INTRODUCTION: Rotator cuff overuse tendinopathy, especially that of the supraspinatus, is a common and disabling condition [1,2]. Previous studies using a rat model of supraspinatus tendon overuse reported degradation of mechanical and histologic properties [2], alterations in inflammatory, angiogenic, and extracellular matrix (ECM) component mRNA levels [3,4], and changes in ECM proteins [5,6]. However, the ability of “rest” to reverse these changes is unknown. In particular, changes in collagens and proteoglycans due to rest represent important biological findings in the possible recovery of overused tendon. Therefore, the objective of this study was to evaluate insertion site and midsubstance ECM profiles of tendons subjected to rest following overuse. Specific hypotheses are: (1) ECM components associated with injury, such as Collagen III and Biglycan, will decrease in the insertion and midsubstance with rest; and (2) cartilaginous components, such as Collagen II and Aggrecan, will remain constant in the insertion but will decrease in the midsubstance with rest.

METHODOLOGY: 40 male Sprague-Dawley rats were used in this IACUC-approved study. Rats were distributed to 2 timepoints of overuse (1 wk, n=25; 8 wks, n=15) and subjected to a previously described overuse protocol [2] consisting of 10° downhill treadmill running for 1h/d, 5d/wk, at a rate of 17 m/min. After the overuse period, rats were either immediately sacrificed to provide baseline overuse data (n=5 per overuse group) or rested (2 wk) for a predetermined length of time (1wk, 3wks, 7wks, and 15wks for the 1wk overuse group, n=5 each; 1wk and 8wks for the 8wk overuse group, n=5 each) and then sacrificed. Supraspinatus tendon, muscle, and bone were isolated, fixed, decalcified, paraffin infiltrated, embedded, and sectioned at 7µm. Sections were immunohistochemically stained using antibodies for Collagen I, II, III, XII, Aggrecan, Decorin, and Biglycan, and developed using a standard ABC/DAB method. Images were acquired at 50x using a 5.0 megapixel camera (Qimaging). Specimen images were divided into insertion and midsubstance regions digitally using a calibrated pixel measurement tool (Photoshop). The insertion was defined as 0.5mm from the fibrocartilage and the midsubstance was the remainder of the tendon. Specimens were graded objectively using a custom DAB intensity measurement program (Matlab). Quartiles were produced using total area DAB intensity ranges, which are the difference between the most and least stained specimens in each target. Each quartile range was assigned a value of undetectable (-), low (+), moderate, (++), or high (+++), and the average intensity for each group was graded accordingly.

RESULTS: Insertion: Following both 1 and 8 wks of overuse, Biglycan decreased with rest, while Aggrecan and Collagen II increased with rest. Collagen III did not show a change with rest following either 1 wk or 8 wks of overuse. Midsubstance: Biglycan and Collagen II decreased with rest, while Aggrecan and Collagen III showed an increase with rest following 1 wk and 8 wks of overuse. Complete results are provided in Tables 1 and 2 and sample stained images are shown in Figure 1.

DISCUSSION: Overuse injuries are thought to include matrix changes with a gradual increase in damage [7]. Therefore, removal of the overuse, through rest, should allow for normal healing and/or remodeling. We therefore hypothesized that Collagen III and Biglycan [8], both injury-associated ECM proteins, would decrease in both the insertion site and midsubstance with rest. Our findings generally support the hypothesis for Biglycan, as protein expression decreased in the insertion and midsubstance with rest. Unexpectedly, Collagen III increased in the midsubstance after rest, possibly due to a delayed anabolic response from the midsubstance in its attempt to adapt to overuse. Additionally, we hypothesized that Collagen II and Aggrecan would not change with rest in the insertion, but would decrease in the midsubstance since these proteins are commonly found in the insertion site but not in the midsubstance in the normal, non-overused tendon. As hypothesized, Collagen II decreased in the midsubstance with rest. The presence of Collagen II in the midsubstance of the supraspinatus tendon in overuse would be expected based on the extrinsic compression the tendon experiences as it passes repeatedly under the acromial arch. Once the stimulus of overuse was removed, Collagen II levels in this location could decrease as there was no additional compression of the tendon. Collagen II and Aggrecan increased in the insertion with rest, also possibly due to a delayed, adaptive response to overuse. Collagen I comprises 65-80% of the dry mass of tendon [9] and Collagen XII is a related protein and could be expected to change in parallel. Interestingly, we observed a decrease in Collagens I and XII in the insertion with 1 wk of rest following 8 wks of overuse, with some recovery after 8 wks of rest. Perhaps, with the sudden withdrawal of an anabolic stimulus like overuse, the equilibrium for these proteins shifted toward catabolism, re-equilibrating with anabolic processes later.

Results of this study begin to help explain the biological consequences of rest in the treatment of overuse injury in the shoulder, and may ultimately lead to development and evaluation of improved treatment modalities for this condition. In addition to further characterization of the tendon changes resulting from rest, studies will be designed to assess the mechanisms responsible for these changes.

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Table 1: Staining intensity of each protein (insertion/midsubstance) after 1 wk of overuse (OV) and overuse + rest

<table>
<thead>
<tr>
<th>ECM Proteins</th>
<th>Twn ov</th>
<th>1 wk ov</th>
<th>1 wk ov+1 wk rest</th>
<th>1 wk ov+2 wks rest</th>
<th>1 wk ov+3 wks rest</th>
<th>1 wk ov+6 wks rest</th>
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</thead>
<tbody>
<tr>
<td>Coll I</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
</tr>
<tr>
<td>Coll II</td>
<td>-/+</td>
<td>-/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
</tr>
<tr>
<td>Coll III</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
</tr>
<tr>
<td>Coll XII</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
</tr>
<tr>
<td>Aggrecan</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
</tr>
<tr>
<td>Biglycan</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
</tr>
<tr>
<td>Decorin</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
</tr>
</tbody>
</table>

Table 2: Staining intensity of each protein (insertion/midsubstance) after 8 wks of overuse (OV) and overuse + rest

<table>
<thead>
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<th>ECM Proteins</th>
<th>8 wks ov</th>
<th>8 wks ov+1 wk rest</th>
<th>8 wks ov+6 wks rest</th>
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<td>+/+</td>
</tr>
<tr>
<td>Coll II</td>
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<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>Coll III</td>
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<tr>
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<td>Aggrecan</td>
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<td>-/-</td>
<td>+/+</td>
</tr>
<tr>
<td>Biglycan</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
</tr>
<tr>
<td>Decorin</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
</tr>
</tbody>
</table>

Figure 1: Representative images depicting an increase in Aggrecan and decrease in Biglycan with rest. (I=insertion, M=midsubstance)