Cyclic Loading Duration and Recovery Dynamics Drive Early Degenerative Programs in Tenocytes

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Introduction: Tendon disorders are highly prevalent, with an estimated 32 million tendon injuries occurring globally each year, and Achilles tendon pathologies affecting ~6% of the U.S. population during their lifetime [1,2]. Repetitive, high-intensity mechanical loading is a major contributor to tendinopathy, which leads to the accumulation of microinjuries that fail to heal properly. Overuse can cause irreversible changes in tendon structure and mechanics, resulting in chronic pain and loss of function. Even with the widespread nature of tendon disorders, the underlying cellular mechanisms that drive disease progression remain poorly understood, and current treatments focus primarily on symptom management rather than addressing root causes. Tendinopathy is characterized by compromised mechanical properties, disrupted collagen architecture, and altered extracellular matrix (ECM) composition [3]. Proper mechanical stimulation is critical for maintaining tendon homeostasis, as both underloading and overloading can perturb tissue organization and gene expression, contributing to tendon degeneration [4]. Thus, understanding how tenocytes, the primary resident cells in tendons, respond to varying mechanical stimuli is essential for better understanding the pathogenesis of tendinopathy. In this study, we investigated how tenocytes respond transcriptionally to variations in mechanical loading duration and recovery. Using a short-term *in vitro* overloading model, we examined the effects of high-intensity cyclic strain applied for 1 or 3 days, followed by a 24-hour recovery period, on the expression of genes associated with tendon identity and matrix remodeling.

Methods: Aligned nanofibrous polycaprolactone (PCL) scaffolds were fabricated via electrospinning and cut into two sizes: large (20 mm × 55 mm) or small (5 mm × 55 mm). Scaffolds were hydrated in ethanol and coated in fibronectin to promote cell adhesion. Juvenile bovine Achilles tenocytes (from four biological replicates) were expanded in 100 mm tissue-culture plates to approximately 80% confluency, then seeded onto the scaffolds at densities of 2×10^6 (large scaffolds) or 7.5×10^5 (small scaffolds) cells per scaffold (**Fig. 1**). Cyclic uniaxial tensile loading was applied to tenocyte-seeded scaffolds at 8% strain amplitude and 2 Hz for 4 hours per day using a custom bioreactor [7]. Large scaffolds were used to compare 1-day vs. 3-day loading durations. Small scaffolds were used to evaluate the effects of a 24-hour recovery period post-loading (**Fig. 3A**). Control scaffolds remained under static, free-swelling conditions in culture media throughout the 3-day period without mechanical stimulation. At the final loading or recovery time point, TRIzol was added to each scaffold for RNA extraction. RT-qPCR was performed to measure expression of tenogenic markers type I collagen (COL1), type III collagen (COL3), tenascin-C (TNC), and the catabolic enzyme matrix metalloproteinase-3 (MMP3), with GAPDH as the reference gene. Statistical significance ($p < 0.05$) was determined using two-sample t-tests or ANOVA.

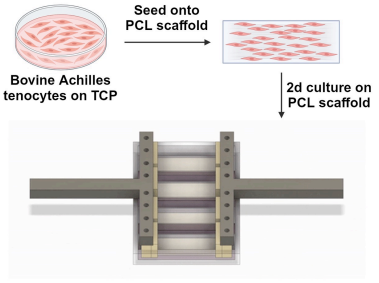

Figure 1. Schematic illustration of the experimental workflow, including scaffold preparation, cyclic loading, and gene expression analysis.

Results: High-intensity cyclic tensile loading (8% strain, 2 Hz, 4 h/day for 3 days) decreased expression of COL1, COL3, and TNC, while increasing expression of the catabolic marker MMP3 (**Fig. 2A**), consistent with a degenerative response [7]. No statistically significant differences were observed between 1-day (1d) and 3-day (3d) loading groups; however, expression trends suggested decreased COL1 and increased COL3 and TNC with prolonged loading. MMP3 expression decreased slightly between 1d and 3d, though levels remained elevated relative to controls (**Fig. 2B**). The effect of a 24-hour recovery period revealed distinct transcriptional patterns. TNC expression increased from 1d to 3d of loading and remained elevated following recovery, though levels declined slightly in the post-recovery groups (1dR, 3dR). Notably, TNC expression in the 1dR group was significantly lower than control levels. In contrast, MMP3 expression progressively increased with loading duration, and was further amplified during recovery, reaching significantly higher levels than nearly all other groups (**Fig. 3B**).

