Collagenous and Non-Collagenous Protein Half-life Differs in Functionally Distinct Tendons

INTRODUCTION:
Tendons transfer force from muscle to bone; in addition some tendons such as the equine superficial digital flexor tendon (SDFT) and human Achilles tendon play a role as an energy store contributing to the efficiency of locomotion. Energy storing tendons need to stretch maximally and recoil for efficient function and have a lower elastic modulus (less stiff material) than positional tendons such as the equine common digital extensor tendon (CDET) (1). We have previously shown that this difference in mechanical properties is a consequence of differences in matrix composition. Efficient energy storage requires the tendon to stretch and therefore energy storing tendons are exposed to high strains; up to 16% in the equine SDFT which is close to the failure strain for this tendon. High strains increase the risk of microdamage occurring to the matrix suggesting that energy storing tendons require a higher capacity for matrix turnover. However our previous findings surprisingly indicated a lower rate of turnover in the SDFT, based on tissue fluorescence measurements (2) and D/L aspartic acid ratios suggested no difference in matrix age between the two tendon types (3).

The SDFT and CDET are subjected to very different physiological mechanical environments in terms of strain level and strain rate, and have an associated difference in matrix composition (1) suggesting a difference in the contribution of collagenous and non-collagenous components to the mechanical response. We therefore hypothesise that there is a difference in the turnover rate of collagenous and non-collagenous matrix components between the SDFT and CDET.

The objectives of this study were to measure collagenous and non-collagenous matrix half life in the functionally distinct SDFT and CDET using the rate of D-Aspartate accumulation as a probe (4) in a group of horses selected with a wide age range.

METHODS:
The mid-metacarpal region of the SDFT and CDET were harvested from 32 horses aged 4 to 30 years euthanased for reasons other than tendon injury. All tendons were normal on gross pathological examination. The matrix was separated into collagenous and non-collagenous components by extracting lyophilised, powdered tendon tissue with guanidine-HCl. The ratio of D to L-Aspartate was measured collag enous and non-collagenous matrix components by extracting lyophilised, powdered tendon tissue with guanidine-HCl. The ratio of D to L-Aspartate was measured by extracting lyophilised, powdered tendon tissue with guanidine-HCl. The ratio of D to L-Aspartate was measured. D-Aspartate measurements were used to estimate the half-life of the collagenous and non-collagenous matrix components (4). Statistical significance was assessed in SPSS using paired t-tests and Pearson Product Moment Correlation and was set at p<0.05.

RESULTS:
Collagen half-life was significantly greater (p<0.001) in the SDFT (197.53±18.23 years) than in the CDET (34.03±3.39 years) and increased significantly with age in the SDFT (p=0.032). Collagen half-life showed no correlation with horse age in the CDET (Fig. 1).

The half-life of the non-collagenous matrix was significantly greater (p=0.036) in the CDET (3.51±0.51 years) compared to the SDFT (2.18±0.41 years) and showed no correlation with horse age in either tendon (Fig. 2). The non-collagenous matrix had a significantly lower (p<0.001) half-life than the collagenous component in both tendons.

Figure 1: Half-life of collagenous matrix plotted against horse age

Figure 2: Half-life of non-collagenous matrix plotted against horse age

DISCUSSION:
The results of this study support our hypothesis and show that the prioritisation of collagenous versus non-collagenous protein turnover differs between the tendon types. The greater collagen half-life in the SDFT supports our tissue fluorescence measurements (3) and indicates a lower rate of collagen turnover in this tendon than in the CDET. It may be that turnover of collagen molecules in high strain tendons is minimal in order to maintain the tendon within the narrow limits of strength and stiffness required for efficient energy storage. A high rate of turnover may weaken the tendon transiently as collagen molecules are degraded and replaced, and the maturation of new molecules may alter the tendon’s material properties. The decrease in collagen turnover in the SDFT with increasing horse age may explain the increased incidence of injury in this tendon with increasing age (6). Based on our previous work, the mechanism does not appear to be a reduction in collagenase levels (3) but may be due to increased resistance of the collagen to enzymatic cleavage due to age related glycation. Turnover of non-collagenous proteins in the matrix (mainly proteoglycans) appears to occur more rapidly in the more injury prone energy storing SDFT than in the CDET. Models of the mechanical response of tendon to load suggest that the viscous component, provided by the proteoglycans, is more influential at higher strain rates (7) as experienced by the SDFT. In support of this we have previously shown that tenocytes in the SDFT produce more mRNA for proteoglycans and some proteoglycan degrading enzymes than their counterparts in the CDET (3).

In conclusion, we have found that turnover of collagen is greater in the low strain positional CDET than in the high strain energy storing SDFT, whereas turnover of the non-collagenous matrix occurs more rapidly in the SDFT than in the CDET. This differential regulation of protein turnover reflects the different mechanical demands and suggests that proteoglycans have a greater influence on mechanical behaviour in energy storing tendons than positional tendons. Further work will be undertaken to determine if cell phenotype is pre-set or whether the cells can alter the synthesis and degradation of matrix proteins when placed in a different strain environment.

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References: