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CHARACTERIZATION OF MUSCULAR MICROSTRUCTURE BY DIFFUSION MAGNETIC RESONANCE

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INTRODUCTION

In muscle, diffusion-weighted nuclear magnetic resonance (DW-NMR) has already proven to be efficient for sizing and counting intramyocellular lipid droplets [1] at high b-values (upper than 10^4 s/mm²). We aim to extend the technique to mitochondria.

MATERIALS & METHODS (figure 1)

The muscles were chosen for their contrasted mitochondrial content. The aims are to :

- Identify signal model (eq. (1)) of water diffusion
- Extract the supposed mitochondrial volume fraction (MVF) (eq. (2))

The model separates muscle fibers in two media :

- The extra-mitochondrial medium
  - diffusion tensor D^f
- The intra-mitochondrial medium
  - isotropic & slow diffusion coefficient D^s

Acquisition starts between 20 and 50 min *post-mortem*.

Parameters are: δ = 3.2 ms, Δ = 10/20 ms, spectral width = 10 kHz, TR/TE = 5000/38.5, T = 13°C and 21 b-values up to 20 000 s/mm².

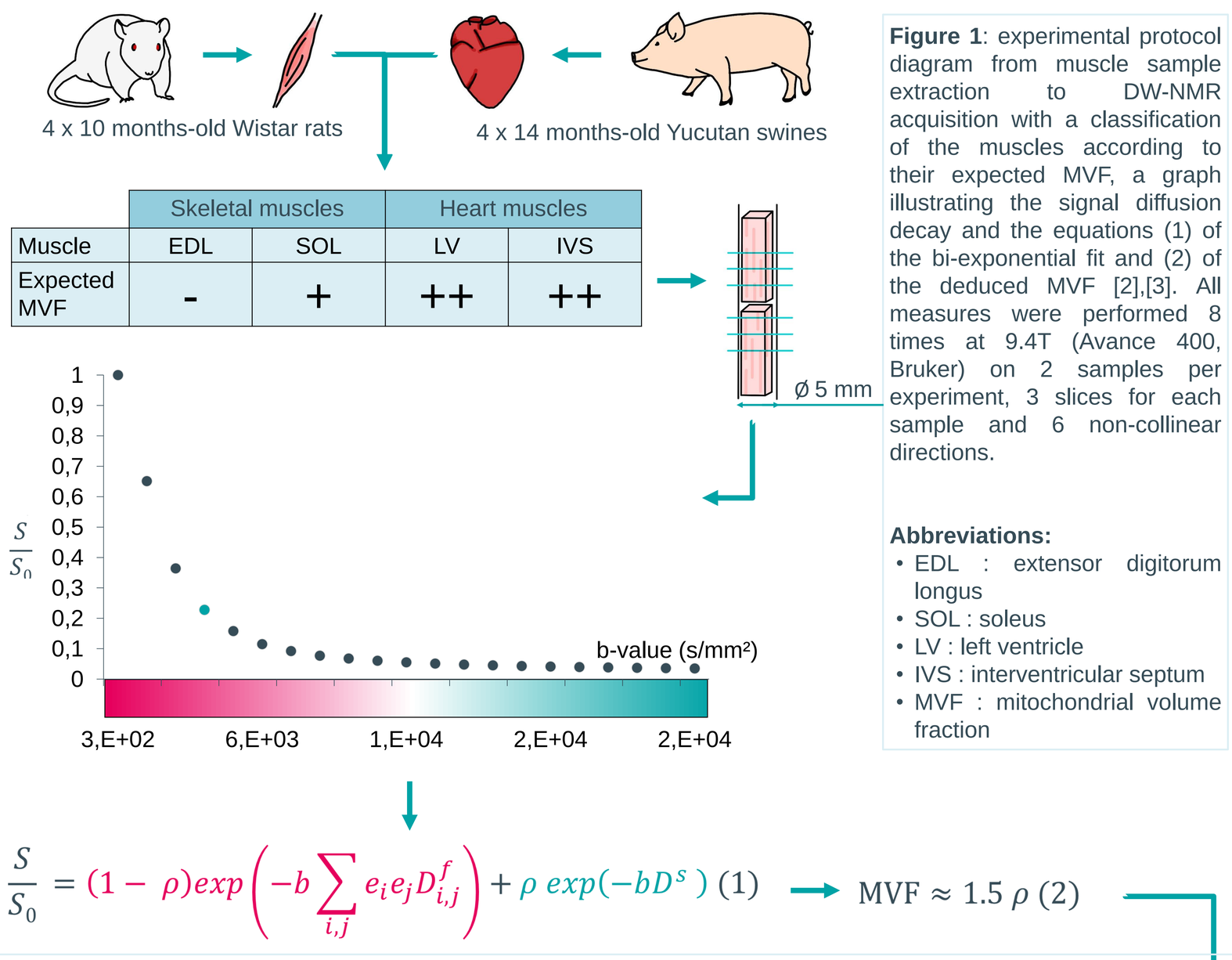

