



Female Rat Supraspinatus Tendon Mechanical Properties Exhibit a Differential Response to Estrogen-Deficiency Depending on Reproductive History

Ashley K. Fung
Snehal S. Shetye
Chantal M. de Bakker
Yihan Li
X. Sherry Liu
Louis J. Soslowsky

McKay Orthopaedic Research Laboratory
University of Pennsylvania

Introduction

The prevalence of rotator cuff tears increases with age and postmenopausal women are at an even greater risk of developing full-thickness supraspinatus tendon tears¹. Previous animal studies have supported these findings, demonstrating that an ovariectomy model of menopause led to reduced bone mineral density of the humeral head and decreased rotator cuff tendon strength^{2,3}. Interestingly, recent preliminary data showed that prior to ovariectomy, reproductive female rats have inferior supraspinatus tendon and proximal humerus trabecular bone properties compared to virgin females; however, when subjected to ovariectomy, they experience a reduced rate of bone loss in the tibia compared to virgin females⁴. While these findings suggest a differential response in bone to estrogen-deficiency depending on reproductive history, how supraspinatus tendons are affected is unknown. Therefore, the objective of this study was to investigate the effect of reproductive history on female rat supraspinatus tendon mechanical properties following ovariectomy. We hypothesized that due to a dramatic decrease in estrogen levels and a high rate of early bone loss, ovariectomy will lead to a reduction in supraspinatus tendon mechanical properties.

Methods

58 female Sprague-Dawley rats (IACUC approved) were divided into virgin and reproductive groups. At age 4 months, reproductive rats underwent three cycles of reproduction, each consisting of a 3-week pregnancy, 3 weeks of lactation, and 3-6 weeks of post-weaning recovery. Virgin and reproductive rats underwent ovariectomy (OVX) surgery at 12 months of age and were sacrificed at 1 month (n = 8/group), 3 months (n = 8/group) or 6 months (n = 5-7/group) post-OVX. Non-OVX, intact 13.5-month old virgin and reproductive female rats (n = 7/group) were used as controls.

Mechanics

Supraspinatus tendons were fine dissected and marked with stain lines for optical strain

tracking. Cross-sectional area was measured using a custom laser device and humeri were secured in polymethyl methacrylate. Right supraspinatus tendons underwent quasi-static tensile testing, consisting of pre-conditioning (10 cycles from 0.5-1% strain), stress relaxation at 5% strain for 600s, dynamic frequency sweep at 5% strain (0.1-10Hz), and ramp to failure at rate of 0.3%/s.

Statistics

Two-way ANOVAs were used to compare the effects of reproductive history and time after OVX with post-hoc Bonferroni corrections. Significance was set at $p \leq 0.05$ and trends at $p \leq 0.1$.

Results

Cross-sectional area increased significantly in the reproductive group and trended towards increasing in the virgin group 1 month post-OVX (Figure 1A). By 6 months post-OVX, however, cross-sectional area was not different from control in either group. While stiffness trended towards increasing in the 3 month OVX reproductive group (Figure 1B), there were no changes in modulus in the reproductive groups. In contrast, modulus was significantly higher in the virgin non-OVX, control group compared to reproductive and significantly decreased in the virgin group at 1 and 3 months post-OVX (Figure 3C). At 6 months post-OVX, modulus in the virgin group increased compared to 1 month post-OVX and was significantly higher than the reproductive group. A similar trend was observed in the virgin group for dynamic modulus across all frequencies, where dynamic modulus was significantly lower at 1 month post-OVX and trended towards decreasing in the 3 month and 6 month groups, compared to control (Figure 1E). In the reproductive group, however, significant viscoelastic differences were observed at 1 month post-OVX. There was a significant increase in percent relaxation at 1 month post-OVX compared to control (Figure 1D), while dynamic modulus across all frequencies was decreased at 1 month compared to control, 3 month, and 6 month post-OVX groups (Figure 1E). $\tan(\delta)$

