Effects of Decorin and Biglycan on Re-Alignment and Mechanical Properties of Aging Supraspinatus Tendons

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Introduction

Tendons exhibit complex mechanical behavior modulated by the tissue's structure and composition, such as the collagen fibers and surrounding matrix. Numerous studies have investigated the mechanical properties of tendons as they age, but results have been inconclusive. One mechanism by which tendon responds to load is by re-alignment of collagen fibers. The ability of tendon to respond in this manner varies throughout development,[1] but has not been studied during aging. In addition, it is unknown how proteoglycans (PGs) affect re-alignment in aged tendons. Therefore, the objective of this study is to measure the tensile mechanical properties and collagen fiber re-alignment of the mouse supraspinatus tendon throughout age and to investigate how this process is affected by the absence of key PGs. We hypothesize that: 1) tendons will get stiffer but will re-align later during the mechanical test as aging progresses and 2) both mechanical and re-alignment changes will be altered in PG-deficient mice.

Methods

Sample Preparation: Supraspinatus (SST) tendons from 106 mice at three ages (90 day, 300 day, 570 day) and three genotypes (biglycan knockout (*Bgn−/−*), decorin knockout (*Dcn−/−*) and wild type (WT)) were isolated. Cross-sectional area was measured and stain lines were placed to denote the tendon insertion and midsubstance. The humerus was fixed in a pot and the tendon was secured in grips.

Mechanical Testing: Samples were loaded in a testing system integrated with a polarized light setup.[2] Tendons were tested in tension as follows: preload, 10 cycles of preconditioning, return to zero displacement, stress relaxation, followed by a 60 second hold and ramp to failure. Images were obtained for strain analysis and image sets were acquired as the polarizers rotated for measurement of fiber alignment during loading.[2]

Data Analysis: Local strain was measured optically. Circular variance (VAR), a measure of the distribution of collagen fiber alignment, was calculated for fiber distributions before preconditioning, after preconditioning, at transition strain (intersection of the toe- and linear-regions, determined using a structural fiber recruitment model at 50% fiber recruitment[3]), and at linear-region strain (at 75% fiber recruitment). Fiber re-alignment during preconditioning was evaluated by comparing VAR values before and after preconditioning. Similarly, fiber re-alignment in the toe- and linear-regions of the stress-strain curve was determined.

Statistical Analysis: Comparisons were made across age and genotype using ANOVA. If significant, post-hoc t-tests with Bonferroni corrections were used. Wilcoxon signed-rank tests were used for the non-normally distributed VAR data. Alignment data is presented as representative samples and mechanics data is presented as mean+SD (*Sig = $p<0.025$).

Result

In WT tendons, stress relaxation was decreased at 300d and 570d compared to the 90d tendons and maximum load was increased from 90d to 300d (not shown). No other mechanical property changes were found in the WT tendons. However, significant changes in re-alignment were found (Fig. 1). At the insertion site, the 90d tendons re-aligned during the preconditioning and linear regions, while the 300d and 570d only re-aligned during the linear region. At the midsubstance, the 90d tendons re-aligned during the preconditioning and linear regions, while the 300d tendons re-aligned only during the linear region and the 570d tendons showed no significant re-alignment throughout the test.

In contrast, mechanical changes were found in the *Dcn−/−* and *Bgn−/−* tendons. Specifically, the *Bgn−/−* tendons at 90d and 570d had a lower modulus and stiffness at the insertion site than the 300d tendons (Fig. 2), while the *Dcn−/−* tendons had an increased insertion site modulus at 300d and 570d compared to 90d. In addition, the *Dcn−/−* tendons had a decreased stress relaxation at 300d and 570d compared to

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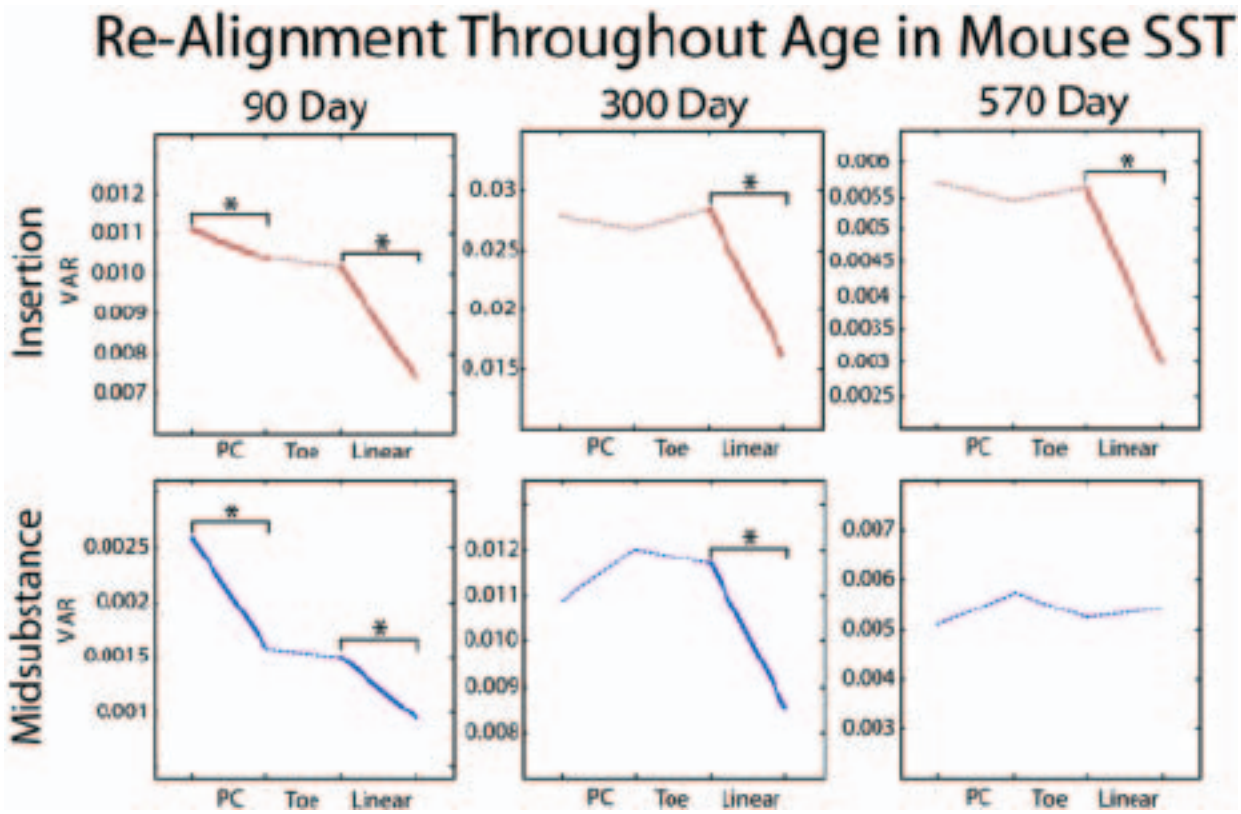

