Dynamic Mechanical Properties of Tendon Repair Tissue are Unaffected by Aging

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Introduction

Tendon injuries are common, especially in the aging population,[1] and incomplete tendon healing is a well-established problem. Furthermore, aging tendons are at increased risk for injury due to changes in their mechanical properties and structural integrity.[2] Age has also been correlated with poorer clinical outcomes for repaired tendons,[3] which may be attributed to an inferior repair response. While a previous study investigating aging and healing in rabbit patellar tendons found no age-related mechanical alterations in uninjured or injured specimens,[4] this study evaluated only quasi-static mechanical parameters. We have recently demonstrated that dynamic properties are more sensitive to changes due to damage from both aging and injury.[5,6] Therefore, the objective of this study was to determine the effect of aging on dynamic properties in the healing patellar tendon in a murine model. We hypothesized that 1) tendons of aged mice will exhibit declining mechanical properties compared to mature mice, and 2) with more advanced age, the healing response will be inferior, leading to decreased mechanical properties in injured tendons.

Methods

Healthy C57BL/6 wild-type mice were sacrificed at 150 (mature), 300 (aged), or 570 (extremely aged) days old (n=14-17 per group). Patellar tendons of additional animals were injured bilaterally (n=11-19 per group) as described.[7] Animal use was IACUC approved. Briefly, a skin incision was followed by longitudinal cuts adjacent to the tendon, under which a rubber-coated backing was passed. This provided support against a 0.75 mm diameter biopsy punch, used to create a full thickness, partial width (~60%) tendon transection. Skin incisions were sutured and mice were allowed cage activity. Injured animals were sacrificed 6 weeks post-operatively. Patellar tendons were subjected to biomechanical testing or RT-qPCR analysis.

For mechanical testing, a single hind limb from each animal was dissected to isolate the tibia-patellar tendon-patella complex. The tendon was stamped into a "dogbone" shape to isolate the repair portion of the tendon. Tendon cross-sectional area was measured with a laser device and the tibia was potted and loaded in an Instron, submerged in a 37°C PBS bath, and tested as follows: 1) preload, 2) preconditioning, 3) stress-relaxation and sinusoidal frequency sweeps at 4%, 6%, and 8% strains, 4) return to gauge length and 5) ramp to failure (0.1%/s). Each frequency sweep was comprised of ten sinusoidal cycles with amplitude of 0.125% at 0.01, 0.1, 1, 5 and 10 Hz. The dynamic modulus $|E^*|$ (the ratio of stress amplitude to strain amplitude) and the tangent of the phase angle $\tan\delta$ (a measure of viscoelasticity equal to the ratio of dissipated to stored energy) were computed at each strain level and frequency. Two way ANOVAs with Bonferroni analyses were performed across frequency and age at each strain level to compare the uninjured and injured states. Significance was set at $p \leq 0.05$ with corrections for multiple comparisons.

For RT-qPCR, total RNA was extracted from patellar tendons of contralateral limbs (n=6-12/group) to assess expression of small leucine-rich proteoglycans (SLRPs) decorin (Dcn), biglycan (Bgn), fibromodulin (Fmod), and lumican (Lum) relative to β-actin. Two way ANOVAs with Bonferroni analyses were performed across age and injury state. Significance was set at $p \leq 0.05$ and a trend was defined as $p \leq 0.1$ with corrections for multiple comparisons.

Results

At 4% strain level, $|E^*|$ of the patellar tendon decreased significantly between P150 to P300 and again between P300 to P570 (Fig. 1a) at all frequencies. $\tan\delta$ increased significantly between P150 to P300 and again between P300 to P570 (Fig. 1b). Results were similar for 6% and 8% strains (data not shown). These alterations were consistent with our hypotheses and suggest a decline in tendon functionality and ability to transfer force throughout aging. Contrary to our hypothesis, healing tendons exhibited no differences in either $|E^*|$ (Fig. 2a)

