

**Introduction:** Rotator cuff tears are prevalent musculoskeletal disorders which most commonly occur clinically following a period of overuse-induced or chronic tendinopathy. The typical laboratory model of rotator cuff repair, however, is based on an acute surgical detachment of an otherwise normal tendon. To best mimic the clinical scenario of an “acute-on-chronic” supraspinatus tendon tear, we have recently utilized a rat model [1] of supraspinatus tendon overuse [2] which induces a tendinopathic condition to precede surgical creation of a tendon tear [3]. However, it is unclear whether this added model complexity is advantageous or even necessary when evaluating the healing response of a repaired tendon. Therefore, the objective of this study was to evaluate the mechanical and histological properties of supraspinatus tendon healing following tendon detachment and repair in a normal tendon or in a tendon subjected to overuse to induce a tendinopathic state prior to surgical injury. We hypothesized that overuse activity prior to surgical detachment and repair would not alter the healing supraspinatus tendon mechanical or histological properties.  

**Methods:** Experimental design: Twenty-three adult male Sprague-Dawley rats (400-450g) were used in this IACUC approved study. Rats in the overuse group (n=9) were subjected to a two week training period followed by four weeks of downhill overuse treadmill activity (17m/min, 1hr/day, 5days/wk, 10° decline) to induce a tendinopathic condition [2], while control rats (n=14) were allowed normal cage activity [1]. All animals were then subjected to bilateral supraspinatus detachment and repair surgery [4]. Following surgery, animals from both groups were allowed cage activity until sacrifice 4 weeks later. The left limbs of each animal were used for mechanical testing while the right limbs were used for histological analysis. Mechanical testing: Supraspinatus tendons were dissected from the shoulder and cleaned of excess soft tissue. Stain lines were placed along the length of the tendon for optical strain measurement. Cross sectional area was measured using a custom laser device. Tendons were then subjected to a mechanical testing protocol consisting of a preload to 0.08 N, ten cycles of preconditioning (0.1-0.5 N at 1% strain/s), a stress relaxation to 5% strain (5%/s) followed by a 600s hold, and a ramp to failure at 0.3%/s. Stress was calculated as force divided by cross sectional area and 2D Langrangian strain was determined optically using custom tracking software. Histology: A subset of muscle-tendon-bone units (n=4 per group) were dissected, processed, paraffin embedded, and sectioned at 7μm using standard histological techniques. Sections were stained with hematoxylin and eosin (H&E) to assess cell shape and cellularity and alcian blue and picrosirius red (ABPR) to assess collagen fiber organization. The insertion site and midsubstance of the tendon were each evaluated.
separately. Cell shape and cellularity were graded by three blinded investigators using a semi-quantitative method. Fiber organization was quantified by measuring the circular standard deviation of collagen fibers from images taken with a polarizing microscope and analysis with custom software, as described previously [4]. Statistical Analysis: Mechanical testing comparisons were made between the two groups using student’s t-tests with significance set at p≤0.05. Histological comparisons were made using Mann-Whitney U tests with significance set at p≤0.05.

**Results:** No statistical differences were found between groups in cross sectional area (Fig. 1A), modulus (not shown), maximum stress (not shown), stiffness (Fig. 1B, or maximum load (Fig. 1C). The overuse group had significantly greater percent relaxation (though only by 5%) compared to the control group (p=0.04, Fig. 1D). In addition, no statistical differences were observed in cell shape and cellularity (Fig. 2) and circular standard deviation (Fig. 3).

**Discussion:** Overall, there were no differences in any of the elastic mechanical properties between the healing tendinopathic and normal tendons following injury and repair surgery. Percent relaxation, a measure of viscoelasticity, was slightly increased following repair of a tendinopathic tendon. This slight but statistically significant increase is consistent with previous findings in response to overuse [2], suggesting that these changes were not masked by the repair. However, no differences were observed between overuse and control groups in the primary study parameters which would directly affect clinical outcomes in this setting, specifically, maximum load and stiffness. We hypothesize that the biological effects of the surgical injury overshadow the relatively milder changes typically evoked by overuse. Because the clinically relevant parameters were not altered in the overuse group, we conclude that when examining healing tendons 4 weeks after a repair, the acute injury model is satisfactory and the more complex tendinopathic model is unnecessary in this rat supraspinatus repair model. Future studies could evaluate early differences in these two models to assess potential biologic differences during the early stages of rotator cuff healing.

**Significance:** This study helps to define an appropriate rat model for assessing healing of the supraspinatus tendon after injury.
Fig. 1 (A-D) Mechanical testing results: (A) No difference was found in cross sectional area. (B) No difference was found in stiffness. (C) No difference was found in max load. (D) Percent relaxation was significantly greater (though only 5%) in the overuse group.

Fig. 2 No differences were observed in cellularity and cell shape.
Circular Standard Deviation

Fig. 3 No differences were observed in collagen fiber organization.

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