FATIGUE LOADING CAUSES A REDUCTION IN THE ABILITY OF TENDON FASCICLES TO RECOIL

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INTRODUCTION

The function of most tendons is to position the limb correctly for locomotion. Specific tendons, including the human Achilles and equine superficial digital flexor tendon (SDFT) also act as energy stores; to fulfil this role, these tendons are subjected to large, repetitive strains. The mechanisms that allow energy storing tendons to extend and recoil rapidly and efficiently in response to these large strains are poorly understood. However, it is well established that energy storing tendons are highly prone to injury, which is thought to occur due to an accumulation of microdamage within the tendon matrix[1]. In our previous work investigating structural specialisations within energy storing tendons, we have observed rotation within fascicles from the equine SDFT in response to applied strain, indicating the presence of helical sub-structures within this tendon[2,3]. Further, we have demonstrated that SDFT fascicles are able to recoil efficiently, which may be due to this helix structure[2]. Finally we have shown that fatigue loading results in decreased rotation within SDFT fascicles, suggesting alterations to this helix[4]. The aim of the current study was to compare the effect of preconditioning with that of fatigue loading on the microstructural strain response of SDFT fascicles and additionally investigate their ability to recoil. **We hypothesise that fatigue loading will decrease the ability of SDFT fascicles to recover following the application of strain.**

METHODS

Sample Preparation

Fascicles (n=6/tendon) were dissected from forelimb SDFTs (n=11) from young horses (age range: 3-6 years) and either imaged using scanning electron microscopy (SEM) (n=18) or subjected to mechanical loading followed by microstructural strain analysis using confocal microscopy (n=48).

Scanning Electron Microscopy (SEM)

Fascicles were fixed in 4% glutaraldehyde in phosphate buffer for 2hrs, then washed overnight in phosphate buffer. A graded dehydration was carried out first in ethanol and then in hexamethyldisilizane. Samples were dried overnight and then mounted on SEM stubs and sputter coated with a gold layer (10nm), before scanning at 10Kv.

Mechanical Loading

Fascicles were stained with 5-dicholorotriazynl fluorescein and then divided into 3 groups: control, preconditioned (PC) or fatigue loaded (FL). Samples in the control group remained unloaded, whereas PC and FL samples were subjected to cyclic testing as follows. PC: 30 cycles to 50% predicted failure stress at a frequency of 1Hz. FL: 1800 cycles to 50% predicted failure stress at a frequency of 1Hz. Hysteresis loss was assessed in the FL group at cycle 30 and cycle 1800.

Confocal Imaging

Each fascicle was then secured in a straining rig, viewed on a confocal microscope and a grid was photobleached onto the fascicle (Fig. 1a). Fascicle recoil was assessed by imaging grids through a series of loading and unloading strains (0% to 4% to 0% to 8% to 0%). Grid deformation was quantified by measuring local longitudinal strains ($x+\Delta x$), transverse strains ($y + \Delta y$), vertical gridline deviation (d_1+d_2) and horizontal gridline rotation ($^{\theta}x$) (Fig.1b&c). In addition, the percent recovery of each grid parameter was calculated after both 4% and 8% applied strain. Significant differences between groups were determined using Kruskal-Wallis tests followed by Dunn's multiple comparison post-hoc analysis. Statistical significance was taken as $p<0.05$. Data are displayed as mean±SEM.

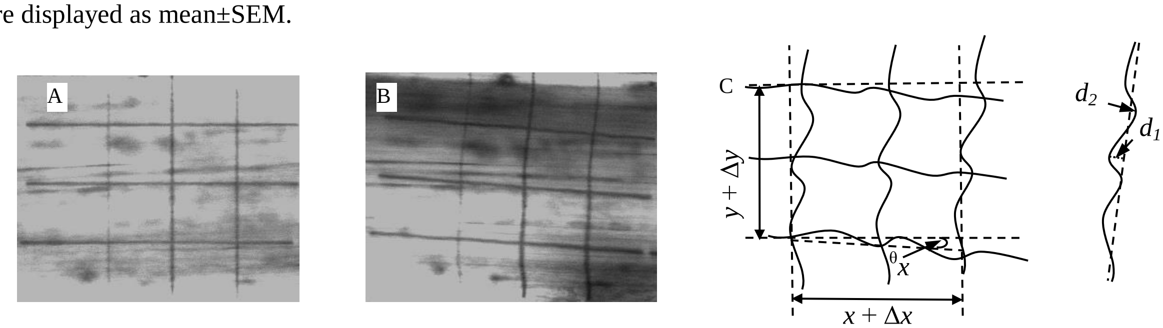

Fig 1. Images of grid at 0% (a) and at 8% (b) strain. Schematic showing calculations of grid deformations (c).

