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

Jaclyn Carlson
Snehal Shetye, PhD[1]
Ashley Rodriguez[1]
Jessica Johnston[1]
Mei Sun[2]
Sheila Adams[2]
David Birk, PhD[2]
Louis Soslowsky, PhD[1]

[1]McKay Orthopaedic Research Laboratory
University of Pennsylvania

[2]University of South Florida
Tampa, FL

Introduction

Classic Ehlers-Danlos syndrome (EDS) patients, who commonly have mutations in the *COL5A1* gene, suffer from connective tissue hyperelasticity, joint instability, and skin hyperextensibility. The role of collagen V in fibrillogenesis in tissues such as skin, cornea, and tendon has been firmly established [1], wherein collagen V haploinsufficiency leads to abnormal tissue development and altered collagen assembly. Recent basic science studies suggest that differences in hormone physiology between sexes may be a factor influencing tendon health [2-4]. Given the joint laxity and tissue hyperelasticity in classic EDS patients, gender-specific changes in hormone levels, the different hormones produced, and their effect on body structure and composition, may further exacerbate the detrimental changes present in pathological tendons. Therefore, the objective of this study was to eva luate the role of gender in the mechanical response of normal and *classic* EDS tendons. We hypothesized that female *classic* EDS mice will have inferior tendon mechanical properties compared to male *classic* EDS mice, but there will only be differences in structural properties due to gender in wild type tendons.

Methods

Adult male and female WT C57/BL6 and HET ***Col5a1***$^{+/-}$ EDS mice (n=60) at 150 days of age were used (IACUC approved). Uninjured patellar tendons were assessed to determine mechanical properties for both genders and genotypes. *Mechanics*. The patella-patellar tendon-tibia complexes were dissected and prepared for mechanical testing [5]. Tendons were subjected to a viscoelastic testing protocol [5,6] 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, gender, and their interaction on mechanical properties. Two-way repeated measures ANOVAs with post-hoc Tukey tests were used to assess the changes in viscoelastic properties. Significance was set at $p \leq 0.05$ and trends at $p \leq 0.1$.

Results

WT male patellar tendons had significantly higher failure load and tissue stiffness when compared with WT females (Fig. 1A,B). WT male tendons also had significantly higher failure stress and tissue modulus when compared with WT females (Fig. 2A,B). The viscoelastic response followed similarly with WT males exhibiting significantly elevated dynamic modulus at 10Hz and across all strains when compared to WT females and trending increases at 0.1 Hz and 1 Hz (Fig. 3, only 3% data shown). HET male tendons had a significantly higher failure load when compared with HET females, with no difference observed in tissue stiffness (Fig 1A,B). WT males had significantly higher failure stress and a trending increase in modulus when compared with HET females (Fig 2A,B). HET male and female mice showed trending differences at 2% strain with frequencies of 0.1

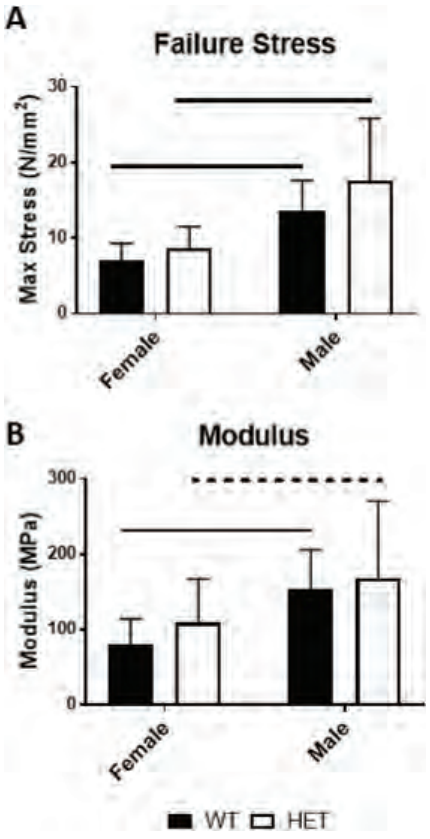

Figure 1. Structural properties of female and male WT and HET Col5a1$^{+/-}$ patellar tendons. WT male patellar tendons failed at higher loads **(A)** and had increased stiffness **(B)**. Solid lines denote significant $p \leq 0.05$ and dashed lines denote trends at $p \leq 0.1$.

