Transcriptomic Responses to Cyclic Loading Reveal Overload-Driven Networks in Early Tendon Degeneration

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Introduction: Mechanical stress is essential for maintaining tendon homeostasis; however, insufficient or excessive loading disrupts tendon structure and function **[1]**. Repetitive overloading can generate micro-tears, disrupt extracellular matrix (ECM) organization, and contribute to chronic degeneration, known as tendinopathy, a prevalent and debilitating musculoskeletal disorder that is common in the Achilles tendon **[2-4]**. Despite its clinical significance, the cellular and molecular mechanisms linking mechanical overload to tendon degeneration are not fully defined, particularly at the level of nuclear regulators. To address this, we profiled transcriptomic changes in bovine Achilles tenocytes subjected to degenerative cyclic tensile loading conditions followed by pathway and upstream regulator analyses to nominate factors involved in responses to excessive loading. This approach enables direct association of mechanical loading parameters with transcriptional responses and provides insight into regulatory networks that may be targeted to prevent or reverse overload-induced tendon degeneration.

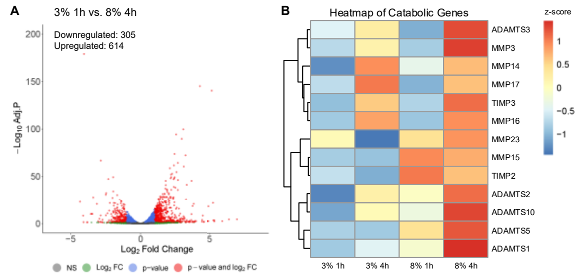

Figure 1. A: Volcano plot of DEGs for 3% 1 h vs. 8% 4 h at 1 Hz, **B:** Z-score heatmap of catabolic genes across 3% or 8% strain for 1 or 4 h loading (n=3).

Methods: Tenocytes isolated from juvenile bovine Achilles tendons were expanded on tissue culture plastic to passage 1 and seeded on aligned poly(ε-caprolactone) (PCL) nanofibrous scaffolds (n=3-4 biological replicates). After 2 days in basal media, the scaffolds were subjected to uniaxial cyclic strain at 3% or 8% for 1 or 4 hours at 1 Hz on 3 consecutive days. Unloaded free-swelling scaffolds served as controls. RNA was extracted immediately post-loading for RNA-sequencing. Differential expression was performed with adjusted p-value < 0.05 and absolute $\log_2$ fold change > 1 considered significant. Differentially expressed genes (DEGs) from 3% 1 h 1 Hz versus 8% 4 h 1 Hz were analyzed using Ingenuity Pathway Analysis (IPA), with expression log ratio and adjusted p-values as observations. IPA Core Analysis identified enriched canonical pathways and predicted upstream regulators. To examine recovery, additional scaffolds were loaded at 8% 4 h 2 Hz for 3 days followed by 3 days of free-swelling culture. RT-qPCR measured expression of type-I collagen (COL-1), type-III collagen (COL-3), tenascin-C (TNC), and matrix metalloproteinase-3 (MMP-3) relative to GAPDH. Statistical analyses were performed using t-test or ANOVA.

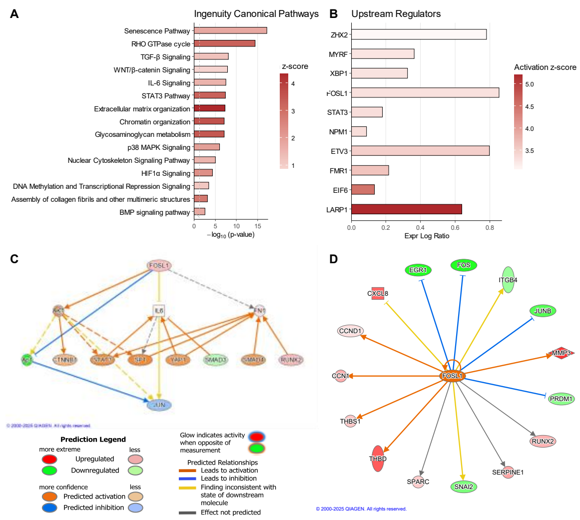

Figure 2. A: IPA canonical pathways enriched in 8% 4 h vs. 3% 1 h, **B:** Top predicted activated upstream regulators, **C:** FOSL1 mechanistic network, **D:** FOSL1 downstream network.