RESULTS

Fascicle Ultrastructure

SEM analysis demonstrated the presence of helix-like structures in fascicles from the SDFT, an example of which is shown in Fig 2.

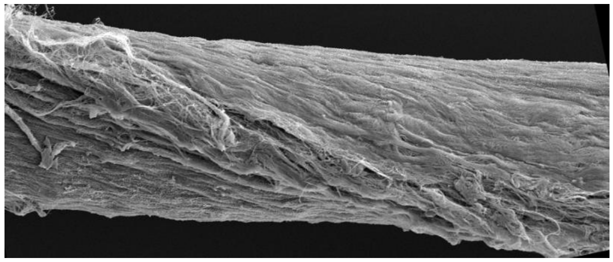

Fig 2. SEM image showing presence of helix like structure in an SDFT fascicle.

Microstructural Strain Response

In agreement with previous results[2.3], local longitudinal strains ($x+\Delta x$), representing fibre extension, were smaller than overall applied strain, and did not differ between test groups (Fig 3a). Large compressive strains ($y + \Delta y$) were observed perpendicular to the loading axis in unloaded samples; these strains were significantly reduced in both PC and FL groups at 8% applied strain ($p<0.01$, Fig 3b). A small amount of vertical gridline deviation (d_1+d_2), representing fibre sliding, was observed in all samples. There was a trend towards increased fibre sliding in FL samples, but this was not significant (Fig 3c). Horizontal gridline rotation (${}^{\theta}x$) was observed in both control and PC groups, but was decreased in FL samples at 8% applied strain ($p<0.05$, Fig 3d).

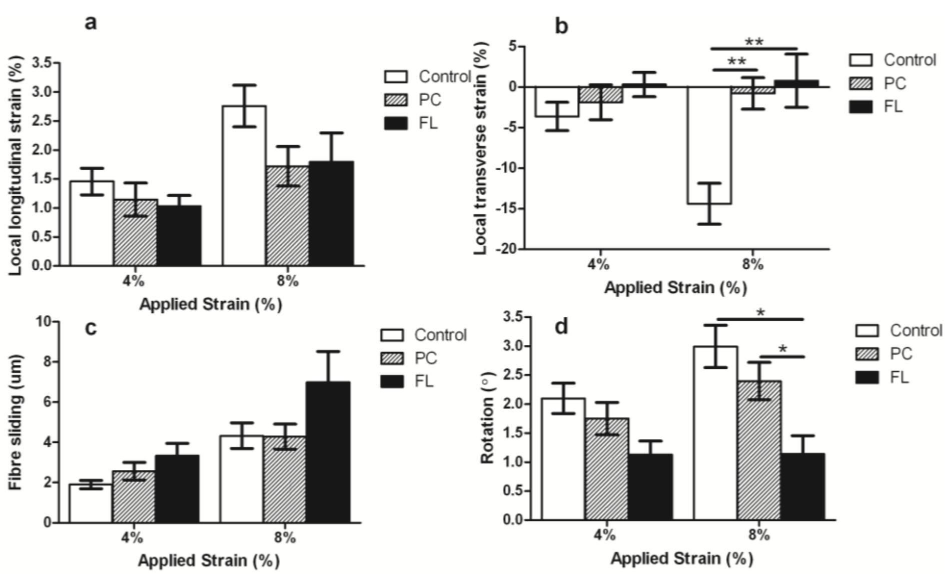

Fig. 3. Local longitudinal strains (a), transverse strains (b), fibre sliding (c) and grid rotation (d) at 4% and 8% applied strain in control, PC and FL groups. Statistical significance: * $p<0.05$, **$p<0.01$.

Recovery of fibre extension was significantly lower in FL samples that in either control or PC samples (Fig. 4a, $p<0.05$). There was no difference in the percent recovery of transverse strain between test groups (Fig. 4b). Recovery of fibre sliding was significantly reduced in PC and FL samples compared to controls (Fig. 4c, $p<0.05$), whilst recovery of rotation was only significantly reduced after fatigue loading (Fig. 4d, $p<0.05$).

