^{1}H MRS TO INVESTIGATE THE HEPATIC PROFILE OF GLUT2$^{-/-}$ MICE

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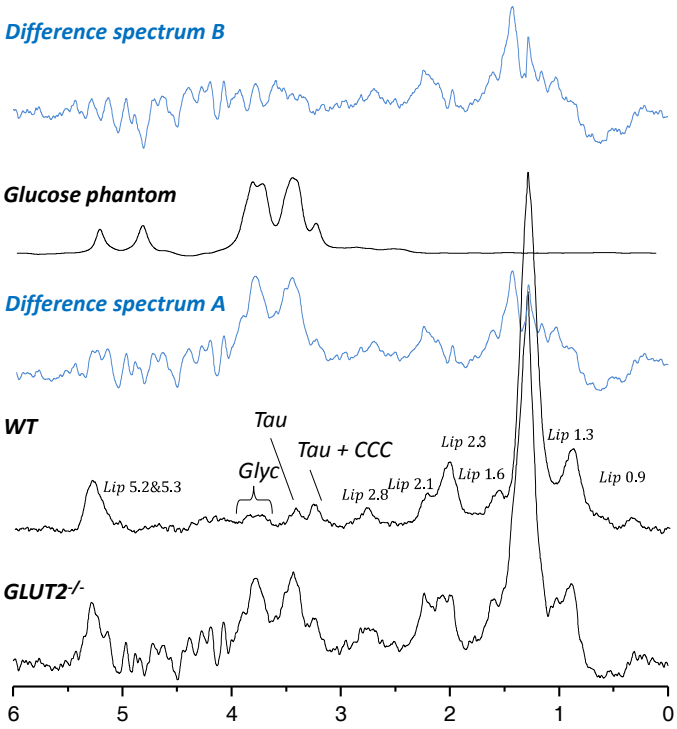
INTRODUCTION: The liver plays an important role in storing energetic fuels from food intake in the form of glycogen or lipids. The liver is also able to produce and release glucose to the blood stream to be used for oxidation in other organs during fasting. The ^{1}H MR spectrum of the mouse liver is dominated by resonances from intra-hepatic lipids. However, under certain conditions, the contribution from other metabolites may become significant. Glucose transport across the liver is facilitated by the glucose transporter 2 (GLUT2). Ablation of GLUT2 in mice results in the lack of net hepatic glycogen breakdown with fasting and is accompanied by persistent high levels of glycogen and glucose-6-phosphate that are nonetheless comparable to those observed in fed control mice [1]. We have performed ^{1}H MRS measurements to assess the hepatic profile of GLUT2$^{-/-}$ mice and non-invasively investigate possible alterations in intra-hepatic metabolite levels in that model. By working at a high field strength (14.1T) we could compensate for the low sensitivity inherent to the small size of the subjects.

METHODS: WT C57BL/6J mice and GLUT2$^{-/-}$ mice under 1-2% isofluorane anesthesia were placed at the supine position with a ^{1}H quadrature surface coil (two 13mm-inner-diameter physically decoupled loops) over the abdomen. MR measurements were performed in a horizontal bore 14.1T-26 cm magnet interfaced to a vnmrj 2.3a console (Agilent Inc.). In the scanner, animals were monitored continuously for their breathing patterns and body temperature through an MR-compatible system (SA Instrument), which also delivered the desired respiratory gating signals (TTL) to the console for MR imaging spectroscopy. Multi-slice GRE images (30×30mm^2, 128×128) were acquired with the respiratory gating signals for anatomical identification of the liver and localized ^{1}H-MR spectra were obtained from a 8 µl voxel with STEAM (TM, 20 ms; TR, 6.5 s; TE, 8 ms; 24-32 scans; 4096 complex points). T_2 corrections were applied for the water and major lipid resonance at 1.3 ppm and the hepatic lipid content (HLC) was expressed as % of total ^{1}H MR signal. To observe intra-hepatic metabolites, STEAM (TM, 20 ms; TR, 5 s; TE, 7.2 ms; 84-128scans; 2048 complex points) was preceded by outer volume saturation and VAPOR to suppress the water signal with seven CHESS pulses and an additional gauss pulse during the TM period [2]. The same MR protocols and parameters were used to perform measurements at 37°C in phantoms of glycogen (200 mM in dPBS) and glucose (100 mM in dPBS).



^{1}H MR spectra from the livers of wild type (WT) and GLUT2$^{-/-}$ mice and the glucose phantom. Spectra in blue were obtained by (A) subtracting WT to GLUT2$^{-/-}$; (B) subtracting glucose phantom to (A)

RESULTS AND DISCUSISON: The figure shows in black lines ^{1}H MR spectra acquire with water suppression from the glucose phantom and the livers of WT and GLUT2$^{-/-}$ mice. Good quality spectra were obtained from the liver of WT mice. These spectra were dominated by resonances from the lipid fatty acyl chains at 0.9, 1.3, 1.6, 2.1, 2.3, 2.8 and 5.3 ppm. In addition, the resonance at 3.4 ppm was attributed to the presence of taurine and that at 3.2 to choline containing compounds (CCC) and taurine. The broad, low intensity resonance at 3.8 ppm, could arise from glycogen protons that experience partial saturation from the VAPOR pulses applied for the water suppression. Unlike the findings for WT mice, spectra acquired for the GLUT2$^{-/-}$ mouse liver showed two peaks whose intensity was well above that of the lipid resonances. This is highlighted on the difference spectrum A in the figure, which results from the subtraction of the spectrum acquired from the WT mouse liver to that of the GLUT2$^{-/-}$. Glycogen could contribute to the resonance at 3.8 ppm. However, quantifications in liver extracts showed twice as much glycogen as that of WT (100 mg/g *vs* 45 mg/g) and this *per se* cannot explain the high intensity peaks observed in GLUT2$^{-/-}$. A major contribution to those resonances is likely to be due to glucose. In fact, as shown in the difference spectrum B (obtained by subtracting the glucose phantom spectrum to the difference spectrum A), the abnormally high resonances in the GLUT2$^{-/-}$ mouse liver could completely be accounted for by glucose. This has not been reported before and may derive from the aggravation of the impaired glucose metabolism with age in this model. Parallel to high glucose levels we observed a lower HLC in GLUT2$^{-/-}$ when compared to age-matched controls (1.1 ± 0.4% *vs* 3.1 ± 0.2%, $P < 0.05$). Our data also suggests that the lipid profile is sensitive to the ablation of GLUT2. This preliminary study showed that in the GLUT2$^{-/-}$ mice it is possible to address by ^{1}H MRS the main energy fuels handled by the liver: carbohydrates and lipids. Future studies are envisaged to comprehensively address the evolution of the hepatic metabolic profile in this model.

CONCLUSION: ^{1}H MRS allowed characterizing the hepatic profile in the GLUT2$^{-/-}$ mice. The longitudinal evaluation of these mice by ^{1}H MRS should contribute to a better understanding of the dynamics between hepatic carbohydrate and lipid metabolism in this model.

REFERENCES:
[1] Burcelin et al (2000) J Biol Chem 275 :10930 [2] Tkac et al (1999) Magn Reson Med 41:649