Fatigue Loading Impacts Multiscale Achilles Tendon Biomechanics During Aging

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INTRODUCTION: Achilles tendinopathy is a common degenerative disease often associated with sub-rupture fatigue loading and age [1]. 82% of Achilles injuries are caused by athletics with the highest rupture rate in males aged 20-39 years [2]. The impact of fatigue loading on tendon structure function properties are not fully understood, with much of the current knowledge derived from end-stage pathologies following tendon biopsy [3,4]. Previous work has demonstrated that aging may decrease mechanical properties in the Achilles tendon, however, most work has focused on the effects of fatigue loading on macroscale elastic tensile properties [5]. The specific role of aging on micro- and macro-scale tendon viscoelastic properties has remained unclear. Therefore, the objective is to identify the effects of fatigue loading on multiscale tendon properties during aging. We hypothesized that both fatigue loading and aging would have significant effects on multiscale tendon properties.

METHODS: An ex-vivo rat model was developed to study the impact of aging and fatigue loading on the Achilles tendons of male F344xBN-F1 rats (IACUC-approved). At 1, 8, or 28 months of age, the rats were euthanized, Achilles specimens were harvested, and cross-sectional area and echogenicity were quantified (Fujifilm Vevo 3100). A mechanical testing protocol was performed: (1) Preload, (2) Precondition, (3) Stress-Relaxation, (4) Frequency Sweep from 0.1 to 10 Hz, (5) Dynamic Cyclic Fatigue Loading at 8-16% failure strain for 1000 cycles at 2 Hz or time equivalent 0% stain hold (control), (6) Stress-Relaxation, and (7) Frequency Sweep from 0.1 to 10hz (Bose Electroforce 3100) (Fig. 1). This protocol was intended to induce a cyclic fatigue injury and assess the mechanical property changes pre vs. post loading. Prior to and following dynamic loading, nanoindentation (KLA iNano) was performed on the posterior surface of the Achilles midsubstance, 5-6 mm from the calcaneus insertion, using a 100 μm flat tip to assess microscale viscoelastic changes. Specimens were then sectioned, fixed, and stained with DAPI. Forward-scatter two-photon imaging was performed at 30x magnification to quantify collagen fiber alignment and cell morphology (Leica Stellaris 8). Statistical analysis was performed using one-way (CSA, Echogenicity and Peak σ) or two-way (Δ|E*|, Δtanδ, circular deviation and aspect ratio) ANOVAs with post hoc tests for multiple comparisons.

RESULTS: Analysis of ultrasound images revealed that aging effects cross sectional area (CSA) and echogenicity with an increase in CSA and decrease in echogenicity in mature specimens (Fig. 2). Macroscale mechanical mechanical analysis also indicated that aging causes an increase in peak stress with a higher peak stress during cyclic loading for mature specimens as compared to juvenile specimens (Fig. 2). Further, while cyclic loading was found to cause a decrease in |E*| and increase in tanδ, aging was found to have no effect on those properties (Fig. 2). No significant change in microscale shear storage modulus or tanδ post loading was found for both fatigue and control groups (data not shown). Histological analysis revealed a decrease in circular deviation due to cyclic loading with aging also shown to further decrease this parameter (Fig. 3). Cell aspect ratio was found to decrease due to cyclic loading for the 1 and 8 month groups, with no significant decrease in the 28 month group (Fig. 3).

DISCUSSION: This study examined the effects of fatigue loading and aging on multiscale biomechanics and collagen organization. We found that the peak stress induced by fatigue loading was higher in tendons from mature vs. juvenile animals. Surprisingly, while fatigue loading significantly decreased |E*|, aging did not further exacerbate this effect. Finally, histological analysis indicates that fatigue loading disrupts collagen fiber alignment, however mature specimens were more resistant to such deformation. Cell aspect ratio findings indicate a change in cell morphology due to cyclic fatigue loading in the 1 and 8 month groups with the 28 month group being resistant to morphological changes.

SIGNIFICANCE/CLINICAL RELEVANCE: Understanding the multiscale evolution and physiological consequences of fatigue injury during aging may further elucidate damage mechanisms, informing potential therapeutic targets. The findings of this study suggest age and fatigue injury dependent structural and macro scale mechanical changes.

