

Local IMCL content within rat TA muscle measured with *in vivo* ^1H MRS correlates with muscle fiber type

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Introduction

^1H NMR spectroscopy (MRS) has proven to be a valuable tool to measure intramyocellular lipid (IMCL) content in human skeletal muscle. Only very recently, this technique has also been applied in rat *in vivo*. It has been shown that the IMCL content differs significantly between rat soleus, tibialis anterior (TA) and extensor digitorum longus muscles as a result of their different fiber type compositions¹. We hypothesize that IMCL content in rat hind limb can vary within one muscle and that this phenomenon correlates with muscle fiber type distribution. Therefore the variability of the IMCL content within the rat TA muscle was studied by *in vivo* ^1H MRS and compared with the muscle fiber type distribution determined by immunohistological staining.

Materials and methods

Materials

- ~12 weeks old male Wistar rats (n = 4)
- 6.3 Tesla horizontal bore Varian MR system
- ellipsoid ^1H surface coil (18/22 mm)

Methods

^1H NMR Spectroscopy

- Transversal images of the midbelly region of the TA were acquired to achieve proper placement of the spectroscopic regions of interest. Voxels of $2 \times 2 \times 2 \text{ mm}^3$ were located at three different positions within the TA muscle (Fig. 1).
- Single-voxel localized ^1H NMR spectra were acquired using the LASER sequence with additional outer volume suppression (TR = 1 s, TE = 28 ms, SWAMP water suppression, 1024 averages).

Immunohistology

- Midbelly region TA tissue was dissected and used for immunohistological analyses.
- Muscle tissue was stained for basal lamina (laminin) and type I and type IIa muscle fibers (myosin heavy chain I and IIa).

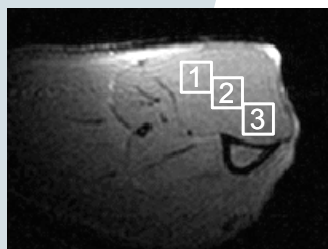


Figure 1: Transversal adiabatic spin echo image of a rat hind limb with voxel positioning in the TA muscle.

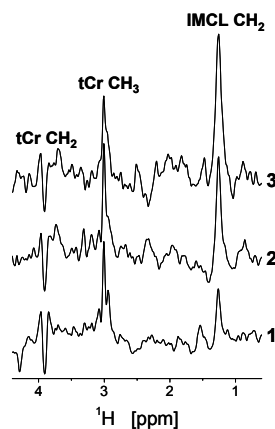


Figure 2: Typical examples of single-voxel localized ^1H NMR spectra from voxel positions 1-3.

Results

Varying the voxel positioning in the TA results in spectra with different IMCL peak amplitudes (Fig. 2). IMCL levels increase going from position 1 to 3 by a factor more than two (IMCL *vs* voxel position: Spearman's $\rho = 0.809$, $p < 0.01$), while the tCr (CH_3) levels tend to decrease in this direction (Fig. 3).

The immunohistological staining of the representative voxel positions shows large differences in fiber type distribution (Fig. 4), revealing areas dominated by type I and type IIa fibers (position 3) and others almost deprived of these specific muscle fiber types (position 1).

The pronounced regionalization of different fiber types within the TA muscle confirms data reported by Wang *et al.*² and is consistent with the NMR results. Highest IMCL levels are found in the medial region, close to the tibia bone, where the amount of oxidative fibers, which use IMCL as a substrate, is highest. Highest tCr levels are found in the lateral region, where the amount of glycolytic fibers is highest.

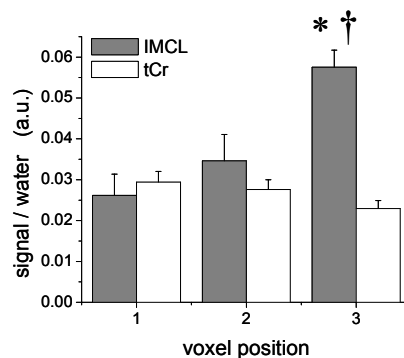


Figure 3: IMCL and tCr content relative to the water signal as a function of voxel position. Data are presented as mean (n = 4) \pm SEM. * $p < 0.05$ relative to voxel position 1, † $p < 0.05$ relative to voxel position 2. (Kruskal-Wallis Test)

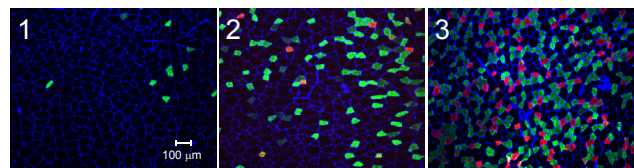


Figure 4: Triple-immunofluorescence assay for representative areas ($1.36 \times 1.07 \text{ mm}^2$) corresponding with voxel positions 1-3. Blue: basal lamina; red: type I muscle fibers (slow oxidative); green: type IIa muscle fibers (fast oxidative).

Conclusions

The variability in rat TA IMCL content correlates with muscle fiber type distribution. Using *in vivo* single-voxel ^1H MRS it is possible to measure the local IMCL content and to study fiber type dependent phenomena within one muscle.

References:

1. Neumann-Haefelin C *et al.* Diabetes 2004;53:528-534.
2. Wang LC *et al.* J Muscle Res Cell Motil 2000;21:587-598.