In vivo fiber tracking of muscle anatomy in rodents (Oryctolagus cuniculus) on a clinical 3T MRI system

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Introduction: The determination of three-dimensional inner muscle architecture is the prerequisite to understand muscle deformation during contraction or to develop realistic finite element (FE) muscle models. Several recent studies have demonstrated that diffusion-tensor MRI (DT-MRI) allows quantitative determination of muscle fiber orientation *in vivo* in humans [1,2] as well as in animals [3,4]. So far, published animal studies were solely performed using MR animal systems. Thus, the purpose of this study was to investigate the feasibility of performing diffusion tensor imaging and fiber tractography on a rabbit using a short scan protocol on a clinical 3T MR system without the need for a special gradient insert system or similar specialized equipment.

Materials and Methods: MR scans were performed on a clinical 3 T MRI system (Tim Trio, Siemens Medical Solutions, Erlangen, Germany) using a small 8-channel multipurpose coil. The coil consists of two elements each containing four small coil loops (CPC, Noras, Höchberg, Germany). A rabbit (Oryctolagus cuniculus, m= 3180g) was anesthetized by using a mixture of Ketamine (10 mg/kg per hour) and Xylazine (1 mg/kg per hour). The legs of the animal were fixed using an adapted custom-made framework and the shanks of the animal were aligned parallel to the magnetic field of the scanner. The two elements of the coil were placed above and below the rabbit's shank, respectively. An anatomical scan was performed by using a single slab 3D T2-weighted TSE sequence with slab selective, variable excitation pulse sequence (SPACE sequence) in sagittal orientation with an isotropic resolution of 0.5 mm³ (FoV: 128 mm; matrix: 256; slices: 192, TR: 2500 ms; TE: 333 ms; TA: 21:02 min). The diffusion tensor scan was performed with a slightly modified EPI sequence provided by the manufacturer and a direction scheme comprising 70 directions each with a *b*-value of 700 s/mm² and six b_0-images. The modification of the sequence allowed a negative slice distance in order to effectively obtain overlapping slices to increase the resolution in slice direction. Sixty slices with transverse orientation and a thickness of 3 mm (FoV: 124 mm; matrix: 82) were acquired with a GRAPPA acceleration factor of 4, resulting in an echo time of 84 ms. The negative slice distance of -50% yielded a resolution of 1.51x1.51x1.5 mm³ for the diffusion tensor data set. The total acquisition time for the diffusion tensor scan with three repetitions was 17:38 min. Tensor reconstruction and fiber tracking were performed using the Diffusion Toolkit [5] and the tracts were evaluated and visualized using Trackvis [5].

Results: For visualization of the major muscle tracts several ROIs in the right shank, including the *M. tibialis anterior* (TA), *M. extensor digitorum longus* (EDL) (*yellow*), parts of the *M. soleus* (SOL*), M. plantaris* (PL) and *M. flexor hallucis longus* (FHL) (*red*) and the *M. gastrocnemius mediale* (GM, *blue*) and *laterale* (*GL*, *brown*) were drawn in the anatomic volume. The fibers which passed these selected ROIs are shown in different views in Fig. 1 along with the anatomic scan.

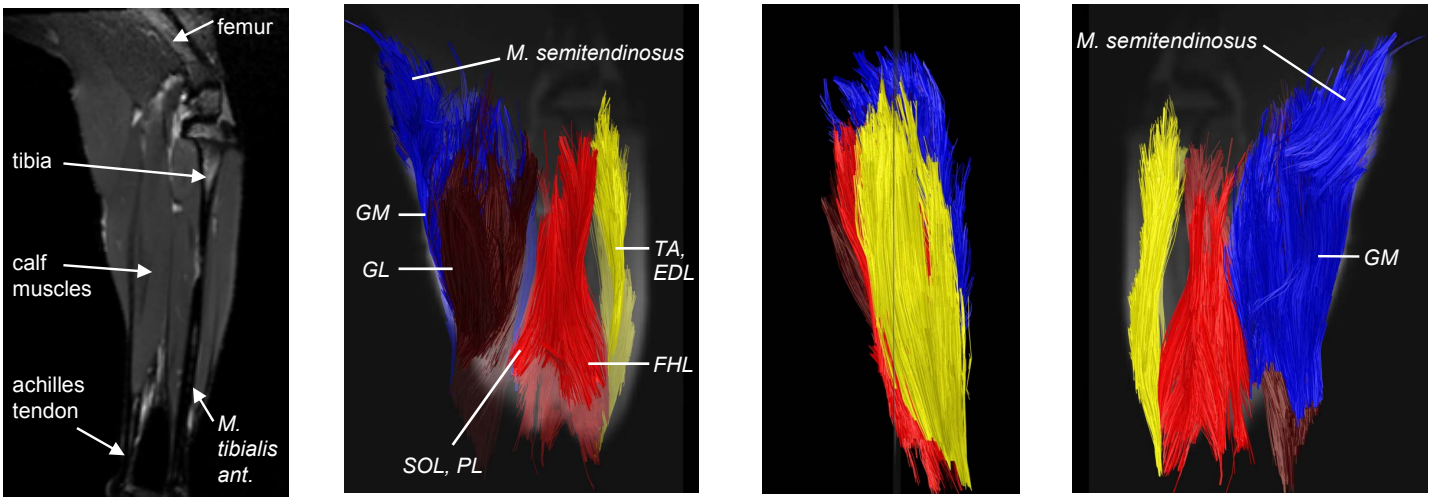

Fig. 1 From *left* to *right*: Sagittal view of the anatomic scan. Lateral, frontal and medial view of the fiber tracts of the right shank revealed from the selected ROIs overlaid on single T_2-weighted slices reconstructed from the 3D T_2-weighted TSE data slab for better anatomic orientation.

Discussion: This study shows the feasibility of *in vivo* fiber tracking of muscle tissue in a rabbit by using standard equipment in combination with a clinical MR system. Based on these first promising results we feel encouraged to investigate further the muscle structure with DTI by using this animal model with the proposed setup and to compare the results with ex-vivo examinations of the muscle fascicles by a manual digitizer (Microscribe MLX). Although we found an adequate mapping of the myoarchitecture, the applied EPI-based acquisition suffers from severe susceptibility artifacts and thus, deforms the architecture of the reconstructed myofibers. Consequently, it could be advantageous to apply 3D DTI sequences (e.g. based on Fast-Spin-Echo techniques) in future studies. Furthermore, from DT-MRI it is difficult to obtain information about the muscle contour. Muscle fibers may cross several muscles resulting in capturing e.g. *M. semitendinosus* and *GM* muscle fibers by defining a ROI within the GM (Fig. 1 right). Therefore it is necessary to include additional information about muscle shape from other measurements (MRI or manual digitizer) to clearly separate the muscles.

References: [1] Lansdown DA, et al. J Appl Physiol. 2007 Aug;103(2):673-81 ; [2] Kan JH, et al. J Magn Reson Imaging. 2009 Mar;29(3):663-70 ; [3] Heemskerk AM, et al. Magn Reson Med. 2005 Jun;53(6):1333-40 ; [4] Zhang J, et al. Exp Neurol. 2008 Aug;212(2):448-57 ; [5] Wang R and Wedeen VJ. Diffusion Toolkit, http://www.trackvis.org (2009)