Deciphering Epigenetic Responses in Tenocytes to Long-Term Dynamic Tensile Loading: Implications for Tendon Degeneration Mechanisms

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Introduction: Tendinopathy, a prevalent musculoskeletal disorder characterized by chronic tendon degeneration, poses a significant clinical challenge, especially in the Achilles tendon. Despite its prevalence, the intricate cellular and molecular mechanisms driving tendinopathy progression remain incompletely understood. Overuse injury, involving repeated tendon strain leading to micro-tears, is a primary cause of tendinopathy **[1]**. Dynamic tensile loading pathologically influences tendon mechanical properties, affecting collagen organization, tissue composition, as well as the expression of anabolic and catabolic genes **[2, 3]**, impacting their phenotype and contributing to pathology. Chromatin organization, governed by epigenetic modifications, plays a crucial role in gene expression and cell differentiation **[4]**. In the context of tendon degeneration, the impact of altered biophysical environments on epigenetic status is an area of growing interest. Thus, this study investigates how repetitive tensile loading, simulating an overuse tendon degenerative model *in vitro*, influences gene expression and histone modifications in bovine Achilles tenocytes. The findings from this research aim to enhance our understanding of the molecular underpinnings of tendinopathy, offering insights to pave the way for targeted therapeutic interventions.

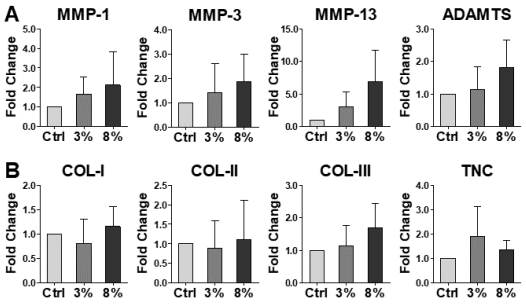

Figure 1. A: Catabolic and **(B)** anabolic gene expression of tenocytes subjected to dynamic loading at 3% or 8% strain for 5 hours (normalized to GAPDH, n=4-5, error bars: mean + SD).

Methods: Tenocytes from juvenile bovine Achilles tendons were isolated and expanded on tissue culture plastic up to passage 1 (P1). To investigate the impact of long-term dynamic tensile loading on gene expression and histone modifications, P1 tenocytes were seeded onto aligned poly(ε−caprolactone) (PCL, 20 × 55 mm^2) nanofibrous scaffolds (2 × 10^6 cells per scaffold) followed by 2 days of culture in basal growth media. A custom tensile loading bioreactor, programmed to sinusoidal waveforms at 3% or 8% strain amplitudes (at 1 Hz), applied cyclic uniaxial force to the tenocyte-seeded scaffolds for 3 or 5 hours. Following dynamic loading, samples were processed for RNA or histone extraction. Relative fold changes in mRNA expressions for type-I collagen (COL-I), type-II collagen (COL-II), type-III (COL-III), tenascin-C (TNC), matrix metalloproteinases (MMPs, MMP-1, -3, and -13), and a disintegrin and metalloproteinase (ADAMTS) was determined using RT-qPCR, using GAPDH as the reference gene. Immunoblotting of H3K9Ac and H3K4me3 (epigenetic activators), H3K27me3 and H3K9me3 (epigenetic repressors), and H3 were performed. Relative fold changes in protein expressions of H3K9Ac, H3K4me3, H3K27me3, and H3K9me3 were normalized to those from no loading condition (Ctrl). To explore the role of inflammation as part of the mechanism behind tendon degeneration, tenocytes seeded on PCL scaffolds were treated with 20 ng/mL of TNFα overnight prior to histone extraction and subsequent immunoblotting. Relative fold changes for histone modification markers were normalized to the control without loading and without TNFα treatment condition.

