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A combined engineering and molecular approach to study the initiation and progression of equine tendinopathy

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How does understanding the structure of tendons assist in injury prevention in the racehorse?

- Tendon injuries are very common in the Thoroughbred. Using a combination of biological and engineering based techniques, we investigated the mechanisms leading to equine tendon injury.

Reasons for this study

- Injury to the superficial digital flexor tendon (SDFT) is common in the racehorse and incidence increases with age so that this injury is most important musculoskeletal problem in National Hunt racehorses

- The SDFT extends and recoils rapidly and repeatedly during gallop, like a spring

- The mechanisms that allow this tendon to function properly as an elastic 'spring' are poorly understood

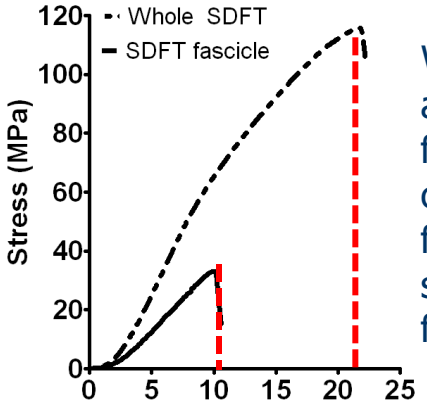
- There are currently few effective treatment options

Aims and Objectives

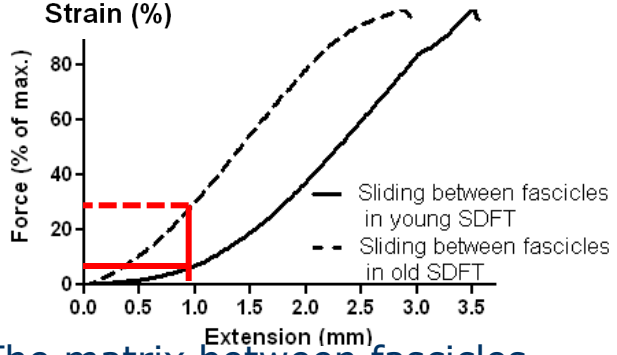
- To characterize extension mechanisms in tendons with different functions
- To determine where within the tendon structure microdamage occurs
- To examine how fatigue loading alters tendon extension mechanisms and local strains at the cell level
- To investigate how fatigue loading alters cell metabolic activity
- To identify age-related alterations in response to fatigue loading

Tendon extension mechanisms

- Extension in the SDFT occurs by sliding between adjacent tendon sub-units (fascicles)
- This allows the large extensions required by the SDFT while protecting the fascicles from damage
- With ageing, the capacity for sliding between fascicles decreases
- This may increase the risk of damage and subsequent injury occurring in tendons from aged (i.e. older) horses



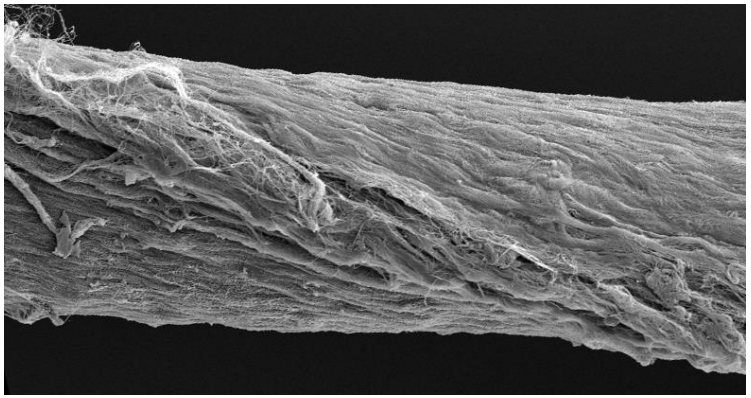
Whole SDFTs are able to extend further than their constituent fascicles due to sliding between fascicles



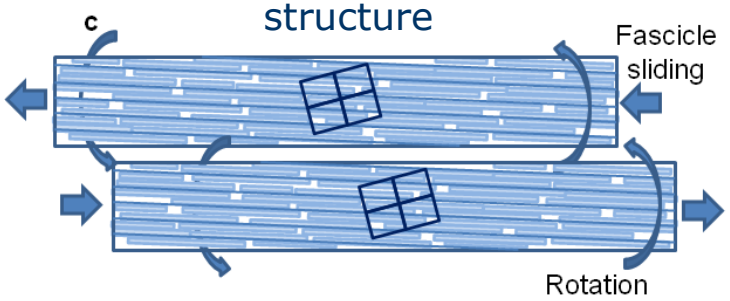
The matrix between fascicles becomes stiffer with ageing, decreasing the capacity for sliding

