Mouse Supraspinatus Tendon Mechanical and Structural Properties Are Dependent on Region and Age

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Introduction

Musculoskeletal pathologies such as rotator cuff tendon disorders of the shoulder are prevalent in the aging population[1]. Dysregulation of tendon homeostasis due to aging can result in premature sub-rupture damage accumulation, degeneration, and ultimately injury. Such changes occur primarily in regions of high and complex stresses, such as at the supraspinatus tendon insertion site of the shoulder[2]. While recent studies in patellar tendons showed inferior dynamic structural response to load, reduced elastic and viscoelastic mechanical properties, and altered fibril structure with aging[3], region and age dependent changes in the rotator cuff of the shoulder remain unknown. Therefore, the objective of this study was to elucidate region-dependent mechanical and structural differences in aging mouse supraspinatus tendon. We hypothesized that aging would result in region-specific mechanical and structural changes, such as inferior elastic and viscoelastic mechanical properties as well as altered collagen fiber realignment and fibril morphology, with larger alterations at the insertion site due to the complex functional demands in this region[2].

Methods

Animals

Forelimbs were collected from male wildtype mice sacrificed at either 300 (P300, n = 20) or 570 (P570, n = 20) days of age (UPenn IACUC approved), corresponding to ~40 and ~65 years of age in human[4], respectively.

Elastic and Dynamic Viscoelastic Mechanics

All mice for mechanical testing were frozen at −20ºC until the day of testing. Mice were thawed at room temperature and the supraspinatus tendon-humerus complex from the left limb of each mouse was carefully dissected to remove extraneous tissue. Stain lines were applied for optical strain tracking of the insertion and midsubstance regions and a laser device was used to measure cross-sectional area of the supraspinatus tendon. The myotendinous junction was placed between two sandpaper tabs with cyanoacrylate glue to prevent slippage. The humerus was secured in a custom construct with polymethyl methacrylate and the construct was mounted on a material testing machine (Instron 5848). All testing was conducted in a phosphate buffered saline bath at 37ºC. Each sample was preloaded to 0.025N. The testing protocol consisted of 10 cycles of preconditioning, followed by stress relaxations at 3%, 5%, and 7% strain. Following each stress relaxation, frequency sweeps of 10 cycles of 0.125% amplitude sinusoidal strain at 0.1, 1, 5, and 10 Hz were performed. Following a 10-minute rest at zero displacement, a quasistatic ramp-to-failure at a strain rate of 0.1%/s was completed. Data were collected at 100Hz. Elastic parameters stiffness, modulus, maximum load, and maximum stress were quantified. Viscoelastic parameters dynamic modulus (E*), phase shift (tan δ), and percent relaxation were quantified for each stress relaxation and frequency sweep.

Fiber Re-Alignment

Throughout mechanical testing, dynamic collagen fiber realignment was quantified using cross-polarization imaging, and regional fiber alignment data was interpolated in MATLAB with a polynomial fit as a function of strain from the load-displacement data. Images were also used to optically measure strain and modulus in the insertion and midsubstance regions.

Transmission Electron Microscopy

Supraspinatus tendons (n = 4/age group) were isolated, fixed, and embedded in epon resin blocks. 85nm sections were cut using an ultramicrotome, stained with uranyless and phosphotungstic acid, and imaged at 60,000x using a transmission electronic microscope (JEOL 1010). Fibril diameter frequency distribution was quantified.

Statistics

Region-specific tendon elastic mechanical properties and collagen fiber realignment were compared using two-way ANOVAs across age and region followed by Bonferroni post-hoc tests. Viscoelastic properties were compared using two-way ANOVAs across age and strain levels followed by Bonferroni post-hoc tests. Fibril

diameter distributions were compared using Kolmogorov-Smirnov tests. Significance was set at $p < 0.05$.

Results

Elastic and Viscoelastic Mechanics (Figure 1)

Cross-sectional area was greater at the insertion than in the midsubstance region in both age groups. Stiffness and modulus were lower at the insertion than in the midsubstance region in both age groups. Midsubstance modulus had an interaction and decreased with age. The viscoelastic response was preserved with aging across strain levels. Specifically, there were differences in stress relaxation and dynamic modulus at 5 and 7% strain relative to 3% strain with aging. P300 tendons exhibited more relaxation at 3% relative to both 5 and 7% strain than P570 tendons. Phase shift was not altered across strain levels for either age group. As expected, all supraspinatus tendon samples failed at their insertion sites.

Collagen Fiber Realignment (Figure 2)

Collagen fiber realignment had a significant interaction with region, but not age, in both age groups. Specifically, normalized circular variance was greater in the insertion than in the midsubstance region (indicative of less fiber alignment) between 3 and 9% strain in both the P300 and P570 age groups.

