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Synopsis

We present an application of diffusion MRI at high b-values to a non-invasive quantification of micron-sized organelles such as mitochondria. The experiments were conducted *ex vivo* on pork muscle and analyzed with a bi-exponential tensorial model, which allows us to estimate the mitochondria content in the muscle. Even though a more systematic comparison between mesoscale diffusion and microscale histology is deserved, this work is a proof of concept and a prerequisite for developing *in vivo* methods for quantifying the content of various organelles in muscles, e.g. for studying mitochondrial dysfunction in aging.

Introduction

We present an application of diffusion MRI at high b-values to a non-invasive quantification of micron-sized organelles such as mitochondria and intramyocellular lipid (IMCL) droplets. Although monitoring the mitochondrial content is of great clinical interest for diagnosis of various diseases related to dysfunctions of mitochondria, their current studies are mainly performed by histology with advanced microscopy (such as TEM) that prohibits *in vivo* measurements. We aim at probing the capacities of diffusion MRI to resolve this challenging problem.

Material

The experiments were conducted *ex vivo* on pork muscle with a temperature regulation at 8°C. The sample of size 16 mm was divided in 32x32 $(0.5\text{mm})^3$ voxels. The protocol was a PGSE sequence at 400MHz with Δ=11.8ms and δ=6.3ms, performed for six non-collinear gradient directions. A fat suppression scheme[1] was employed to reduce the contribution from lipids. The b-values ranged from 0 to about 32000 s/mm^2 and the noise/signal ratio was estimated at below 0.005.

Method

The signal has a "fast" contribution from the intracellular water almost freely diffusing in large (~50μm) muscle cells, and a "slow" contribution from restricted diffusion of mitochondrial water and possibly from residual lipids in IMCL[2]. We employ thus a bi-exponential tensorial fit

$$S = S_0 \cdot \left[(1-\rho) \exp\left(-b \sum_{i,j} e_i D^f_{i,j} e_j \right) + \rho \exp(-bD^s) \right], \quad (1)$$

where S_0 is the reference signal, 1-ρ is the volume fraction of the intracellular water, D^f is the apparent diffusion tensor for the intracellular water inside the anisotropic fibrillar structure of the muscle cells, e_i is the unit direction of the gradient, and D^s is the apparent diffusion coefficient of the "slow" component. In a first approximation, mitochondria can be treated as isolated micron-sized compartments, in which the motional narrowing regime is expected, with

$$D_s = \frac{2\zeta_{-1} L^4}{D\delta(\Delta - \delta/3)}, \quad (2)$$

where L is the size of the compartment, and ζ_{-1} is a numerical shape-dependent coefficient[3]. Since this signal is very sensitive to the size L, only relatively small organelles (whose size is inferior to 7μm) can provide a non-negligible signal at high b-values that excludes e.g. blood vessels. As mitochondria are filled with around 65% of water[4], the mitochondria volume fraction (MVF) would be given by $MVF = 1.5 \cdot \rho$, if the contribution from the IMCL was fully eliminated. In practice, $1.5 \cdot \rho$ is the upper bound of the MVF.

Results

Figure 1 shows an example of data fitted by Eq. (1). Note that the slowly decreasing part of the curve is much higher than the estimated noise level (SNR > 5). If this residual signal comes only from the mitochondrial water, one can access the map of the MVF (Fig. 2). The map shows some darker areas, which would correspond to the slow-oxidative fibers, whereas the brighter areas would correspond to the fast-glycolytic fibers. The obtained values of the MVF range between 3% and 6% (see Ref. [5] for comparison with mouse and dolphin). The bi-exponential tensorial fit reveals anisotropy of the medium with a nearly constant main eigenvector of D^f over the whole sample that corresponds to the muscle fibers direction. The corresponding eigenvalue is around $1.3 \cdot 10^{-9} m^2/s$, which is consistent with the diffusion coefficient of water at 8°C: $D = 1.6 \cdot 10^{-9} m^2/s$ (the slight difference being attributed to intracellular crowding). Approximating mitochondria by spheres (with ζ_{-1}=8/175) the slow diffusion coefficient $D^s \approx 2 \cdot 10^{-11} m^2/s$ yields via Eq. (2) an

estimate of the effective radius, $L \approx 2\mu m$, which is twice higher as compared to the standard values. This quantitative discrepancy can be attributed to (i) the effect of the ignored low permeability of the mitochondrial membranes (given that mitochondria are deformed and their membranes are altered by post mortem proteolytic systems[6,7]) which would lead to a faster signal attenuation and thus to a greater value of D^s than expected from Eq. (2); (ii) non-spherical shape of mitochondria; (iii) the use of the intrinsic water diffusion coefficient D that ignores molecular crowding; and (iv) residual contribution of IMCL and noise (the SNR being around 5).

Discussion

The mitochondria are the natural candidates to explain the observed residual signal at high b-values, given that the contribution of lipids was reduced by the fat suppression scheme. Even if this reduction was not perfect, the observed value of D^s is much higher than the diffusion coefficient of lipids[2], $D^{lip} \approx 6.6 \cdot 10^{-12} m^2/s$, so that diffusion of lipids alone cannot explain the observed experiment. Even though a more systematic comparison between mesoscale diffusion and microscale histology is deserved, this work is a proof of concept and a prerequisite for developing in vivo methods able to quantify the content of various organelles in muscle, e.g. for studying mitochondrial dysfunction in aging.

Acknowledgements

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Figures

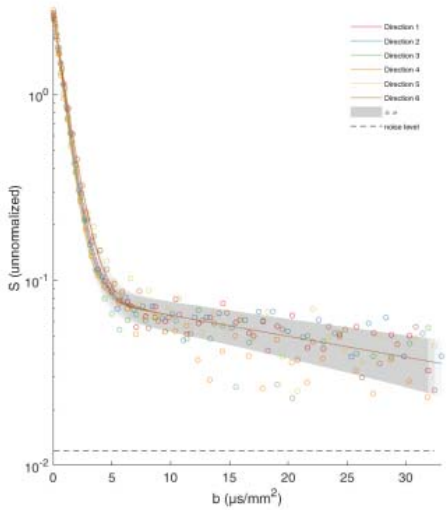

Figure 1: Example of a bi-exponential tensorial fit of the data for the six gradient directions. The value of ρ is 2.9% and thus MVF is 4.4%. The level of noise (dashed line) is about 0.5% of the reference signal. Gray shadowed region indicates the 67% confidence interval (fitted curves ± noise level).

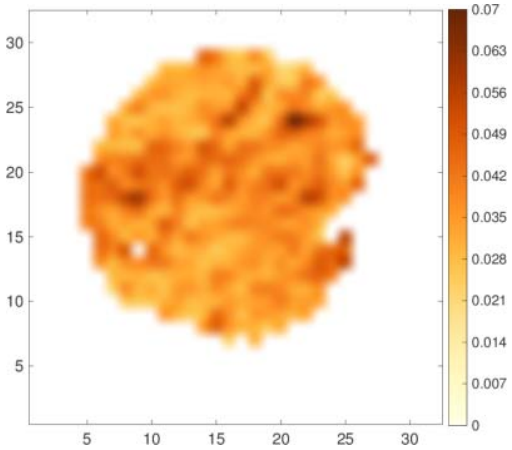

Figure 2: Map of the estimated mitochondria volume fraction. The image is not homogeneous, the values range typically from 3% to 6%.