Results: RNA-seq analysis identified 8% 4 h loading as the overload regimen, with the largest DEG set compared to free-swelling control, while 3% 1 h represented a mild condition **[5]**. Direct comparison of 3% 1 h and 8% 4 h at 1 Hz yielded 305 downregulated and 614 upregulated genes **(Fig. 1A)**. The z-score heatmap revealed robust induction of catabolic genes at 8% 4 h and relative suppression at 3% 1 h, while 3% 4 h and 8% 1 h showed partial induction **(Fig. 1B)**. IPA Canonical Pathways were enriched for senescence, inflammation, ECM remodeling, mechanotransduction, and chromatin pathways, including TGF-β, WNT/β-catenin, IL-6, STAT3, p38 MAPK, HIF1A, nuclear cytoskeleton, and glycosaminoglycan metabolism pathways **(Fig. 2A)**. Upstream Regulator analysis **(Fig. 2B)** nominated predicted activated regulators, including cytokine and stress nodes (STAT3, XBP1), an AP-1 family member (FOSL1), a transcriptional repressor (ETV3), and translational factors (NPM1, EIF6, LARP1). Focusing on FOSL1 revealed a directional cascade linking upstream drivers (IL-6, STAT3, SMAD3/4, YAP1, AKT, SP1, CTNNB1) to AP-1 and FN1, indicating cooperative drivers of the 8% 4 h expression profile **(Fig. 2C)**. The FOSL1 downstream network predicted activation of CCND1, CCN1, THBS1/THBD, and MMP3, and inhibition of EGR1, FOS, JUNB, and PRDM1 **(Fig. 2D)**. Building on our previous observation that higher frequency loading (2 Hz) exacerbates catabolic activity while suppressing anabolic gene expression **[5]**, we examined recovery following overload. 8% 4 h 2 Hz loading for 3 days (3d) resulted in unchanged COL-1, decreased COL-3 and TNC, and increased MMP-3 expression **(Fig. 3A)**. Following 3 days of free-swelling recovery (3dR), COL-3 expression approached control, TNC exceeded control, and MMP-3 declined toward levels observed in control, indicating partial recovery.

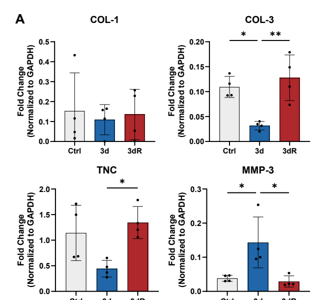

Figure 3. A: Gene expression after 8% 4 h 2 Hz loading for 3 days with 3 days of free-swelling recovery (normalized to GAPDH, *p<0.05, **p<0.01, n=4, mean ± SD).

Discussion: This study underscores the impact of repetitive high-intensity mechanical loading on tenocytes, revealing coordinated transcriptional changes that resemble early tendon degeneration. Excessive loading induced stress, inflammatory, mechanotransductive, and ECM remodeling pathways. Enrichment of chromatin organization and DNA methylation terms, together with predicted regulators that connect signaling to transcriptional repression, suggests nuclear-level control of these responses. Network analysis indicates convergence of IL-6, STAT3, SMAD, YAP1, AKT, SP1, and β-catenin on AP-1 and FN1, positioning these nodes at the interface of inflammatory signaling and matrix turnover. Within the AP-1 family, subunit balance appeared shifted, with FOSL1 predicted to be active while several immediate-early members (FOS, JUNB) were suppressed, a configuration that favors catabolic outputs. Downstream of FOSL1, predicted activation of MMP3, CCND1, and CCN1 supports roles in matrix remodeling, adhesion signaling, and proliferation, while inhibition of EGR1, FOS, JUNB suggests selective suppression of immediate-early AP-1 components and specific adhesion or fate regulators. These findings indicate AP-1 subunit rebalancing, rather than uniform AP-1 activation, is a key determinant of overload-induced programs. The recovery experiment demonstrated that elements of this response are reversible, and this plasticity underscores the potential for strategies to mitigate overload-induced degeneration. Ongoing studies will directly test causality by perturbing FOSL1 during loading and assess recovery dynamics comprehensively through combined RNA-seq and ATAC-seq to define persistent or reversible regulatory features.

Significance: This study defines upstream regulatory networks linking mechanical overload to catabolic and remodeling responses in tenocytes. These insights reveal mechanisms of overuse-driven tendon degeneration and support therapeutic strategies aimed at preventing or reversing of tendinopathy.

References: [1] Cook+, *BJSM* 2009; [2] Glazebrook+, *J Orthop Res* 2008; [3] Maeda+, *Connect Tissue Res* 2010; [4] Freedman+, *Sci Rep* 2018; [5] Heo+, *ORS* 2025

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