

Effects of a Low Protein Diet on Tendon Healing in a Mouse Patellar Tendon Injury Model

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Introduction: Protein malnutrition affects millions of individuals worldwide. In developed countries, it particularly affects the elderly, a group commonly presenting with orthopaedic complaints related to tendon and ligament injuries. While protein malnutrition has been shown to have detrimental effects on bone and dermal wound healing following injury [1,2,3], the effect on tendon or ligament healing has not been studied. Therefore, the objective of this study was to examine the effects of a protein-deficient diet on tendon healing in an established mouse model of patellar tendon injury. We hypothesized that there would be decreased tendon biomechanical properties reflective of a diminished healing response in animals subjected to a low protein diet compared to normal.

Materials and Methods: Twenty-six C57BL/6 mice at ten weeks of age were used (IACUC approved). Mice were randomly assigned to two diet groups. Eighteen mice were provided a low protein diet (6% protein) and the other eight mice received a normal protein diet (23% protein). Diets were isocaloric and animals were allowed to consume *ad libitum*. Mice were weighed at the beginning of the study and weekly thereafter.

After five weeks on the assigned diet, bilateral patellar tendon injuries were created as described [4]. Briefly, an incision was made adjacent to each tendon. A rubber backing was placed underneath the tendon to provide support, and a 0.75 mm diameter biopsy punch was used to create a full thickness, partial width (~60%) central tendon defect. Skin incisions were sutured, and mice were allowed unrestricted cage activity. Following surgery, nine mice on the 6% protein diet remained on the low protein diet (Low Protein group) while the other nine were switched to the 23% protein diet (Reassigned group). The eight mice on the normal protein diet pre-operatively remained on the same diet after surgery (Normal Protein group). All mice were sacrificed six weeks post-operatively. One tendon from each animal was selected randomly for biomechanical testing.

For biomechanical testing, patellar tendons were dissected from the selected limb of each mouse, leaving the patella-tendon-tibia complex intact. Tendon cross-sectional area was measured using a custom laser device [5]. The tendon was then stamped into a dumbbell shape and cross-sectional area was again measured for use in calculation of material properties. The tibia was potted in PMMA and placed in a custom fixture. Specimens were submerged in a 37°C PBS bath and tensile tested as follows: preload, preconditioning, stress relaxation for ten minutes at 5% strain, return to zero strain for one minute, and ramp to failure at 0.1% strain/s. Tissue strain was measured optically [6].

Measured animal weights were normalized to initial weights for comparison. Pre-op weights between the two groups were compared using an unpaired t-test. Comparisons of post-op normalized weights and biomechanical properties were made between the three diet groups using a one-way ANOVA (significance at $p \leq 0.05$, trend at $p \leq 0.1$).

Results: Normalized weight at five weeks after diet initiation (time of surgery) was significantly less in the low protein diet group ($p=0.02$). However, there was no difference at the end of the eleven week study. Cross-sectional area, peak stress and modulus were not different between the three experimental groups. There was a trend in percent relaxation, with the normal protein group having the highest value ($p=0.1$).

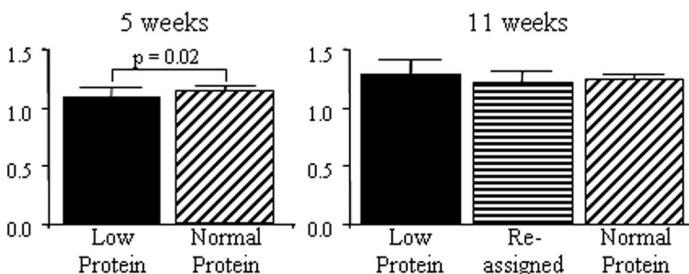


Figure 1. Normalized weight was significantly different at 5 weeks (surgery) but not at 11 weeks (sacrifice).

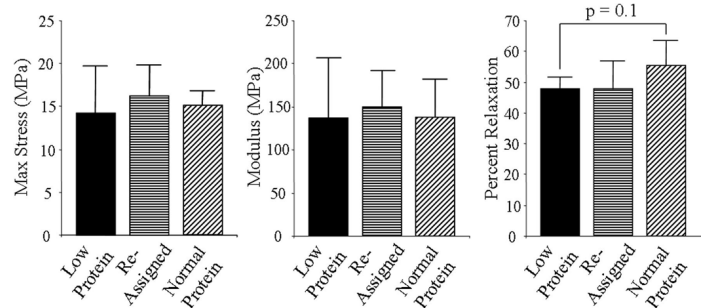


Figure 2. No difference was found between groups for max stress or modulus. A trend towards significance was seen in percent relaxation.

Discussion: This is the first study that specifically evaluates tendon healing following low protein diet consumption. Healing was assessed in patellar tendons in mice that consumed low (6%) and normal (23%) protein diets. An additional group that received the low protein diet pre-operatively and was reassigned to the normal protein diet post-operatively was also evaluated. We hypothesized that tendon biomechanics would be decreased in the low protein group, highest in the normal protein group, and between the two for the reassigned group. However, we did not find uniform differences indicative of altered tendon healing, which is in contrast to results of bone and dermal wound healing studies that used similar diets in the setting of protein malnutrition. Unexpectedly, the only trend found was a higher percent relaxation in the normal protein group as compared to the low protein group.

In this initial study, ten week old mice were used. All mice gained weight throughout the study. Since food was consumed *ad libitum*, the amount of each diet consumed was calculated at the end of the study and then corrected for the number of days mice received either diet. This analysis revealed no difference in the amount of food consumed by either group. Therefore, it is unlikely that the similarity of weights at the end of the study was due to over-eating in the low protein group. Future studies could evaluate the effects of a low protein diet on weight in elderly mice, a group more likely to display weight loss in the setting of protein malnutrition. Additionally, previous studies investigating various effects of low protein diets in mice have used weight loss alone as an indication of protein malnutrition [3,7]. Further investigation of measures for verification of protein malnutrition such as total serum protein and liver size could be useful. Future studies could also include additional mice in each group, with biomechanical and histologic evaluation over more varied post-operative periods to further investigate the findings observed in this study as well as potential differences between groups over time.

References: 1) Day et al: J Orthop Trauma 15:47-53, 2001. 2) Hughes et al: J Bone Joint Surg 88A:2386-94, 2006. 3) Lim et al: J Nutr Biochem 17:328-36, 2006. 4) Lin et al: J Biomech 39:61-9, 2006. 5) Favata, PhD Thesis, 2006. 6) Derwin et al: J Biomech 27:1277-85, 1994. 7) Nakagawasa et al: Behav Brain Res 167:111-7, 2006.