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Fascicles and the interfascicular matrix show adaptation for fatigue resistance in energy storing tendons

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ABSTRACT

Tendon is composed of rope-like fascicles, bound together by interfascicular matrix (IFM). Our previous work shows that the IFM is critical for tendon function, facilitating sliding between fascicles to allow tendons to stretch. This function is particularly important in energy storing tendons, which experience extremely high strains during exercise, and therefore require the capacity for considerable inter-fascicular sliding and recoil. This capacity is not required in positional tendons. Whilst we have previously described the quasi-static properties of the IFM, the fatigue resistance of the IFM in functionally distinct tendons remains unknown. We therefore tested the hypothesis that fascicles and IFM in the energy storing equine superficial digital flexor tendon (SDFT) are more fatigue resistant than those in the positional common digital extensor tendon (CDET). Fascicles and IFM from both tendon types were subjected to cyclic fatigue testing until failure, and mechanical properties were calculated. The results demonstrated that both fascicles and IFM from the energy storing SDFT were able to resist a greater number of cycles before failure than those from the positional CDET. Further, SDFT fascicles and IFM exhibited less hysteresis over the course of testing than their counterparts in the CDET. This is the first study to assess the fatigue resistance of the IFM, demonstrating that IFM has a functional role within tendon and contributes significantly to tendon mechanical properties. These data provide important advances into fully characterising tendon structure-function relationships.

Statement of Significance

Understanding tendon-structure function relationships is crucial for the development of effective preventative measures and treatments for tendon injury. In this study, we demonstrate for the first time that the interfascicular matrix is able to withstand a high degree of cyclic loading, and is specialised for improved fatigue resistance in energy storing tendons. These findings highlight the importance of the interfascicular matrix in the function of energy storing tendons, and potentially provide new avenues for the development of treatments for tendon injury which specifically target the interfascicular matrix.

1. Introduction

Energy storing tendons, such as the human Achilles and patellar tendons, play an important role in locomotory efficiency, decreasing the energetic cost associated with movement [1,2]. To enable this function, energy storing tendons have distinct mechanical properties, such as greater extensibility and elasticity leading to improved energy storage and return, when compared to tendons that are purely positional in function, such as the anterior tibialis tendon [1,3–5]. Energy storing tendons also have superior fatigue resistance, withstanding a greater number of loading cycles prior to failure than positional tendons in mechanical tests using the whole tendon [6,7].

Tendons are hierarchical fibre-composite materials, in which collagenous units are grouped together, forming subunits of increasing diameter [8]. At the higher hierarchical levels, the collagen is interspersed with a less fibrous, highly hydrated matrix, traditionally referred to as the ground substance [9]. The largest tendon subunit is the fascicle; with a diameter of approximately

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300 μm, fascicles are visible to the naked eye and can be isolated by cutting longitudinally through the tendon. Fascicles are bound together by the interfascicular matrix (IFM), which is the largest hierarchical level of ground substance, and is also referred to as the endotenon. The IFM is rich in glycoproteins, elastin and collagens [9–11].

In order to fully understand tendon structure-function relationships, it is important to characterise the specialisations that result in enhanced energy storage in specific tendons. Our previous studies have demonstrated specialisation of both fascicles and IFM in energy storing tendons. The additional extensibility in energy storing tendons is provided by the IFM, which enables greater sliding between adjacent fascicles, resulting in higher levels of extension in the tendon as a whole [3]. In addition, both fascicles and the IFM are more elastic in energy storing tendons, demonstrating less hysteresis and stress relaxation during cyclic loading than in positional tendons [12,13]. We have also shown that fascicles from energy storing tendons are more fatigue resistant than those from positional tendons, both in the bovine and equine model [13,14], however no previous studies have assessed the fatigue resistance of the IFM and how this differs between tendons with differing functions.

In the current study, we adopted the equine model to assess the fatigue response of functionally distinct tendons. The horse is a relevant and accepted model for tendon research, as it is an athletic species which maximises energy efficiency by storage and release of elastic energy in the limb tendons. The predominant energy store in the horse is the forelimb superficial digital flexor tendon (SDFT), which has an analogous function to the Achilles tendon [15–17]. Indeed, tendon injuries in the SDFT show a very similar epidemiology, aetiology, and pathology to those seen in the human Achilles tendon [16,17]. The anatomically opposing equine common digital extensor tendon (CDET) is an example of a positional tendon, functionally comparable to the human anterior tibialis tendon [18]. We tested the hypothesis that the IFM in the energy storing SDFT is more fatigue resistant than the IFM in the positional CDET, similar to the difference between the fascicles in the two tendon types.

