Mechanical Unloading via Sciatic Nerve Resection Delays Proliferative and Matrix-Driven Achilles Tendon Growth during Early Postnatal Development

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INTRODUCTION: Musculoskeletal tissues, including tendons, are highly responsive to mechanical cues. Both excessive and insufficient loading can alter tendon composition, and drastic shifts in the mechanical environment are implicated in tendon pathology [1]. While the influence of loading on adult tendons and tendon healing is well established, much less is known about how the forces that arise with the activities of daily living regulate tendon growth in early postnatal development, a period marked by a shift from cell proliferation to increasing extracellular matrix (ECM) accrual. To investigate the role of mechanical unloading during early postnatal growth, we employed surgical sciatic nerve resection (SNR) at P1 to reduce hindlimb loading. Our approach targets the time when both proliferative and ECM-driven tendon growth are active. We hypothesize that mechanical loading is a critical driver of both proliferative-driven growth and ECM-driven growth in the Achilles tendon.

METHODS: All animals and procedures were IACUC approved. Sciatic Nerve Resection. Unilateral left sciatic nerve resection (SNR) was performed on post-natal day 1 (P1) in male and female *Col1a1(3.6 kb)-CFP* reporter mice with evaluation at post-natal days 14, 42, and 84 (N=28, 12 males, 16 females). SNR results in sustained gait abnormalities as we reported previously [2]. Cryohistology. Hindlimbs were harvested, formalin-fixed, embedded in OCT, and cryosectioned (8 μm) in the transverse plane. Fluorescent imaging with a nuclear counterstain, followed by brightfield imaging with toluidine blue staining were performed. Scanning Electron Microscopy was used to assess collagen fibril diameter. Sections were fixed, dehydrated, and coated with 6 nm thick platinum-palladium mixture prior to imaging. Image Analysis. Achilles tendon cross-sectional area and cell density were quantified using Fiji. Statistics. SNR limb vs. contralateral limb groups were compared via paired t-tests at α=0.05.

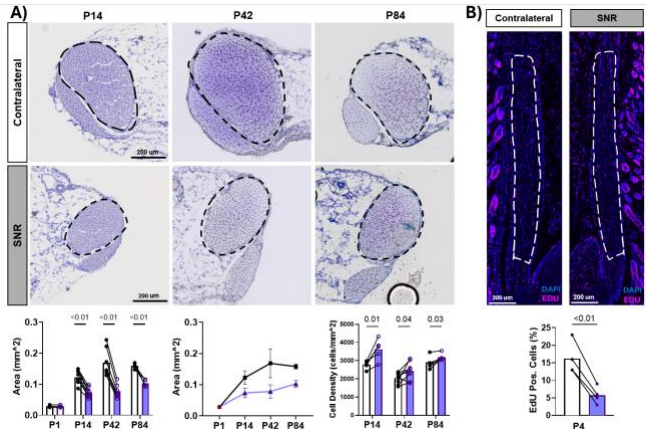

Fig. 1: Neonatal SNR significantly decreased Achilles tendon cross-sectional area (CSA, shown in rep. images) while increasing cell density (A) P1 samples were prior to surgery*. Cell proliferation was also reduced at P4 (B).

RESULTS: SNR resulted in smaller Achilles tendons. Our results indicate that the sustained gait abnormalities arising from SNR [2], alters the growth of the Achilles tendon in neonatal mice. Neonatal SNR significantly decreased Achilles tendon cross-sectional area (CSA) in the SNR limb at all timepoints (**Fig. 1A**, p<0.01). The contralateral limb exhibited steady CSA expansion from P14 to P42, whereas the SNR limb showed minimal growth between P14 and P42. Interestingly, the SNR limb displayed a surge in growth between P42 and P84, achieving ~25% of its total growth during this interval. The CSA reduction in the SNR limb was accompanied by a significant increase in cell density in the SNR tendon at all timepoints (**Fig.1A**, p<0.05). To determine whether early deficits in tendon growth corresponded with altered cell proliferation, we administered EdU injection at the time of SNR surgery. At P4, EdU labeling revealed a reduction in proliferating cells in the SNR tendon compared to the contralateral limb (**Fig. 1B**, p<0.01). SNR delays temporal decline in (Col1a1 3.6kb) expression: At P14, the contralateral limb displayed significantly higher CFP intensity than the SNR limb (**Fig. 2,** p < 0.001), consistent with reduced CSA at this time point. By P42, however, the SNR limb exhibited significantly higher CFP intensity compared to the contralateral side (p < 0.001). At P84, the contralateral limb showed a steeper reduction in signal, whereas the SNR limb maintained higher CFP intensity at this later point **(Fig. 2)**. SNR reduces collagen fibril diameter: Fibril diameters were significantly smaller in the SNR tendon compared to contralateral controls at P14 (9.5% reduction) and P42 (29.4% reduction), with the effect more pronounced at P42 (p < 0.001, **Fig. 3**).

