Shear wave speeds track fatigue-induced damage accumulation and failure in tendon fascicles

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INTRODUCTION: Tendinopathy is a painful condition where the fibrous structure of tendon is locally disrupted and is commonly linked to overuse.[1] Repetitive loading through daily tasks, like walking, can lead to damage accumulation in the tendon microstructure[2] and a tissue-level rupture. Current methods for assessing fatigue-induced damage are limited because they are either invasive or cannot be performed outside of the laboratory. Shear wave tensiometry is a technique for gauging tissue elasticity and axial stress in tendons based upon the shear wave speed (SWS).[3] A recent study has found that regional SWSs track the regional axial stress in fibrous soft tissues when subjected to axial tension.[4] This may indicate that SWSs are sensitive to local disruption of tendon microstructure that alters inherent material properties or loading. The objectives of this study were to (1) investigate the relationship between SWS and axial stress in tendon fascicles and (2) determine whether SWSs can track fatigue-induced damage to the tendon structure during cyclic stress relaxation and creep, both of which may occur *in vivo*. Our hypotheses were (1) that SWSs would increase linearly with tension/axial stress in fascicles, and (2) that fatigue loading would induce microdamage in fascicles, resulting in a greater local stress and an increase in the SWS in the tissue.

METHODS: ***Experiment:*** We dissected n = 24 rat tail tendon fascicles from eight, 32-month F334xBN rats. We secured fascicles in waveform grips in a custom loading device (backlash repeatability ± 2 µm) in a 1X PBS bath (Fig. 1a). We first preloaded fascicles to a 0.05 N tension and cyclically loaded from either 3-6% grip-to-grip strain (stress relaxation) or from 10-25 MPa (creep) at 1 Hz for 1000 cycles or until failure. We excited shear waves in the fascicle using a custom tapping device and transverse motion was tracked using two laser Doppler vibrometers. SWS was measured using the time delay in wave arrival between successive laser points.[5] We confirmed microdamage using second harmonic generation (SHG) imaging (Fig. 1b). ***Statistical Analysis:*** To test our first hypothesis, we assessed the SWS-axial stress relationship using linear regressions,[3] where the dependent variable was the axial tension, and the independent variable was the squared SWS. To test our second hypothesis, we evaluated the relationship between number of fatigue loading cycles and fascicle stiffness and SWS-stress slope using repeated measures mixed models with post hoc pairwise comparisons across fatigue loading cycles for both stress relaxation and creep. We evaluated relative SWS for low (10-15 MPa), medium (15-20 MPa), and high (20-25 MPa) loads using repeated measures mixed models to examine the effect of fatigue loading cycles only. For all analyses, significance was set at $\alpha = 0.05$.

RESULTS: We observed increased fibril kinking and interfibrillar spacing with increasing fatigue loading cycles (Fig. 1b) and a decrease in the tangent stiffness throughout fatigue loading in all fascicles ($p < 0.001$ for both stress relaxation and creep). Stress was proportional to SWS^2 for a given loading cycle in all fascicles ($R^2 > 0.99$ on average) (Fig. 1c,e). However, SWSs increased with fatigue loading such that the slope of the stress-SWS^2 relationship declined ($p < 0.001$ for stress relaxation, $p < 0.01$ for creep) (Fig. 1d,f). The increase in SWS was particularly marked near failure, increasing by an average of 14.6 m/s (14.1% increase, Fig. 1g). We did not detect a difference between increases in SWS at low, medium, and high loads ($p = 0.90$).

