Reproduction and Lactation Lead to Long-Term Changes in Supraspinatus Tendon and Humeral Trabecular Bone Properties in a Rat Model

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Introduction

Physiological changes due to pregnancy increase the risk of developing musculoskeletal complications such as shoulder, lower back, and knee pain[1]. Altered levels of estrogen and relaxin during pregnancy increase knee joint and ligament laxity, compromising joint function, and these changes persist years after pregnancy[2]. Further, tibial and lumbar vertebral trabecular bone structure in reproductive female rats has been shown to be inferior compared to virgins but not different when compared to male, suggesting that reproduction and lactation induce bone loss that is not fully recovered post-weaning[3]. However, the long-term effects of reproduction on tendons and bones of the shoulder have not yet been studied. Therefore, the objective of this study was to evaluate the supraspinatus tendon mechanical response and humeral trabecular bone properties of male, virgin female, and reproductive female rats. We hypothesized that reproduction and lactation would induce long-term changes leading to inferior supraspinatus tendon properties and humeral trabecular bone microstructure in reproductive females as compared to virgin females.

Methods

20 Sprague-Dawley rats (IACUC approved) across three groups were used in this study: male (n = 9), virgin female (n = 6) and reproductive female (n = 5). At age 6 months, reproductive female rats underwent two reproductive cycles, each consisting of a 3-week pregnancy, 3 weeks of lactation, and 6 weeks of post-weaning recovery. Rats were sacrificed at 12-14 months of age, and shoulders were harvested for supraspinatus tendon mechanical testing and trabecular bone analysis. *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, stress relaxation at a 5% strain hold for 600s, a dynamic frequency sweep at 5% strain (0.1-10Hz), and ramp to failure at rate of 0.3%/s. Left supraspinatus tendons underwent fatigue testing, consisting of pre-conditioning and fatigue loading until failure at 2Hz between loads corresponding to 7% and 40% maximum stress, as determined from quasi-static testing. Fatigue parameters, including peak cyclic strain, secant modulus, tangent modulus, hysteresis, and laxity, were recorded at two breakpoints marking the ends of the primary (BP1) and secondary (BP2) phases of a triphasic fatigue life curve. *Trabecular bone analysis:* Left proximal humeri were scanned using μCT (10.5μm, μCT35, Scanco Medical). A 100-slice volume of interest proximal to the humeral growth plate was identified for trabecular bone microstructure analysis. *Statistics:* Comparisons across groups were made using one-way ANOVAs with Bonferroni post-hoc corrections. Significance was set at $p \leq 0.05$ and trends at $p \leq 0.1$.

Results

Male tendons exhibited significantly higher stiffness compared to virgin and reproductive female tendons (Figure 1A). However, reproductive females had significantly lower

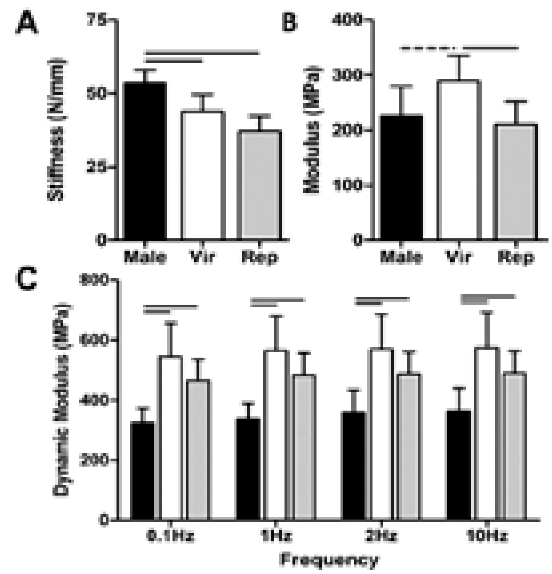

Figure 1. (A) Males had higher stiffness, while virgin females had increased (B) linear modulus and (C) dynamic modulus. Solid lines denote significance at $p \leq 0.05$ and dashed lines denote trends at $p \leq 0.1$.

