Reduced Hindlimb Loading Following Sciatic Nerve Resection Disrupts Achilles Tendon Mechanics, Cell Density, and Gene Expression in Wild-Type and Tendon-Targeted Collagen XII Knockout Mice

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Disclosures: None

INTRODUCTION: Collagen XII is a Fibril-Associated Collagen with Interrupted Triple Helices (FACIT) expressed during growth and development [1] that is responsive to mechanical stress and forms ECM complexes that absorb or transduce mechanical signals to interact with collagen I fibrils and cell surfaces in tendon [2]. Recent data in tendon-targeted collagen XII deficient (ScxCre;*Col12a1*$^{f/f}$; KO) mice demonstrated disruption of tendon structural and functional properties, suggesting that collagen XII regulation is critical in tendon development [3]. Studies involving murine tenocyte tensioning and de-tensioning demonstrated that accumulation of collagen XII increases and decreases with applied loading and unloading, respectively [4]. However, the roles of altered loading and collagen XII expression on the development of tendons remain unknown. Therefore, the objective of this study was to elucidate the role of reduced loading and altered collagen XII expression on the development of Achilles tendon (AT) mechanics, cellular properties, and gene expression. We hypothesized that reduced mechanical loading of the hindlimb via sciatic nerve resection (SNR) would impair tendon mechanics and lead to alterations in cellular properties and gene expression, with less notable effects in KO mice due to the absence of mechanosensitive collagen XII.

METHODS: Animals: Male and female postnatal day (P) 42 KO and wild type Cre(-) littermate control mice (WT) were used (IACUC approved). SNR: Unilateral SNR was performed as described [5] in P3 mice. Tendon Mechanics (n=9/group): ATs were measured with a laser device to quantify cross-sectional area (CSA) and underwent our mechanical testing protocol [3]. Cellular Properties (n=6-7/group): Whole ankle and knee joints were fixed, decalcified, paraffin embedded, sectioned in the sagittal plane, and stained with DRAQ5 to assess cell density and nuclear aspect ratio. Gene Expression (n=7/group): AT RNA was isolated and converted to cDNA, pre-amplified, and loaded into a Standard BioTools 96.96 Dynamic Array. Statistics: SNR and contralateral limbs were compared for each genotype via paired t-tests with significance at $p \leq 0.05$.

RESULTS: Tendon Mechanics: SNR limbs exhibited reduced CSA (**Fig. 1A**), maximum stress (**Fig. 1B**), stiffness (**Fig. 1C**), and modulus (**Fig. 1D**) relative to contralateral limbs similarly in both WT and KO mice. SNR limbs also demonstrated reduced dynamic modulus (**Fig. 1E**), increased phase shift (**Fig. 1F**), and stress relaxation (**Fig. 1G**) at 5% strain relative to contralateral limbs in WT and KO mice. Cellular Properties: Representative DRAQ5-stained sagittal histology sections (**Fig. 2 Left**) demonstrated that reduced hindlimb unloading via SNR resulted in decreased AT cell density and no changes in nuclear aspect ratio (**Fig. 2 Right**) compared with contralateral tendons similarly in both WT and KO mice. Gene Expression: SNR induced marked expression profile changes in both P42 WT (**Fig. 3A**) and KO (**Fig. 3C**) ATs, as assessed by Principal Component Analysis. Additionally, volcano plots demonstrated differential gene expression profiles between contralateral and SNR tendons in WT (**Fig. 3B**) and KO (**Fig. 3D**) mice.

DISCUSSION: As hypothesized, SNR altered Achilles tendon mechanics, cell density, and gene expression in WT and KO mice. Previous studies noted similar changes in Achilles tendon CSA and mechanical properties with SNR [5], and the observed decrease in cell density in SNR tendons is supported by studies showing similar findings with dramatically reduced cell numbers in unloaded tendons [6]. One striking finding from this study was the robust transcriptional response to SNR observed at P42. We observed decreased expression of genes involved with ECM synthesis and regulation (*Col1a1*, *Col1a2*, and *Fmod*) and increased expression of genes involved with matrix catabolism and remodeling (*Mmp3*, *Mmp13*, and *Adamts17*). While many ECM and remodeling genes were differentially expressed in both genotypes, several exhibited attenuated or even reversed fold-changes in KO tendons. For example, *Mmp13* was upregulated in WT SNR tendons but not in KO SNR tendons, suggesting that collagen XII may influence the catabolic response to altered mechanical loading during tendon development. Altogether, these changes mirror a shift toward a degradative matrix environment [7], which may contribute to the reductions in mechanical properties observed in SNR tendons. Contrary to our hypothesis, altered loading via SNR affected Achilles tendon mechanics and cellular properties in both WT and KO mice similarly, suggesting that either that the effects of SNR are so large that they dominate the tendon response or that collagen XII may not be required for cellular or mechanical adaptation to unloading in this model. However, given the distinct gene expression responses between genotypes, collagen XII may still play a regulatory role in matrix remodeling during tendon development under altered loading conditions. Future studies will use additional structural, functional, and compositional analyses at multiple postnatal timepoints to evaluate the roles and potential relationships between mechanical loading and collagen XII expression during development.