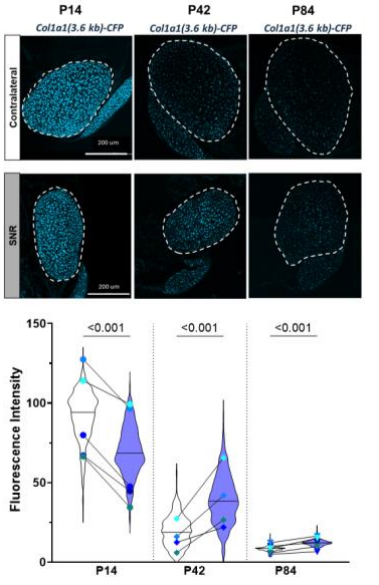

Fig 2: Col1a1(3.6kb)-CFP expression was reduced in SNR tendons at P14 however the SNR limb exhibited significantly higher CFP intensity at later time points (P42 & P56) compared to the contralateral limb.

DISCUSSION: We targeted periods of active cell proliferative- and matrix-driven growth using an SNR model and found that mechanical unloading acutely suppresses cell proliferation, collagen expression, and fibril thickening, ultimately impairing tendon growth. Early unloading via SNR led to rapid reductions in tendon CSA and cell number by P14, aligning with the known window of active cell proliferation. While the SNR tendon remained significantly smaller than the contralateral tendon at all time points, SNR may actually extend the growth period of the Achilles tendon since CSA of the contralateral tendon plateaued at P42, whereas the SNR tendon continued expand through P84. The altered temporal dynamics of Col1-CFP expression, which correlate with changing rates found in tendon CSA, reinforce that mechanical loading is a key regulator of tendon cell phenotype during postnatal growth. The slower decline of Col1a1 expression in the SNR limb suggests that mechanical unloading extends the period of this matrix-synthesizing cell phenotype. The reduced fibril diameter suggests that diminished loading limits fibril thickening, leading to progressive architectural defects. This corresponds with reduced material properties that we previously reported [3].

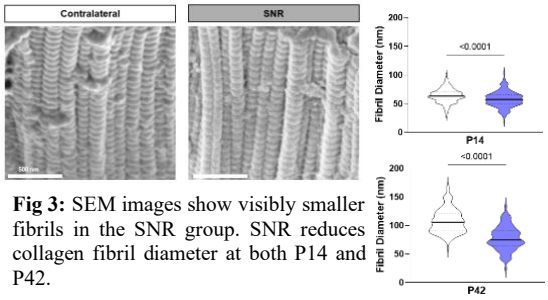

Fig 3: SEM images show visibly smaller fibrils in the SNR group. SNR reduces collagen fibril diameter at both P14 and P42.

SIGNIFICANCE/CLINICAL RELEVANCE: Together, these findings demonstrate that mechanical cues are required to sustain both the proliferative and matrix-accumulating phases of postnatal tendon growth. The marked reductions in structural and material properties confirm that SNR is an effective model for unloading the Achilles tendon during a mechanically sensitive developmental window, offering a powerful platform to study how chronic unloading influences tendon maturation and long-term mechanical integrity.

REFERENCES: 1. Lavagnino, JOR 2015; 2. Fogarty, JOR 2023; 3. Johnson, ORS 2024.

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