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The mechanical properties of tail tendon fascicles from lubricin knockout, wild type and heterozygous mice

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ABSTRACT

The purpose of this study was to analyze the effects of lubricin on tendon stiffness and viscoelasticity.

A total of 36 mice were tested with 12 mice in each of the following groups: lubricin knock-out (−/−), heterozygous (+/−) and wild-type (+/+). A ramp test was used to determine the elastic modulus by pulling the fascicles to 2.5% strain amplitude at a rate of 0.05 mm/s. Then, followed by a relaxation test that pulled the fascicles to 5% strain amplitude at a rate of 2 mm/s. The fascicles were allowed to relax for 2 min at the maximum strain and a single-cycle relaxation ratio was used to characterize viscoelastic properties.

There was no significant difference in the Young's modulus between the three groups ($p > 0.05$), but the knockout mice had a significantly ($p < 0.05$) lower relaxation ratio than the wild type mice.

Based on these data, we concluded that lubricin expression has an effect on the viscoelastic properties of tendon fascicles. The clinical significance of this finding, if any, remains to be demonstrated.

1. Introduction

Lubricin, also known as proteoglycan 4 or superficial zone protein, has many biological functions. These include cytoprotection, lubrication and anti-adhesion (Rhee et al., 2005; Sun et al., 2006). Lubricin improves tendon gliding (Taguchi et al., 2008), and is present between tendon fascicles as well (Sun et al., 2006). Recent evidence (Kohrs et al., 2011) suggests a role for lubricin in interfascicular gliding, but the effect of lubricin on tendon stiffness and viscoelasticity is unknown.

The function of the tendon is to efficiently transfer forces from the muscle to the skeleton, minimizing energy loss during the load transfer as well as allowing enough extension to avoid injury (Gupta et al., 2010; Ker, 2007; Screen, 2008; Yin and Elliott, 2004). Tendons consist of bundles of collagen fibers, or fascicles, which play an important role in their tensile properties (Bensamoun et al., 2006). Collagen makes up 70–80% of a tendon or ligament's dry weight (Yamamoto et al., 1999). Tendons also consist of proteoglycans, which play a dominant role in the viscoelastic behavior and strain transfer within the tendon (Yin and Elliott, 2004). Proteoglycans are the major crosslinking elements between collagens. Loss of proteoglycans in tendon leads to altered assembly of collagen fibrils and changes the tendon mechanical properties (Danielson et al., 1997; Kuc and Scott, 1997; Pins et al., 1997; Robinson et al., 2005). It is also clear that age affects the material properties of tendons and tendon fascicles (Bensamoun et al., 2006).

The purpose of this study was to analyze the effects of lubricin on tendon mechanical properties by comparing tail fascicles from wild, heterozygous and lubricin knockout (Kohrs et al., 2011) mice. The hypothesis was that KO mice fascicles would have a higher stiffness and a lower relaxation ratio.

2. Materials and methods

2.1. Fascicle isolation and preparation

Animals were obtained after use in other studies approved by the Institutional Animal Care and Use Committee (IACUC). A total

Abbreviations: L_0, initial length; TIEG, TGF-β inducible early gene-1; KO, knockout.

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of 36 mice were used with 12 mice in each of the following groups: Lubricin knock-out (−/−), heterozygous (+/−) and wild-type (+/+). After sacrifice, the mouse tails were resected and stored frozen at −80 °C. This process has been shown not to affect tendon mechanical properties (Graf et al., 1992; Lee et al., 2009). The tails were then thawed, dissected and tested at room temperature. The age range for all mice was between 10 and 13 weeks. The gender was random; the specific male–female distribution was 7–5, 4–8 and 6–6 for the wild type, heterozygous and lubricin knock-out groups, respectively. The mouse tails were transected 60 mm from the distal end of the tail. The skin was removed 1 mm from the proximal end to provide space to find an appropriate fascicle. The tail was transected again at a level 33 mm from the distal end, leaving a 27 mm tail piece for fascicle isolation (Fig. 1). Fascicles were carefully removed from the proximal end of this section using forceps. The only fascicles used in these tests were those that slid out smoothly with minimal resistance. We considered the experimental unit to be the animal, not the individual fascicle, and we also considered that there might be some variability in the diameter of individual fascicles within the same animal. Four fascicles – two fascicles from each of the left and right dorsal tendons (Fig. 2) – were used for mechanical evaluation, and the data was averaged to yield a single data point (Bensamoun et al., 2006; Kohrs et al., 2011). During dissection and testing, the fascicles were kept moist with saline.

