IN VIVO ^{1}H MRS AT 14.1T FOR THE ACCURATE CHARACTERIZATION OF THE LIPID PROFILE OF THE MOUSE LIVER

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TARGET AUDIENCE: Spectroscopic scientists; basic scientists of animal models of metabolism.

PURPOSE: The major challenges of ^{1}H MRS studies of hepatic metabolism in mice are respiration motion and poor sensitivity due to the small sample size. We aimed to demonstrate that at high magnetic field (14.1T), the hepatic lipid content and saturation profile of the fatty acyl chains can be accurately characterized by *in vivo* ^{1}H MRS from small voxels placed within the mouse liver.

METHODS: C57BL/6J mice under 1-2% isofluorane anesthesia were placed at the supine position with a ^{1}H quadrature surface coil 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 (Fig. 1) . Localized ^{1}H-NMR spectra were obtained from a 8-15 µl voxel with STEAM (TM, 20 ms; TR, 6.5 s; TE, 8-35 ms; 18-25 scans). All MR spectra were corrected for B_0 drift and phase, summed and analyzed with LCModel. T_2 correction was done by mono-exponential fit of peak areas as a function of TE. HLC was estimated as the T_2-corrected area of the 1.3 ppm-lipid resonance (Lip 1.3) relative to that of the water plus Lip 1.3. To assess the lipids' unsaturation profile, water suppression [1] was applied allowing for a better characterization of the fatty acyl resonances, including the olefinic protons at 5.3 ppm. The quantification method as well as that used to estimate the unsaturation profile (Table 1) were validated in soybean oil emulsion phantoms. For comparison, the *in vitro* spectrum of the soybean oil emulsion was acquired in a Bruker DRX-600 spectrometer equipped with a cryo-probe tuned to ^{1}H. Data are expressed as mean ± SEM.

Table. 1 *Lipid profile as determined from the ^{1}H NMR spectra. The equations for the several parameters are shown. db, double bound; PUdb, poly-unsaturated db; UFA, unsaturated fatty acyl chain; SFA, saturated fatty acyl chain; FA, fatty acyl chain. *the theoretic value of 1.5 was assumed since the Lip 5.3 resonance is affected by the water suppression pulse in the spectrum acquired* in vitro*.*

	Saturation Index $\frac{L1.3\times3}{L0.9\times2}$	*Mean db per FA* $\frac{L5.3\times3}{L0.9\times2}$	*Mean PUdb per FA* $\frac{L2.8\times3}{L0.9\times2}$	*Mean PUdb per UFA* $\frac{L2.8\times4}{L2.1\times2}$	*UFA (% of total FA)* $\frac{L2.1\times3}{L0.9\times4}\times100$	*SFA (% of total FA)* $100-UFA$
Phantom in vivo	9.2 ± 0.40	1.25 ± 0.23	0.66 ± 0.03	0.88 ± 0.07	76 ± 7%	24 ± 7%
Phantom in vitro	7.9	1.5*	0.54	0.71	76%	24%
Mouse liver in vivo	7.9 ± 0.79	0.86 ± 0.01	0.50 ± 0.08	0.81 ± 0.11	62 ± 2%	38 ± 2%

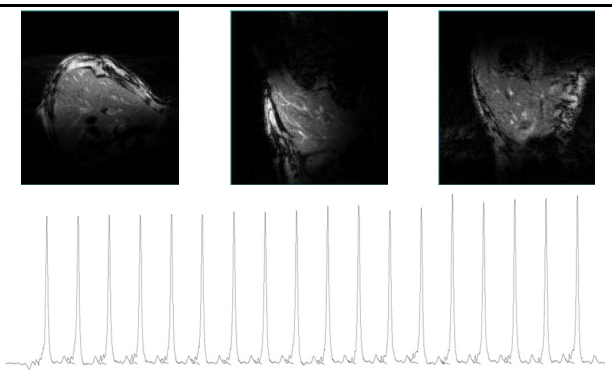

Fig. 1 *Typical anatomical MR images (top) and water signals (bottom) from the volume of interest.*

RESULTS: Respiration-gated MRI and MRS acquisition yielded well-defined anatomical structure of mouse liver and very stable inter-scans signal intensity (Fig. 1). HLC was 1.1±0.1% in young adult mice. Water T_2 was 8.4±0.3 ms, slightly shorter than previous reports of ~11 ms at lower fields [2,3]. The T_2 values of the several lipid resonances were within the same range (12-19 ms) and comparable with those determined in the phantoms (15-20 ms). Fatty acyl resonances were well resolved in the water-suppressed spectra acquired from the mouse liver *in vivo* (Fig. 2A) and choline-containing compounds could be identified at 3.2 ppm. Because of the narrow range of T_2 values, at an ultra-short TE the relative signal intensities of the lipid resonances were preserved when comparing with the *in vitro* spectrum of the soybean oil emulsion. The lipid resonances around the water signal (4-5.5ppm) were clearly affected by a broadband water-suppression pulse in the *in vitro* spectrum. Nevertheless, the lipid profile derived from spectra acquired with TE of 2.8 ms was in good agreement with that determined *in vitro* in the soybean oil emulsion (Table 1).

DISCUSSION AND CONCLUSION: We report that highly stable localized ^{1}H-MRS of the mouse liver can be achieved at 14.1T. The enhanced sensitivity at 14.1T allowed for accurate determination of HCL from small voxels confined to hepatic tissue. Furthermore, the saturation profile of the fatty-acyl chains can be determined by using an ultra-short TE STEAM thus minimizing signal variability due to T_2 and J-evolution effects. This approach is suited to study mice models of impaired lipid metabolism or liver diseases.

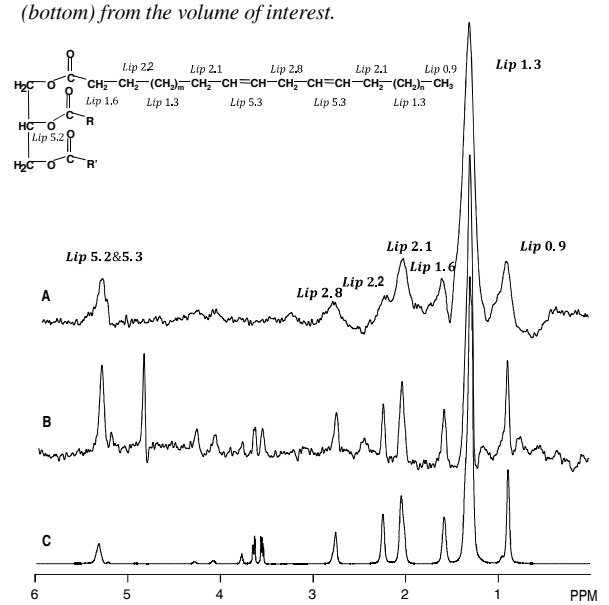

Fig. 2 In vivo *^{1}H NMR spectra (weight parameters: gaussian 0.05, gaussian shift 0.02 and no line broadening) acquired with VAPOR water suppression with TE 2.8 ms of* ***(A)*** *the mouse liver and* ***(B)*** *a soybean oil emulsion phantom.* ***(C)*** In vitro *^{1}H NMR spectra of a soybean oil emulsion sample. The general structure of a triglyceride is shown on top and ^{1}H NMR resonances assigned to the respective groups.*

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