Mouse skeletal muscle architecture as measured using three dimensional diffusion tensor imaging

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Introduction:

Mechanisms of force loss that occur during progressive muscular diseases are increasingly studied using genetically modified mice. Such diseases generally cause both a decrease in maximal tension and progressive muscle wasting which results in changes in muscle architecture. Since this architecture is a main determinant of the mechanical behavior of skeletal muscle [1], one must know this architecture in order to determine the actual loss in muscle performance. The muscle organization is characterized by various parameters, including fiber length, pennation angle, and physiological cross-sectional area (PCSA). The determination of these parameters by traditional anatomical reconstruction is destructive and extremely time consuming. Therefore, the aim of this study was to develop methods for determining the *in vivo* three-dimensional architecture of mouse skeletal muscle, using diffusion-tensor MRI.

Materials and methods:

Specimen: Male C57BL/6 mice (n=6) were anaesthetized with isoflurane (1.0-1.5% in air). The body temperature was maintained at 36-39 °C and the animal's respiration was continuously monitored.

MRI: MR was performed with a horizontal 9.5 cm bore, 6.3 Tesla MRI scanner using a 1.5 cm solenoidal RF coil. A 3D diffusion-weighted fast spin echo sequence with fat suppression was used. The diffusion gradients were applied along 6 non-collinear directions and one reference image was recorded without diffusion weighting. Scan parameters were: FOV=15x15x30 mm^3, matrix size=60x60x128 (zero filled to 64x64x128), TE=10 ms, ETL=6, NSA=2 and TR=1 s (total scan time 2:20 hours) and Δ=20 ms, δ=10 ms and b-value=0 or 584 s/mm^2. Initial analysis was done using Mathematica (Wolfram Research). The pixel intensities of the 3D DTI dataset were fitted to obtain the six elements of the diffusion tensor. For every dataset, we have drawn three ROIs in the tibialis anterior from which the means of the eigenvalues, ADC (Trace(D)/3) and fractional anisotropy (FA) were calculated.

Fiber tracking: Fiber tracking was performed using a visualization tool for DTI data as described by Vilanova et al. [2]. The fiber paths were calculated starting from a user defined seed point. A minimal fractional anisotropy (FA) of 0.2 and a maximal angle change of 10 degrees per integration step were used as stop criteria.

Several structural parameters were determined from the tibialis anterior (TA) and the extensor digitorum longus (EDL). This determination was done three times to obtain the user induced variation. The PCSA was determined by selecting a ROI that includes all fibers attached to the distal tendon plate. The dot product of this ROI and the corresponding principal eigenvectors provided the PCSA. The range of TA fiber lengths was determined from a group of fibers running from the distal to the proximal tendon plate. The pennation angle of the TA muscle fibers was determined from fibers running approximately through the midsagittal plane. The pennation angle was defined as the maximal angle between the projection of the tendon plate and the fibers on the plane of view.

Results and Discussion:

For the TA the means (± SD) of the three eigenvalues λ_1, λ_2 and λ_3 were (1.76 ± 0.10)x10^{-3}, (1.12 ± 0.05) x10^{-3} and (0.77 ± 0.05)x10^{-3} mm^2·s^{-1}, respectively, while the mean ADC and FA were found to be (1.22 ± 0.08)x10^{-3} mm^2·s^{-1} and 0.39 ± 0.02, respectively. These values are similar to values reported previously [3].

The possibilities of 3D DTI and fiber tracking are depicted in Figure 1. Figure 1a shows the fiber reconstruction in four muscle groups. Figures 1b-1d illustrate the procedure for estimating pennation angle, fiber length and PCSA, respectively. Fiber tracking in the whole leg was possible and the fiber orientation corresponded with known fiber architecture.

Table I summarizes the structural parameters for the different mice. The variations in the PCSA between the different mice are mainly caused by the large variation in muscle mass. The smaller values for the calculated fiber lengths compared to the DTI-based fiber lengths are probably caused by the inclusion of shorter EDL muscle fibers. The architectural parameters, as determined using the present non-invasive approach, are in agreement with values determined with dissection [1].

Conclusion:

We have shown that with DT-MRI and fiber tracking the overall muscle structure, fiber length, pennation angle and physiological cross sectional area of mouse skeletal muscle can be determined in vivo. These methods enable detailed longitudinal studies of muscle architecture in wild-type and transgenic mice.

References:

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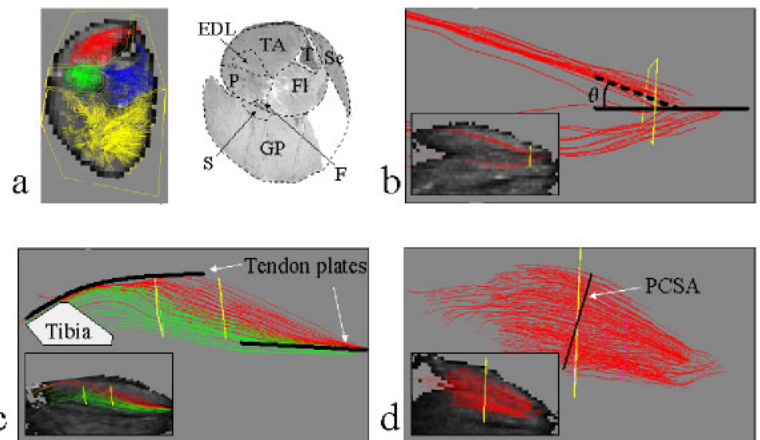
Fig. 1. Representative examples of fiber tracking in the mouse hind limb. Fiber tracking was started from the ROIs indicated with the yellow lines. The insets in b-d show the fibers projected on a 2D MRI slice for anatomical reference. (**a**) Transversal slice showing fiber tracking in different muscle groups. The right image shows an anatomical reference (tibialis anterior (TA), extensor digitorum longus (EDL), peroneus (P), gastrocnemius and plantaris (GP), soleus (S), flexors (FL), semimembranosus (Se), tibia (T) and fibula (F)). (**b**) Determination of the pennation angle (θ). (**c**) Tracking of fibers in the TA muscle, from which the fiber length between the two tendon plates was determined. (**d**) Determination of the PCSA (the black solid line).

Table I. Muscle architectural parameters (mean ± SD) obtained by fiber tracking. FL$_{PCSA}$ = fiber length calculated from the PCSA and the combined masses (weighed after dissection) of TA and EDL, FL$_{DTI}$ = fiber length determined from the length of the tracked fibers, θ = pennation angle.

mouse	Muscle mass (mg)	PCSA (mm^2)	FL$_{PCSA}$ (mm)	FL$_{DTI}$ (mm)	θ (deg.)
1	28.4	5.4 ± 0.3	5.2 ± 0.3		21 ± 1
2	53.1	7.5 ± 0.4	7.1 ± 0.4	7.8 ± 0.6	21 ± 1
3	46.0	9.1 ± 0.4	5.0 ± 0.2	6.6 ± 0.7	21 ± 2
4	63.2	8.1 ± 0.5	7.9 ± 0.4		22 ± 2
5	38.9	6.2 ± 0.4	6.2 ± 0.4	6.8 ± 0.5	24 ± 2
6	37.7	6.0 ± 0.3	6.3 ± 0.3	5.8 ± 0.6	24 ± 3