Introduction: We have shown in previous in vivo experiments that ligament length changes occur by the movement of collagen fibers past one another during growth and contracture and that this phenomenon occurs diffusely throughout the entire ligament length. The “sliding fibril hypothesis” requires that discontinuous fibrils have a reversible mechanism of fiber to fiber bonding which releases to allow fiber sliding, then reattaches. Schmidt, et al showed that a pentapeptide, NKISK, competitively inhibits binding of fibronectin to decorin by mimicking the sequence in the decorin molecule which is presumed to be the fibronectin binding site. Unpublished work from our laboratory has shown that stressed tendons immersed in NKISK or in relaxin (a hormone effective in relaxing ligaments and other connective tissues) stretch significantly more than control tendons and that free, intact fibrils can be isolated after exposure of ligament to NKISK. This study focuses on the effect of NKISK and/or relaxin as well as the possible additive effect of the two agents (in combination) on interfibrillar bonding and on subsequent collagen fiber sliding in the stressed rat tail tendon model. By applying a covalently bonding, fluorescent collagen dye, we were able to label collagen fibers and observe subsequent movement during tendon strain.

Methods: The test solutions were 1 mM NKISK and 46 units/ml porcine relaxin in commercially prepared phosphate buffered saline (PBS), pH 7.4, to which 0.03% NaN₃ had been added. PBS was the control solution. Rat tail tendons were harvested from frozen 500-g Sprague Dawley rat cadavers sacrificed for unrelated, IRB approved experiments. Similar sized, single tendons were marked with India ink at 15 mm intervals. Tendons were placed individually in glass tubes filled with the test solutions and the assemblies were suspended (this assembly was weighed at the end of the experiment to determine the load applied to the tendon). There were 4 models (control, NKISK, Relaxin, and NKISK Relaxin mixture) each consisting of 10 tendons. The distance between the India ink marks were measured with a caliper accurate to 0.02 mm at the beginning of the suspension period and again after 3, 6, 12, 27, 36 and 48 hrs. The experiment was repeated using the above procedure in a second rat tail with the same 4 models, each consisting of 4 tendons. In the second experiment, the tendons were stained perpendicularly between the ink marks with a thin line using a 10-0 nylon suture dipped in the fluorescent collagen dye dichlorotriazinyl fluorescein (DTAF – 5mg/ml in fresh 0.2-M sodium bicarbonate). A picture, demonstrating the DTAF stain initially and again at 27 hrs. The initial and final fluorescent marking widths were blindly measured on the 16X photomicrographs using the caliper and the percent increase in width of the mark was calculated. In both experiments, the India ink mark measurements were used to calculate the percent creep at each of the time points. At the end of the experiment the portion of tendon between the India ink marks was air dried for three days and then weighed in order to calculate the cross sectional area of the tendon. Differences between models were evaluated for statistical significance using a Bonferroni t-test.

Results: There is moderate variability between rat tails as well as individual tendons. The first experiment consists of 40 tendons (10 per model) without photomicrographs and the second experiment 16 tendons (4 per model) with photomicrographs. In the first experiment, the mean tendon stress was 0.516 +/- 0.049 (SE) Mpa with similar mean values in all four groups. Figure 1 displays the induced creep in experimental models compared to that of the control model in the first experiment with all experimental values being statistically significant (p<0.05) from the 3 hour time point on. In both experiments, NKISK and Relaxin treated tendons consistently demonstrated significantly more creep than the controls from the same tail. Figure 2 shows representative initial and final photomicrographs of a DTAF mark. The final photomicrograph shows collagen fibers protruding from either side of the mark as would be expected with collagen fiber sliding. The percent increase in DTAF marking widths (+/- SE) were 15.4 +/- 1.7%, 16.4 +/- 1.8%, and 22.1 +/- 2.3% respectively in the NKISK, Relaxin, and mixture models compared to only 9.5 +/- 8.1% in the control, but only the mixture model was significantly different from the control.

Discussion: Tendons exposed to NKISK and Relaxin consistently stretched significantly more than the control tendons at each time interval. NKISK is reported to inhibit decorin-fibronectin binding and postulated to interfere with interfibrillar bonding. In this study both NKISK and Relaxin potentiated tendon creep with changes in length most likely occurring as a result of collagen fiber sliding as evidenced by photomicrography. No bundle ruptures were present as would have been indicated by sliding of large bundles in the photomicrographs. We had postulated that if NKISK and Relaxin worked by different mechanisms they would have an additive effect when combined. Such an additive effect was not present. This study provides further support for the “sliding fibril hypothesis” for length changes in collagenous tissues.


Acknowledgements: Support by Aileen Stock Orthopaedic Research Fund.