

Reduced Postnatal Loading after Sciatic Nerve Resection Impairs Achilles Tendon Growth and Maturation.

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INTRODUCTION: Mechanical forces are key regulators of musculoskeletal tissue formation and maintenance. Load bearing tissues, such as tendons, respond to mechanical loading events to maintain tensional homeostasis by fine tuning the formation and degradation of extracellular matrix proteins [1]. Both excessive and insufficient loading are implicated in the disease etiology of tendons. For example, overuse tendinopathies, which commonly inflict the Achilles tendon, account for 7% of musculoskeletal disorders in the US [2]. Manipulation of mechanical loads via rehabilitative loading exercises promotes repair processes that may culminate in improved restoration of normal tendon structure and function [3]. While loading has been shown to influence tendon composition in adults and healing tendons, it is less clear how mechanical forces influence Achilles tendon growth during early postnatal stages when there is a rapid accrual of extracellular matrix. Growth of the Achilles is predominantly governed first by cell proliferation during embryonic stages and then transitions to extracellular matrix (ECM) production shortly after birth. Here, we performed sciatic nerve resection (SNR) in neonatal mice on post-natal day 1 (P1) to explore how reduced muscle loading impacts Achilles tendon growth and maturation during this critical time window. We hypothesized that reduced mechanical loading of the hindlimb after SNR would result in reduced growth and development of the Achilles tendon, resulting in changes in tissue morphology and mechanical properties.

METHODS: All animals and procedures were IACUC approved. Sciatic Nerve Resection Procedure. Unilateral left sciatic nerve resection (SNR) was performed on post-natal day 1 (P1) in male and female CD1 mice (**Fig. 1a**) with evaluation at post-natal day 42 (P42). Gait Analysis. The CatWalk XT system was used to measure gait parameters in SNR and control mice. MicroCT. Excised hindlimbs from each group of mice were scanned on a Scanco μ CT45 scanner to measure tibia length. Biomechanical Testing. Achilles tendons were dissected, cross-sectional area was measured using a custom laser device, placed in custom grips, preconditioned, and loaded to failure (0.1%/sec). Structural and material properties were calculated using custom MATLAB scripts. Cryohistology. Hindlimbs were harvested, formalin-fixed, embedded in OCT, and cryosectioned (8 μ m) in the transverse plane using Cryofilm 2C. Fluorescent imaging with a nuclear counterstain followed by brightfield imaging with toluidine blue staining was performed. Image Analysis. Achilles tendon cross-sectional area and cell density were quantified using Fiji. Statistics. Gait parameters between SNR and control groups were compared via one-way ANOVA with Tukey's HSD post-hoc comparisons ($p < 0.05$). SNR limb vs. contralateral limb were compared via paired t-tests for all other assays ($p < 0.05$).

RESULTS: SNR resulted in sustained gait impairments. CatWalk XT analysis at P42 was used to assess changes in gait parameters that would inform hindlimb loading patterns. SNR mice exhibited marked gait abnormalities with over 50% of strides being below the detectable threshold in the operated limb compared to 100% detection in contralateral limbs and control limbs. Additionally, SNR resulted in marked reductions in maximum stride intensity (**Fig. 1b**) and paw print width (**Fig. 1c-d**) compared to unoperated control limbs ($p < 0.0001$), a hallmark of sciatic denervation. SNR resulted in smaller posterior musculature and bone shortening. Gross microscopic images revealed smaller posterior musculature in the hindlimbs (data not shown). As previously reported [4], we also found that the tibiae in the SNR limbs were shorter than unoperated controls (16.7 ± 0.1 vs. 17.8 ± 0.4 , $p < 0.01$). SNR resulted in smaller Achilles tendons. The Achilles tendons in the SNR limbs were shorter (**Fig. 2c**) with reduced birefringence and cross-sectional (**Fig. 2d**) ($p < 0.05$). The total number of cells per cross section was also reduced (**Fig. 2e**, $p < 0.05$) while there was an increasing trend in cell density (**Fig. 2f**, $p = 0.08$) in the SNR tendons. SNR resulted in impaired mechanical properties of the Achilles tendons. To further examine the role of early postnatal loading in Achilles tendon growth and maturation, we performed uniaxial tensile testing. The stiffness, failure force, and modulus were all significantly reduced in the Achilles tendon of the SNR limb (**Fig. 3a-c**, $p < 0.05$, Figure 4), with a decreasing trend in maximum stress (**Fig. 3d**, $p = 0.1$).