2. Materials and methods

2.1. Sample collection and preparation

Forelimbs, distal to the carpus, were collected from horses aged 3–7 years (n = 4) euthanased at a commercial equine abattoir, as a by-product of the agricultural industry. Specifically, the Animal (Scientific Procedures) Act 1986, Schedule 2, does not define collection from these sources as scientific procedures. The SDFT and CDET were harvested from the forelimbs within 24 h of euthanasia. Whilst it was not possible to obtain a full exercise history for the horses, none of the tendons had clinical or macroscopic evidence of tendon injury. Tendons were wrapped in tissue paper dampened with phosphate buffered saline (PBS) and then in tin foil and stored at −80 °C. On the day of testing, tendons were thawed and fascicles, approximately 30 mm in length, were dissected from the mid-metacarpal region of the tendon as previously described (n = 6–8 per tendon) [19]. In addition, groups of two fascicles, bound together by IFM were also dissected from the same region (n = 6–8 per tendon) [3]. Fascicle hydration was maintained by storing the samples on tissue paper dampened with Dulbecco's modified eagle medium (DMEM).

2.2. Determination of fascicle fatigue properties

Fascicle diameter was determined using a laser micrometer, measuring continuously along a 10 mm length in the central portion of the fascicle and taking the smallest diameter to calculate cross-sectional area, assuming a circular cross section [3]. Fascicles were secured in custom made individual loading chambers [20], with a grip to grip distance of 10 mm, and fascicle fatigue properties were determined using an Electroforce 5500 mechanical testing machine, equipped with a 22 N load cell (TA instruments, Delaware, USA), housed within a cell culture incubator (37 °C, 20% O_2, 5% CO_2). A pre-load of 0.1 N was applied to remove any slack within the samples. We have previously shown that fascicle failure strain is more consistent between samples than failure stress [3], Accordingly, one loading cycle to a displacement of 1 mm (10% strain, equivalent to 50% of predicted fascicle failure strain [19]) was applied to establish an appropriate and consistent peak load for cyclic fatigue testing. This peak load was subsequently applied to the fascicles in a cyclic manner at a frequency of 1 Hz until sample failure. Load and displacement data were recorded continuously throughout the test at a frequency of 100 Hz. In addition, the maximum and minimum load and displacement were recorded for each cycle.

2.3. Determination of IFM fatigue properties

Samples were prepared for IFM fatigue testing as previously described [3,21]. Briefly, transverse cuts were made in the opposing ends of 2 fascicles bound together by IFM, leaving a consistent IFM length of 10 mm. The intact end of each fascicle was secured in the loading chambers and IFM fatigue properties were determined using an Electroforce 5500 mechanical testing machine, equipped with a 22 N load cell, housed within a cell culture incubator (37 °C, 20% O_2, 5% CO_2). A pre-load of 0.02 N was applied to remove any slack within the samples. IFM failure extension is more consistent between cycles than failure force [3], therefore one loading cycle of 1 mm displacement was applied, which is equivalent to 50% of the predicted failure extension [3], to find the peak load. This load was subsequently applied to the IFM in a cyclic manner at a frequency of 1 Hz until sample failure. Load and displacement data were recorded continuously throughout the test at a frequency of 100 Hz. In addition, the maximum and minimum load and displacement were recorded for each cycle.

2.4. Data analysis

For each test, the number of cycles to failure was recorded. The maximum and minimum displacement data were used to plot creep curves to failure (Fig. 1a) and the gradient of the maximum and minimum displacement curves during secondary creep were calculated.

The load and displacement data were used to plot force extension curves (Fig. 1b). Hysteresis over cycles 1–10, 11–20, the middle 10 cycles and the last 10 cycles prior to failure was calculated by dividing the area between the loading and unloading curves (energy dissipated) by the area under the loading portion of the curve (energy input), and expressed as a percentage. In addition, the maximum loading and unloading stiffness was calculated for cycle 1, cycle 10, the mid-test cycle, 10 cycles prior to failure and the last cycle prior to failure.

Fascicle elongation was calculated at cycle 10 and at the cycle prior to failure by subtracting the maximum extension at cycle 1 from the maximum extension in these cycles. It was not possible to calculate IFM elongation, relative to the first cycle, as the low forces involved in this load controlled experiment required several cycles to fully stabilise, therefore the elongation between cycle 10 and the cycle prior to failure was calculated.