Figure 1: experimental protocol diagram from muscle sample extraction to DW-NMR acquisition with a classification of the muscles according to their expected MVF, a graph illustrating the signal diffusion decay and the equations (1) of the bi-exponential fit and (2) of the deduced MVF [2],[3]. All measures were performed 8 times at 9.4T (Avance 400, Bruker) on 2 samples per experiment, 3 slices for each sample and 6 non-collinear directions.

Abbreviations:
- EDL : extensor digitorum longus
- SOL : soleus
- LV : left ventricle
- IVS : interventricular septum
- MVF : mitochondrial volume fraction

RESULTS

- The minimum signal to noise ratio obtained from a whole slice was above 100.
- ρ might be underestimated due to permeation of mitochondria to the exterior medium.

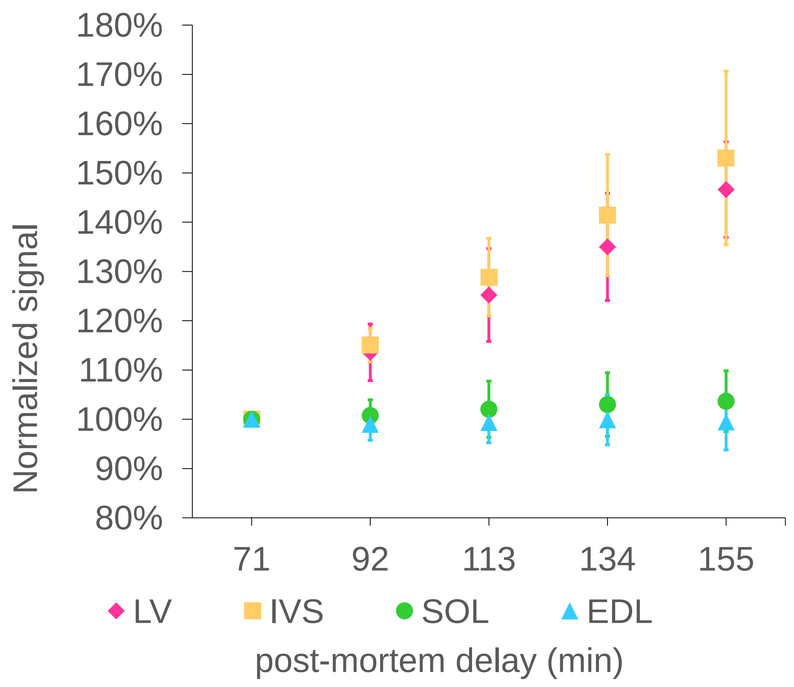

Figure 2: mean signal ± standard deviation from each muscles. Δ = 20 ms and b = 20 000 s/mm². The signal is averaged on six non-collinear gradient directions and on all samples then normalized by the signal at the first comparable *post-mortem* delay = 71 min.

