INTRODUCTION:
The growth/differentiation factors, GDFs 5, 6, and 7 in particular, appear to play a role in tendon and ligament biology and may have the potential to be used clinically to augment tendon/ligament repair in humans [1]. Mice deficient in GDF5 have altered tendon composition and impaired mechanical behavior [2, 3]. GDF7 deficient mice have a subtle tendon phenotype, although there is some evidence that this may be due to overcompensation by the related family member, GDF5 [4, 5]. Preliminary evidence suggests a tendon phenotype in 4-week-old GDF6 deficient mice as well, but this strain has been challenging to work with due to high postnatal mortality rates, particularly in females [6]. We have recently improved the longevity of the GDF6 mouse line by out-crossing onto a mixed background strain, thus enabling a more comprehensive baseline characterization of the effect of GDF6 deficiency in tendon. The objective of the present study was thus to establish the tendon phenotype in mice deficient in GDF6 by examining the composition and material properties of male and female GDF6 --/-- and +/- Achilles and tail tendons at 4 and 16 weeks of age.

METHODS:
Experimental animals consisted of 4 and 16 week old male and female mice deficient in GDF6 (--/--) and their wild type (+/+) littermates (n=10 per group). Due to high postnatal mortality rates in --/-- animals from the original line [7], mice were out crossed to increase colony vigor, resulting in a mixed 129SV/J x C57BL6/J x CBA background. Animals were sacrificed via CO2 inhalation in accordance with IACUC guidelines. Compositional analysis and tensile mechanical testing were performed on Achilles tendons and tail tendon fascicles. Right Achilles tendons were digested in papain solution and processed for compositional analysis of DNA, glycosaminoglycan (GAG), and hydroxyproline (Hypro, indicative of total collagen). Left Achilles tendons were tested in tension to failure at a strain rate of 100%/sec to determine structural and material properties. From the tail, one tendon fascicle bundle was used for compositional analysis (GAG:DNA; Hypro:DNA). For material property determination, 5 individual tendon fascicles were randomly chosen by alternating between the 3 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 can be found in [4] for Achilles tendons, and [5] for tail tendons. All dependent variables were analyzed separately for males and females using a two-factor ANOVA within each single sex cohort with genotype (--/-- and +/-) and age (4, 16 wks) as the two independent variables. A cutoff value of p < 0.05 was used for statistical significance. The 4-week-old +/- female Achilles tendon mechanical testing data were rendered unreliable by a load cell malfunction, and were not included in the analysis.

RESULTS:
GDF6 deficiency resulted in lower animal body mass and smaller Achilles tendons, with this size effect being more pronounced in males than in females. Male GDF6 --/-- tail tendons had significantly less GAG:DNA and Hypro:DNA compared to +/- littermates, while among female mice, genotype did not have a statistically significant effect on tail tendon composition (Fig. 1 & 2). In association with lower compositional parameters, male GDF6 --/-- tail tendons had significantly lower levels of tissue material strength, modulus of elasticity (material stiffness), failure strain, post-yield strain, and strain energy density (Table 1). For female tail tendons, genotype did not have a significant effect on any material property that was measured (data not shown).

In Achilles tendons, the effect of genotype on composition was similar to what was seen in the tail tendon site (Fig. 3 & 4). In males, GDF6 --/-- Achilles tendons had less Hypro:DNA and GAG:DNA (compared to +/-), but the difference in GAG:DNA was not statistically significant. Compositional differences were not large enough to have a significant effect on male Achilles tendon material properties, however (Table 2). Genotype did not have a significant effect on female Achilles tendon composition (Fig. 4). Based on the 16-week-old data, genotype had no significant effect on female Achilles tendon material properties (data not shown).

DISCUSSION:
This study in male and female mice at 4 and 16 weeks of age demonstrates for the first time that animal sex modulates the effect of GDF6 deficiency on murine tendon. Male (but not female) tendons exhibit lower levels of collagen and glycosaminoglycan compared to wild type littermates. Compositional differences in male GDF6 --/-- and +/- tendons were large enough to have an effect on tissue level material properties in the tail, but not in the Achilles site. No compositional or material property effects of GDF6 deficiency were seen in female tendons at either site. These data support the growing body of evidence, which suggests that GDFs 5, 6, and 7 play a role in tendon.

REFERENCES:

ACKNOWLEDGEMENTS:
This work was funded in part by NIH R01AR049745.