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# Strain-Induced Collagen Fibril Deformation is Diminished with Advanced Age in Mouse Supraspinatus Tendon

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## Introduction

Age-related tendon degeneration increases the risk of rotator cuff injuries which can lead to significant pain and disability.[1] The supraspinatus tendon, as part of the rotator cuff, exhibits region-dependent mechanical properties that change with advanced age which are likely a contributing factor to the increased risk of rupture in the elderly population.[2,3] While these age-related changes to bulk tissue properties in the supraspinatus tendon have been demonstrated,[2,4] tendon is a complex hierarchical tissue that dynamically responds to mechanical loading though changes in structural organization at multiple length scales. Despite this, it is unclear how aging affects the relationship between bulk tissue strain and collagen fibril deformation on the nanoscale level.[5] Therefore, the objective of this study was to determine how collagen fibrils deform with applied strain in different regions of the supraspinatus tendon at two distinct ages. We hypothesized that collagen fibrils would experience deformation earlier in older tendons because of a reduction in early strain attenuation mechanisms such as uncrimping and changes in fiber alignment.

## Methods

Supraspinatus tendon-humerus complexes were harvested from p300 and p570 male wild-type C57BL/6 mice (IACUC approved). Tendon cross-sectional area was measured using a laser displacement sensor. After preparation for mechanical testing, samples were subjected to 10 cycles of preconditioning between 0.02 and 0.04 N followed by a one-minute rest and then a ramp to a randomly assigned strain (1%, 5%, or 9%; n = 5-6/group) at a rate of 0.1% strain per second. The tendon was immediately flash frozen after reaching the target strain, removed from the test fixture, and embedded in optimal cutting temperature compound while keeping the tissue frozen to maintain the applied strain.[6,7] Cryosections of the tendons were collected at 20 μm thickness and fixed in formalin. Nanoscale topographical images of the sections were acquired using tapping-mode atomic force microscopy (AFM) to visualize collagen fibrils. Five 2x2 μm images were acquired in both the insertion region (within 1 mm of humeral insertion) and midsubstance region (1-2mm away from humeral insertion) across multiple tissue sections for each sample (Figure 1). Collagen fibril d-period was measured using Fourier transform analysis.[8] The average d-period length, local variance (average variance in d-period length within individual images), and global variance (variance in d-period length across entire sample) were calculated for the insertion and midsubstance regions of each sample. Data for p300 and p570 samples were analyzed independently using two-way ANOVAs including the main effects of region, strain, and their interaction with Tukey-adjusted post-hoc testing within significant main effects.

## Results

The applied strains of 1%, 5%, and 9% corresponded to the early toe, early linear, and early yield portions of the stress-strain curves, respectively, in both ages (Figure 2a,b,f,g). Average d-period length increased from 67.8 nm to 68.7 nm with applied strains of 1% to 5% in p300 samples, corresponding to a fibril strain of approximately 1.3% (Figure 2c). However, d-period length was not different between applied strains of 1% and 9%. In contrast with the p300 data, fibrils from p570 samples experienced no strain-induced changes in d-period length (Fig 2h). Moreover, local and global variance in d-period length