- The signal increases at high b-values with *post-mortem* delay in muscles rich in mitochondria

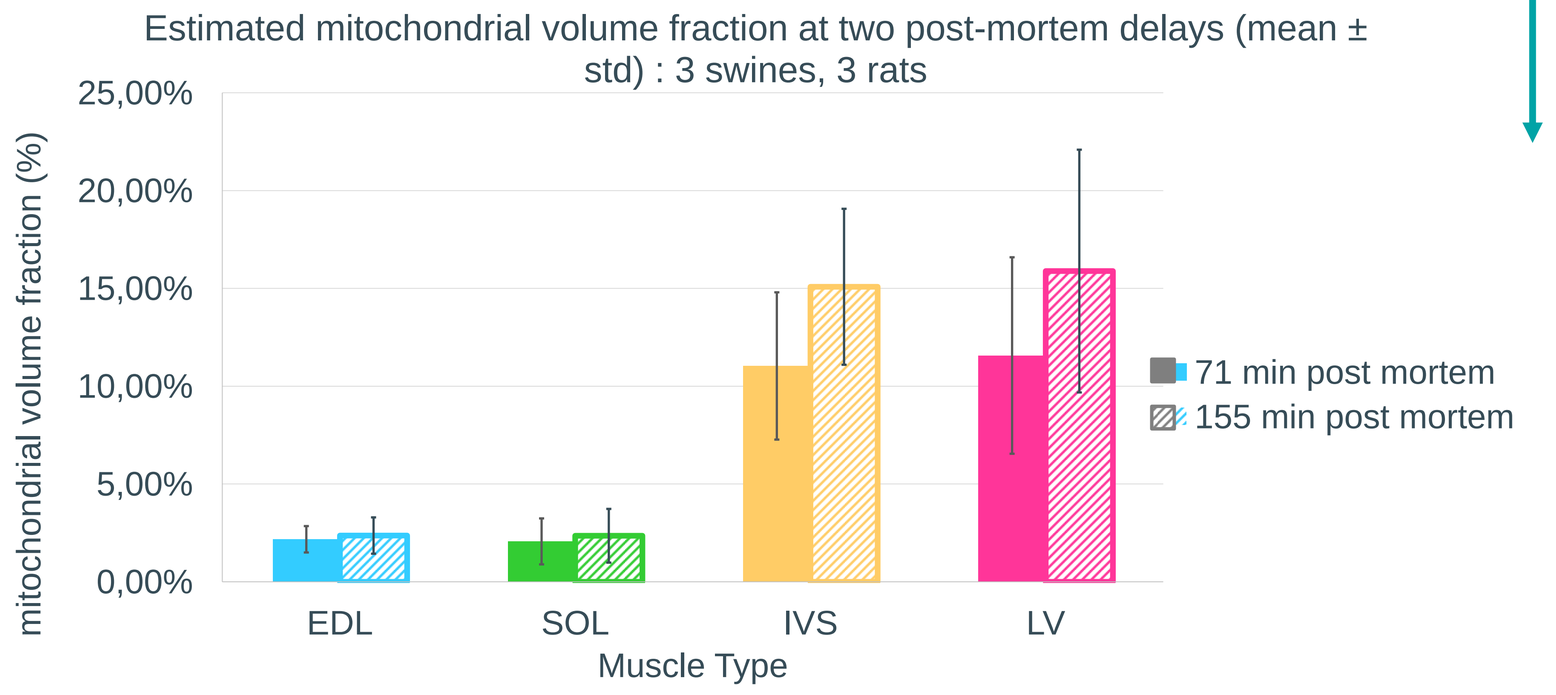

Figure 3: mean MVF ± standard deviation from each muscles on 3 swines and 3 rats at two distinct *post-mortem* delays. The MVF was obtained from eq. (2, fig. 1) after applying the fit from eq. (1, fig. 1). Note that note that 1 rat was not taken into account due to a lack of signal in 2 slices, and 1 pig was not taken into account due to its diet, different from other swines.

- The estimated MVF is greater in heart muscle than in skeletal muscle
- The estimated MVF increases with *post-mortem* delay, which could be interpreted as a mitochondria swelling.

CONCLUSION

Significant differences were observed between cardiac and skeletal muscles in terms of *post-mortem* signal evolution and deduced mitochondrial fraction. These observations are in line with our hypothesis and will be confronted to the analysis of electron microscopy images. This work also reflects the importance of taking into account the *post-mortem* delay in the modeling when seeking to study the microstructure of a biological sample. This work is a first step towards dynamic characterizations of mitochondria morphometric parameters by DW-NMR *in vivo*, in a non-destructive manner. As these parameters have already been associated with particular physiological conditions, the potential applications in perspective of this work appear promising.

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