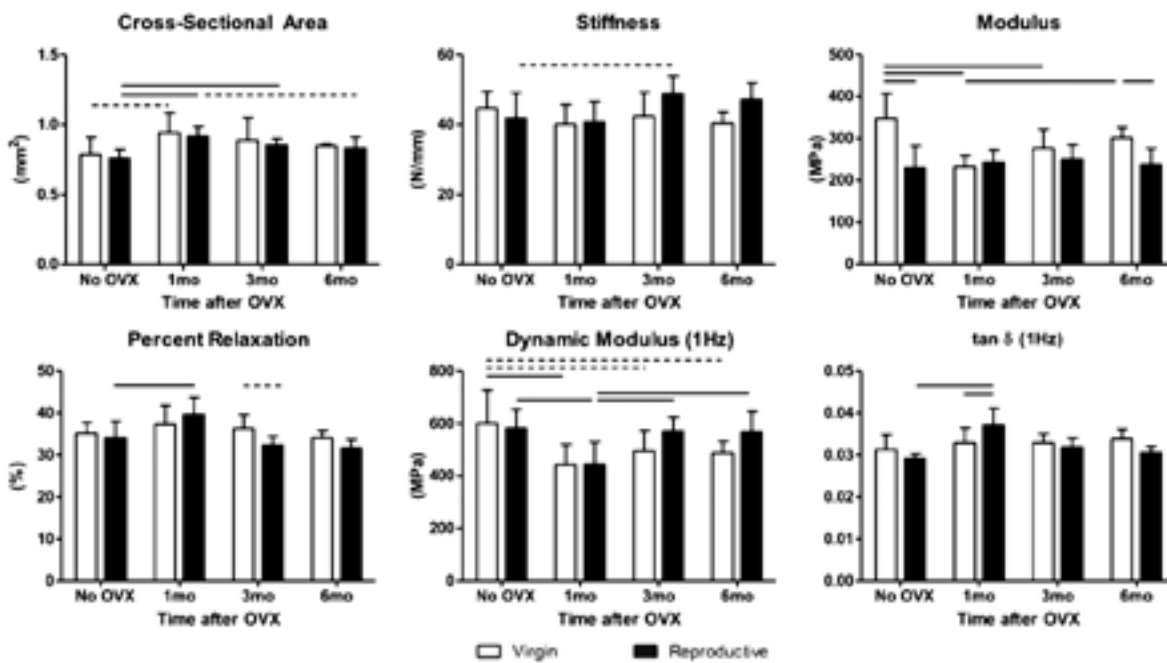


Figure 1: (A) Cross sectional area increased in the reproductive group at 1 month post-OVX but was not different from control by 6 months post-OVX. (B) Stiffness in the reproductive group trended towards increasing 3 months post-OVX, (C) but there were no differences in modulus in the reproductive rats. However, modulus significantly decreased in the virgin group at 1 and 3 months post-OVX. At 1 month post-OVX, the reproductive group exhibited (D) increased stress relaxation (E) decreased dynamic modulus and (F) increased $\tan(\delta)$. Solid lines denote significance for $p \leq 0.05$ and dashed lines for trends $p \leq 0.1$.

increased at 1 month post-OVX and was significantly higher than the virgin group at this time point (Figure 1F) with no differences at 3 months and 6 months.

Discussion

This study investigated the effect of reproductive history on tendon properties following ovariectomy. Higher modulus in the virgin control group compared to the reproductive group was consistent with recent preliminary data for rats that had undergone 2 cycles of reproduction. Modulus had a significant interaction effect, where the virgin group experienced a reduction but the reproductive group exhibited no differences in response to OVX. Previous studies showed that rotator cuff tendon strength decreases with ovariectomy^{2,3} and attributed these results to reduced structural integrity of the mineralized fibrocartilaginous insertion site. Other *in vitro* studies also found that estrogen plays a role in collagen synthesis and maintaining tissue elasticity^{5,6}. Our findings suggest that, similar to bone, supraspinatus tendons of virgin and reproductive rats respond differently to estrogen deficiency. Interestingly, recent studies in bone uncovered several adaptation mechanisms, including redistribution of bone mass toward load-bearing compartments and increased bone mechano-sensitivity in

reproductive rats, which may account for the protective effect of reproductive history on bone when subjected to estrogen deficiency⁷. Decreased viscoelastic parameters at 1 month post-OVX but recovery by 6 months in reproductive tendons suggest that additional tendon adaptation mechanisms developed during the course of reproduction may have altered the tendon response to estrogen deficiency later in life. Further studies are necessary to explore the mechanisms behind tendon adaptations in these models.

This study highlights the importance of considering reproductive history during the diagnosis and treatment of rotator cuff injuries in post-menopausal women, particularly in the early stages of menopause where tendon properties were observed to change substantially.

References

1. Abate et al, 2014, *Menopause*.
2. Cadet et al, 2010, *J Orthop Res*.
3. Chen et al, 2015, *PLoS One*.
4. de Bakker et al, 2018, *J Bone Miner Res*.
5. Torricelli et al, 2013, *Age*.
6. Maman et al, 2016, *Connect Tissue Res*.
7. Li et al, 2018, *ORS Meeting*, 0695.