Pathologic innervation in models of tendon overuse

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INTRODUCTION: Peripheral afferent neurons terminate at the tendon surface, transmitting nociceptive and mechanoreceptive signals. Nerve ingrowth within the tendon proper has been found as a feature of tendinopathy, indicating pro-inflammatory, nociceptive, and hypertrophic tissue responses[1]. Chronic tendinopathy, marked by enduring tendon pain, impaired function, and structural alterations, constitutes a substantial healthcare challenge capable of exerting profound effects on both the well-being of individuals and athletic prowess. Overuse triggers chronic tendinopathy by subjecting the tendon to repetitive mechanical stress, which can lead to microtrauma and impaired tissue healing[2]. Our prior work suggested after acute tendon injury, peripheral afferent nerves innervate the damaged tendon site and induce a regenerative response. However, whether and how peripheral neurons play a role in the chronic tendinopathy remains uncertain. To this end, our objective is to investigate the aberrant innervation patterns in chronic tendinopathy through mouse tendon overuse models and propose potential therapeutic insights for human chronic tendinopathy.

METHODS: All animals experiments were conducted under approval of the Johns Hopkins Animal Care and Use Committee. Human biceps tendon biopsies were identified at Johns Hopkins University surgical pathology archives under IRB approval. The slides were interpreted using the semiquantitative grading scale of Movin. We applied dorsiflexion immobilization (DI) model, in which ankles of mice were fixed, to mimic the overstrain of Achilles tendons[3]. Immobilization devices were crafted from TempAssure 0.5-mL PCR tubes by removing caps and bottoms, drilling dual holes, and affixing with iron wire, inducing dorsiflexion and talipes calcaneus positioning. 8-week-old scleraxis (Scx)-GFP and nerve growth factor (NGF)-eGFP were used. The devices were applied on left feet of mice to maintain dorsiflexion 6 hr/day, 5 day/week for 1 and 4 weeks, alongside non-immobilized contralateral limb for control. In addition, a four-week intensive treadmill running (ITR) model was conducted on 10-week-old Scx-GFP mice to mimic Achilles tendon overuse[4]. Routine histological staining was used to assess tendinopathy. Nerve distribution and vasculogenesis within Achilles tendons was documented using immunohistochemistry for Beta III Tubulin (TUBB3) and CD31. Unpaired two-tailed Student t-test was used for a two-group comparison.

RESULTS: The Achilles tendon was examined for chronic tendinopathy at 4 weeks post DI. Histological analysis revealed heightened safranin O-stained glycosaminoglycan, pronounced collagen fiber disorientation and remodeling, shown as reduced collagen fiber length (36.0% reduction) and increased collagen width 9.4% increase) in Picrosirius Red (PSR) staining (**Fig.1A, B**). PSR result indicated subtle alterations in collagen fiber orientation 4 weeks post ITR, as well as disturbances in width. Significant increase in TUBB$^+$ nerve innervation (9.5-fold increase) and CD31+ vascularization (4.5-fold increase) in DI animals. A similar uptrend was observed in animals after ITR. Furthermore, nerves and blood vessels ingrowth into the tendon body was observed in animals underwent DI (**Fig.1A**). Substantial upregulation of NGF reporter activity in NGF-eGFP animals was observed 1 week after DI (37.2% increase) (**Fig. 2A, B**). Finally, histologic analysis of human biceps tendons revealed increasing innervation within the tendon body and peritenon with increasing degree of tendinopathy (**Fig. 3A, B**).

DISCUSSION: Approximately half of sports injuries result from overuse, with a notable focus on tendon-related issues. Achilles tendinopathy affects roughly 30% of athletes, with an annual incidence of 7-9%. Overuse-related tendon injuries present a significant hurdle in sports medicine, currently lacking effective treatments. Our study suggested that the severity of human tendinopathy exhibited a positive correlation with innervation. In mouse DI and ITR models, we revealed abnormal innervation patterns under pathological conditions. Abnormal nerve sprouting in tendinopathy amplified inflammatory and metabolic pathways during tendon regeneration, facilitated by neurotrophic factors like NGF[1]. Continued research aims to investigate nerve modulation as a potential therapeutic strategy for addressing chronic tendinopathy.

SIGNIFICANCE/CLINICAL RELEVANCE: This study presents a comprehensive investigation of innervation within the framework of tendon overuse model, suggesting future therapeutic relevance for neuromodulation in human chronic tendinopathy.

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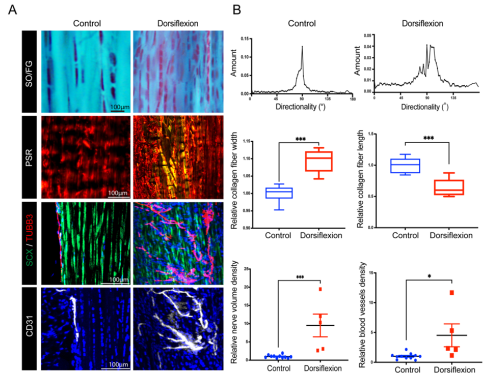

Fig1. Pathologic innervation in models of mouse tendon dorsiflexion immobilization. (A) Pathologic innervation of the Achilles tendon after 4 weeks dorsiflexion immobilization depicted by Safranin O/Fast green staining, Picrosirius red (PSR) staining, TUBB3, and CD31 immunofluorescent staining. (B) Frequency distribution of tendon fiber orientation measured by an OrientationJ plugin of ImageJ. Collagen fiber diameters analyzed by CT-FIRE. N>5. Data presented as mean ± 1 SD. *P<0.05, **P<0.01, and ***P<0.001.

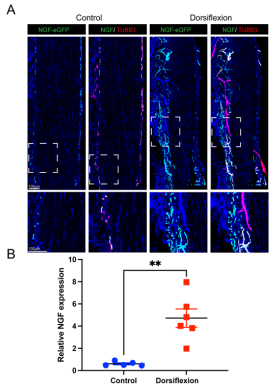

Fig2. NGF expression enhanced after 1 week dorsiflexion immobilization (A) Representative TUBB3 staining of NGF-eGFP mice after 1 week dorsiflexion immobilization. (B) NGF expression significantly upregulated after dorsiflexion immobilization. N=6. Data presented as mean ± 1 SD. *P<0.05, **P<0.01, and ***P<0.001.

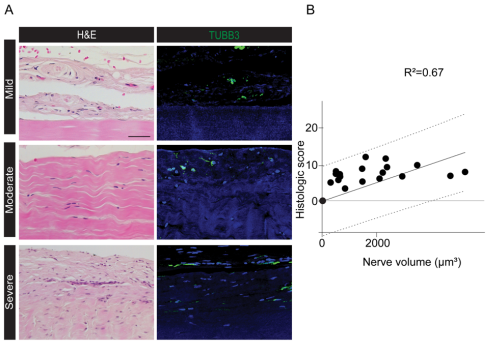

Fig3. Human tendinopathy is associated with innervation. (A) Representative H&E and TUBB3 staining performed on sagittal sections of human tendon, demonstrating the innervation of mild, moderate, and severe tendinopathy. (B) Linear correlation and 95% prediction band between histologic score and nerve volume quantified in human tendon samples. Each dot represents a single tendon image analyzed. A higher histologic score indicates more severe tendinopathy. N=20.