Introduction: Tendinosis is believed to begin as a focal area of intratendinous degeneration. Often caused by overuse, these conditions lead to microtrauma and weaken the structural and vascular elements of the tendon. As tendinosis progresses and the intrinsic reparative ability of the tendon is overwhelmed, the tissue degenerates, often leading to tendon rupture, joint instability and degenerative arthritis. Little is known about the mechanisms responsible for tendinosis and this lack of knowledge has hindered therapeutic advances in treatment for tendinopathy. In this study, we used a rat overuse model [1] to profile the changes associated with repetitive tendon injury at the cellular and molecular level. We hypothesize that by understanding the biological events that cause tendon degeneration, we can identify surrogate markers of this condition, allowing for early diagnosis and treatment.

Methods: Male Sprague-Dawley rats were subjected to a previously published repetitive exercise protocol consisting of treadmill running on a decline to model overuse injury to the rotator cuff [1]. Rats were sacrificed after 4, 8, 13 and 16 weeks of treadmill running. Three rats from each time point and 3 cage activity controls were randomly selected for histologic analysis. The supraspinatus tendon from both the right and left shoulders were removed, processed for histology, stained with toluidine blue, and analyzed by light microscopy. For molecular analysis, both supraspinatus tendons from 5 rat runners and 5 cage activity controls were pooled and total RNA was extracted using the TRIspin method [2]. Northern blot analysis was carried out using 10 µg of total RNA. Blots were consecutively hybridized with rat cDNA probes specific for collagen type I, collagen type III, biglycan, decorin, TGF-β1, and GAPDH.

Results: A detailed histological time course of the overuse injury in rats run for 4 to 16 weeks was performed. Control supraspinatus tendons showed low cellularity, long spindle shaped cells, and well organized linear collagen fibers (fig 1.). Tendons from rats run for 4 weeks showed some slight changes in these areas but did not allow identification of early events in the disease process. Tendons from rats run for 8 weeks showed more degenerative changes, potentially reflecting the progression of tendinosis. Large numbers of tendon cells were rounded up and bunched together, and the collagen fiber network was disorganized and wavy. Tendons from rats run for 13 to 16 weeks showed focal areas of damage as well as an increasing number of blood vessels in the body of the tendon, consistent with what is seen in later stages of human tendinopathy (fig 1.). Next, Northern blot analysis was performed on tendons from rats run from 4 to 13 weeks. Changes in expression of collagen type I, collagen type III, biglycan, decorin, TGF-β1 were evaluated. In contrast to our histological data, we found significant gene expression changes in tendons from rats run for only 4 weeks. In these rats, collagen type I, collagen type III, and TGF-β1 were downregulated while biglycan and decorin levels remained unchanged (fig 2). Similar changes were seen in tendons from rats run for 8 weeks. In tendons from rats run for 13 weeks, levels of collagens I and III remained repressed, while biglycan levels began to fall and TGF-β1 expression levels returned to that of control values (fig 2).

Discussion: Our histological time course evaluation of overuse in the rat revealed tendon degeneration which was clearly evident after 8 weeks of exercise. In the injured tendons, we observed increases in cell number, changes in cell shape, and disorganization and damage in collagen fibers. Our Northern analysis revealed several significant changes beginning at 4 weeks of running, before any overt histological signs of tendinosis. The profile of extracellular matrix gene products observed correlated with the apparent tissue degeneration in these tendons, as most transcript levels decreased over time. This may be reflective of a tissue that is repeatedly being damaged and cannot respond to injury by altering gene expression to successfully mount an appropriate repair response. We did detect an increase in the levels of TGF-β1 in 13 week exercise tendons and this may be a consequence of wound healing of the tendon injury. The molecular data presented in our study is consistent with previous biomechanical data from this model which showed decreased mechanical properties (maximum stress and modulus) of the exercised tendons between 4 and 16 weeks [1]. In that study, the exercised tendons showed an increase in the amount of tissue present over time, suggesting an increase in the production of matrix components [1]. This fact was not reflected in our gene expression analysis as most of the matrix mRNAs examined were downregulated. One potential explanation for this discrepancy is in the limited number of genes we evaluated. This finding warrants further molecular analysis with a more extensive set of markers. Our data suggest that the lack of overt histological changes early in tendon injury is not reflective of the mechanical or molecular properties of this tissue. It appears tendons are damaged early in the course of overuse and fail to regain the material properties and gene expression profile of the original tissue. This may be one reason why tendons subjected to overuse are unable heal themselves. We believe molecular and cellular analysis will be useful for identifying factors important in the development and progression of tendinosis, allowing for intervention in the early phases of this condition which may help prevent further degeneration and ultimately promote healing.

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References:

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![Figure 1. Histology of control, 4 and 13 week supraspinatus tendons](image1.png)

![Figure 2. Northern Blot analysis of control, 4 and 13 week supraspinatus tendons](image2.png)