Aged Tendons Exhibit Muted Remodeling Responses to Varying Levels of Cyclic Tensile Strain

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INTRODUCTION: Musculoskeletal injuries disproportionally effect aging and elderly populations, yet the biological drivers of age-related tendon degeneration are unknown. Healthy tendons sense changes in their mechanical environment and remodel their extracellular matrix (ECM) to meet the functional needs of the tissue [1]. We have previously documented that young tendon explants display an adaptive remodeling response to various levels of cyclic and static strain *ex vivo* [2]. We found that 3% cyclic strain serves as a mechano-setpoint for young flexor tendon explants, maintaining matrix composition by increasing tenocyte metabolic activity and protein synthesis. It is hypothesized that this mechanically-driven adaptive response is lost with aging, and that dysfunctional mechano-sensing and turnover capacity puts aged tissues at increased risk for injury. In a recent study, we documented divergent responses of young and aged tendon explants to an altered mechanical stimulus. Despite similar properties at baseline, aged tendons exhibited reduced metabolism, proliferation, and matrix synthesis in response to stress deprivation [3]. However, we have not yet explored the remodeling capacity of aged tendon explants to cyclic tensile strain. Therefore, the objective of this study was to investigate the response of aged tendon explants to various levels cyclic tensile strain and identify age-specific mechanisms of mechanically-driven remodeling. We hypothesized that aged tendon explants would display a muted adaptive response to changes in tensile strain and exhibit a shifted homeostatic mechano-setpoint from that of young tendons.

METHODS: Flexor digitorum longus (FDL) tendon explants were harvested from aged (22-month) male C57BL/6J mice (BU IACUC approved) [3]. Explants were gripped at a 10mm gauge length and cultured in an incubator-housed tensile loading bioreactor. Explants were subjected to stress deprivation (SD) as well as 1%, 3%, 5% and 7% cyclic strain (CS). CS tendons were preloaded to a 0% strain of 20g and loaded using a displacement-controlled waveform at 1 Hz for 1-hour followed by a 5-hour hold at 0% strain. This loading protocol was repeated 4 times a day for 7-days of culture. Metabolism was measured via a resazurin reduction assay. Biochemical assays were performed for DNA content (PicoGreen), GAG content (DMMB), and total collagen content (OHP) [3]. Matrix biosynthesis was assessed using 24-hr incorporation of radiolabels ^{3}H-proline (10 μCi/ml) and ^{35}S-sulfate (20 μCi/ml) to measure synthesis of total protein and sulfated glycosaminoglycans (sGAG), respectively [3]. Assays were normalized to tendon dry weight to account for tendon size. Generic MMP activity in spent culture medium was assessed using a fluorometric detection kit (AnaSpec). Statistical evaluation was performed using one-way ANOVAs with post-hoc Bonferroni corrected t-tests, significance at $p<0.05$ (solid lines) and trends at $p<0.1$ (dashed lines). Significance from day 0 ($p<0.05$) is marked with an asterix (*). All data is presented as mean ± 95% confidence interval.

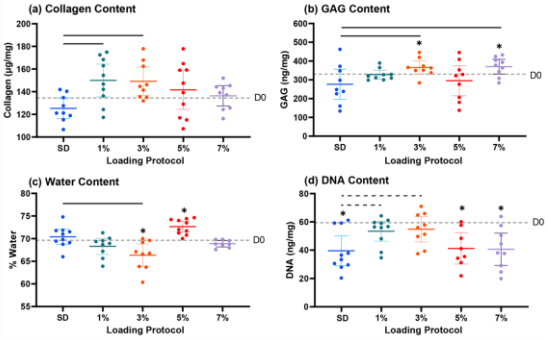

Figure 1. (a) Collagen, (b) GAG, (c) water, and (d) DNA content for stress-deprived (SD), 1%, 3%, 5%, and 7% cyclically-strained aged tendon explants. Solid bar indicates significant difference from SD ($p<0.05$) and dotted line indicates trend ($p<0.1$). Day 0 data shown as grey dotted line with asterisk (*) indicating significant differences ($p<0.05$).