DISCUSSION: We found that SWSs were an excellent predictor of tension or axial stress in rat tail tendon fascicles. More notably, we found that SWSs increased in concert with fatigue loading cycles, indicating that SWSs likely predict microdamage. Based on our findings, it is apparent that SWSs are tracking changes in tendon mechanics in the plastic deformation region near failure. The increases in shear wave speed near failure were on average 14.6 m/s (Fig. 1c). This increase in SWS would predict an increase in axial stress of only 0.2 MPa near failure, which may indicate that tensiometry is very sensitive to microstructural changes near failure. Limitations include the fascicle width being greater measurement width of our laser Doppler vibrometers, which may result in a region-specific wave profile across the width of the fascicle that over- or under-estimates SWSs as microdamage accumulates.[4] Additionally, we evaluated SWSs without considering dispersion, which can cause phase differences in SWS due to the small thickness of the fascicle.[6] Our <u>*key takeaway*</u> is that SWSs are likely tracking the Cauchy stress in fascicles, which increases dramatically near failure when the loadbearing cross-sectional area decreases due to individual fibril failures. Future studies will examine SWSs during fatigue loading in energy-storing tendons that are often injured, such as the Achilles tendon, and establish the feasibility of making these measured in larger tendons *ex vivo.*

SIGNIFICANCE/CLINICAL RELEVANCE: Increased lifespans and activity levels in the older population[7] will make tendon injuries more challenging to address leading to an increased burden on society.[8] Noninvasive measures of tendon microdamage have tremendous potential to identify overuse and enhance therapeutic strategies preceding tendon rupture to keep people active and maintain their overall health.

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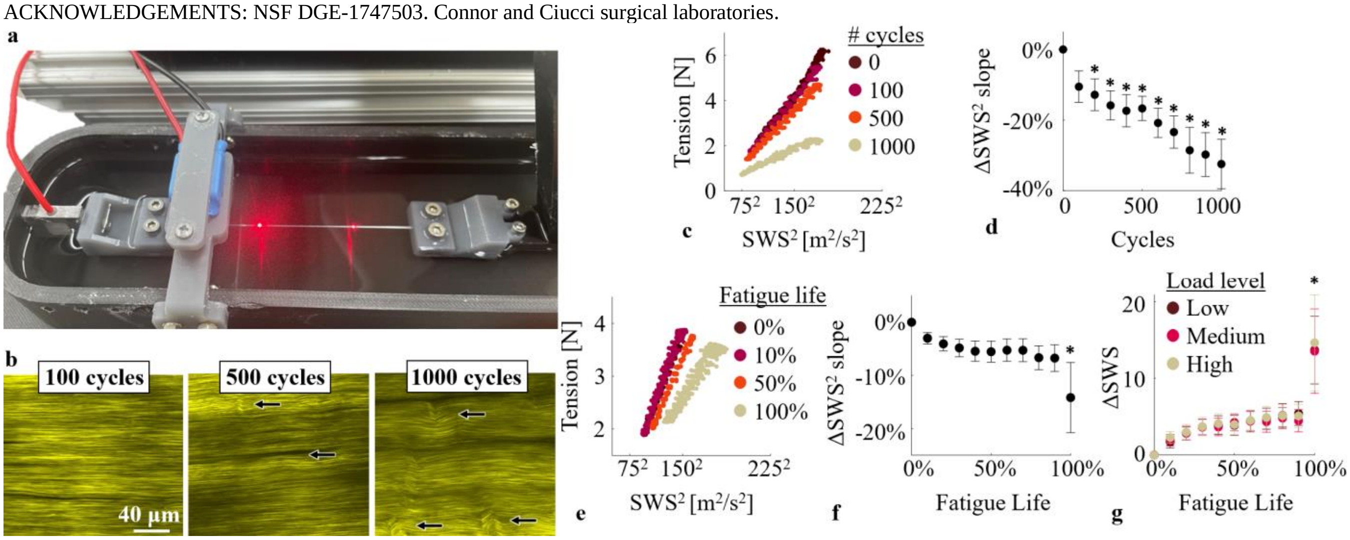

Figure 1: (a) Tabletop testing setup. (b) SHG images revealed microstructural damage with increasing fatigue loading cycles. (c-d) Cyclic stress relaxation revealed a sustained high SWS with increasing fatigue cycles. (e-f) Cyclic creep demonstrated an increase in shear wave speed for a given tension value across fatigue life. Both loading protocols showed that the SWS-axial stress slope decreases across fatigue life and near failure ($p < 0.001$). (g) Shear wave speed increased by an average of 14.6 m/s near failure. Error bars = SE. Asterisks indicate statistically significant differences from baseline (0% fatigue life).