Fascicle extension mechanisms

- Fascicle rotation occurs during extension, suggesting that fascicles in SDFT have a helical structure.
- This helical structure acts as a spring, providing the ability to recoil and recover efficiently
- The amount of rotation that occurs decreases with ageing, suggesting the helix is altered in aged individuals
- This results in decreased ability to recoil



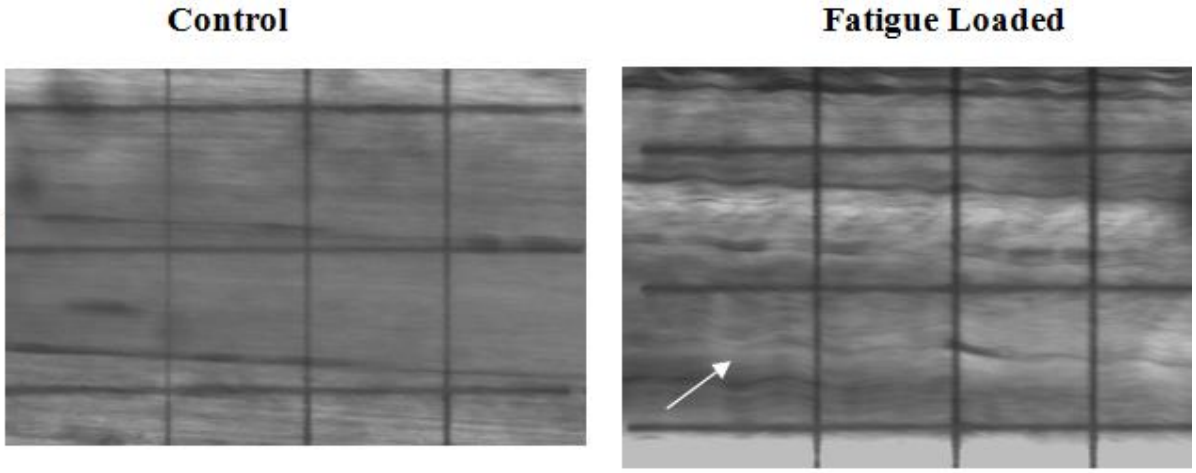
SEM image of SDFT fascicle showing helix structure



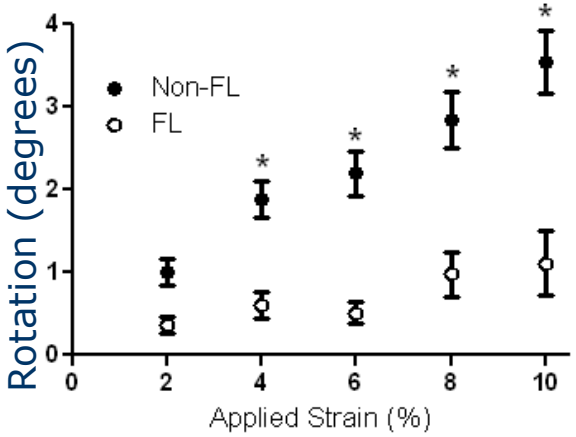
Schematic showing extension mechanisms in SDFT. (Thorpe et al., 2013, Acta Biomater, 9:7948-56)

Effect of repetitive loading

- Repetitive loading results in mild damage, with kinking of the collagen fibres observed (white arrow)
- This is accompanied by decreased rotation in response to applied load, and a decreased ability to recover when load is removed



Images showing fibre kinking in repetitively loaded fascicles



Fascicle rotation is decreased as a result of repetitive loading

Effect of ageing & repetitive loading

- In samples from older horses, repetitive loading results in more severe damage, mainly occurring between the collagen fibres
- This is accompanied by increased fibre sliding in response to applied load and a decrease in number of cycles to failure

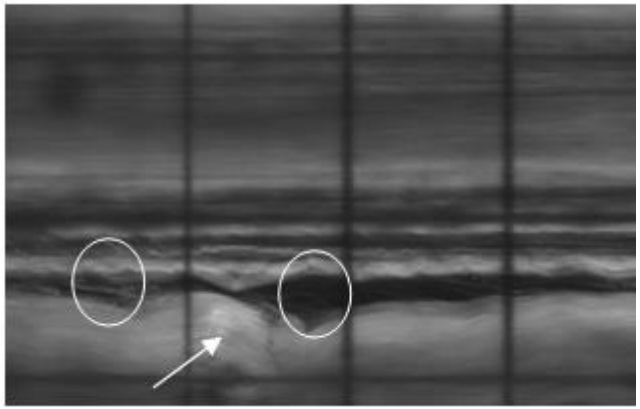
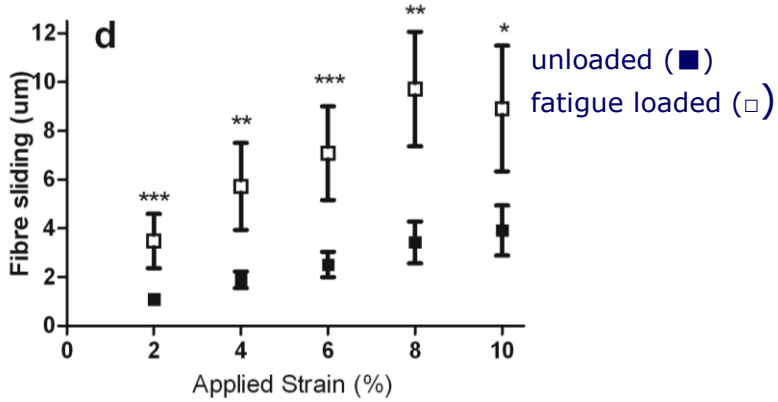