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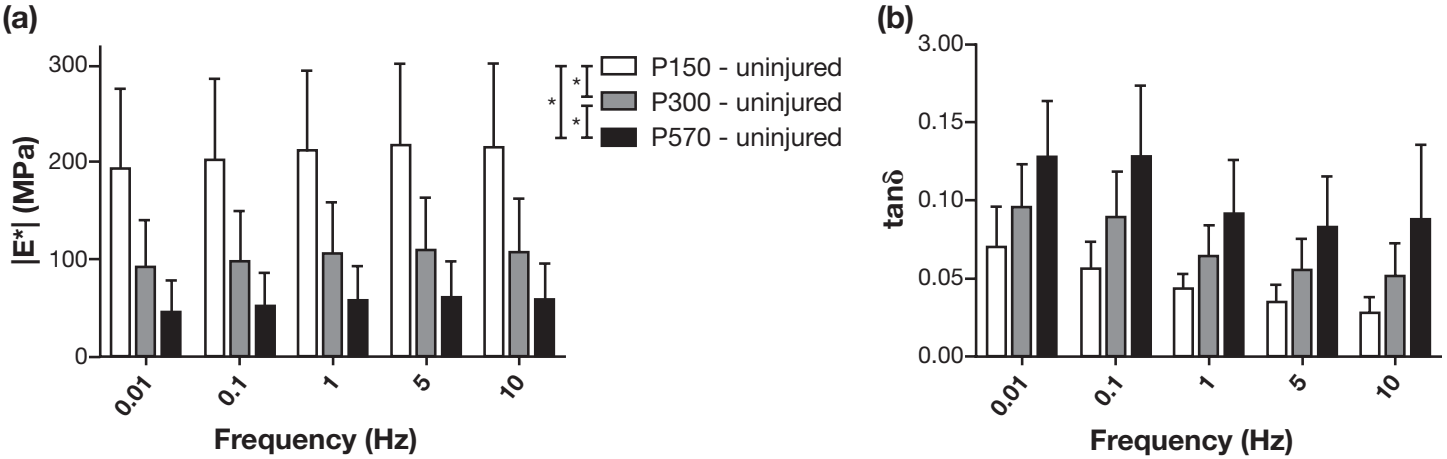

Figure 1. a) |E*| was significantly decreased in uninjured tendons with age while b) tanδ was significantly increased. * shown in legend denotes p≤.05/2 at each frequency for both |E*| and tanδ.

or tanδ (Fig. 2b) with age. Expression of Dcn had a decreasing trend at all ages post-injury, and Bgn expression significantly decreased after injury at 120 and 540 days. Expression of Fmod and Lum was maintained.

Discussion

Contrary to our hypothesis, this study demonstrates that despite age-related differences in uninjured tendons, there are no significant differences in injured tendon tissue after 6 weeks of healing across age groups. Interestingly, the values of the tested parameters in the injured tendons are roughly equal to those in the aged (P570) uninjured group, suggesting that the post-operative repair tissue at any age is approximately as damaged as a normal tendon of advanced age.

Post-injury levels of SLRPs decorin and biglycan had a decreasing trend compared to uninjured; expression of these SLRPs is essential to tendon development. Reduced levels of these SLRPs could impact repair and affect biomechanical outcomes.

Our results are consistent with literature examining the repair response in tendon as a function of age,[4,8,9] and further supports the notion that tendon healing is not fundamentally age-dependent. In this study, the injured central portion of the tendon was isolated from the lateral and medial struts by stamping. In vivo, this injured region is flanked by these uninjured struts. The properties of the uninjured struts likely influence the loading environment of the injured portion. Thus, in an aged tendon, the injured portion is flanked by inferior tissue, bears a greater portion of the load, and is therefore at an increased risk of reinjury.

This study provides significant insight into the effect of aging as well as into the repair response to injury at any age, and is instructive to both basic science efforts and to clinical care. Future work will investigate an earlier time point after the injury as well as the roles of molecular constituents in an attempt to understand the mechanisms governing the findings presented here.

Significance

This study demonstrates that tendon's repair response to injury does not deteriorate with age. Rather, inferior tendon healing in the aging population may be due to the quality of the tendon as a whole rather than due to its repair potential.

