Local intramyocellular lipid content within rat tibialis anterior muscle measured with *in vivo* ^{1}H MRS correlates with muscle fiber type composition

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Introduction

^{1}H 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 compositions[1]. However, within individual rat hind limb muscles also large and systematic regional differences in the relative densities of fiber types have been observed[2]. We hypothesize that the 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* ^{1}H MRS and compared with the muscle fiber type distribution determined by immunohistological staining.

Materials & Methods

In vivo NMR experiments were performed on ~12 weeks old male Wistar rats (n = 4), anesthetized with 2.0% isoflurane. All NMR experiments were performed on a 6.3 Tesla horizontal bore Varian MR system, using an ellipsoid ^{1}H surface coil (18/22 mm). Transversal images of the midbelly region of the TA were acquired using an adiabatic spin echo sequence (TR = 2 s, TE = 24 ms) to achieve proper placement of the spectroscopic regions of interest. Voxels of $2 \times 2 \times 2$ mm^3 were located at three different positions within the TA muscle: (1) lateral, (2) in the center, and (3) medial, close to the tibia bone (Fig. 1a). Single-voxel localized ^{1}H NMR spectra were acquired using the LASER sequence with additional outer volume suppression (TR = 1 s, TE = 28 ms, SWAMP water suppression, 1024 averages). Unsuppressed water spectra were recorded from the same voxels and used as internal reference. The spectra were fitted in the time domain by using a nonlinear least squares algorithm (AMARES) in the jMRUI software package, yielding integrals for the IMCL CH_2 peak (1.28 ppm), the total creatine (tCr) CH_3 peak (3.02 ppm) and the unsuppressed water peak. Data are presented as mean ± SEM. The Kruskal-Wallis Test was performed to detect differences between voxel positions ($p < 0.05$) using the Mann-Witney U test for post-hoc pair-wise analyses. The correlation between voxel position and IMCL and tCr content was calculated using the Spearman correlation coefficient. From one animal, 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).

Results & Discussion

Figure 1a shows a typical example of the positioning of the three voxels within the TA muscle and figure 1b shows the corresponding localized ^{1}H NMR spectra. The IMCL/tCr ratio clearly differs for the three positions. In the lateral region of the TA, the IMCL intensity is smaller than the tCr intensity, whereas medially, close to the tibia bone, an inverse relation is found. IMCL and tCr signals were referenced to the water signal (Fig. 1c), as both are known to vary with fiber type composition. 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 levels tend to decrease in this direction. This relation was present in all midbelly region cross sections of the muscle. Figure 1d shows the immunohistological staining for three areas of the TA muscle corresponding with the three voxel positions. The top panel of figure 1d (position 1) does not contain a single type I fiber and only a few type IIa fibers. This part of the TA muscle is mainly composed of the non-oxidative type II fibers. The middle panel (position 2) contains a few type I fibers and some more type IIa fibers, but is still dominated by non-oxidative fibers. The bottom panel (position 3) is dominated by the oxidative type I and type IIa fibers.

The pronounced regionalization of fiber type composition 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. These relationships have been shown before, when comparing different muscles, e.g. TA and soleus. However, this is the first time that a correlation between local IMCL and tCr levels measured by *in vivo* ^{1}H NMR and muscle fiber type distribution within one muscle has been found. When studying IMCL levels during an intervention (e.g. fasting), different positions of the voxel might give different results, as the effects are likely to depend on fiber type composition.

Conclusions

It is concluded that the variability in rat TA IMCL content correlates with muscle fiber type distribution. Using *in vivo* single-voxel ^{1}H 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.

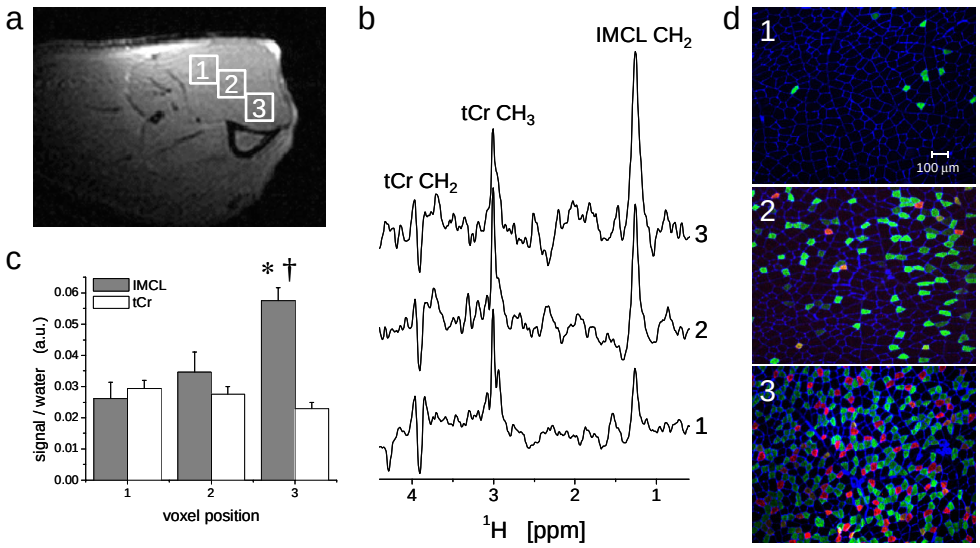

Figure 1. a: Transversal adiabatic spin echo image of a rat hind limb with voxel positioning in the TA muscle. The rat was positioned sideways in the MR scanner. **b:** Typical examples of single-voxel localized ^{1}H NMR spectra from voxel positions 1-3. **c:** IMCL and tCr content relative to the water signal as a function of voxel position. Data are presented as mean (n = 4) ± SEM. ∗ $p < 0.05$ relative to voxel position 1, † $p < 0.05$ relative to voxel position 2. **d:** Triple-immunofluorescence assay for representative areas (1.36×1.07 mm^2) corresponding with voxel positions 1-3. Blue: basal lamina; red: type I muscle fibers; green: type IIa muscle fibers.