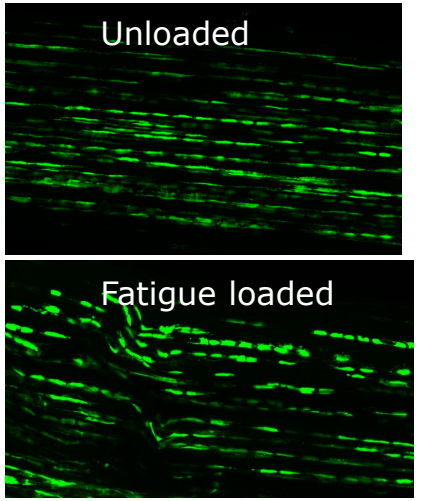
Image showing damage in repetitively loaded fascicles from older tendon



Levels of sliding between collagen fibres increased after repetitive loading in older samples

Cell response to repetitive loading

- Repetitive loading causes cells within tendon fascicles to change shape, becoming rounder rather than long and thin
  - This will affect how cells respond to further loading
- Loading also causes changes in cell metabolic response
  - There is increased degradation of some matrix proteins
- With ageing, cells become more elongated, and show an enhanced rounding response to loading
- Ageing also results in changes in the levels of some matrix proteins



Images showing stained cells in unloaded and repetitively loaded fascicles

Results & Conclusions

- Two key mechanisms enable efficient function in the SDFT
  - Sliding between fascicles allows large extensions while protecting fascicles from damage
  - Helical 'spring-like' fascicles provide the ability to recoil efficiently
- These mechanisms are both compromised in SDFTs from older horses
  - This is likely to increase the risk of injury occurring with age
- Repetitive loading results in alterations to helix structure
  - This is accompanied by matrix damage, reduced ability to recoil, changes in cell shape and cell metabolic activity
  - The response is more severe in aged samples, with greater damage observed

Impact on the Thoroughbred

- The structural specialisations identified in the SDFT help to explain how it functions
  - This information is important when developing methods to restore tendon properties following injury.
- Changes observed as a result of repetitive loading and ageing gives us a better understanding of the initiation of tendon injury
  - This will allow the development of more effective preventative methods and treatment strategies
- The specific alterations in cell response provide targets for future research, with the ultimate aim of limiting injury risk by prevention, early injury identification and more effective treatment options

Potential next steps

- Characterize matrix between fascicles that allows sliding behaviour
  - Identify proteins specific to this matrix and their function
  - Identify how this matrix affects whole tendon fatigue properties
  - Identify ageing changes
  - Potential targets for preventative & treatment strategies

- Investigate fascicle helix
  - Characterize in young & old SDFTs
  - Changes as a result of loading
  - Develop methods to image *in situ* & *in vivo*

- Cell response to loading
  - Further investigate changes in protein levels
  - Effect of ageing
  - Methods to prevent degradative response

Find out more about tendon and ligament injuries in racehorses and this project

- PD Clegg, Musculoskeletal disease and injury, now and in the future.
  - Part 2: Tendon and ligament injuries http://onlinelibrary.wiley.com/doi/10.1111/j.2042-3306.2012.00563.x/full
- Research carried out by Dr Chavaunne Thorpe, winner of the prestigious Savio-L-Y. Woo Young Researcher prize
  - Helical sub-structures in energy-storing tendons provide a possible mechanism for efficient energy storage and return http://www.hblb.org.uk/documents/blog/Thorpe%20tendon%20research.pdf
  - To find out more info about Dr Thorpe visit 'In the Press' section of Racehorse health
- Thorpe, C., Spiesz, E., Chaudhry, S., Screen, H. and Clegg, P., (2015) Science in brief: Recent advances into understanding tendon function and injury. *Equine Vet. J.,* **47**, 137-140.

  http://onlinelibrary.wiley.com/doi/10.1111/evj.12346/abstract

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