INTRODUCTION:
Non-enzymatic glycation of connective tissue matrix proteins is a major contributor to the pathology of diabetes and ageing. Biochemical and morphological alterations due to glycation of collagen in tendon have been well characterized. Further, non-enzymatic glycation induced cross-links have been shown to influence the biomechanics of tendon, with increased stiffness and more brittle failure. This leads to reduced functionality of aged and diabetic tendon making them more susceptible to damage and injury, and interfering with healing processes [1]. However, it remains unknown on what hierarchical level glycation induced cross-links affect tendon mechanics, thereby making it difficult to understand repercussions on mechano-transduction, subsequent tissue remodeling, and the design of novel treatments. To fill this gap, the present study investigates glycation induced alterations to the tendon meso-scale. For that reason tendon were stretched under a multiphoton confocal microscope after cell nuclei staining as markers to separate fibrillar sliding and fibrillar stretch. Methylglyoxal (MG), a naturally occurring reagent inducing glycation and consecutive cross-linking was used to induce cross-linking. We hypothesized that MG cross-linking would change the way tendon reacts to load at the fiber level.

METHODS:
Tails from skeletally mature rats (at least 17 weeks old) were removed and thawed after sacrifice according to local and federal regulations. Approximately 6 mm long rat tail tendon fascicles (RTTF) were carefully extracted from the middle part of the tail. Cell nuclei were stained in Syto13 solution for 12 hours. 5 fascicles were then incubated for 4 hours in MG crosslinking solution. 5 additional fascicles were used as control samples.

Specimens were then mounted on a custom designed tensile straining device (Fig.1A), to load the fascicles while on the stage of a multiphoton confocal microscope (Olympus FV1000MPE). The stretching device consisted of two linear stepper motors (Zaber NA11B30) and a load cell (Inelta KMM20). Samples were immersed in a petri-dish filled with PBS throughout the stretching process. 0.05N of preload was applied to measure initial length that allowed strain calculations, followed by 10 cycles of preconditioning between 0-2% strain. 100µm deep image stacks were taken at 5 µm depth resolution using a 25X magnification lens. Strains from 0-6% were applied to the samples at 0.5% increments. 3D stacks were taken at each strain increments (60s after stretch) and combined to form 4D (3D+ stretch) movies. The 60s were necessary to reposition the tendon to guarantee measurements are of the same location within the tendon.

Cell nuclei movements were tracked using Imaris 7.2 (Fig.1B). Displacements were then exported to matlab (v7.9) for further analysis. Cell nuclei were clustered using k-means clustering algorithm into groups (Fig.1C) where each group of cells belongs to a single fiber according to their positions and displacements. Intra fiber strains and sliding in between fibers (represented as percentage of the field of view) were calculated and averaged using a custom algorithm. Force data immediately after each stretch was also recorded for stiffness comparison. An unpaired t-test was used to compare stiffness between MG cross-linked and control samples.

RESULTS:
Cell tracking results showed MG cross-linked tendon had higher intra fiber strain, but comparable fiber sliding compared to control samples. (Fig.2A and 2B). Forces at corresponding strains were generally higher in MG cross-linked samples compared to untreated ones, which resulted in significantly higher stiffness in the linear part of the stress-strain curve of the MG treated samples (p<0.01) (Fig.2C).

DISCUSSION:
To the best of our knowledge, this is the first study to investigate glycation related functional alterations in tendon at the fiber level. We have found that in MG cross-linked tendon, fibrillar stretch occurs more predominately compared to fibrillar sliding, in contrast to what occurs in normal tendon. In addition, higher total strains (summed intra and inter fiber strain for a given applied strain) due to increased fibrillar extension in the MG cross-linked tendon, suggests less relaxation in MG cross-linked tendon compared to controls.

A major limitation of this study is that image stacks could only be taken 60s after each stretch increment - and results only represent what happens to tendon after relaxation.

SIGNIFICANCE:
This study investigates the difference in mechanical response to loading at fiber level for ageing tendon, with the aim to better understand the root cause in age-related tendon diseases.

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REFERENCES: