INTRODUCTION:
Despite the well-documented sex-bias in non-contact human ACL injury rates [1], surprisingly little comprehensive analysis has been performed examining the establishment of sex-based differences in tendon and ligament properties during growth. The primary objective of this study was to test the hypothesis that animal sex has a significant effect on murine tendon composition and material properties by examining animals from a broad range of ages between weaning and young adulthood. A secondary objective was to assess whether such differences, if present during growth, are ubiquitous or, instead, depend on anatomical location.

METHODS:
10 male and 10 female C57/BL6J mice at each of 4, 6, 9, 12, and 15 weeks of age were sacrificed via CO2 inhalation in accordance with IACUC guidelines. Right Achilles tendons were digested in papain solution and processed for compositional analysis of DNA, glycosaminoglycan (GAG), and hydroxyproline (OHP, indicative of total collagen content). Left Achilles tendons were tested in tension to failure at a strain rate of 100%/sec to determine structural and material properties. One tail tendon fascicle bundle was used for compositional analysis. For material property determination, 5 individual tendon fascicles were randomly chosen by alternating between the three remaining tendon bundles of the tail and tested to failure in tension at a strain rate of 50%/sec in PBS, with average values used for each mouse. Complete methods for compositional analysis and material property determination can be found in [2] for Achilles tendons, and [3] for tail tendons. All dependent parameters were analyzed statistically with a deterministic cutoff value of p < 0.05 was used for statistical significance.

RESULTS:
In Achilles tendons, animal sex significantly affected GAG/DNA [Figure 1], with female levels ranging from 10-30% less than that of males. Collagen content (as indicated by OHP/DNA) was also significantly affected by sex, with female OHP/DNA ranging from 30-45% less than that of males after 4 weeks of age [Figure 2]. Despite these substantial differences in composition, females exhibited comparable material strength and material stiffness values when compared to males [Figures 3 & 4]. Sex did not have a significant effect on tendon failure mode (i.e. mid-substance vs. avulsion). Female Achilles tendons did, however, have a significantly higher failure strain than males, which was associated with significantly greater energy absorption per unit volume (data not shown).

In conclusion, our results demonstrate that animal sex does indeed have a significant effect on murine tendon composition, but not on material strength or stiffness, during growth. These differences depend on anatomical location in a way that may relate to mechanical loading environment, although further investigation is required to address this question.

REFERENCES:

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ACHILLES TENDON DATA

TAIL TENDON DATA