In Vivo Measurement of Membrane Permeability and Fiber size in Calf Muscle Using Time-dependent DWI

E. Fieremans[1], D. S. Novikov[1], E. E. Sigmund[1], K. Liu[2], J. H. Jensen[1], and J. A. Helpern[1,3]
[1]Center for Biomedical Imaging, Department of Radiology, New York University School of Medicine, New York, New York, United States, [2]Siemens Medical Systems, United States, [3]Center for Advanced Brain Imaging, Nathan S. Kline Institute, Orangeburg, NY, United States

Introduction

The diffusivity measured in tissue *in vivo* depends on the diffusion time and is sensitive to tissue microstructure. Novikov *et al.* [1] recently showed that the presence of randomly oriented permeable flat membranes results in a time-dependent diffusion coefficient $D(t)$ that approaches the tortuosity limit as $1/\sqrt{t}$. From this dependence, values for the free diffusion coefficient D_0, the membrane permeability κ, membrane surface-to-volume ratio S/V, and the mean cell diameter $a = 2d \cdot V/S$ in d dimensions can be estimated. Time-dependent diffusion has been observed in rat brain gray matter before and after ischemic stroke [2] and *ex vivo* in the calf tongue and heart [3]. Here we measure the time-dependent diffusion *in vivo* in the human calf muscle. Assuming cell membranes of muscle fibers as predominant restrictions to water motion, we determine their membrane permeability, the free diffusion coefficient, and mean fiber diameters from the diffusion eigenvalues transverse to the fibers, based on ref [1].

Methods

Imaging was performed on the right calf muscle of a 28 y/o female and the left calf muscle of a 28 y/o male using a 3T wide-bore Siemens Verio system with an 8-channel knee coil. DWI images were acquired along 6 gradients directions for b = 0, 100 and 500 s/mm^2. The DWI was performed at a minimum of 8 different time points by using a Stejskal-Tanner or twice-refocused spin echo diffusion preparation for the shortest times and a stimulated echo diffusion preparation with mixing times from 10 ms up to 1.5 s at longer times. Other imaging parameters were: TR > 6s, TE $\geq$ 32ms, matrix = 64 x 64, FOV = 190 x 190 mm^2, thickness = 5 mm (female) and 10 mm (male). Total scan time was 50 min for each volunteer session.

DTI parametric maps were calculated for all diffusion times using in-house developed software in Matlab. The corrected b-matrix, as provided in the vendor sequence, was used to include the contributions of both applied diffusion gradients and imaging gradients. To verify the accuracy of the acquisition, the protocol was first tested on several liquids in which the diffusion coefficient was shown to be independent and isotropic over the range of diffusion times used in the muscle scans. Maps were generated of the mean diffusivity, fractional anisotropy (FA) and diffusion eigenvalues (λ_1,λ_2,λ_3). Regions of interest (ROIs) were manually outlined on T_2-weighted anatomical images to study the time-dependent diffusion in the Anterior Tibialis (AT), Extensor Digitorum Longus (EDL), Gastrocnemius Medialis (GM), Gastrocnemius Lateralis (GL), Peroneous Longus (PL), Posterior Tibialis (PT) and Soleus (SOL). The time-dependence of the transverse diffusivity $D_\perp(t) = (\lambda_2 + \lambda_3)/2$ is used to fit to the model [1] both in these ROIs and for individual voxels to create parametric maps.

Results

For all ROIs, the largest eigenvalue λ_1 in the direction parallel to fibers remained relatively constant over time, whereas the values for λ_2 and λ_3 decreased markedly with time. The time-dependence of $D_\perp$ in each muscle group agrees well with theory [1], with all R^2-values $\geq$ 0.98. The fitted values for the free diffusivity D_0, cell permeability κ and mean fiber diameter $a = 4V/S$ are listed in the Table for each ROI, together with the corresponding mean FA-value at long times (t = 1 s). In addition, voxel-wise fitting was performed to create the parametric maps as shown in the Figure, whereby for each subject D_0 was fixed to its mean value over all ROIs in order to reduce the noise in the parametric maps.