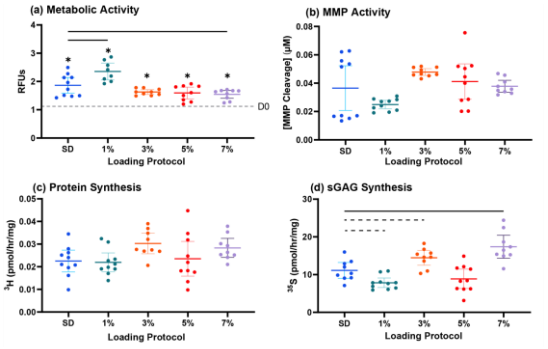

Figure 2. (a) Metabolic activity, (b) MMP activity, (c) total protein synthesis, and (d) sGAG synthesis for stress-deprived (SD), 1%, 3%, 5%, and 7% cyclically-strained aged tendon explants. Solid bar indicates significant difference from SD ($p<0.05$) and dotted line indicates trend ($p<0.1$). Day 0 data shown as grey dotted line with asterisk (*) indicating significant differences ($p<0.05$).

RESULTS: Collagen content increased for 1% and 3% CS compared to SD group; however, no loading protocol elicited significant differences from baseline conditions (Fig. 1a). GAG content was significantly higher in 3% and 7% CS groups compared to SD and baseline conditions (Fig. 1b). While 3% CS decreased tendon hydration and 5% CS increased tendon hydration, SD, 1%, and 7% CS maintained baseline water content (Fig. 1c). Only 1% and 3% CS sustained baseline DNA content while SD, 5%, and 7% CS led to significant declines (Fig. 1d). All groups displayed elevated metabolic activity from baseline but metabolic activity was found to be highest at 1% CS and lowest at 7% CS (Fig. 2a). While no significant differences are found in MMP activity, 1% CS tendons showed consistently low responses (Fig. 2b). No significant differences were found in total protein synthesis (Fig. 2c). 1% CS decreased sGAG synthesis compared to SD while 3% and 7% increased sGAG synthesis (Fig. 2d). The response of aged tendons was compared to previously published data in young (4-month) explants (Fig. 3) [2]. Young tendons display increased protein synthesis with significant differences between loading protocols (Fig. 3a). Metabolic activity is higher in young explants and peaks at 3% CS (Fig. 3b). MMP activity is found to be significantly higher at 5% and 7% CS (Fig. 3c).

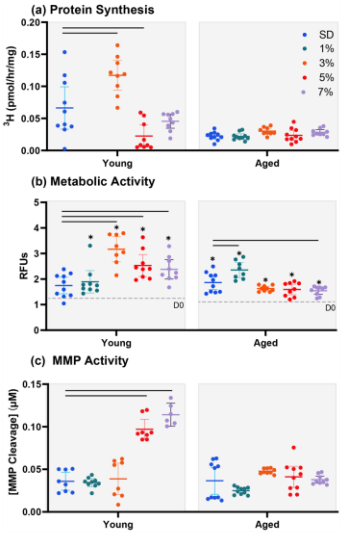

Figure 3. (a) Protein synthesis, (b) metabolic activity, and (c) MMP activity for stress-deprived (SD), 1%, 3%, 5%, and 7% cyclically-strained young and aged tendon explants. Solid bar indicates significant difference from SD ($p<0.05$). Day 0 data shown as grey dotted line with asterisk (*) indicating significant differences ($p<0.05$).

DISCUSSION: This work demonstrates an altered mechano-set point and muted mechano-response for aged tendon explants compared to young. Overall, the response to various levels of mechanical loading was found to be less adaptive in aged tissues. Even at optimal loading, aged tendons demonstrate reduced biosynthesis capacity. At higher strains (5-7%), young tendons show significant increases in MMP activity, indicating a degradatory profile; however, aged tendons fail to elicit a similar response. While smaller differences were found between loading conditions in aged explants, it appears as though 1% CS best maintains baseline conditions and leads to the highest metabolic activity with low MMP activity. This indicates a shift in the mechano-set point to lower magnitudes of cyclic strain for aged tendons, which is supported by literature reporting age-related decreases in muscle mass and force generation, resulting in reduced *in vivo* loads [4]. Future work will explore the biological mechanisms behind this altered response through gene expression and proteomic analysis. We are also specifically investigating the contribution of cellular senescence to altered mechano-responses in aged tendons [5].

SIGNIFICANCE/CLINICAL RELEVANCE: This work furthers our understanding of the regulation of tissue homeostasis in aged tendons, which can inform clinical rehabilitation strategies for treating elderly patients.

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