Effect of Immobilization and Immediate Mechanical Load on Healing of the Tendon-to-Bone Interface in a Rat Model

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INTRODUCTION:
Joint motion is often prescribed following surgery involving repair of tendon to bone, such as rotator cuff repair. It is known that mechanical stimulus is essential for optimal tendon biomechanical properties. However, the effect of relative tendon-bone motion is currently poorly understood. We hypothesize that excessive tendon-bone motion applied in the immediate post-operative period causes sustained inflammation due to repetitive micro-injury at the healing interface, preventing or delaying tendon-to-bone healing. The goal of this study is to examine how an immediate post-operative applied load affects tendon healing to the surface of a bone.

METHODS:
After obtaining approval from our Institutional Animal Care and Use Committee, we used a validated Sprague Dawley rat patellar tendon model of tendon-to-bone surface healing (Figure 1) to compare immobilization to immediate loading of a tendon-to-bone repair site.

The patellar tendon was detached and repaired in 37 rats. An external fixator was used to immobilize the knees. The rats were then divided into an immobilization group (N=20) and an immediate load group (N=17). Each group was further divided into a 2 week or 4 week time point. In the loaded group, we controlled the load history of the tendon-bone interface using a custom loading apparatus. The animal was lightly anesthetized and then we applied 50 cycles of 3N axial tensile load at 0.17 Hz load to the healing repair site.

Biomechanical testing, micro CT, and histology were done on all groups. Independent samples t-tests were used to analyze the differences in the three variables between the loaded group and the non-loaded group. This was completed for the animals at both 2-weeks and again for the animals at 4-weeks. Paired samples t-test was then used to assess the change in the variables between 2-weeks and 4-weeks. This was done for both the load group and the non-load group.

RESULTS:
At 2 weeks, the average load to failure of the immobilized group (N=8) was 28.4 ± 6.1 N and of the immediate load group (N=9) was 25.5 ± 10.1 N (p=0.5). At 4 weeks, the average load to failure of the immobilized group (N=12) was 52.4 ± 7.8 N, and of the immediate load group (N=8) was 46.6 ± 8.6 (p=0.1). There was no significant difference in load to failure at either 2 or 4 weeks between the immobilized and loaded groups. However, there was a significant increase in load to failure between 2 and 4 weeks for both the immobilized (p <0.001) and the immediate load (p =0.001) group suggesting a healing enthesis with increasing biomechanical strength.

Micro CT analysis evaluating new bone formation can be reported as a ratio of the bone volume formed over the total volume of the bone, marrow and porous space at the tendon-to-bone healing site. At 2 weeks, the average ratio of new bone formation for the immobilized group (N=2) was 0.3 and for the loaded group (N=1) was 0.4. While the sample size does not allow for statistical analysis, the numbers do suggest an increase in bone formation over time in both groups.

At 2 weeks, specimens stained with safranin O show relative grayscale brightness for the immobilized group (N=2) to be 35.2, and for the loaded group (N=2) to be 43.2. At 4 weeks, the relative grayscale brightness for the immobilized group (N=3) was 51.8, and for the loaded group was 38.2. While sample size limitations prevent statistical analysis here as well, our results suggest that collagen fiber continuity can continue to improve over time in immobilized animals. Such improvements in collagen fiber continuity may contribute to the increased tendon attachment strength over time.

DISCUSSION:
This data shows that, in this tendon-to-bone healing model, there is no difference in load to failure strength at 2 or 4 weeks between interfaces which are immobilized or immediately loaded. Although the data is limited by small sample sizes at this point, several points worthy of further analysis are noted. Immediate loading appears to produce relatively more new bone formation, more proteoglycan content, and more collagen continuity than the immobilized group in the early post-operative period up to 2 weeks. However, the new bone formation and proteoglycan content appear to be no different by 4 weeks. Of note, collagen fiber continuity appears to improve over time even in the immobilized group. It is possible that excessive or prolonged loading adversely affects the healing interface, possibly by causing partial microscopic failure. Interestingly, new bone formation increases over time in both the presence and absence of loading, which may explain why both groups have equivalent loads to failure at 4 weeks.

Limitations of this study include the small sample sizes of the groups evaluated by micro CT and histology. Further analyses using this model are ongoing in our laboratory.

REFERENCES:
2. Rodeo et al. AJSM 2006