Sex Differences Are Present in Uninjured Achilles Tendon and Muscle

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Introduction

Achilles tendon ruptures are common and devastating injuries. Although men and women both experience Achilles tendon ruptures at similar ages and sport participation times, it is surprising that 84% of all ruptures occur in men.[1] Differences in hormone physiology between sexes may influence Achilles tendon and gastrocnemius muscle homeostasis and injury risk.[2,3] However, it is unknown whether the primary effects of hormone differences act specifically on tendon or on its origin muscle.[4,5] Therefore, the objective of this study was to determine mechanical, structural and histological properties of uninjured Achilles tendon and muscle in female, ovariectomized (OVX) female, and male rats. We hypothesized that female and OVX rats would exhibit equal tendon mechanical, structural, and histological properties but decreased muscle fiber size compared to male rats.

Methods

Experimental design and sample preparation: Achilles tendon-foot complexes from 54 age-matched adult male (n = 16), female (n = 16), and OVX (6-weeks after OVX) (n = 16) Sprague Dawley rats were harvested (IACUC approved). Tendons were then either fixed for histological analysis or frozen until preparation for high-frequency ultrasound (HFUS) analysis and mechanical testing as described.[6] All tendons were randomized to blind a single dissector at time of fine dissection and subsequent testing and analysis. Additionally, gastrocnemius muscle tissue was excised, embedded in optimal cutting temperature (OCT) compound, and flash frozen for histological analysis. *Tendon histology:* Samples (n = 8/group) were processed using standard techniques, sectioned sagitally at 7 μm, and stained with hematoxylin & eosin or safranin-o/fast green. Images were graded on a scale of 1-3 by three blinded investigators for cell shape, cellularity, and proteoglycan staining intensity. *Muscle histology:* Samples (n = 8/group) were sectioned axially at 10μm, co-stained with DAPI and laminin, imaged, and analyzed for average fiber cross sectional area (CSA) using the SMASH application.[7] *HFUS:* Tendons (n = 12/group) were loaded at 1N in a PBS bath while a series of sagittal images were acquired using a 40MHz scanner (Vevo 2100, MS550D; VisualSonics) and analyzed for fiber alignment. *Mechanical testing:* Tendons (n = 8/group) were measured for CSA and underwent a ramp to failure at 0.1%/sec. Tendons used for HFUS were subjected to a separate mechanical testing protocol consisting of stress relaxation at 6% strain, a low-load dynamic frequency sweep (ranging from 0.1 to 10 Hz), and fatigue testing at 2 Hz using a sinusoidal waveform (from ~10-40% of ultimate strength) until failure. From these tests, quasi-static mechanical properties (toe and linear modulus, and percent relaxation) and dynamic mechanical properties (modulus, tan(δ), hysteresis, and laxity) were computed. *Statistics:* One-way ANOVAs were used to compare between groups for all assays except tendon histology. Significant relationships were evaluated using post-hoc tests with Bonferroni corrections ($\alpha = 0.05/3$). Kruskal-Wallis tests were used to evaluate differences in tendon histological properties between groups ($\alpha = 0.05$).

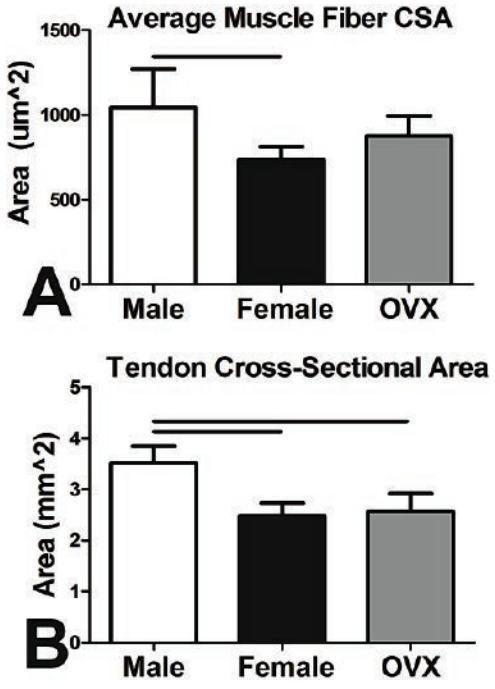

Figure 1. (A) Achilles muscle fiber area and (B) tendon area were both increased in males. Solid lines indicate significant differences ($p<0.017$). Data presented as mean and standard deviation.