Results: The RT-qPCR results demonstrated an increasing trend in the expression of catabolic genes (MMP-1, MMP-3, MMP-13, ADAMTS) in tenocytes under 3% and 8% strain for 5 hours loading (**Fig. 1A**). However, interestingly, there were no significant changes in the expression of anabolic genes (COL-I, COL-II, COL-III) as well as TNC (**Fig. 1B**). These data suggest heightened structural protein and extracellular matrix remodeling in response to prolonged overloading. Application of 3% strain loading for 3 hours to tenocytes led to an approximately two-fold decrease in H3K9Ac and H3K4me3 expression, indicative of gene activation, and a reduction in H3K27me3 and H3K9me3 expression, markers of gene repression (**Fig. 2A, B**). This suggests that specific histone modifications governing both gene activation and repression contribute to the response to repetitive tensile overloading. Additionally, treating tenocytes on PCL scaffolds with TNFα without loading mirrored the levels observed with 3-hour 3% cyclic loading, reducing H3K9Ac, H3K27me3, and H3K9me3 (**Fig. 3A, B**). Given that TNFα is a pro-inflammatory cytokine released in response to the pathological tendon environment, inducing anabolic and catabolic events **[5, 6]**, this indicates that cyclic tensile overloading simulates conditions similar to pathological tendon loading.

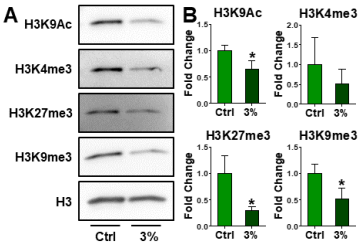

Figure 2. A: Immunoblot of histone markers extracted from tenocytes subjected to dynamic loading at 3% strain for 3 hours and **(B)** quantification (normalized to H3, *p<0.05 vs. control, n=3, error bars: mean + SD).

Discussion: This study unveils intricate molecular responses in tenocytes to long-term dynamic tensile loading, providing insights into tendon degeneration mechanisms. The elevated expression of catabolic genes (i.e., MMP-1, -3, -13, ADAMTS) in response to prolonged long-term cyclic loading indicates a heightened state of remodeling within tenocytes. This observation aligns with the paradigm of overuse injuries, where repeated mechanical stress induces micro-tears and triggers a cellular response for tendon repair and remodeling **[1]**. The observed reduction in histone modifications associated with both gene activation (i.e., H3K9Ac, H3K4me3) and repression (H3K27me3, H3K9me3) in response to 3% strain for 3 hours underscores the complex regulatory role of epigenetic modifications in tenocytes. This suggests that these specific histone markers may orchestrate the expression of different genes in response to degenerative mechanical loading environments in tendons. Ongoing studies are more focused on exploring the impact of mechanically regulated histone modification status on gene expression in tenocytes through ChIP-seq. Moreover, the similarities in histone modification levels between the cyclic loading and TNFα treatment without loading imply that the long-term cyclic tensile loading mimics conditions akin to pathological tendon loading. The reduction of histone modification markers in response to TNFα aligns with the known pro-inflammatory effects of this cytokine, emphasizing its role in driving anabolic and catabolic events in the tendon microenvironment. Current work is determining parameters for physiological and pathological *in vitro* loading in conjunction with identifying how mechanically induced changes in histone modifications correlate with altered gene expression by profiling accessible chromatin through ATAC-seq and by understanding the impact of the epigenetic landscape through ChIP-seq.

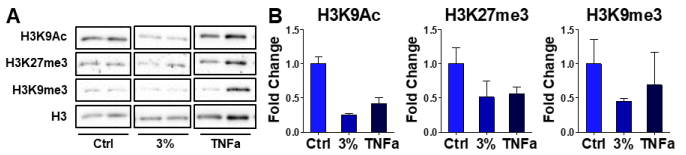

Figure 3. A: Immunoblot of histone markers extracted from tenocytes subjected to dynamic loading at 3% strain or 20 ng/mL TNFα overnight treatment and **(B)** quantifications (normalized to H3, n=2, error bars: mean + SD).

Significance: This study advances our understanding of the molecular and epigenetic responses of tenocytes to dynamic tensile loading, offering potential targets for therapeutic interventions in tendinopathy. The identification of specific histone modifications and gene expression patterns associated with overuse conditions provides a foundation for future research aimed at developing targeted strategies for tendon regeneration and repair.

References: [1] Aicale+ 2018, *J Orthop Surg Res;* [2] Freedman+ 2018, *Sci Rep*; [3] Maeda+ 2009, *J Appl Physiol*; [4] Bannister+ 2011, *Cell Research*; [5] John+ 2010, *J Orthop Res*; [6] Ellis+ 2022, *J Immunol Regen*.

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