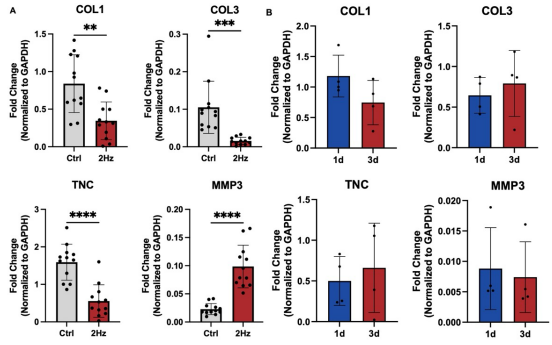

Figure 2. A: Decreases in tenogenic gene expression relative to baseline (free-swelling control) in tenocytes exposed to 3 days of cyclic loading (8% strain, 2 Hz, 4 h/day) (n = 12 biological replicates; mean ± SD; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001). **B:** Gene expression in tenocytes subjected to 1 or 3 days of loading (n = 4 biological replicates; mean ± SD).

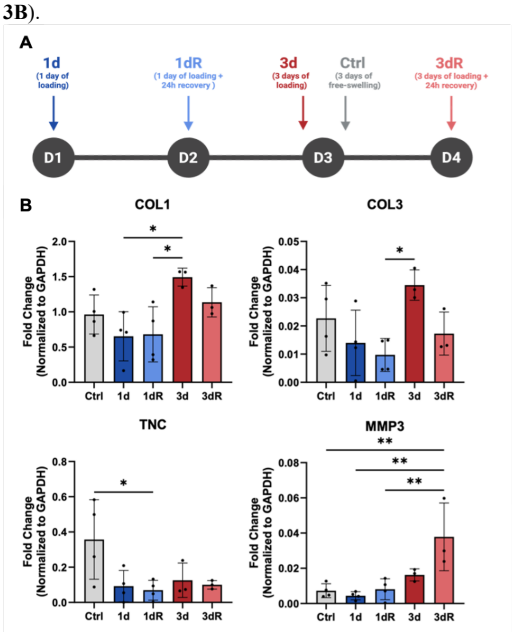

Figure 3. A: Schematic of the recovery study timeline for RNA extraction. **B:** Gene expression of tenocytes subjected to 1 day (1d), 1 day + 24 h recovery (1dR), 3 days (3d), and 3 days + 24 h recovery (3dR) of cyclic loading (n = 4 biological replicates; mean ± SD; *p < 0.05, **p < 0.01).

Discussion: Experimental patterns are consistent with an early cellular response to overloading, marked by a shift from strong COL1 expression toward more compliant COL3, reflecting rapid matrix remodeling [3]. Elevated TNC expression, commonly associated with tissue injury, may indicate an attempted reparative response [5]. Although increased MMP3 expression was anticipated between 1 and 3 days of loading due to its role in ECM degradation [6], the modest transcriptional changes suggest that protein abundance and enzymatic activity may diverge from mRNA levels. Future studies examining protein activity and other MMP family members will be important to clarify this relationship. The recovery experiments revealed that MMP3 expression continued to rise even after mechanical stimulation ceased, indicating that 24 hours of rest was insufficient to restore tenocyte homeostasis. Instead, a degenerative transcriptional profile persisted, with elevated catabolic markers and reduced tenogenic expression. Together, these findings suggest that high-intensity loading induces degenerative changes after only 1 day, which are sustained through 3 days and remain unresolved after short-term recovery. Longer recovery intervals and integration of molecular and protein-level analyses will be critical for future studies to fully define the dynamics of tenocyte recovery and degeneration.

Significance: This study highlights the critical role of loading duration and recovery timing in regulating tenocyte responses, offering foundational insight for developing *in vitro* models of tendon degeneration. These findings may guide the design of targeted regenerative therapies and rehabilitation strategies, ultimately improving clinical outcomes for patients with tendon disorders.

References: [1] Citro+, *J. Tissue Eng.* 2023.; [2] Patch+, *JAAPA* 2023.; [3] Wang, *J. Biomech.* 2006.; [4] Stańczak+, *Cell. Physiol. Biochem.* 2024.; [5] Järvinen+, *Scand. J. Med. Sci. Sports* 2000.; [6] Sakai+, *J. Biol. Chem.* 2025.; [7] Heo+, ORS 2025.

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