DISCUSSION: The role of mechanical loading in maintaining Achilles tendon homeostasis is well established and chronic aberrant loading can contribute to tendinopathies. The effect of applied loading during periods of active cell proliferative- and matrix-driven growth are not well understood. Using a surgical denervation model, we found persistent gait alterations with smaller posterior musculature and tibia shortening, which is consistent with previous findings [4]. We also found that the reduced loading had profound effects on the growth and maturation of the Achilles tendon, yielding smaller tendons with reduced mechanical properties (structural and material). These results suggest that the cells are producing less and possibly inferior extracellular matrix, which we plan to investigate in future studies. Cell proliferation also contributes to postnatal growth and we interestingly found a trending increase in cell density in the SNR limbs, which warrants future study.

SIGNIFICANCE/CLINICAL RELEVANCE: Understanding how mechanical loading regulates tendon growth and maturation may inform novel therapies to treat disease and injuries.

REFERENCES: [1] Wang JH, et al, *J Biomech.* 2006; [2] Rutland M, et al, *N Am J Sports Phys Ther.* 2015; [3] Langberg H, et al, *J Phys.* 1999 [4] Killion CH, et al, *Mol. Biol. Cell.* 2017.

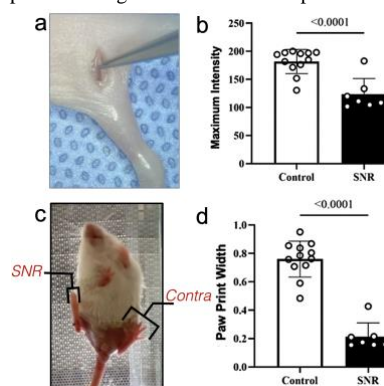


Fig. 1: SNR procedure (a) resulted in reduced maximum stride intensity (b) and paw width (c-d) for detectable strikes measured via the CatWalk XT gait analysis system.

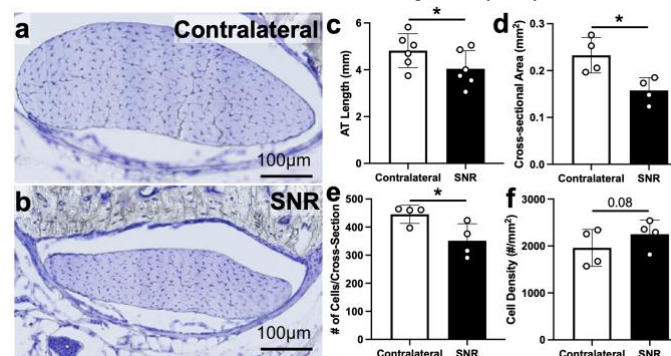


Fig. 2: SNR Achilles tendons (ATs) were markedly smaller than contralateral ATs (representative images in a-b). AT length (c) and cross-sectional area (d) were significantly reduced. The # of cells/cross section was also reduced (e) and there was a trending increase in cell density (f). * $p < 0.05$.

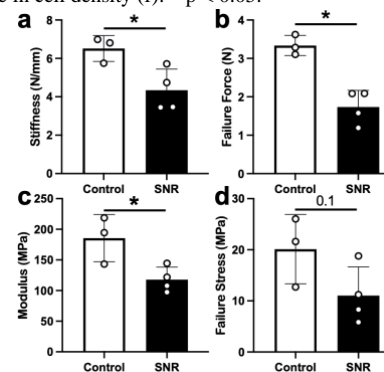


Fig. 3: SNR Achilles tendons displayed inferior mechanical properties: stiffness (a), failure force (b), modulus (c), and failure stress (d). * $p < 0.05$.