Hepatic glycogen metabolism in mice by in vivo 13C MRS at 14T

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Introduction: Hepatic carbohydrate metabolism, involving glycolysis, gluconeogenesis, glycogenesis and glycogenolysis, is essential for normal blood glucose homeostasis. *In vivo* ^{13}C MR spectroscopy has been successfully applied in rats and mice with time resolved detection of the incorporation of carbons into glycogen at field strengths up to 9.4T *[1-2]*. The contributions of direct and indirect glycogen synthesis can be studied upon steady state infusion of [1-^{13}C] glucose using temporally resolved detection of the incorporation of ^{13}C-label into specific positions of glycogen *[1]*. The application of this technique at higher field strengths allows for a better spectral resolution, but requires alternative ways to overcome the larger chemical shift dispersion. The aim of this study was to determine hepatic glucose uptake and simultaneous detection of glycogen ^{13}C resonances in mouse liver by localized ^{13}C MRS at 14T.

Materials and Methods The pulse profile was tested on a phantom containing 250mM glycogen and 5.5mM [1,6-$^{13}C_2$] glucose in dPBS. MRS measurements were performed on a 14.1 T horizontal bore magnet. A quadrature ^{1}H coil was used for imaging and shimming (FASTMAP). ^{13}C MR spectra were acquired with a 9 mm three turn surface ^{13}C coil in a ~300 µl volume using optimized outer volume suppression for localisation *[3],* a 0.5ms adiabatic excitation pulse, 1000ms TR, 128 averages, NOE and Waltz-16 ^{1}H decoupling. As the adiabatic 90º pulse has a relatively narrow bandwidth for applications of ^{13}C detection at 14T, signal intensities of glucose C1 and C6 were measured by placing the transmit frequency (TX-freq) at 100 or 58 ppm in an interleaved mode. In order to detect natural abundance signals we inverted the sweep of the at60.n29 pulse, which mirrored the asymmetric pulse profile when applied at 60 ppm. After an overnight fasting ^{13}C incorporation into carbon positions of hepatic glycogen was measured in C57Bl6 mice upon >4hr *i.v.* infusion of [1-^{13}C]-glucose via the femoral vein under isoflurane anesthesia. Signals of glycogen and glucose were fitted with AMARES using jMRUI.

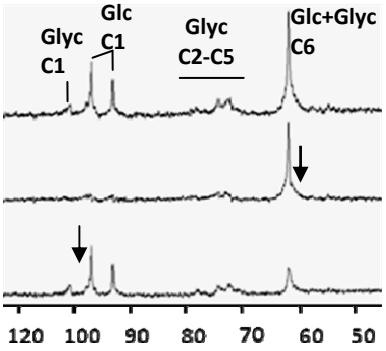

Fig.1: ^{13}C MR spectra of phantom (with 1,6-$^{13}C_2$ glc and NA glycogen) Bottom: TX frequency at 100ppm Middle: at 60ppm(inverted sweep). Top: sum of both. (↓ indicate TX freq.)

Results and discussion Fig. 1 demonstrates the signal losses due to the limited bandwidth of the adiabatic RF pulse shape. The signal intensity of glc C6 was reduced with 40% when the TX frequency was placed for optimal signal at the C1 resonances of glc and glyc (bottom spectrum), compared to that of the spectrum with the TX positioned at 60ppm (middle). The summed spectrum at the top shows an enhanced signal intensity at the resonances between 70-80 ppm, reflecting the C2-C5 signals of glucose and glycogen. The conversion of glucose into glycogen at the C1 position in the mouse liver was detected during ~4 hrs of [1-^{13}C]-glucose infusion. The stack plot in Fig 2 shows the resonances of glucose C1β (96.8ppm), C1α (92.6ppm) and Gly C1 (100.5 ppm) acquired with the TX-frequency at 100ppm. The fitted signal intensities of these spectra at a higher temporal resolution show an immediate increase in glucose followed by a constant glucose level during the entire experiment. The inverted sweep of the excitation pulse allowed for additional detection of natural abundance C2-C5 resonances *in vivo* (Fig.3).

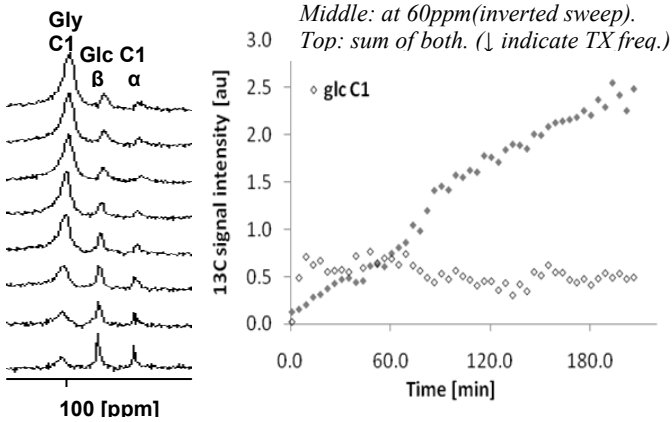

*Fig.3. In vivo ^{13}C MR spectra of mouse liver showing label incorporation into 1-^{13}C-glycogen (Gly C1), Summed spectra: 6*128 acquistions. Left: Signal intensities (per 4.5 min of glucose and glycogen C1 in mouse liver during 4 hrs of [1-^{13}C] glucose infusion.*

The use of high field strengths is beneficial for the distinction between glycerol C1+C3 (62.5ppm) and glycogen C6 (61.4 ppm) resonances. However, we showed that the need for a short adiabatic excitation pulse for localized glycogen detection (T_2 ~ 6 ms) at 14T leads to major signal losses as the bandwidth is not large enough to cover the 6kHz chemical shift difference between the C1 and C6 position at this field strength. We overcame this problem by interleaved acquisition at two TX-frequencies. This did not hamper sufficient temporal resolution and made corrections for the adiabatic pulse profile unnecessary.

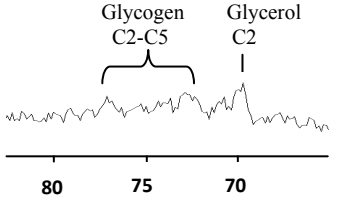

Fig. 3. Localised ^{13}C MR spectra in mouse liver at 14T showing the glycogen C2-C5 region (72-77 ppm) acquired with the TX-freq at 61ppm.Summed over 4 hr.

Conclusion We optimized a protocol for *in vivo* ^{13}C MRS at 14T to study glycogen synthesis and detection of ^{13}C-label scrambling at the triose level, which opens new windows to study alternative pathways of glycogen synthesis in mouse models *[4]*.

***References** : [1] Kunnecke et al. MRM 2000, Biochim Biophys Acta 1991 [2] Choi et al, Eur J Biochem 2002, [3] Choi et al. MRM 2000, [4] Guillam et al. PNAS 1998.* ***Acknowledgements:** Supported by Centre d'Imagerie BioMedicale (CIBM) of the UNIL, UNIGE, HG, CHUV, EPFL and the Leenaards and Jeantet Foundations.*