SIGNIFICANCE/CLINICAL RELEVANCE: This study elucidates the roles of mechanical loading and collagen XII expression in regulating Achilles tendon function, cellular properties, and gene expression to inform strategies to treat injury and disease.

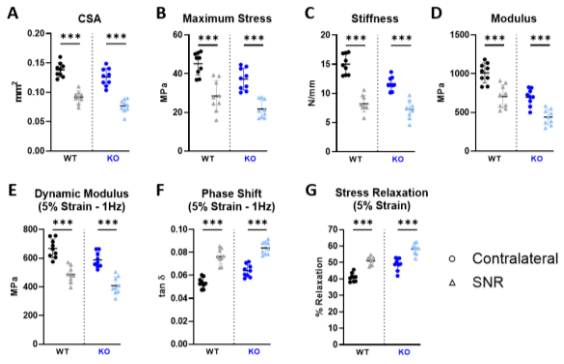

Figure 1. Achilles Tendon Mechanics. SNR limbs exhibited reduced (A) cross-sectional area (CSA), (B) maximum stress, (C) stiffness, and (D) modulus in WT and KO mice. SNR limbs also demonstrated reduced (E) dynamic modulus, increased (F) phase shift, and increased (G) stress relaxation relative to contralateral limbs in WT and KO mice. Data as mean ± standard deviation (***p≤0.001).

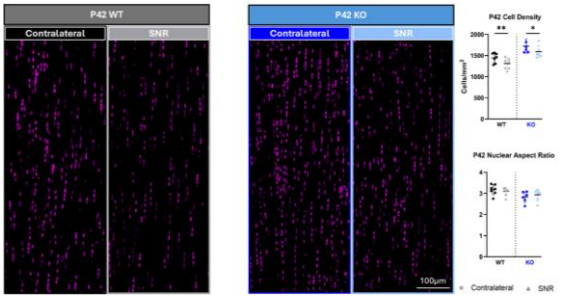

Figure 2. Achilles Tendon Cellular Properties. (Left) Representative DRAQ5-stained sagittal histology sections for the midsubstance regions of contralateral and SNR Achilles tendons of P42 WT and KO mice. (Right) P42 SNR Achilles tendons demonstrated decreased cell density and no changes in nuclear aspect ratio compared with contralateral tendons in WT and KO mice. Data as mean ± standard deviation (*p≤0.05, **p≤0.01).

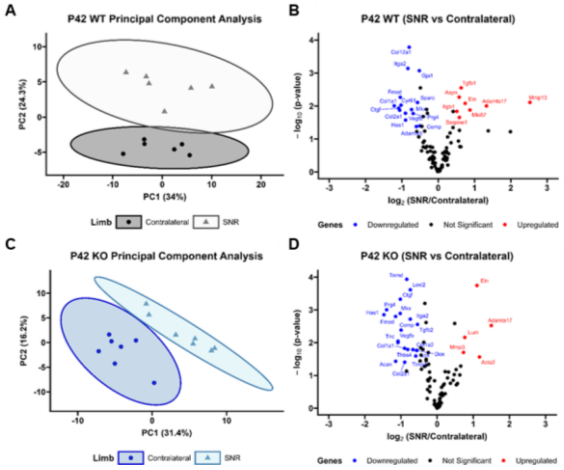

Figure 3. Achilles Tendon Gene Expression. (A) P42 WT and (C) KO SNR and contralateral Achilles tendons demonstrated significant clustering via Principal Component Analysis. (B) Volcano plots show differential gene expression with upregulated ($p \leq 0.05$ and log2 (fold change)≥0.5) and downregulated ($p \leq 0.05$ and log2 (fold change)≤-0.5) genes in WT and (D) KO SNR tendons relative to contralateral tendons.

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