AGE RELATED CHANGES TO MECHANICAL AND MATRIX PROPERTIES IN HUMAN ACHILLES TENDON

Introduction
Achilles tendon rupture is a common and debilitating injury occurring not only in ‘elite’ athletes but also in individuals with a ‘normal’ lifestyle. Recent studies have shown that the incidence of injury is increasing and that injuries are more prevalent in middle and old age with a peak occurring in the fourth and eighth decade of life. This suggests that the mechanical integrity of the tendon may decline with age. Previous studies of age related changes have been inconclusive with some showing an increase in stiffness while others report no change. In this study we test the hypothesis that ageing results in a deterioration of material properties, which is specific to energy storing tendons, such as the Achilles tendon.

Methods
Human Achilles (n=10) and anterior tibialis (n=5) tendons (non-energy storing) were retrieved post mortem (stored frozen at -20°C, n=4) and from amputated limbs (fresh, n=11) from female (n=4) and male (n=5) donors aged 48 to 84 years. Cross sectional area (CSA) was measured in the mid-region of the tendons, which corresponds to the site most often injured in the Achilles tendon (approximately 3 cm from the insertion on the calcaneus). Tendons were moulded in dental embedding paste (Blueprint Cermix, Claudius Ash), a digital image taken of the mould and image analysis software (Image-Pro Plus) used to calculate CSA. Tendons were mechanically tested to failure in a hydraulic materials testing machine (Dartec). Tendons were mounted using cryoclamps with the level used for CSA measurement centred and a gauge length of 3 cm. A pre-load of 25 N (Achilles) or 12 N (ant. tib.) was applied and tendons conditioned using 20 loading cycles from 25 N to 1 kN (Achilles) or 12 N to 0.5 kN (ant. tib.) at 0.5 Hz. Following conditioning, the tendon was loaded at 100%/sec to failure. A load/displacement curve was plotted and the ultimate strength, ultimate stress (force per unit area at failure), stiffness and elastic modulus (force per unit area per unit extension in the linear region) were calculated for each tendon.

Following the destructive test, a sample of tissue (n=11) was taken from the mid-region of Achilles and anterior tibialis tendons (6 donors) for matrix analysis. Water content, collagen content (hydroxyproline assay), total sulphated glycosaminoglycan (GAG) content (dimethylmethylen blue assay), DNA content (fluorometric assay using Hoechst 33258) and collagen-linked fluorescence were determined. Statistical significance was evaluated using a paired t-test and correlations using a Spearman’s correlation (SPSS software).

Results
The Achilles tendon had a significantly (p = 0.039) lower elastic modulus than the anterior tibialis tendon from the same limb. However, ultimate stress values were not significantly different between Achilles and anterior tibialis tendons (Fig. 1). Achilles tendon tissue had a significantly (p = 0.006), higher GAG content (p = 0.010), higher collagen-linked fluorescence values (p = 0.018), higher DNA content (p < 0.001) but no difference in total collagen content compared to the anterior tibialis tendon (Table 1).

Table 1 Matrix composition of Achilles (n = 6) and anterior tibialis (n = 5) tendons. Data are presented as mean ± SD. * denotes a significant difference relative to anterior tibialis tendon.

<table>
<thead>
<tr>
<th></th>
<th>Achilles</th>
<th>anterior tibialis</th>
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<tbody>
<tr>
<td>% Water content</td>
<td>69.0 ± 2.9</td>
<td>56.7 ± 6.3</td>
</tr>
<tr>
<td>% Collagen content (dry wt)</td>
<td>77.2 ± 1.3</td>
<td>76.8 ± 3.7</td>
</tr>
<tr>
<td>Fluorescence (units/mg coll)</td>
<td>588 ± 128 *</td>
<td>540 ± 131</td>
</tr>
<tr>
<td>GAG content (µg/mg dry wt)</td>
<td>8.17 ± 1.49 *</td>
<td>5.30 ± 1.72</td>
</tr>
<tr>
<td>DNA (µg/mg dry wt)</td>
<td>0.61 ± 0.05 *</td>
<td>0.18 ± 0.05</td>
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The ultimate stress values decreased significantly (p = 0.007) with age in both tendons (Fig. 1). The GAG content increased with age and showed a significant (p = 0.025) correlation with tendon ultimate stress values.

Discussion
These results demonstrate a difference in the matrix properties between human Achilles tendon and anterior tibialis tendon. These differences are reflected in their mechanical properties and may relate to physiological function. In previous work, we have noticed similar differences in energy storing and non-energy storing tendons in the horse. Ageing results in a decrease in the strength of tendon tissue in the Achilles tendon (energy storing) and the anterior tibialis tendon (non-energy storing). These results may explain the increased incidence of tendon injury in older age. The changes observed in the tendon matrix with age are similar to changes we have observed in degenerated equine tendons. Comparable changes have been observed in ligaments following disuse, thus the changes observed may result from relative inactivity in older people. Determination of the age at which this deterioration begins and the effect of exercise on the process are important areas to focus on in the future.

Conclusion
Tendons which are functional distinct have specific mechanical and matrix properties, suggesting that tenocytes are responsive to their mechanical environment. However, the effect of ageing is similar for both energy and non-energy storing tendons.

References