

# Non-invasive detection of fatigue-induced microstructural changes in tendon using diffusion tensor imaging

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**INTRODUCTION:** Soft tissue tears represent a large portion of musculoskeletal injuries in the United States [1] and are hypothesized to be caused by fatigue damage to the collagen matrix [2]. Diffusion Tensor Imaging (DTI) can characterize soft tissue microstructure by measuring water diffusion properties through the tissue [3] and has been used to assess ligament and tendon pathologies [4-6]. However, whether DTI is capable of differentiating tissues damaged from fatigue prior to bulk failure remains unknown. We have shown that in structurally analogous and mechanically similar tissue-mimicking fiber constructs, microstructural disruptions caused by fatigue loading result in increased axial diffusivity (AD) and radial diffusivity (RD), which are DTI metrics that are sensitive to the separation between physical barriers that restrict water diffusion [7]. Thus, AD and RD may be potential biomarkers of fatigue-induced damage in soft connective tissues. Therefore, the objective of this study was to evaluate whether DTI could be used to detect fatigue-induced changes in tendon. We hypothesized that fatigue loading would lead to an increase in AD and RD.

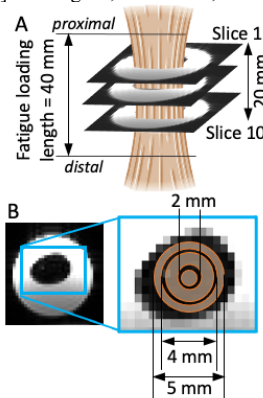
**METHODS:** Five 100 mm long sections from five porcine deep digital flexor tendons were harvested. First, DTI scans of the central region (length = 20 mm) were obtained using a diffusion-weighted spin-echo sequence (9.4T, TE/TR = 18/800 ms, 12 directions, 15 averages,  $b = 400 \text{ s/mm}^2$ , diffusion gradient duration = 4 ms, diffusion gradient separation = 10 ms, slice thickness = 2 mm, in-plane resolution =  $0.5 \times 0.5 \text{ mm}^2$ , field of view =  $20 \times 20 \times 20 \text{ mm}^3$ ). Next, the tendons underwent uniaxial cyclic tensile loading for 10,000 cycles or to failure, whichever came first (100 – 273.2 N, test length = 40 mm, strain rate = 0.6%/s, sampling frequency = 100 Hz). Following fatigue, a second DTI scan was conducted and the two scans were spatially co-registered using MRI visible markers attached to an anatomical landmark. The percent change in stiffness between the initial and final 500 cycles of the fatigue test was computed. To evaluate whether changes from fatigue were homogeneous within a cross-section, each MRI slice was divided into three radial regions of interest (ROI) and the raw voxel data was filtered and averaged (Fig 1). For each ROI, the diffusion tensor, AD, and RD were computed using a weighted linear least squares method [8]. Paired t-tests and ks-tests (for non-normally distributed data) were used to evaluate pre- and post-fatigue AD and RD of each ROI ( $\alpha=0.05$ ). DTI metrics are described using median and median absolute deviation.

**RESULTS:** All tendons reached the secondary phase of creep fatigue loading. The median change in stiffness from fatigue was +13.3% [range= -29:19%]. Overall, fatigue loading caused larger changes in RD compared to AD and were radially heterogeneous. RD increased by 9.3% in the central region of the tendons from  $1.18 \pm 0.12 \times 10^{-3} \text{ mm}^2/\text{s}$  to  $1.29 \pm 0.33 \times 10^{-3} \text{ mm}^2/\text{s}$  ( $p<0.001$ , Fig 2D), and by 9.7% in the mid-radial zone from  $1.24 \pm 0.18 \times 10^{-3} \text{ mm}^2/\text{s}$  to  $1.36 \pm 0.18 \times 10^{-3} \text{ mm}^2/\text{s}$  ( $p=0.006$ , Fig 2E). AD increased by 4.7% in the mid-radial zone from  $1.48 \pm 0.18 \times 10^{-3} \text{ mm}^2/\text{s}$  to  $1.55 \pm 0.17 \times 10^{-3} \text{ mm}^2/\text{s}$  ( $p=0.01$ , Fig 2B). Changes in RD following fatigue were also heterogeneous across the length of the tendon. Within the mid-radial zone, RD increased in slice 1 by  $0.35 \pm 0.15 \times 10^{-3} \text{ mm}^2/\text{s}$  and decreased in slice 10 by  $-0.18 \pm 0.06 \times 10^{-3} \text{ mm}^2/\text{s}$  (Fig 3A). There was no significant correlation between changes in stiffness and changes in the mean RD of the entire tendon ( $R^2 = 0.45$ ,  $p = 0.22$ , Fig 3B).