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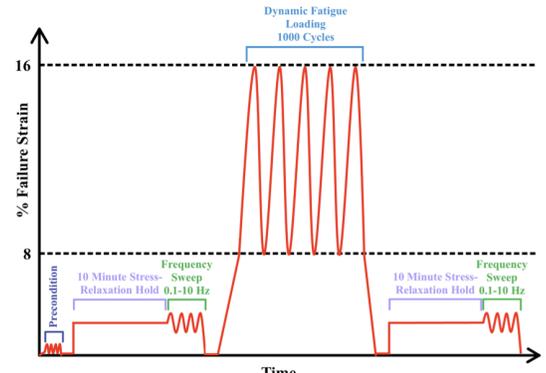
Figure 1: *Mechanical testing protocol for fatigue loaded specimen: (1) Preconditioning, (2) 10 Minute Stress-Relaxation Hold, (3) Frequency Sweep at 0.1-10 Hz, (4) Dynamic Fatigue loading at 8-16% failure strain for 1000 cycles, (5) 10 Minute Stress-Relaxation Hold, (6) Frequency Sweep at 0.1-10 Hz.*
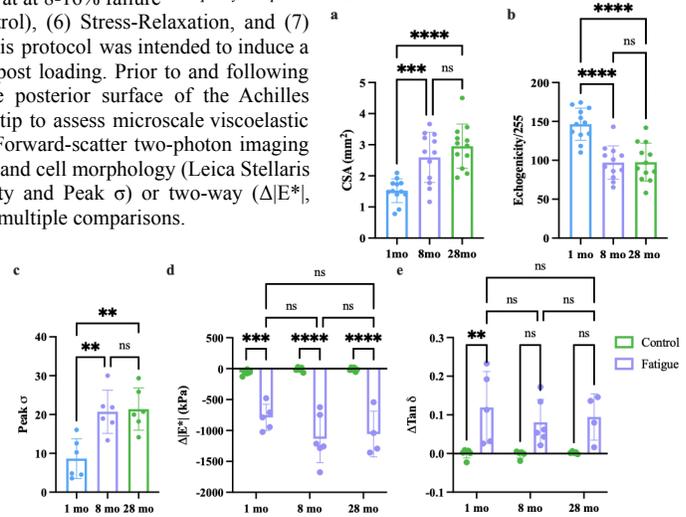
Figure 2: *Fatigue Injury and Aging Impacts Tendon Structure and Macroscale Tendon Mechanical Properties* as determined by **(a)** cross sectional area, **(b)** echogenicity, **(c)** peak stress, **(d)** change in |E*|, **(e)** and change in tan δ pre vs. post fatigue loading. Data shown as mean ± S.D.
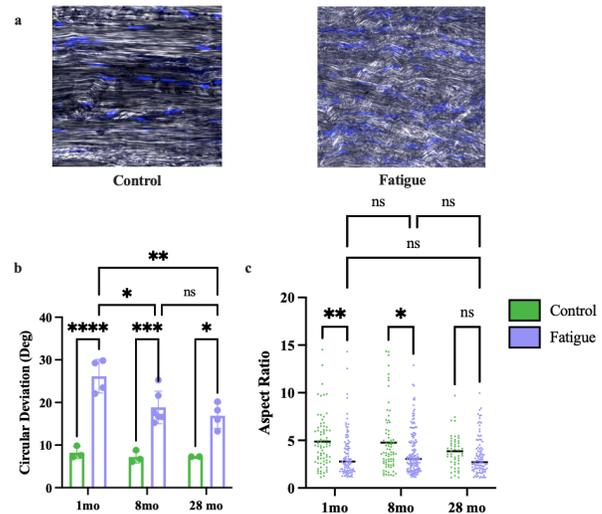
Figure 3: *Fatigue Injury and Aging Impacts Tendon Structure and Cell Shape* as evaluated by **(a)** analysis of multiphoton imaging for **(b)** circular deviation and **(c)** cell aspect ratio. Data shown as mean ± S.D.