Fibril Morphology (Figure 3)

Fibril distributions were significantly different across region and age with smaller diameter fibrils at the insertion compared to the midsubstance within each age group. Insertion region fibrils had narrower distributions compared to the

Discussion

We studied regional properties in aging mouse supraspinatus tendons. Supporting our hypothesis, insertion regions exhibited inferior elastic mechanical properties and reduced collagen fiber realignment compared to midsubstance regions. Additionally, insertion region fibril size distributions

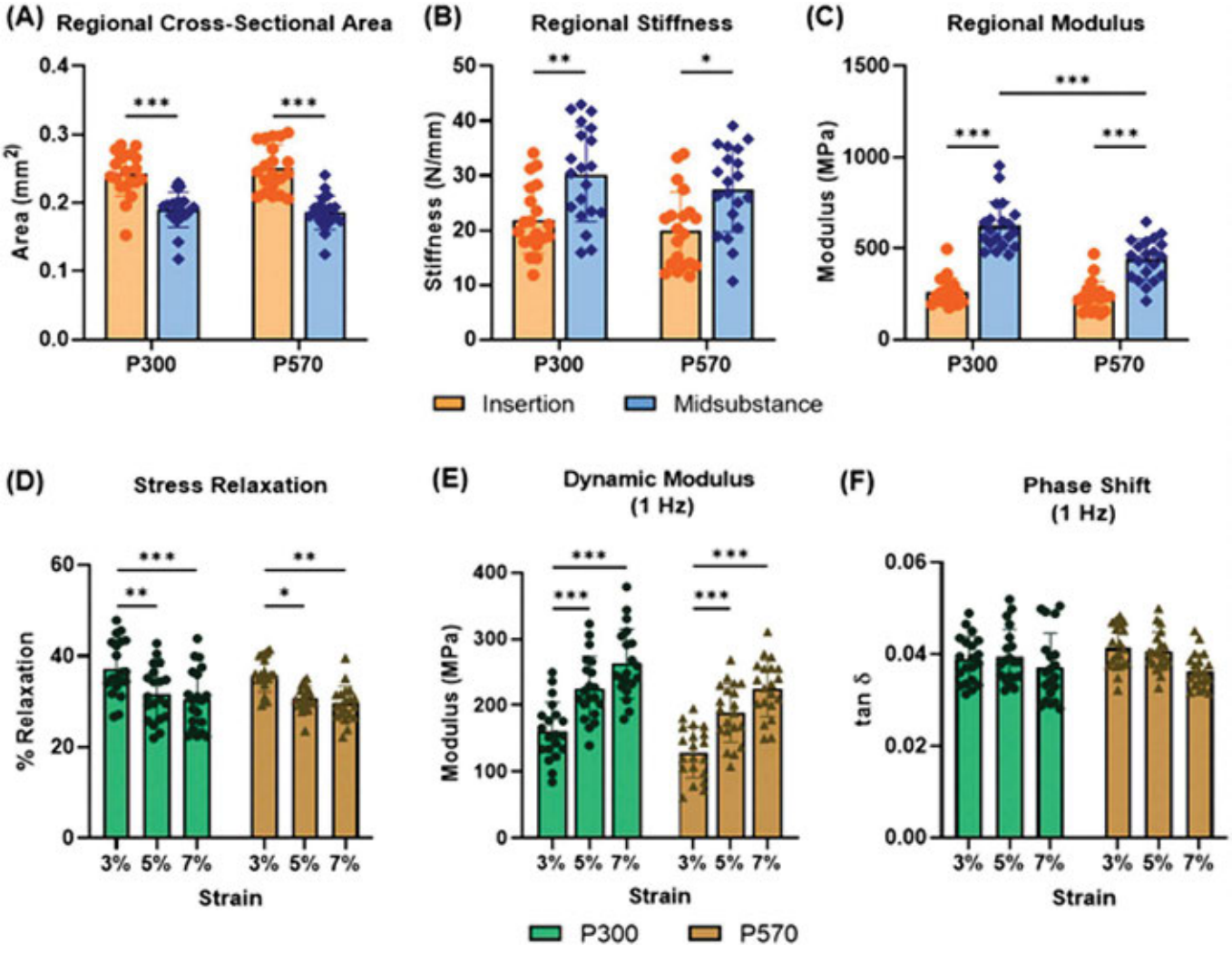


Figure 1. Insertion regions demonstrated greater **(A)** cross-sectional area while midsubstance regions had greater **(B)** stiffness and **(C)** elastic modulus for both age groups. Elastic modulus was **(C)** reduced with aging. Viscoelastic properties were conserved with aging with similar differences across strain levels in **(D)** stress relaxation and **(E)** dynamic modulus and no differences across strain level in **(F)** phase shift in both age groups. Data as mean ± standard deviation (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$).

