w PI, however, gender differences in these properties were seen in HET tendons 6w PI. This could be explained by a diminished late healing response in HET females compared to males, obscuring the increased effect of reduced collagen V on male tendons versus female tendons at

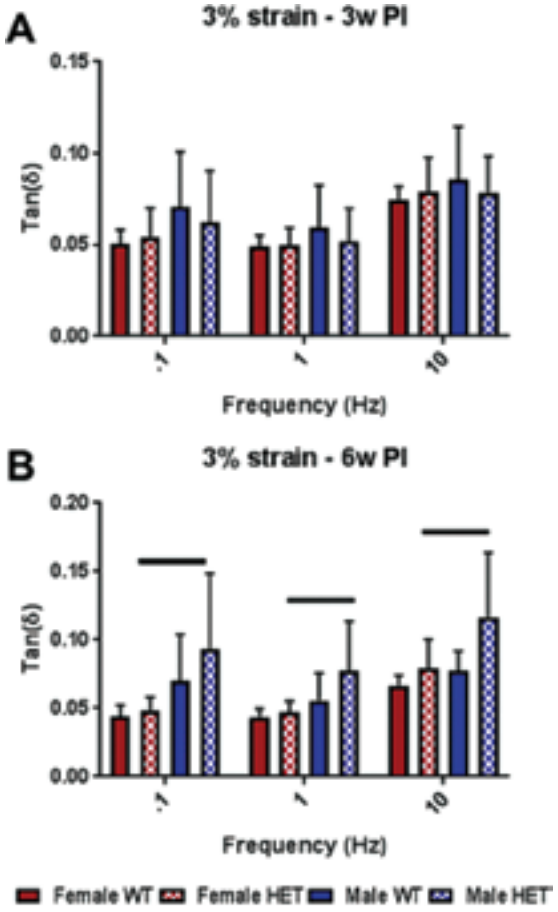

Figure 3. Tan(δ) of female and male WT and HET patellar tendons. HET male mice showed no differences in tanδ) from HET female mice at 3w PI **(A)** but had increased tan(δ) measurements across all frequencies at 3% strain, 6w PI **(B)**.

the 6w time point. Male patellar tendon properties are more dependent on collagen V than female tendon properties and the reduction of collagen V affects the healing response of male and female tendons in differing capacities. Future work may include further characterizing the healing response through histological analysis to understand cellular differences that could explain these mechanical differences.

This study demonstrates that gender-specific effects play an explicit role in tendon injury and healing and can influence the degree to which tendon properties of *c*EDS mice are affected.

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