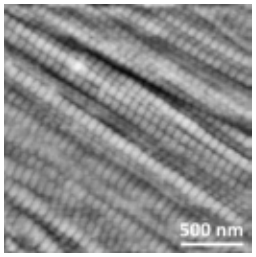


**Figure 1.** Representative image

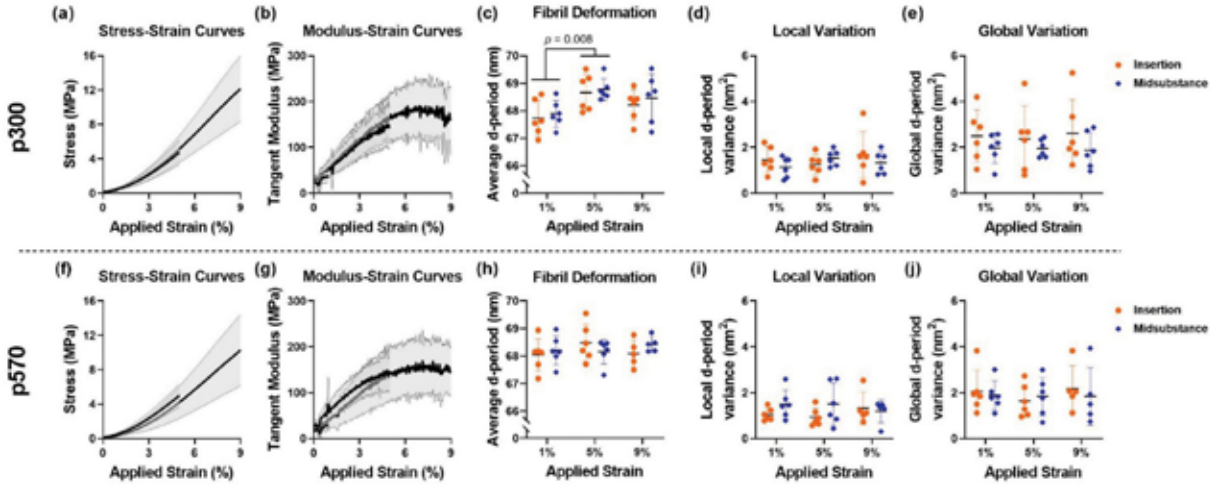


**Figure 2.** Average stress-strain curves **(A,F)** and modulus-strain curves **(B,G)** from p300 and p570 samples. Fibril deformation was significantly increased from 1% to 5% applied strain independently of region in p300 samples **(C)** but was unaffected by strain and region in p570 samples **(H).** Local and global variation were unaffected by strain and region in both p300 and p570 samples **(D,E,I,J)**.

showed no effect of strain or region in both p300 and p570 ages (Figure 2d,e,i,j).

## Discussion

Significant deformation of collagen fibrils was observed in p300 supraspinatus tendons. Unexpectedly, this fibril deformation occurred at the lower applied strain of 5%. Therefore, some of the applied strain is transmitted from the bulk tissue level to the collagen fibrils between the early toe and early linear regions of the loading curve despite the uncrimping and reorganization that would be expected concurrently between these strains.[9] At the larger applied strain of 9%, the d-period length was no longer different than the 1% strain baseline value. Because the tissue exhibited early yield behavior (i.e., a reduction in modulus) at 9% strain, these data suggest that tissue yielding may result from early damage to the extracellular matrix that causes the collagen fibril d-period to begin to return to its initial length. Similar strain-dependent changes, with larger fibril deformations at intermediate applied strains, were found previously in supraspinatus tendons from younger p150 mice.[6,7] Contrary to our hypothesis, no fibril deformation was observed in supraspinatus tendons from p570 mice in this study. At this advanced age, the lack of strain transmission from the bulk tissue scale to the fibril scale indicates that smaller-scale mechanisms are likely dominated by structural reorganization such as uncrimping, sliding, and/or realignment rather than deformation of collagen fibrils.[9,10] Identifying the interplay between, and combination of, these mechanisms which become prominent with advanced age is a promising area for future study. In addition to measuring fibril deformation, this study also investigated the variation in d-period lengths. Even with changing d-period length in p300 samples, the variation remained similar for all strains. Therefore, the increase in d-period length was homogenous across all fibrils in the tissue, rather than the alternative where some fibrils would experience deformation while others would not. However, it should be noted that while fibril deformation was homogeneous in this controlled experiment, more complex mechanical loading of the supraspinatus tenon in situ could result in heterogenous fibril engagement.

## Significance

Results from this study provide insights regarding nanoscale mechanisms that influence age-related degeneration and changes in mechanical properties of supraspinatus tendon.

## Acknowledgment

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