modulus compared to virgin females but no difference compared to males (Figure 1B). Males had significantly lower dynamic modulus for all frequencies compared to both female groups (Figure 1C) but no difference in percent relaxation or tan(δ) (not shown). For fatigue properties at BP1, virgin females had significantly higher tangent and secant modulus compared to males and trended towards increasing compared to reproductive females (Figure 2A). However, there were no differences in secant or tangent modulus at BP2. No differences in hysteresis were observed at BP1, but reproductive females had significantly increased hysteresis compared to males at BP2 (Figure 2B). Cycles to failure and peak cyclic strain at BP1 was significantly higher in males compared to both female groups, and there were no differences in laxity at either breakpoint (not shown). Additionally, trabecular bone analysis revealed reduced bone volume fraction (BV/TV) and trabecular number (Tb.N) in reproductive females compared to virgin females but no difference compared to males (Figure 3A,B). Trabecular separation (Tb.Sp) in reproductive females was significantly increased compared to virgin females but trended towards a decrease when compared to males (Figure 3C). While trabecular thickness (Tb.Th) was significantly higher in males, there was no difference between female groups (not shown).

Discussion

This study identified substantial differences in supraspinatus tendon and proximal humerus trabecular bone properties based on sex and reproductive history. Proximal humerus bone microstructure was superior in virgin females, consistent with previous findings in the tibia and vertebra. Previous research has linked ovariectomy to decreased failure stress of rotator cuff tendons and a less pronounced tidemark at the enthesis[4,5], and a similar mechanism may govern irrecoverable reproductive bone loss. Fatigue results also indicate that virgin and reproductive females experience a greater reduction in moduli and capacity to store energy, respectively, and together, these results suggest that females, regardless of reproductive history, may be more susceptible to early tendon degeneration. During pregnancy, hormonal fluctuations induce increased pelvic ligament laxity in preparation for parturition. Though the mechanisms are still unclear, several clinical studies have found sustained biomechanical changes in these ligaments despite a return to pre-pregnancy hormone levels[6,7]. Therefore, supraspinatus tendons following reproduction may be synergistically influenced by a direct effect of hormone changes and an indirect effect of bone loss near the insertion site. Shoulder pain after pregnancy has been associated with frequent breastfeeding that places added stress on the upper extremities. However, these findings suggest that biological changes during reproduction may inherently increase the risk for rotator cuff injury. Future studies will explore transient changes during pregnancy and investigate the mechanisms underlying long-term changes in tendon and bone properties following reproduction.

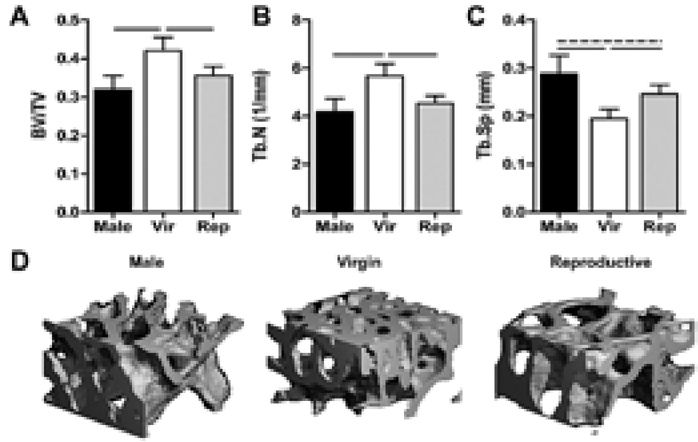

Figure 3. Virgin rats exhibited (A) higher bone volume fraction, (B) higher trabecular number, and (C) lower trabecular spacing compared to male and reproductive rats. Solid lines denote significance at $p \leq 0.05$ and dashed lines denote trends at $p \leq 0.1$. (D) Representative 3D humeral trabecular bone images.

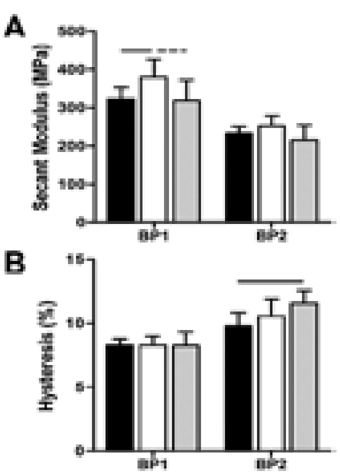

Figure 2. (A) Secant modulus was higher in the virgin group at BP1 and (B) reproductive females had increased hysteresis compared to males at BP2.

Significance

This study identifies long-term changes in supraspinatus tendon and humeral trabecular bone properties that result following pregnancy and lactation, highlighting the importance of considering reproductive history in the diagnosis and treatment of shoulder injuries.

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