2.2. Fascicle cross-sectional area

Once removed, each fascicle was secured with Loctite 401 cyanoacrylate adhesive to thin sheets of Nitrile rubber on both ends, exposing 15 mm of fascicle between sheets (Fig. 3). Care was taken to ensure there was no adhesive on the exposed 15 mm section. Each fascicle was mounted onto a custom fixture which allowed it to be rotated 90° about the long axis while being submerged in saline. Eight scaled measurements of the diameter were taken under 200× magnification at 0° and 90° and rotated around the long axis for a total of 16 measurements. The cross-sectional area was calculated using the average diameter and assuming a round cross-section. Before being mounted on the testing apparatus, the initial length (L_0) corresponding to 0% strain (under a 2 g preload) was measured with a digital caliper (Interapid, Brown & Sharpe, North Kingstown, RI). Strain for each specimen was calculated based on this measured initial length.

2.3. Mechanical testing

The fascicles were mounted onto a custom-designed mechanical test system (Fig. 4), which includes two clamps, a 150-g transducer (Transducer Techniques, Temecula, CA), and a stepper-motor-driven linear actuator (Servo Systems, Montville, NJ). Data was collected at a sampling rate of 100 Hz. Fascicles were kept submerged in a saline bath at room temperature throughout testing.

2.4. Ramp test

There was no cyclic preconditioning of the fascicles. Each fascicle was subjected to a preload of approximately 2 g at the start of the ramp test. The fascicle was then pulled to 2.5% strain amplitude at a rate of 0.05 mm/s and returned to the initial position at the same speed. The fascicle was then maintained at L_0 for 60 s prior to the relaxation test. The elastic modulus was calculated from the slope of the linear region of the stress–strain curve.

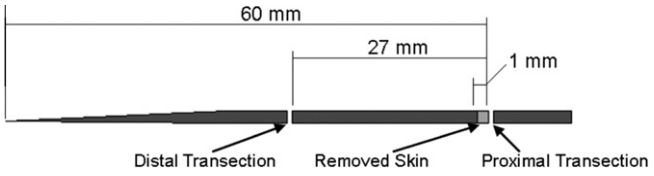

Fig.1. A mouse tail diagram showing the transection cuts.

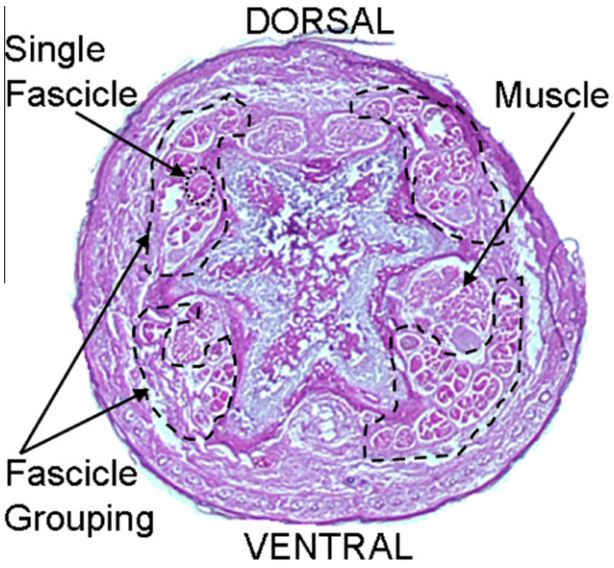

Fig.2. A stained cross-section of a mouse tail showing the four tendons and the fascicle grouping within the tendons.

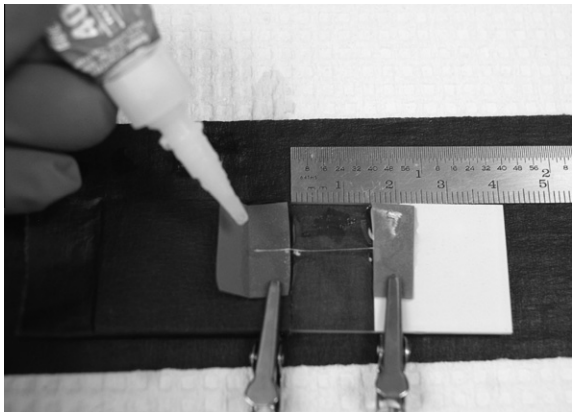

Fig.3. A photograph showing the process of attaching a fascicle to the medium material (Nitrile rubber) with glue (Loctite 401 cyanoacrylate adhesive). The fascicle is kept moist in saline throughout the process.

2.5. Relaxation test

Following the ramp test, each fascicle was once again preloaded to approximately 2 g and then pulled to 5% strain amplitude at a speed of 2 mm/s. The fascicles were allowed to relax at this maximum strain amplitude for 2 min. Based on pilot data, 2 min was determined to be a suitable relaxation period to achieve a static stress. The elastic modulus was again calculated from the slope of the linear region of the stress–strain curve. The viscoelastic properties of the fascicles were characterized by defining a stress relaxation ratio (Eq. (1)), a ratio of the stress at 5% (maximum) strain, σ_p, to the static stress after 2 min of relaxation, σ_r (Fig. 5). The preload was subtracted from the maximum and static stresses before calculating this ratio.