Tendon Extracellular Matrix Remodeling is Dependent on Sex Hormone Signaling

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INTRODUCTION: Remodeling of the extracellular matrix (ECM) is required for the proper healing, strengthening, and maintenance of tendon. Thus, it is important to investigate the factors which regulate the delicate balance of synthesis and breakdown. There are well documented sex differences in tendon injury rates[1,2] and healing outcomes,[2,3] but the influence of sex on ECM remodeling is not well understood. Sex differences are often attributed to innate differences in tissue and signaling[4] or to sex hormones,[5] including estrogen and progesterone, but these factors are rarely decoupled. The objective of our study is to separate the variables of sex and hormones to determine their impacts on tendon ECM remodeling and how they contribute to the sex differences presented in literature. We hypothesized that innate sex differences will be present where males will have greater ECM protein content, synthesis rates, cellularity, and metabolism compared to females. Furthermore, we expected the addition of estrogen and progesterone to increase protein synthesis, content, and cellularity in both sexes.

METHODS: Flexor digitorum longus (FDL) tendon explants were harvested from young adult (4 month) male and female C57B/6J mice. The explants were cultured in stress-deprived conditions for one week in either no hormone (89% DMEM + 10% FBS + 1% PSA, 'NH'), estrogen-supplemented (1nM 17β-estradiol, 'E'), or progesterone-supplemented (1nM progesterone, 'P') medium. On the last day of culture, ^{35}S-sulfate and ^{3}H-proline were added for 24 hours to measure the synthesis of sulfated glycosaminoglycans (sGAG) and ECM proteins, respectively. Explant metabolism was measured by a resazurin reduction assay. Wet and dry weights were then taken for each explant before digesting with Proteinase K overnight. The sample digests were then assessed for cellularity (PicoGreen assay), GAG content (DMMB assay), and total collagen (OHP assay). Protein synthesis, sGAG synthesis, cellularity, collagen content, and GAG content were normalized to dry weights. Explant metabolism was normalized to day 0 readings from explants of the same sex and hormone treatment. Statistical evaluation was performed through two-way ANOVAs with Tukey's multiple comparisons tests where appropriate. Significance is reported at $p<0.05$.

RESULTS: There were no differences with sex or hormones in sGAG synthesis (Fig. 1A). Comparing the NH conditions, males had increased protein synthesis, increased GAG content, and decreased collagen content compared to females (Fig. 1B-D). In the aspect of tendon health, male tendons had higher explant metabolism than females in the absence of hormones (Fig. 2A). The addition of hormones in males only had impacts on cellularity and explant metabolism, where cellularity decreased with both hormone conditions and metabolism decreased with estrogen (Fig. 2). In females, the addition of estrogen resulted in increased protein synthesis, increased GAG content, and decreased collagen content (Fig. 1B-D) and the addition of progesterone led to an increase in protein synthesis and decreases in both collagen content and cellularity (Fig. 1B, D & Fig. 2B).

DISCUSSION: In support of previous work, we found innate sex differences in most measured biosynthetic parameters. Contrary to our hypothesis, the addition of hormones led to sex-dependent changes in the tendon explants. This is an important finding as many studies in the literature use male models to assess the impact of female sex hormones on tendon.[6–8] However, our results suggest that the influence of estrogen and progesterone is not comparable across sex in tendon tissue. When adding estrogen to female tissues, we see more similarities to the male tissue than to the female NH condition (Fig. 3). This indicates that estrogen may compensate for or reverse some of the innate sex differences we saw in tendon. Progesterone seems to play a substantial role in female collagen synthesis, increasing levels above that of males. This may be responsible for the decrease in connective tissue injuries in females during the luteal phase of the cycle, when progesterone is high.[9] The decrease in both tendon health measures with estrogen in males may indicate decreased tendon viability in response to hormone addition. This could be responsible for the lack of response to estrogen. Additionally, the decreased cellularity of both male and female tendons in response to progesterone warrants further study to determine the impact of the hormones on culture conditions. These data support the strong influence of sex and hormone signaling on ECM synthesis. However, the breakdown of the proteins is not represented, and is the focus of ongoing studies. In addition, these studies were performed under stress deprivation, and therefore future work will focus on the interplay between mechanical loading and hormones as stimuli for ECM remodeling.

SIGNIFICANCE/CLINICAL RELEVANCE: This work will inform research and clinical practice in the treatment of tendon injury, especially for those who experience changes in hormones due to cycling or hormone therapies.

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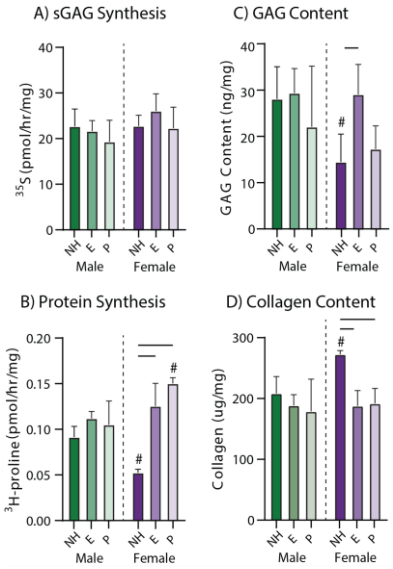

Figure 1. Male and female explant protein synthesis and content results at 7 days stress deprivation with either no hormone (NH), estrogen supplemented (E), or progesterone supplemented (P) culture medium. Data is presented as mean +95% CI, the lines indicate statistical significance within sex, and # notate sex differences at $p<0.05$.

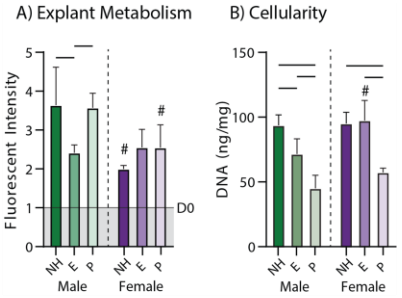

Figure 2. Explant health results at 7 days stress deprivation with either no hormone (NH), estrogen supplemented (E), or progesterone supplemented (P) culture medium for male and female tendons. Data is presented as mean +95% CI, where lines indicate within sex comparisons, and # indicate between sex comparisons, both at $p<0.05$.

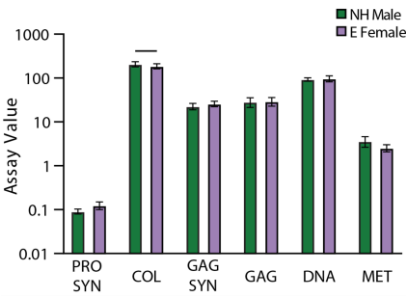

Figure 3. Comparison between male explants with no hormone and female explants with estrogen. Data is presented as mean ±95% CI, where lines indicate $p<0.05$.