

Aging Disrupts Non-Collagenous Mediators of Tendon Structure During Mechanical Adaptation

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INTRODUCTION: Aging is a primary risk factor for degenerative tendon injuries and is strongly linked to dysfunctional extracellular matrix (ECM) remodeling. Prior work demonstrates that aged tendons exhibit impaired remodeling to various mechanical stimuli including complete mechanical unloading [1,2], acute compression [3], and cyclic tensile strain [4]. These studies report muted responses in aged tissues suggesting a loss of mechanically-sensitive adaptations, yet the underlying mechanisms remain unknown. To investigate this, we utilized genome-wide transcriptomics to identify genes and regulatory pathways altered in aged tendon explants during long-term mechanical loading. We then employed our recently developed exercise protocol [5] to precisely modulate the tendon mechanical environment *ex vivo*. Using a multiscale assessment of ECM turnover, we aimed to detect specific ECM adaptations impaired or absent in aged tendons. We hypothesized that age-related differences in remodeling would involve non-collagenous matrix components, which show heightened sensitivity during dynamic remodeling.

METHODS: Flexor digitorum longus (FDL) tendon explants were harvested from 4-month (young) and 24-month (aged) male C57BL/6J mice (BU IACUC approved) and cultured in a tensile-loading bioreactor [4]. Initial studies were conducted in males to compare to previous data; studies in females are ongoing. Explants were subjected to 1% cyclic strain as a control loading condition [4]. Samples for transcriptomic analysis were collected at baseline (day 0; n=4/age) and after 14 days of *ex vivo* loading (day 14; n=6/age). Differentially expressed genes (DEGs) were filtered using a false discovery rate (FDR) < 0.01 and an absolute fold change (FC) > 2. Baseline differences were defined as Aged D0 – Young D0. Age-related differences in loading adaptation were calculated as the difference in loading-induced change: (Aged D14 – Aged D0) – (Young D14 – Young D0). Significant DEGs were compared to the matrisome gene set [6]. Separately, we investigated the response of young and aged tendon explants to an *ex vivo* exercise protocol (Fig. 1) [5]. Adaptations were assessed at day 14 under control (Ctl) and exercise (Ex) loading. qPCR (n=4/group) was used to quantify the relative gene expression of ECM remodeling targets. Incorporation of radiolabels ³H-proline and ³⁵S-sulfate (n=5/group) was used to measure real-time synthesis of total protein and sulfated glycosaminoglycans (sGAG) [4]. Biochemical assays (n=5/group) were performed for GAG content (DMMB) and total collagen content (OHP) [4]. Protein levels of secreted MMPs and TIMPs in day 12 culture media (n=5/group) were quantified using a custom-designed multiplex ELISA. All data are presented as Log₂(Ex/Ctl) to indicate adaptations to exercise, where positive values indicate increases with exercise and negative values indicate decreases. Statistical significance was assessed using two-tailed t-tests for loading and age effects (*p<0.05).

RESULTS: Transcriptomic analysis revealed distinct gene expression profiles between young and aged tendons at baseline (Fig. 2, left) and during the response to mechanical loading (Fig. 2, right). Ten genes differed significantly both at baseline and in the loading response. In response to exercise, *Coll1a1* (Fig. 3a) and *Col3a1* (Fig. 3b), as well as total collagen content (Fig. 3d), were unchanged and did not differ between ages. Total protein synthesis increased with exercise in both ages, with a greater increase in young tendons (Fig. 3c). *Fmod* (Fig. 3e) expression increased in young only, while *Bgn* (Fig. 3f) increased in both ages. sGAG synthesis (Fig. 3g) increased with exercise for young tendons, but GAG content (Fig. 3h) was unchanged. *Il6* (Fig. 3i) expression was lower in aged tendons, while *Tgfb1* (Fig. 3j) was upregulated with exercise only in young tendons. *Mmp3* (Fig. 3k) expression was reduced in aged tendons while *Mmp13* (Fig. 3l) was downregulated in young only. On the protein level, MMP-3 (Fig. 3m) was unchanged, MMP-13 (Fig. 3n) increased in young, and TIMP-1 (Fig. 3o) decreased in aged samples.

