The typical IMCL distribution pattern in rat tibialis anterior muscle remains unchanged after high-fat diet or fasting

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Introduction

^{1}H NMR spectroscopy (MRS) is a valuable tool to measure intramyocellular lipid (IMCL) content in human as well as in rat skeletal muscle. It has been shown that the IMCL content differs significantly between different muscles. This could be ascribed to their different fiber type compositions, because oxidative and glycolytic fiber types are known to have different lipid storage capacities. Recently, we showed that IMCL within rat TA muscle is inhomogeneously distributed and correlates with the typical fiber type regionalization within this muscle (1). The present study reports on high-fat diet and fasting as dietary interventions to manipulate IMCL levels and to investigate the effect on the typical pattern of IMCL distribution in the rat TA muscle.

Materials & Methods

In vivo NMR experiments were performed on ~16 weeks old male Wistar rats (n = 11), anesthetized with 2.0% isoflurane. The animals were subjected to two ^{1}H single-voxel MRS measurements, separated by 7 days. Six animals were put on a high-fat diet (~16% fat, ~50% carbohydrate, ~18% protein) ad libitum, during the 7 days after the first measurement. The other 5 animals stayed on normal chow but fasted for 15 hours preceding the second measurement.

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 or soleus muscle (SOL) 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. 1). One voxel of $1.5 \times 1.5 \times 2$ mm^3 was placed in the SOL. 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 for TA voxels, 2048 averages for SOL voxel). 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.

Results

Figure 1 shows a typical example of the positioning of the three voxels within the TA muscle. Localized water suppressed spectra originating from the three voxel positions are shown in figure 2a, confirming the earlier reported typical IMCL distribution in rat TA (1). Figure 2b displays spectra from the same rat after the high-fat diet, showing that IMCL is significantly increased in all three voxels. Figure 3a and 3b show the IMCL content of the different voxels of the TA and the voxel of the SOL before and after high-fat diet (n = 6) and fasting (n = 5), respectively. The IMCL contents measured before the dietary interventions (Fig. 3a and 3b, black bars) confirm quantitatively the typical IMCL distribution shown in figure 2a. The high-fat diet resulted in a substantial increase in IMCL for all voxels in the TA (post-DIET vs. pre-DIET; TA1: +115%, p = 0.008; TA2: +120% p = 0.001; TA3: +111%, p = 0.002) as well as in the SOL (post-DIET vs. pre-DIET; SOL: +67%, p = 0.036) (Fig. 3a). In contrast, the fasting procedure did not induce any significant changes in IMCL for the voxels in the TA (Fig. 3b). The fasting procedure did result in a decrease of IMCL in the SOL (post-DIET vs. pre-DIET; SOL: -40%, p = 0.004) (Fig. 3b).

Discussion

We investigated whether a dietary intervention which affects IMCL levels also changed the typical IMCL distribution within the TA. Seven days of high-fat feeding increased the IMCL content in the TA by more than 100% and the SOL by 67%. However, IMCL within the TA was homogeneously increased and therefore the distribution pattern of IMCL was not changed. Fifteen hours of fasting decreased the IMCL content in the SOL by 40%, but did not induce a significant change in the IMCL levels in the TA. In contrast, Neumann-Haefelin *et al.* reported an increase of IMCL in glycolytic muscles (TA and extensor digitorum longus) and a constant level of IMCL in oxidative muscle (SOL), measured at 12, 24, 48, and 72 hours of starvation (2).

Conclusion

The characteristic IMCL distribution within the rat TA muscle reported earlier (1) was confirmed in this study. Fasting did not result in a change in IMCL levels, in contrast to the high-fat diet which induced an increase of the IMCL levels in the rat TA. However, the typical IMCL distribution in the rat TA was not changed due to the high-fat diet.

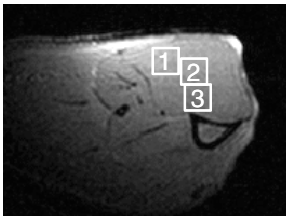

Figure 1. Transversal adiabatic spin echo image of a rat hind limb with voxel positioning in the rat TA muscle.

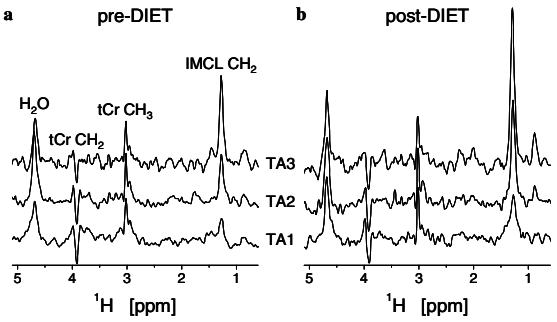

Figure 2. Typical examples of single-voxel localized ^{1}H NMR spectra from voxel positions 1-3 within the TA, from the same animal (**a**) before and (**b**) after 7 days of high-fat diet.

References
1) De Feyter H.M.M.L et al. Proc. ISMRM 2005, #2034.
2) Neumann-Haefelin C. et al. Diabetes 2004;53:528-34.

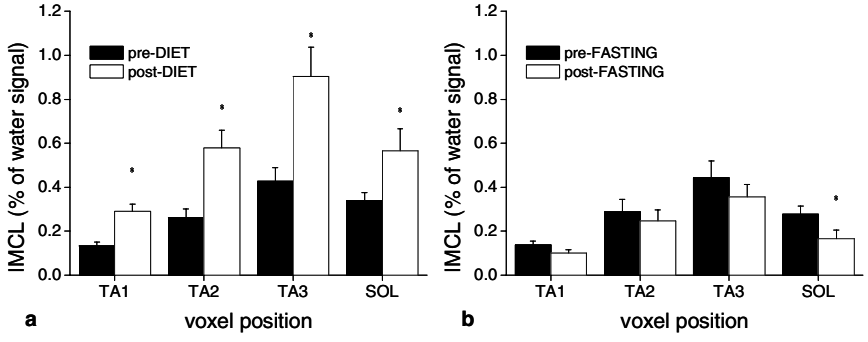

Figure 3. IMCL content of the different voxel positions in the TA and the SOL (**a**) before and after 7 days of high-fat diet (n = 6) and (**b**) before and after 15 hours overnight fasting (n = 5). * $p < 0.05$ (T-test) vs. IMCL content before dietary intervention.