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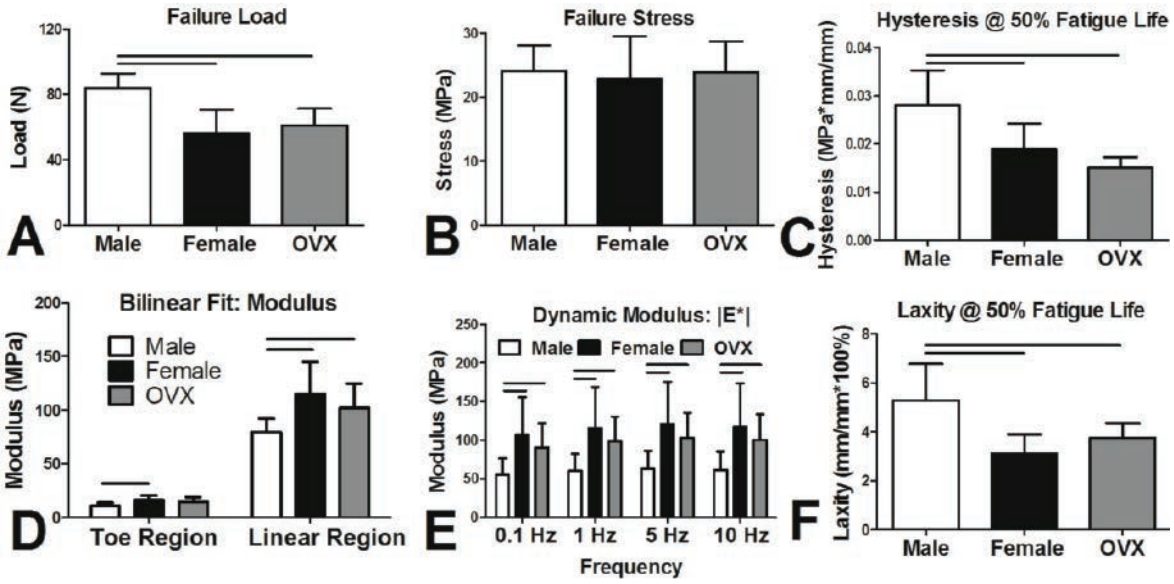

Figure 2. Achilles tendon mechanical properties. (A) Failure load was increased in males, (B) failure stress was not different between groups, (C) hysteresis was increased in males, (D) toe and linear modulus were both increased in females and OVX, (E) dynamic modulus was increased in females and OVX at 0.1, 1, 5, and 10 Hz, and (F) laxity was increased in males. Solid lines indicate significant differences ($p<0.017$). Data presented as mean and standard deviation.

Results

Tendon histology: There were no differences between groups in cell shape, cellularity, or proteoglycan content (not shown). *Muscle histology:* The average fiber CSA (Figure 1A) was significantly increased in male compared to female muscle samples. There were no differences between OVX muscle samples and the other two groups. *HFUS:* Circular standard deviation of the mean fiber orientation (a measure of collagen alignment) did not differ between groups (not shown). *Mechanical testing:* Tendon CSA (Figure 1B) and failure load (Figure 2A) were significantly greater in male tendons compared to female and OVX tendons. Failure stress (Figure 2B) was not different between groups. Percent relaxation (not shown) and hysteresis (Figure 2C) were significantly increased in male compared to female and OVX tendons but there were no differences in tan(δ) between groups (not shown). Toe and linear stiffness, as well as transition strain, were not different between groups (not shown). Conversely, toe modulus was significantly decreased in male compared to female tendons and nearly significant compared to OVX tendons ($p = 0.02$) (Figure 2D). Similarly, the linear modulus was significantly decreased in male compared to female and OVX tendons. Dynamic modulus (Figure 2E) was significantly decreased in male tendons at all frequencies tested and during fatigue testing (not shown), Tendon laxity (Figure 2F) was significantly increased in males.

Discussion

Contrary to our hypothesis, sex differences were found in both uninjured Achilles tendon and muscle. Female and OVX tendons exhibited increased moduli (quasi-static and dynamic) compared to male tendons, which suggests that female and OVX tendons have superior material properties. Further, the decreased percent relaxation and hysteresis in female tendons suggest that they may be more efficient at returning stored elastic energy following deformation. Taken together with the decreased muscle fiber size compared to males, these mechanical differences may, in part, explain the apparent decreased susceptibility to tendon rupture that is observed clinically in women despite their lower failure load. Interestingly, there may be a dose-dependent response with respect to estrogen levels for various mechanical and histological properties (e.g., muscle fiber CSA, tendon modulus, laxity), given that mean values for OVX rats generally fall between those of males and females. Further investigation is required to elucidate the mechanism governing the superior mechanical properties observed in female tendons, especially given that several previous studies implicate estrogen as an inhibitor of collagen production.[5] Future work will study the effect of tendon matrix composition and analysis of protein markers for muscle strength and fiber type, and investigate sex differences in the context of tendon healing.

Significance

This study identified inferior tendon mechanical properties and increased muscle fiber size as a potential explanation for the increased susceptibility for Achilles tendon damage observed clinically in men compared to women.

Acknowledgements

This study was supported by NIH/NIAMS (R01AR064216S1), the NIH/NIAMS supported Penn Center for Musculoskeletal Disorders (P30 AR050950), the NIH/NCATS (TL1TR000138), and the NSF GRFP. The authors thank D. Choi and C. Riggin for their contributions.

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