Figure 1. Re-alignment occurred during the preconditioning (PC) and linear regions in both locations of the wild type tendons at 90d (left). At 300d (middle), re-alignment occurred during the linear region in both locations. At 570d (right), the insertion re-aligned in the linear region but no significant re-alignment occurred in the midsubstance.

the 90d tendons (not shown). At the insertion of the *Bgn−/−* tendons, re-alignment occurred during the preconditioning and linear regions at 90d, while re-alignment only occurred during the linear regions at 300d and 570d (similar to the WT insertion). However, at the midsubstance of the *Bgn−/−* tendons, re-alignment occurred only during the linear region at all ages. In the *Dcn−/−* tendons, the midsubstance re-aligned in the same fashion as the WT insertion and the *Bgn−/−* insertion, but the insertion site re-aligned only during the linear region at all ages.

Discussion

While previous studies have reported broad changes in mechanical properties of WT tendons with age, this study did not support those findings. However, changes in re-alignment in the WT tendons did show a decreased response to load with age. Tendons at 300d and 570d do not re-align their collagen fibers until the linear region, a later response than 90d tendons. This result, seen well in the WT tendon midsubstance (Fig. 1), is indicative of a breakdown of the structural organization of tendon over time and mirrors the response of tendon during development.[1]

The PG-deficient tendons showed altered mechanical properties with age, predominantly at the insertion site (Fig. 2). However, changes in re-alignment throughout age were not found in the midsubstance of the *Bgn−/−* tendons or at the insertion of the *Dcn−/−* tendons. While a diminished capacity at those locations is present when comparing the knockout tendons to the WT 90d tendons, this capacity does not degrade further after 90d. This suggests that decorin and biglycan may play a role in the aging process in this tendon and that the lack of these PGs may shield the tendon from deteriorating effects. Although changes in mechanical properties with removal of

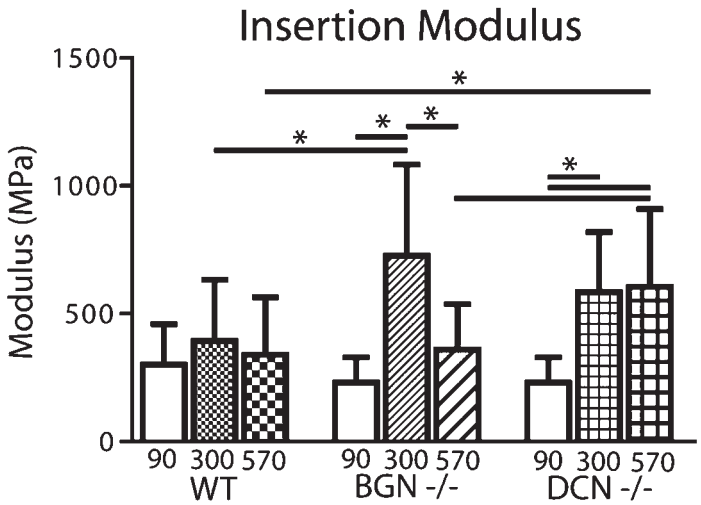

Figure 2. Changes were found in the insertion modulus of the PG-knockout tendons, but not in WT, with age.

PG and glycosaminoglycans have been debated,[4] this study shows that decorin and biglycan contribute to tendon's response to load, in particular with re-alignment of collagen fibers. Finally, changes in mechanical properties did not occur in concert with changes in collagen fiber re-alignment, suggesting that typical mechanical property measurements alone are insufficient to describe how structural alterations affect tendon's response to load.

Significance

Collagen fiber re-alignment and mechanical properties are altered with aging in the mouse supraspinatus tendon, and proteoglycans play an important role in regulating this process.

Acknowledgements

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