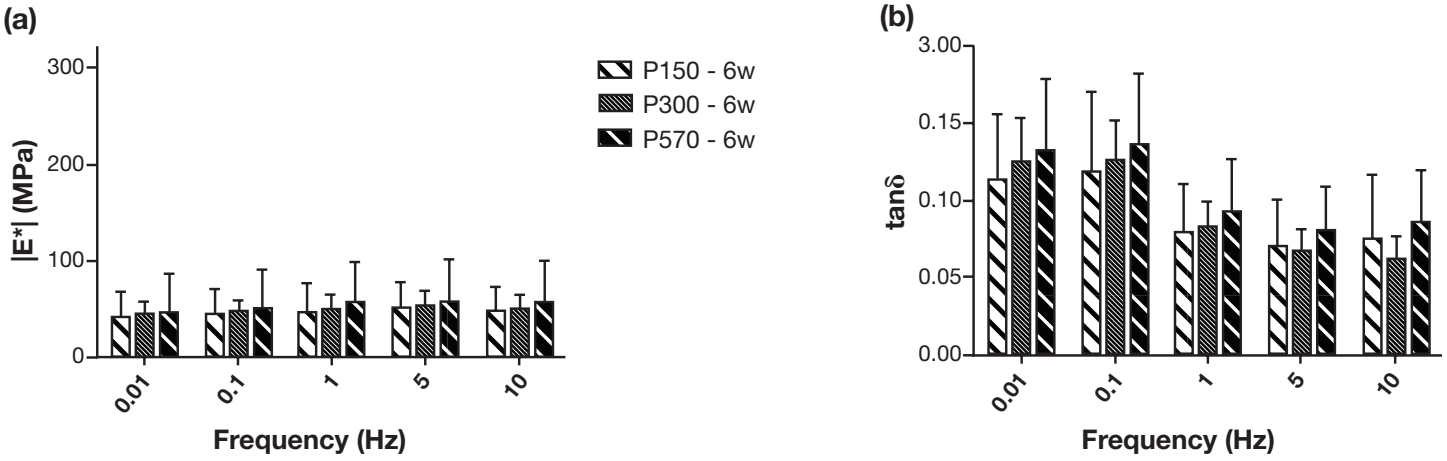

Figure 2. Neither a) |E*| nor b) tanδ of injured tendons differed with age.

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References

1. Maffulli N, Waterston SW, Squair J, et al. Changing incidence of Achilles tendon rupture in Scotland: a 15-year study. *Clin J Sport Med* 1999;9:157-60.

2. Gillis C, Sharkey N, Stover SM, et al. Effect of maturation and aging on material and ultrasonographic properties of equine superficial digital flexor tendon. *Am J Vet Res* 1995;56:1345-50.

3. Oh JH, Kim SH, Kang JY, et al. Effect of age on functional and structural outcome after rotator cuff repair. *Am J Sports Med* 2010;38:672-8.

4. Dressler MR, Butler DL, Boivin GP. Age-related changes in the biomechanics of healing patellar tendon. *J Biomech* 2006;39:2205-12.

5. Buckley MR, Pathmanathan L, Mienaltowski MJ, et al. Age-Related Changes in Tendon Mechanical Properties are Not Enhanced by the Absence of Biglycan and Decorin. *Trans Orth Res Soc* 2012;37:1308.

6. Kumar A, Dunkman AA, Buckley MR, et al. Tendon Repair Response to Injury is Affected by the Absence of Biglycan and Decorin. *Trans Orth Res Soc* 2012;37:158.

7. Lin TW, Cardenas L, Glaser DL, et al. Tendon healing in interleukin-4 and interleukin-6 knockout mice. *J Biomech* 2006;39:61-9.

8. Sklenka AM, Levy MS, Boivin GP. Effect of age on collagen fibril diameter in rabbit patellar tendon repair. *Comp Med* 2006;56:8-11.

9. Dressler MR, Butler DL, Boivin GP. Effects of age on the repair ability of mesenchymal stem cells in rabbit tendon. *J Orthop Res* 2005;23:287-93.