INTRODUCTION:
The mechanism through which ligaments and tendons change length during growth and contracture is unclear. It has been hypothesized that there is a reversible “interfibrillar bond” that when broken allows the sliding of collagen fibrils past one another during length changes. NKISK, a small polycationic pentapeptide with two lysine residues, competitively inhibits the binding of fibronectin to decorin by mimicking a sequence in the decorin molecule that is presumed to be the fibronectin binding site. Previously, rat patellar tendons increased in length with progressive increases in NKISK concentration and treatment time. The tendons were then tested biomechanically, and no statistically significant difference between the groups in terms of maximum load, ultimate strength, structural stiffness, or elastic modulus was found. Our current study is a further examination of the in vivo effect of NKISK in the rat patellar tendon model with respect to even higher concentrations of NKISK, the length of treatment, residual effect, and a biomechanical analysis.

METHODS:
NKISK was synthesized by the Peptide Synthesis Facility at the University of North Carolina at Chapel Hill. Experimental solutions of 1 mM, 5 mM, and 10 mM of NKISK were prepared in commercially available sterile phosphate buffered saline (PBS). The control solutions were PBS or a 5 mM solution of QKTSK, a “nonsense” molecule of similar molecular weight and charge to NKISK. Male Sprague-Dawley rats, 350-600 grams, were divided into seven groups (n = 7, 7, 10, 12, 7, 7) Under sterile conditions and brief ether anesthesia, the rats’ knees were clipped and then the patellar tendons were percutaneously injected superficially and deep using a 27-gauge needle with 1 ml of different concentrations of NKISK, QKTSK, or PBS. Each day after injection the rats in all groups were placed in a water bath for two to five minutes of swimming to facilitate distribution of the injected solution and increase tensile loading of the tendon. All the animals were sacrificed 24 hours after their last injection, (except one group which were sacrificed seven days after their last injection). Rat patellar tendons have very sharply demarcated origins and insertions, which were measured three times under 1.5X magnification with a caliper accurate to 0.02 mm. The lengths of the experimental tendons were compared to the contralateral controls using a paired t-test. Using the stress-deformation data, the maximum load, the energy to yield, structural stiffness (in linear region between load limits of 25-75% max. load), elastic modulus, displacement and strain at maximum load were calculated, and the experimental tendons were compared to the control tendons using a one way analysis of variance.

RESULTS:
Treatment with NKISK led to a progressive increase in the length of the patellar tendons with increases in both concentration and treatment time. 5 mM QKTSK did not result in a significant change in length as compared to PBS, 0.98%, p = 0.76. 5 mM NKISK produced significant increases in length vs 5 mM QKTSK, 10.68%, p < 0.001 and vs PBS, 12.4%, p < 0.001. 5 mM NKISK produced a significant increase in length, 10.84%, p = 0.05 over 1mM NKISK. 10mM NKISK produced a significant increase in length 5.24%, p = 0.02 over 5mM NKISK. Two more NKISK regimes compared to the control PBS in the opposite knee, one looking at a seven day delayed sacrifice to evaluate a prolonged effect of 5mM NKISK, and one extending the treatment time with 5mM NKISK from 7 to 14 days. Both produced significant increases in length equaling 8.96%, p < 0.001, and equaling 13.56%, p < 0.001, respectively. Biomechanical studies were undertaken on the patellar tendons, and were compared to the PBS controls. There were no statistically significant differences between the groups in terms of load at maximum load, strain at maximum load, energy to yield point, elastic modulus, stiffness, or displacement at maximum load. Despite the p-values, there appears to be a trend with the 10mM NKISK solution to have a smaller ultimate load, lower modulus, lower stiffness, and more displacement.

DISCUSSION:
In the current study, we continue the evaluation of the in vivo effect of NKISK on dense collagenous tissues. The “nonsense” peptide, QKTSK, did not produce a change in length, proving the specificity of the NKISK sequence.

Figure 1 extrapolates our data comparing the different concentrations of NKISK. This shows that the dose response curve was steepest between 1mM and 5mM NKISK solutions, and began to flatten between 5mM and 10mM NKISK. In evaluating treatment time, there was only a 1.16% increase in length going from 7 to 14 days, and it was not statistically significant. Studying the prolonged effect of NKISK by delaying sacrifice for 7 days, appeared to show a loss of some of the length gained with a percent change in length of 8.96%, as compared to 12.4% for the straight seven day specimens, but this difference was not statistically significant. The findings are compatible with the hypothesis that NKISK allowed the fibrils to slide past one another and “rebond” in a lengthened position. The biomechanical analysis of the different treatment regimens demonstrated no statistically significant differences in the tendons treated with the NKISK. These findings support our hypothesis that NKISK plays a role in the sliding fibril model and can produce in vivo length changes without significant mechanical damage to the tendon. In conclusion, we have confirmed that NKISK allows lengthening in patellar tendon in an in vivo model and demonstrated that there is a dose response curve to the peptide. The effect persists after stopping administration and has minimal if any effect on the biomechanical properties of the tendon. The precise mechanism for this lengthening cannot be elucidated at this time, however it is believed to occur through competitive, specific inhibition of the decorin-fibronectin or decorin-decorin binding sites allowing collagen fibers to slide. Further study is warranted to investigate this model fully.

REFERENCES:

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