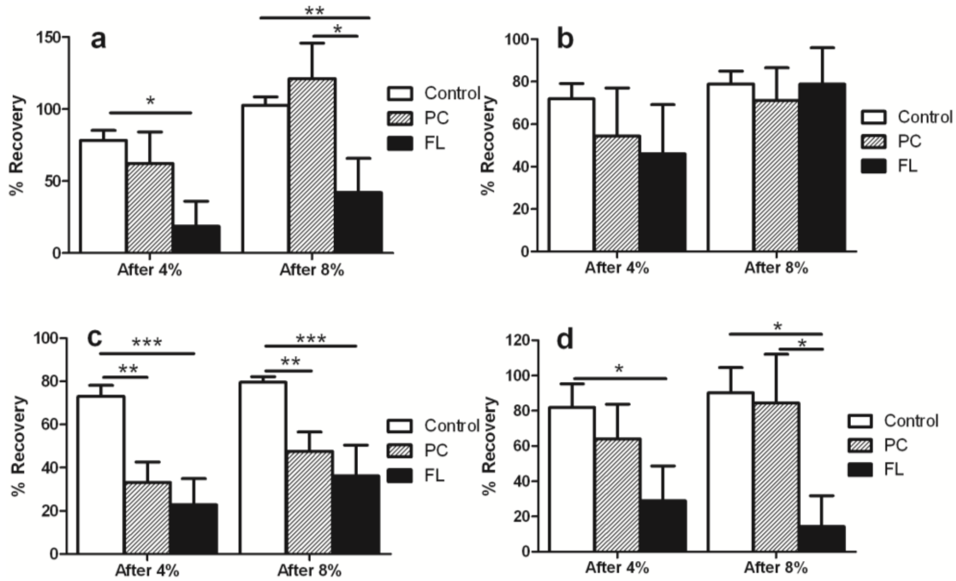

Fig.4. Percentage recovery of local longitudinal strains (a), transverse strains (b), fibre sliding (c) and grid rotation (d) after 4% and 8% applied strain in control, PC and FL groups. Statistical significance: * p<0.05, **p<0.01, ***p<0.001.

Hysteresis loss was significantly greater at cycle 1800 than at cycle 30, increasing from an average of 10.9±1.7% to 16.8±1.4% (Fig 5, p<0.01).

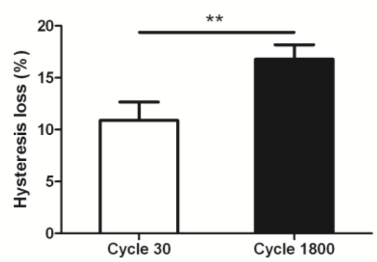

Fig 5. Percentage hysteresis loss in SDFT fascicles after 30 cycles and 1800 cycles of cyclic loading. Statistical significance: **p<0.01.

DISCUSSION

Our previous data[2-4] indicate the presence of helices at the fascicle level within the SDFT, which act as springs, allowing the tendon to extend and recoil efficiently and rapidly. In the current study, we have provided visual evidence of this helix using SEM. In addition, using a combination of cyclic fatigue testing and confocal microscopy we have investigated SDFT fascicle micromechanics before and after loading, and assessed how samples recover from the loading conditions. Overall, fascicles appear to exhibit a gradual change from fibre extension to fibre sliding with FL, which is accompanied by a reduced recovery of both parameters. In addition, there is a large reduction in the compressive transverse strains, which occurs almost immediately once the sample is loaded, suggesting that water is moved out of the samples after a very small number of loading cycles.

Interestingly, we also observed a large decrease in rotation in response to applied strain in FL samples, indicating alterations to the helix structure. This is accompanied by a decreased ability to recover and increased hysteresis loss in FL fascicles. Combined, these results suggest that FL causes alterations to the helix substructure identified in fascicles from the energy storing SDFT, which appears to result in a decreased ability of these 'springs' to recoil efficiently. This may result in increased susceptibility to damage and subsequent injury during further bouts of loading. It is therefore important to fully characterise this helix structure within energy storing tendons, and determine how alterations in helix parameters affect tendon fatigue resistance to further understand the initiation and progression of tendon injury.

ACKNOWLEDGEMENTS

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