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To cite this version:

Isabella Silva Barreto, M Pierantoni, M Hammerman, E Törnquist, S Le Cann, et al.. Characterization of collagen structural response to in situ loading of the rat Achilles tendon. XXVIII Congress of the International Society of Biomechanics, Jul 2021, Stockholm (virtual), Sweden. hal-03242445

HAL Id: hal-03242445
https://hal.science/hal-03242445

Submitted on 31 May 2021

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Characterization of collagen structural response to *in situ* loading of the rat Achilles tendon

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Summary
The collagen response in rat Achilles tendons to *in situ* tensile loading was studied using small-angle X-ray scattering (SAXS), to evaluate the relationship between the elastic and viscoelastic behavior of the tissue and collagen fibrils. The fibril strains were substantially lower than the applied tissue strains. Fibril strains increased linearly before yielding and breaking prior to tissue failure.

Introduction
The mechanical properties of tendons are depending on a complex hierarchical design, where collagen is arranged into fibrils in a periodic, quarter staggered pattern at an intermolecular distance referred to as d-spacing. This study aimed to characterize the Achilles tendon's mechanical response to *in situ* loading at the nanoscale and how it relates to the resulting macroscopic tissue mechanical behavior.

Methods
Achilles tendons (n=16) were harvested from Sprague Dawley rats (10-14 weeks) and stored frozen in NaCl until measurements at the SAXS beamline (cSAXS, Swiss Light Source, PSI) (energy 12.4keV, beam size 150µm). The tendons were loaded using a custom-made uniaxial tensile device (Fig 1) [1] while SAXS patterns (q=0.05-1.45nm^{-1}) were acquired simultaneously from the center of the tendon. A radiation damage test was conducted prior to testing which ensured that all analysis was conducted below 28kGy. The tendons were loaded (5mm/min) in ramp to failure (N=5), cyclic loading (N=5) or stress relaxation (N=6). From collagen peaks identified from the azimuthally integrated scattering intensity over the angular sector θ (Fig 1.A), local fibril strain and collagen structural parameters were extracted [2,3]. All analysis was performed using MATLAB.

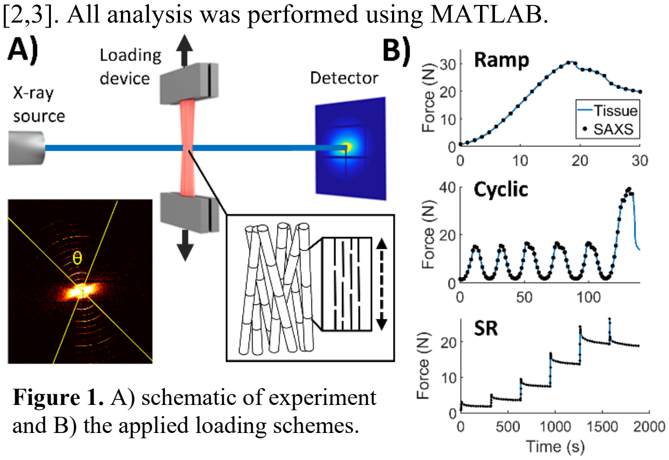

Figure 1. A) schematic of experiment and B) the applied loading schemes.

Results and Discussion
For all loading schemes, the fibril strain, strain heterogeneity and overlap followed the macroscopic load. During ramp to failure, the fibril strain increased linearly with tissue strain in the elastic region (Fig 2.A). The fibrils in the beam path yielded and broke (fibril strains 1.3±0.3%) prior to global tissue failure, which could be the result of an accumulation of nanoscale ruptures throughout the specimens. With increasing load, the fibril d-spacing increased and the overlap region decreased, which indicates fibril stretching and sliding simultaneously. During cyclic loading, the tissue hysteresis decreased with number of cycles (Fig 2.B, i). Interestingly, while the d-spacing at maximum load remained constant for all cycles (0.9±0.5%), it decreased sequentially at the end of each cycle (Fig 2.B, ii). This suggests that the initial decline in tissue hysteresis is accompanied by an increase in fibril recovery and stretchability. During stress relaxation, most of the collagen parameters experienced transient relaxation simultaneously as the tissue (Fig 2.C).

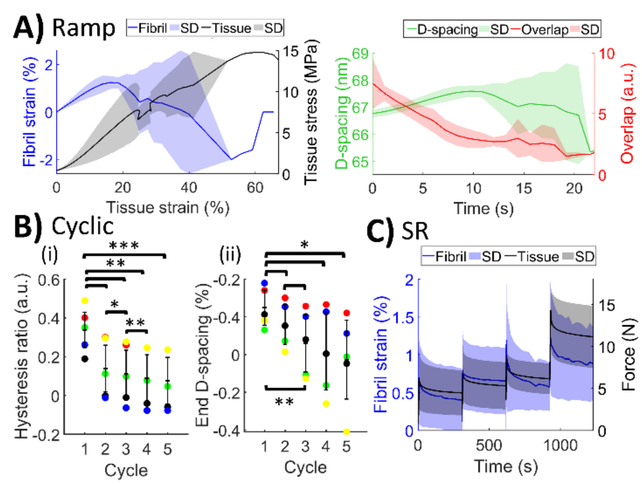

Figure 2. Changes in fibril parameters during A) ramp to failure, B) cyclic loading and C) stress relaxation.

Conclusions
Different loading scenarios enabled linking of the nano and macroscale in the Achilles tendon in terms of mechanics, such as accumulation of local fibril ruptures before global failure and adaptation of the nanostructure to the applied load.

Acknowledgments
This project was funded by the European Union's Horizon 2020, No 731019 (EUSMI) through beamtime at cSAXS beamline, PSI, and by the Knut and Alice Wallenberg Foundation.

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