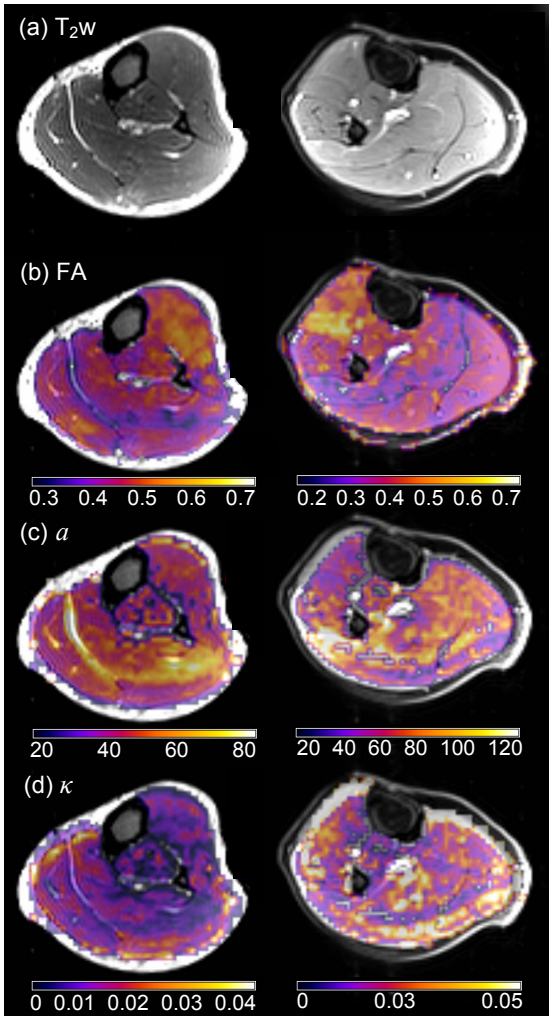

Figure: (a) Anatomical T_2w- MRI FatSat map; (b) FA for t = 1s; (c) fiber diameter a; and (d) permeability κ of the calf muscle of a female (left) and male (right) subject.

Discussion

The fitted values for D_0, κ and a are consistent with our assumption that the predominant restrictions to molecular motion are the cell membranes of muscle fibers, rather than smaller intracellular substructures. Indeed, the fitted values for the mean inter-membrane spacing a fall within the expected range of muscle fiber diameters [4], and the membrane permeability values are within the expected range for cell plasma membranes in eukaryotic cells, e.g. a permeability value of 0.013 µm/ms was found in the sarcolemma of male rat calf skeletal muscle cells [5].

The mean fiber diameters of the male subject are in good agreement with literature, e.g. a would be on average 60 µm based on ref. [4]. Interestingly, the fiber diameters of the female subject were found to be smaller, which might be explained by the fact that the mean fiber cross sectional area and type II muscle fiber diameters tend to be smaller in females [6,7]. For both subjects, the smallest fibers are found in the EDL and largest fibers in the SOL (see Table and Figure), which agrees with histological findings [4]. A strong inverse correlation is also observed between the FA at long times and a.

To conclude, we present here a novel *in vivo* method of quantifying cell size and membrane permeability from time-dependent diffusion measurements. This tool may be useful in clinical applications requiring distinction between cell morphology (e.g. mean diameter) and integrity (permeability).

Table: Fitting results for the free diffusivity values D_0, permeability κ, fiber size a, as well as the mean FA value for t = 1 s in the muscle groups in the female (F-left) and male (M-right) subject.

	FA (1s)		a [µm]		κ [µm/ms]		D_0 [µm^2/ms]	
ROI	F	M	F	M	F	M	F	M
AT	0.49	0.44	37	58	0.020	0.018	1.59	1.36
EDL	0.49	0.49	32	41	0.028	0.030	1.60	1.56
GL	0.42	0.37	32	63	0.037	0.019	1.58	1.53
GM	0.43	0.36	43	69	0.020	0.016	1.52	1.51
PL	0.44	0.38	36	68	0.030	0.018	1.58	1.53
PT	0.43	0.41	40	45	0.025	0.027	1.63	1.65
SOL	0.41	0.34	51	85	0.018	0.015	1.59	1.52
mean	**0.45**	**0.40**	**38**	**61**	**0.03**	**0.02**	**1.58**	**1.52**
std	**0.03**	**0.05**	**6**	**14**	**0.006**	**0.005**	**0.03**	**0.08**

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