High Resolution Diffusion Tensor MRI of Rabbit Tendons and Ligaments at 11.7T

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INTRODUCTION

Tendon and ligament injuries such as sprains and ruptures are a major source of musculoskeletal disability. Magnetic resonance imaging (MRI) is a reliable tool for detecting large and complete tears of these tissues; however, conventional T1 and T2-weighted grayscale images exhibit poor contrast and a low signal-to-noise ratio which makes identification of low-grade injuries more challenging to delineate. Diffusion tensor imaging (DTI) is a well established MRI approach for assessing tissue microstructural organization by quantifying the 3D diffusion of water molecules within tissues. DTI has been widely used to quantify the orientation of white matter tracts in the brain and more recently has been adapted to characterize the matrix organization of skeletal tissues such as muscle, intervertebral disc and articular cartilage.[1-5] The highly-organized, anisotropic, hierarchical composite structure of tendons and ligaments is likely well suited for DTI; however, very few DTI data have been reported for these tissues. The current study presents results of high field MRI analyses of rabbit medial collateral ligament (MCL) and semitendinosus (SemiT) tendon, with a focus on quantifying DTI metrics and fiber tractography.

METHODS

Specimen Preparation: Contralateral pairs of SemiT tendons from three and MCLs from two male, skeletally mature New Zealand White rabbits were studied. A custom made tissue holder was used to secure the tissues inside the NMR tube. The proximal and distal aspects of each tissue were glued to the specimen holder at an approximate angle of 55° to the direction of B_0, the applied magnetic field, to increase signal intensity due to the magic angle effect.[6] The holder was then placed in a 10 mm diameter NMR tube containing perfluorinated oil (Krytox GPL-102 PFPE oil, DuPont, NJ, USA) to maintain the hydration state of the tendons as well as to minimize proton signals from the bathing medium. The tube was then placed in a RF coil and inserted into the bore of the magnet.

Image Acquisition: Scans were conducted at 11.74T in a 56mm vertical bore magnet using a Bruker DRX Avance spectrometer (Bruker BioSpin, Billerica, MA, USA). Images were acquired using a Bruker Micro 5 imaging probe with triple axis gradients (maximum strength 200 G/cm) and a 10 mm diameter RF saddle coil[7]. Each specimen received DTI, T1 and T2-weighted sequences. DTI was performed using a 3D spin echo DTI sequence (MCLs: TR = 1s, TE = 14 ms, δ/Δ = 1/9 ms, NEX = 2, FOV = 12.8 x 12.8 mm, six diffusion directions, 100 µm thick coronal slices, b= 600 s/mm^2, in-plane resolution=100 µm, scan time= 8 hrs/sample; SemiTs: TR = 1s, TE = 15 ms, δ/Δ = 2/8 ms, NEX = 1, FOV = 9.6 x 3.2 mm, six diffusion directions, 200 µm thick axial slices, b= 600 s/mm^2, in-plane resolution=50 µm, scan time= 2 hrs/sample). T1 was measured using a saturation recovery spin echo sequence in 12 steps with TRs from 105 to 5000 ms (TE = 8 ms, slice thickness = 200 µm). T2 was measured using a CPMG spin echo sequence with 16 echoes and 5.3 ms echo spacing (TR = 3 s, slice thickness = 200 µm).

Data Analysis: Fractional anisotropy (FA) and mean diffusivity (MD) values were extracted from the experimental data using Bruker's ParaVision 4.0 software. These values were computed from the ROIs drawn on the middle slice of the image data acquisition set for each tissue. FA is a scalar metric that describes the directionality of the diffusion tensor and has values ranging between 0 (isotropic) and 1 (anisotropic). MD is a non-directional measure of free translational diffusion and provides an index of general tissue integrity. DTI-Studio version 3.0.1 (John Hopkins University, Baltimore, MD, USA) was used to generate collagen fiber tracts for both types of tissues using the Fiber Assignment by Continuous Tracking (FACT) algorithm. Tractographic analysis of DTI data is based on the assumption that the primary eigenvector of the diffusion tensor coincides with the local fiber orientation.

RESULTS

The average T1 and T2 relaxation times for the four MCL samples studied were 1375 ± 21 and 25.7 ± 1.7 ms, T1 and T2 values for the six SemiT samples studied were 1350 ± 84 and 32.9 ± 1.6 ms, respectively. FA and MD (x10^{-6} mm^2/s) results for these tissues are presented in Tables 1 and 2. Average FA and MD values for the six SemiTs were 0.66 and 1388x10^{-6} mm^2/s respectively, and corresponding values for the four MCLs were 0.47 and 1255x x10^{-6} mm^2/s respectively. Coronal slices of diffusion weighted images (Fig 1a) of the ligaments show parallel collagen fiber bundles running along the major axis of the tissue. This is supported by the 3D tractography image showing the spatial distribution and orientation of individual fiber tracts in the tissue (similar in both SemiTs and MCLs) (Fig 1b).

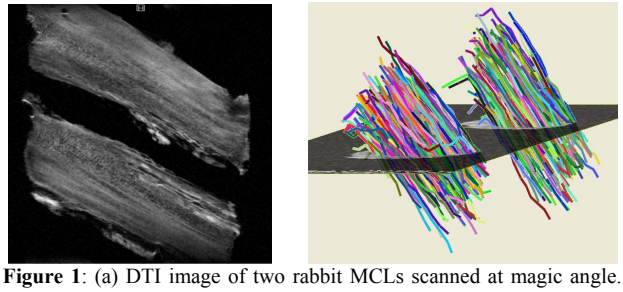

Figure 1: (a) DTI image of two rabbit MCLs scanned at magic angle. (b) Tractography showing collagen fiber tracts of two semitendinosus tendons generated using FACT algorithm from DTI Studio.

Sample#	FA	MD (x10^{-6} mm^2/s)
1	*0.63±0.17*	*1490±339*
2	*0.61±0.16*	*1540±343*
3	*0.67±0.18*	*1370±342*
4	*0.68±0.17*	*1290±336*
5	*0.73±0.19*	*1320± 403*
6	*0.68±0.17*	*1380±345*

Table1: FA and MD values (mean ± SD) from six rabbit Semitendinosus tendons.

Sample#	FA	MD (x10^{-6} mm^2/s)
1	*0.45±0.09*	*1290±134*
2	*0.48± 0.1*	*1180±115*
3	*0.48±0.09*	*1260±156*
4	*0.48± 0.1*	*1290±166*

Table 2: FA and MD values (mean ± SD) from four rabbit MCL ligaments.

DISCUSSION

To our knowledge, this is the first study that investigates tendon and ligament microstructure using 3D DTI at high magnetic fields. Our preliminary results for fractional anisotropy and mean diffusivity show excellent repeatability among tissues, and imaging results (Figure 1) confirm the known microstructural organization of collagen bundles in tendons and ligaments. The FA and MD values (Table 1, 2) are, respectively, higher and lower than corresponding reported values for articular cartilage, anulus fibrosus and skeletal muscle[3-5], consistent with the highly organized collagenous structure of tendons and ligaments. The quantitative and graphical capabilities of DTI are likely to provide more rigorous data regarding tissue structural integrity in comparison to conventional MRI. Ongoing work in our laboratory is examining the sensitivity of DTI metrics to mechanically-induced damage in tendons and ligaments.

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