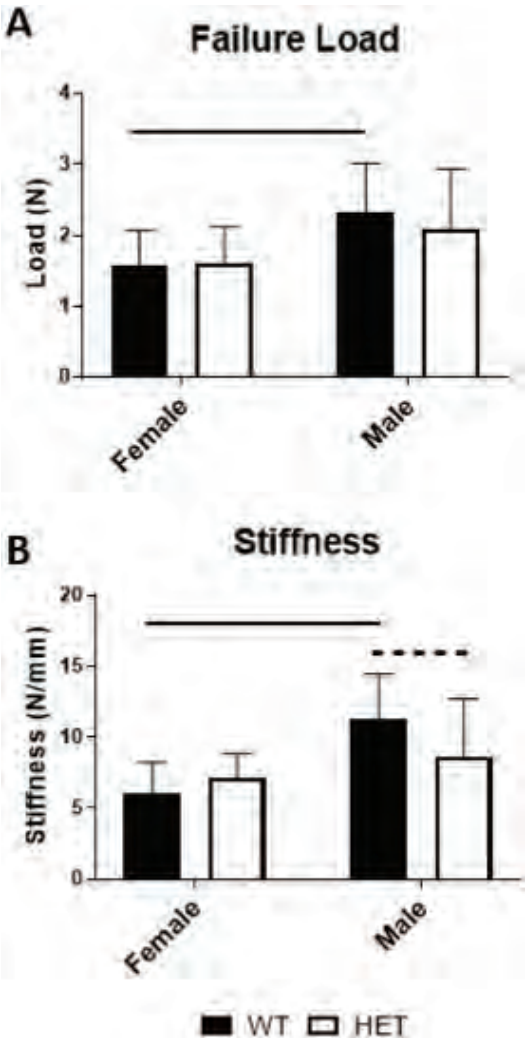

Figure 2. Material properties of female and male WT and HET Col5a1$^{+/-}$ patellar tendons. WT and HET male patellar tendons failed at higher stress **(A)** and WT male patellar tendons had an increased modulus **(B)**. Solid lines denote significant $p \leq 0.05$ and dashed lines denote trends at $p \leq 0.1$.

and 1 Hz, and at 4% strain, 1 Hz (data not shown). Male HET tendons trended towards a decrease in stiffness compared to WT tendons, with no other difference between genotypes. Male WT and HET tendons had a significantly larger area than female mice of the same genotypes (data not shown).

Discussion

WT male patellar tendons demonstrate superior material and structural properties compared to WT female patellar tendons. As female patellar tendons were significantly smaller than male patellar tendons, it is not surprising that their properties would be decreased. Conversely, it is surprising that although HET male patellar tendons were significantly larger in cross-sectional area than female patellar tendons, the same differences in structural properties seen in WT tendons were not present. This contrasting finding indicates that the structural properties of HET male tendons were affected by the reduction of type V collagen to a greater degree than the structural properties of HET female tendons, after

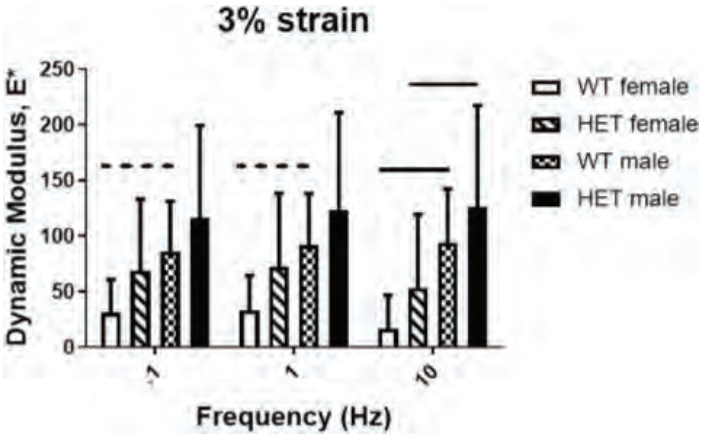

Figure 3. Viscoelastic properties of female and male WT and HET Col5a1$^{+/-}$ patellar tendons. At a frequency of 10 Hz and strain of 3%, WT and HET male patellar tendons dynamic moduli were significantly increased when compared to WT and HET female patellar tendons. Similar findings were seen at 2% and 4% strains across all frequencies.

considering the inherent gender-differences. We hypothesized a negative effect of female sex hormones on patellar tendon health, yet our results indicate the opposite. This finding is extremely interesting as previous studies have reported an influence of sex hormones on tendon health [2-4], without a clear determination of how hormones would affect tendon function. Furthermore, although HET ***Col5a1***$^{+/-}$ EDS mice have a 50% reduction in fibril number [1], this did not result in significant differences between HET and WT material, structural or viscoelastic properties. However, a trend toward a significant difference in stiffness between male WT and HET mice supports our conclusion that a reduction in type V collagen content affects male tendon mechanical properties to a greater degree than female properties. This indicates a gender specificity of the effects of collagen V on patellar tendon mechanical properties. Future work may include histological analysis of HET male and female patellar tendons to understand the cellular differences that could explain these mechanical differences.

Significance

This study demonstrates that sex hormones and gender-specific effects of hormone expression play a tendon-specific role in tendon health, and can influence the degree to which tendon properties of *classic* EDS mice are affected.

Acknowledgements

This study was supported by AR065995, AR044745 and the Penn Center for Musculoskeletal Disorders (P30 AR069619).

References

1. Wenstrup RJ, *et al.* *J Biol Chem.* 279:53331-7, 2004.
2. Bridgeman JT, *et al.* *Foot Ankle Int.* 31:1081-4, 2010.
3. Liu SH, *et al.* *J Orthop Res.* 14:526:33, 1996.
4. Romani WA, *et al.* *Eur J Appl Physiol.* 113:2503-10, 2013.
5. Dunkman AA, *et al.* *Matrix Biol.* 32:3-13. 2013.
6. Miller KS, *et al.* *J Biomech Eng.* 134:031007. 2012