DISCUSSION: In agreement with prior work, transcriptomic profiling revealed altered ECM-associated gene expression between native young and aged FDL tendons [7]. We also identified age-specific differences in loading-induced expression, indicating regulatory changes that occur independently of baseline transcriptional differences. Notably, matrix metalloproteinases *Mmp3*, which mediates non-collagenous ECM breakdown, and *Mmp16*, whose role is less well defined but likely involves collagen and glycoprotein turnover, were differentially expressed, suggesting impaired ECM clearance capacity. Additionally, *Lox* and *Loxl4*, which catalyze collagen crosslinking to stabilize the fibrillar ECM, were differentially expressed, which may directly influence tendon mechanical properties. We also observed differential regulation of genes associated with TGF- β activation, cell-cell and cell-ECM adhesion, and cellular stress responses. In all, transcriptomic data revealed distinct age-specific, loading-induced expression profiles involving ECM support and organization molecules, suggesting impaired collagen fibril assembly and compromised matrix architecture. We then compared the adaptive responses of young and aged tendons to increased strain. Consistent with prior work [5], young tendons exhibited exercise-induced ECM adaptations; however, many of these responses were diminished or absent in aged tendons. Specifically, aged tendons showed dysregulated proteoglycan turnover and altered exercise-associated signaling, suggesting impaired sensing and a diminished capacity for adaptive ECM remodeling. We also observed that TIMP-1 protein production was significantly reduced in exercised aged tendons. As TIMP-1 is a broad-spectrum inhibitor of MMPs, decreased production could increase MMP activity, imbalance in TIMP/MMP ratios, and accelerated ECM degradation. Interestingly, collagen synthesis and content were unaffected by aging or loading, suggesting that collagen turnover may not be the primary driver of age-related tendon dysfunction. Instead, this highlights the significance of signaling molecules and organizational regulators in mediating loading-induced adaptations that lead to collagen incorporation and remodeling. Future work will determine if similar age-related impairments extend into female tendons and will further investigate ECM organization through analysis of crosslinking and fibril morphology.

SIGNIFICANCE/CLINICAL RELEVANCE: This work furthers our understanding of exercise-induced remodeling in aged tendons, which can inform clinical rehabilitation strategies for treating elderly patients.

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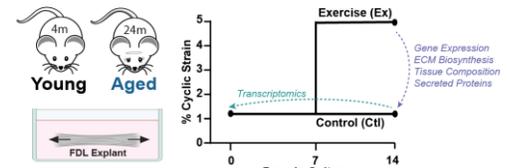


Figure 1. Young and aged FDL tendon explants were subjected to either control (Ctl) or exercise (Ex) *ex vivo* strain protocols. Age-related remodeling under control loading was assessed using transcriptomic analysis. Separately, relative gene expression, ECM biosynthesis, tissue composition, and secreted protein measurements were performed to evaluate age-related adaptations to exercise.

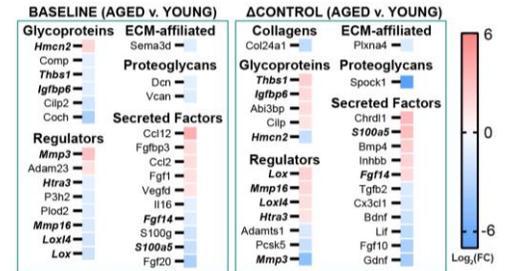


Figure 2. Heat map of Log₂(FC) of extracellular matrix-associated DEGs (FDR<0.01 & |FC|>2) between aged and young tendons at baseline (left) and for the control loading-induced change (right). Genes in bold are significantly different in both contrasts.

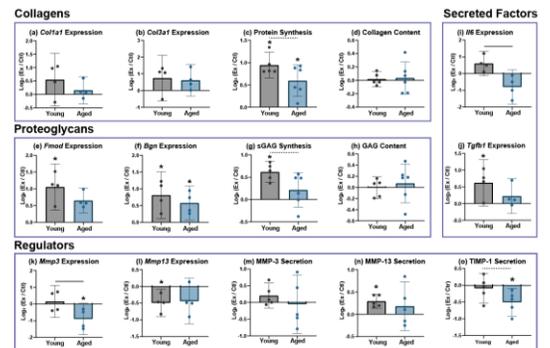


Figure 3. Synthesis of the collagenous matrix was assessed by (a) *Col1a1* and (b) *Col3a1* gene expression, as well as (c) total protein synthesis and (d) collagen content. Synthesis of the matrix proteoglycans was evaluated by (e) *Fmod* and (f) *Bgn* gene expression, along with (g) sGAG synthesis and (h) GAG content. Additional matrix turnover mechanisms were examined via gene expression of secreted factors (i) *Il6* and (j) *Tgfb1*, and gene and protein production of ECM regulators: (k) MMP-3, (l) MMP-13, and (o) TIMP-1. Data are shown as Log₂(Exercise/Control), where positive values indicate increases and negative values indicate decreases with exercise. Significant differences between exercise and control are indicated with an asterisk (*, p < 0.05). Age-related differences in adaptation are indicated with a solid line (—, p < 0.05) or dotted line (---, p < 0.1).