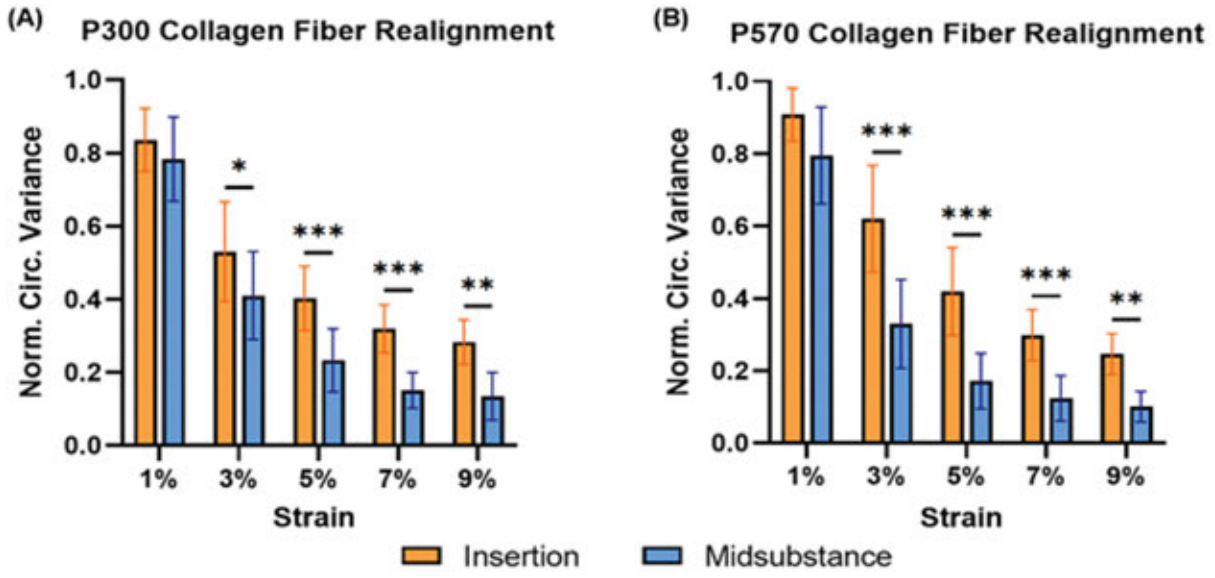
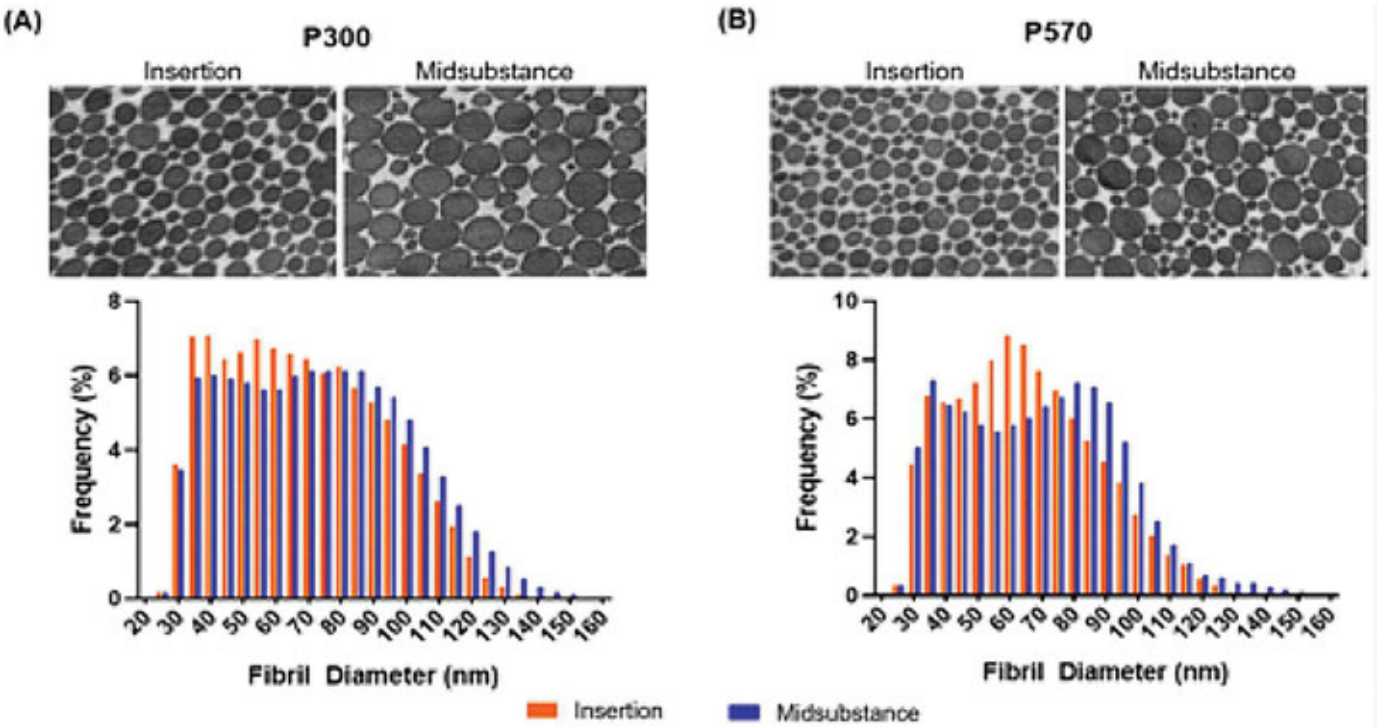


