

# Functional Changes to Achilles Tendons in a Mouse Model of an Adolescent Masculine Gender-Affirming Hormone Treatment

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## DISCLOSURES: None

**INTRODUCTION:** Gender differences exist in tendon and ligament injury risk have been associated with changes in sex steroid levels and are prominent during puberty (1). Tendons express estrogen receptors (2), and estrogen plays a critical role in collagen synthesis in tendon. For example, estrogen deficiency in rats following ovariectomy can lead to ~30% reduction in collagen content in Achilles tendon (3). Testosterone contributes to increased tendon stiffness in men by increasing collagen turnover and content (1). Furthermore, testosterone may indirectly reduce tendon and ligament laxity by downregulating the expression of relaxin receptors, a modulator for joint elasticity (4, 5). While these studies show a role of sex steroids in mature tendon adaptation, the impact of sex steroids on tendon function during pubertal growth has not been explored. Gonadotropin release hormone (GnRH) is released by the hypothalamus, stimulating the release of pituitary gonadotropins to activate the production of estrogen and testosterone. Inhibition of the hypothalamo-pituitary gonadal (HPG)-axis leads to hypoestrogenia and, when combined with testosterone treatment, can mimic current clinical treatment for gender-affirming therapy in adolescents. We have recently reported the effects of transmasculine gender affirming hormone treatment on skeletal growth in which puberty suppression results in longer femurs and testosterone treatment results in shorter femurs in female-born mice. In this study, we examined how tendon function is influenced by GnRH agonist (GnRHa) treatment, which inhibits the HPG-axis, with and without testosterone during peripubertal growth in female born mice.

**METHODS:** All work was approved by IACUC. C57BL/6N female mice (N=23) were randomly assigned to one of four experimental groups and experiments were started at postnatal age 26 (P26). The study design is illustrated in Figure 1A. Groups included sham surgery (control; n=7), GnRHa-only treatment (GnRHa, n=6), Testosterone-only treatment (Testosterone, T; n=5), and GnRHa + T treatment (GnRHa+T; n=5). Mice in the GnRHa and GnRHa + post-T groups were subcutaneously implanted with a GnRHa (3.6mg) depot in silastic tubing at P26. Control and post-T groups underwent a sham surgery at the same time point. At 3-weeks post-implantation of GnRHa or sham surgery, mice received implants with testosterone (10 mg) or vehicle (for GnRHa and control groups), and mice were euthanized at P89. Untreated male mice (N=3) were also euthanized at P89. Mice were weighed and carcasses were stored frozen at -20C until testing. Achilles tendons were thawed, dissected, and imaged using photogrammetry (to measure cross-sectional area, CSA). Tendon-bone (calcaneal) attachments were kept intact, and muscle was removed prior to uniaxial biomechanical testing in a temperature-controlled saline bath (0.1mm/min; Biomomentum, Mach-1 v500c). Material properties were determined from load-displacement data using R (v4.2.2). Stiffness was determined using the piecewise linear segmentation by dynamic reprogramming recursion package (dpseg). Mechanical properties were determined by converting load-displacement to engineering stress-strain using the CSA and initial gauge length. Mouse weights were compared using a one-way ANOVA. CSA and biomechanical data were compared using two-way ANOVAs (puberty suppression; testosterone treatment) with multiple comparisons (Tukey's multiple comparisons tests; Prism v10; Graphpad, CA).

**RESULTS:** All groups of female-born mice had similar mass and weighed significantly less than age-matched male mice. Treatment with testosterone significantly influenced variation in max load and stiffness, and treatment with GnRHa also significantly influenced variation in max load (Figure 1B). We also identified a significant interaction between GnRHa and testosterone treatment related to max load. No significant differences in CSA were found between female-born groups. However, when accounting for changes in the CSA of tendons, we did not find any significant differences between groups related to material properties (stress, tangent modulus).

**DISCUSSION:** In this study, we identified how pubertal suppression with and without testosterone can influence tendon properties in mice. Alone, pubertal suppression using GnRHa did not influence overall weight for female-born mice, however the ability of tendon to carry load before failure was significantly increased. Treatment with testosterone led to increased variation in tendon size, and tendons were most similar to age-matched male mice. Both testosterone and delayed puberty, together or by themselves, can induce changes in tendon function which may be related to collagen organization and crosslinking, which will be explored further at the microstructural level using histology. These changes in tendon function may be driven by changes in skeletal muscle strength and size, however we did not explore this in the current study. Future work will identify if and how GnRHa and testosterone together and alone can influence functional outcomes of connective tissue during key phases of peripubertal growth and sexual maturation.

**CLINICAL RELEVANCE:** Therapy in transmasculine youth currently consists of puberty suppressors (e.g., GnRHa) and androgen treatment (e.g., testosterone) (7). It is important to understand the potential effects of pubertal suppression on functional properties of musculoskeletal tissues, like tendon, to provide guidance on training and injury recovery for adolescent and young adult transgender patients.

**REFERENCES:** 1. Hansen and Kjaer, *Advances in Experimental Medicine and Biology*, 2016; 2. Leblanc et al. *J Steroid Biochem Mol Biol*. 2017; 3. Ramos et al. *Scan J Med Sci Sports* 2012; 4. Miller et al. *J Appl Phys* 2007; 6. Dehghan et al. *Int. J. Mol. Sci* 2014; 7. Salas-Humara *Curr Probl Ped Adol Health Care* 2021.

**ACKNOWLEDGEMENTS:** National Science Foundation GRFP (LAH), NSF CAREER (MLK), NIHR01AR079367 (MLK)

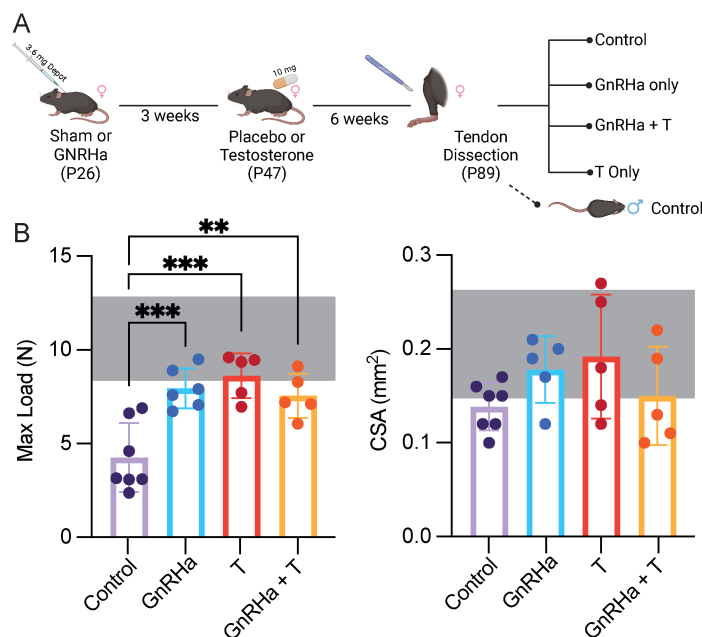


Figure 1. (A) Study design to assess gender-affirming hormone therapy on tendon biomechanical properties. (B) All treatments resulted in increased strength (max load, N) compared to age-matched control mice. Testosterone alone (T) resulted in biomechanical properties similar to age-matched male mice (range shown in gray shaded region). No significant differences were found in CSA between groups. Data are biological replicates. \*\* p<0.01; \*\*\* p<0.001.