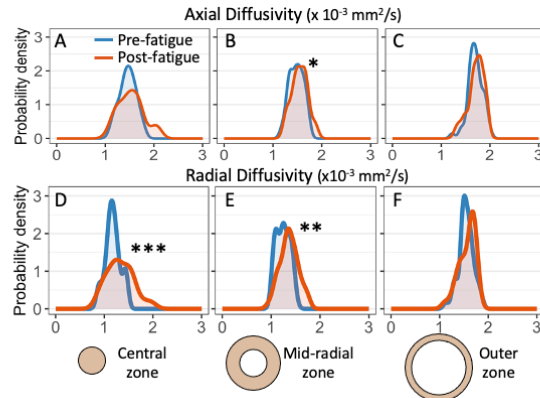
**DISCUSSION:** Fatigue loading resulted in heterogeneous changes in DTI metrics. Fatigue caused increases in RD in the central region of tendons while the mid-radial zone resulted in increases in both AD and RD (Fig 2). These findings suggest that fatigue loading resulted in local reorganization, disruption, or removal of diffusion-mediating barriers or fibers in the tissue, predominantly in the radial direction. Our findings of spatially specific changes in DTI metrics agree with studies that identified localized areas of fatigue-induced damage in tendon [2,9]. The increases in AD and RD were higher in regions that correspond to the proximal tendon (slice 1) and suggests that fatigue may have induced larger microstructural disruptions in the proximal region (Fig 3A). Notably, the correlation between changes in RD and changes in stiffness at the bulk level was likely dominated by one sample. Changes in stiffness are likely associated with fiber reorganization and disruption and our results highlight that fatigue-induced changes are spatially specific in nature. Current work includes assessment of the DTI-inferred microstructural changes with ground-truth histology to provide a better understanding of the diffusion-altering microstructural mechanisms caused by fatigue loading. With these results, we show that DTI metrics AD and RD are promising non-invasive biomarkers of fatigue-induced microstructural damage in tendon and potentially other connective soft tissues.

**SIGNIFICANCE/CLINICAL RELEVANCE:** These results highlight the ability of DTI metrics AD and RD to quantify fatigue-induced changes in tendon, thus advancing the potential use of DTI as a clinical tool to non-invasively assess fatigue-induced damage in patients at risk of connective soft tissue injuries.

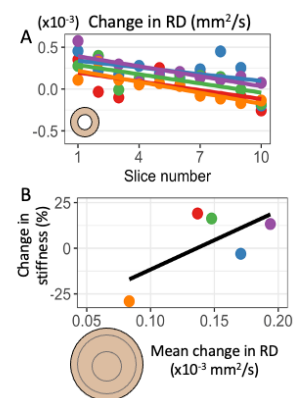
**REFERENCES:** [1] BMUS, US Bone and Joint Initiative, 2014 [2] Szczesny+, J Biomech, 2018 [3] Damon+, NMR Biomed, 2016 [4] Wengler+, Magn Res Med, 2018 [5] Liu+, J Magn Reson Imaging, 2020 [6] Van Dyck+, J Ortho Res, 2020 [7] Pineda Guzman+, engrXiv, 2023 [8] Damon+, J Biomech, 2021 [9] Firminger+, J Biomech, 2021



**Figure 1.** Testing length and DTI scan field of view of the tendons (A), and the slice's radial regions analyzed (B).



**Figure 2.** Probability density functions showing the distribution of AD (A-C) and RD (D-F) in each radial region of the tendon pre- and post-fatigue loading. \* $p<0.05$  \*\* $p<0.01$  \*\*\* $p<0.001$



**Figure 3.** A) Fatigue-induced changes in RD in region R2 along the tendon length. B) Change in cyclic stiffness vs mean bulk change in RD.