Figure 2. Insertion regions demonstrated lower collagen fiber realignment (greater normalized circular variance) at strain values between 3 and 9% in the (A) P300 and (B) P570 age groups. Data as mean ± standard deviation (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001).



	Insertion	Midsubstance
Mean	68.4 nm	72.9 nm
Median	66.4 nm	71.9 nm
Q1	47.9 nm	50.6 nm
Q3	86.9 nm	92.7 nm

	Insertion	Midsubstance
Mean	64.9 nm	68.6 nm
Median	63.2 nm	68.5 nm
Q1	47.6 nm	47.1 nm
Q3	79.9 nm	86.6 nm

Figure 3. Fibril diameter distributions for **(A)** P300 and **(B)** P570 age groups are similar with shifts towards smaller diameter fibrils in the insertion compared with the midsubstance region. Aging also resulted in a shift towards smaller fibrils in the midsubstance.

shifted towards smaller fibril diameters. Previous studies in flexor tendons demonstrated that mechanical properties and fibril diameter distributions can differ from the bone-tendon junction to the myotendinous junction[5]. Interestingly, aging did not influence regional and whole tendon elastic and viscoelastic properties and collagen fiber realignment but did influence fibril morphology.

Multiscale regression analyses have shown that two strong predictors of mechanical properties at the insertion and midsubstance regions were collagen fiber realignment and fibril diameter[6]. Our results suggest potential multiscale structure-function mechanisms relating macroscale tissue mechanical behavior to microscale collagen fiber realignment and nanoscale fibril morphology. Specifically, in the insertion regions, decreased collagen fiber realignment, indicative of a reduction in dynamic structural response to load, in conjunction with smaller diameter fibrils unable to withstand the same loading magnitude results in inferior mechanical properties relative to the midsubstance region. This induces earlier accumulation of damage at the fiber and fibril levels propagating to the macroscale, ultimately leading to premature tendon failure at the tendon insertion[2], as observed in all supraspinatus tendon samples. Clinically, these structure-function mechanistic findings may further explain why supraspinatus tendon tears predominantly occur at its insertion on the proximal humerus[7].

Significance

This study reveals critical mechanical and structural differences in supraspinatus tendon region and age. Future studies will consider additional region-specific multiscale structural, functional, and compositional mechanisms in aging supraspinatus tendons.

Acknowledgments

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References

1. Buckwalter JA, Heckman JD, Petrie DP. An AOA critical issue: aging of the North American population: new challenges for orthopaedics. *Journal of Bone and Joint Surgery* 2003;85-A(4):748-58.

2. Connizzo BK, Han L, Birk DE, *et al.* Collagen V-heterozygous and -null supraspinatus tendons exhibit altered dynamic mechanical behaviour at multiple hierarchical scales. *Interface Focus* 2016;6(1):20150043.

3. Dunkman AA, Buckley MR, Mienaltowski MJ, *et al.* Decorin expression is important for age-related changes in tendon structure and mechanical properties. *Matrix Biology* 2013;32(1):3-13.

4. Dutta S, Sengupta P. Men and mice: Relating their ages. *Life Sciences* 2016;152:244-248.

5. Watanabe T, Imamura Y, Hosaka Y, *et al*. Graded arrangement of collagen fibrils in the equine superficial digital flexor tendon. *Connective Tissue Research* 2007;48(6):332-337.

6. Connizzo BK, Adams SM, Adams TH, *et al.* Multiscale regression modeling in mouse supraspinatus tendons reveals that dynamic processes act as mediators in structure-function relationships. *Journal of Biomechanics* 2016;49(9):1649-1657.

7. Bell R, Taub P, Cagle P, *et al.* Development of a mouse model of supraspinatus tendon insertion site healing. *Jounral of Orthopaedic Research* 2015;33(1):25-32.