EVOLUTION OF THE HEPATIC LIPID PROFILE OF THE ADULT MOUSE - *IN VIVO* AND *IN VITRO* ^{1}H MRS ASSESSMENTS AT 14.1T

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TARGET AUDIENCE: Basic researchers on animal models of hepatic metabolism

PURPOSE: Localized ^{1}H MRS can be employed to non-invasively assess the biochemical composition of selected organs in living animals. We aimed to quantitatively measure the lipid content and saturation profile of the fatty acyl chains from the mouse liver using short echo time STEAM [1] at high magnetic field, i.e. 14.1T. Furthermore, the *in vivo* data from three different groups were studied and compared with those from *in vitro* ^{1}H NMR analysis of tissue extracts.

METHODS: All experiments were performed with the approval by the local ethic committee. C57BL/6J mice were studied at 4 months (male, young adults) and at 7 months (males and females, adults). A ^{1}H quadrature surface coil (two 13mm-inner-diameter physically decoupled loops) placed right above the abdomen was used as a transceiver. Animals were scanned in the supine position, under 1-2% isofluorane anesthesia, in a horizontal bore 14.1T magnet (Agilent). The animals' breathing patterns and temperature were monitored through an MR-compatible system (SA Instruments), which also delivered the necessary TTL signals to the console for respiratory gating. All MR pulse sequences were applied with respiratory gating signals to avoid unwanted motion artifacts. Multi-slice GRE images were acquired for anatomical identification of the liver. Localized, ^{1}H MR spectra were acquired from a 8-15 µl voxel with STEAM (TM 20 ms; TR 6.5 s; TE 8 ms; 18-25 scans). To assess the fatty acyl chains, VAPOR water suppression [1] was applied to eliminate water signal (Fig. 1A) and a TE of 2.8 ms was used to minimize T_2 and J-evolution effects on the signal intensities. All spectra were corrected for B0 drift and phase, summed for LCModel analysis. The hepatic lipid content (HLC) was expressed as % of total ^{1}H MR signal. The unsaturation profile of the lipids was calculated as shown in Fig. 2. After the *in vivo* MR measurements, the animals were sacrificed, the livers excised and treated with Chloroform:Methanol (2:1) to extract the lipid fraction [2], which was further analyzed by ^{1}H-NMR spectroscopy *in vitro* in a Bruker DRX-600 spectrometer. Data are expressed as mean ± SEM.

RESULTS: *In vivo* MR studies of the different mice groups indicated that HLC increased with age and this tendency was aggravated by obesity (Table 1). These measurements were in good correlation with those performed in the respective lipid extracts *in vitro*. Water-suppressed short echo ^{1}H MR spectra from mouse liver (Fig. 1A) preserved the lipid profile, similar to that found in the *in vitro* samples (Fig. 1B,C). Further quantification of such quality data allowed resolving the contribution of unsaturated and saturated fatty acyl chains (UFA and SFA) to the hepatic lipids as well as the polyunsaturation degree of such FA (Fig. 2). The mean number of total double bonds and poly-unsaturated double bounds (PUdb) was higher in the lipids of the hepatic extracts relative to the assessments performed *in vivo*. In the hepatic extracts, it was found that the contribution of UFA to the total lipids increased with HLC but the mean poly-unsaturation decreased, thus implying an increased contribution from mono-unsaturated FA (MUFA) to the UFA fraction. Notably there was strong negative correlation between the PUdb per UFA and the UFA fraction of total lipids (Fig. 3).

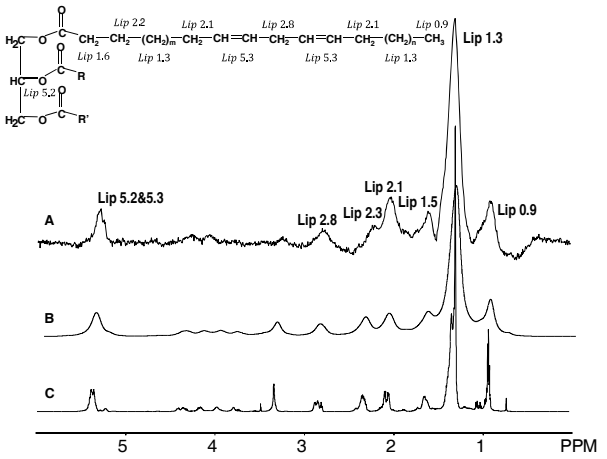

Fig. 1 (A) *Representative* in vivo *^{1}H NMR spectrum of the mouse liver, the water signal was suppressed and the fatty acyl resonances can be appreciated.* ***(B, C)*** in vitro *^{1}H NMR spectrum of the lipids extracted from the liver, line broadening was applied* ***(B)*** *to match the* in vivo *peak line shapes. On top, the general structure of a triglyceride is shown and the different fatty acyl groups identified by their ^{1}H NMR chemical shift assignments.*

Table 1 *Hepatic lipid content (HLC) determined in vivo*

	Young adult male mice	Adult male obese mice	Adult female mice
HLC (%)	0.99 ± 0.14	6.69 ± 0.36	2.02 ± 0.46

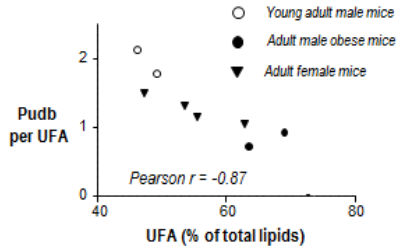

Fig. 3 *Mean UFA Poly-unsaturation as a function of the UFA fraction.*

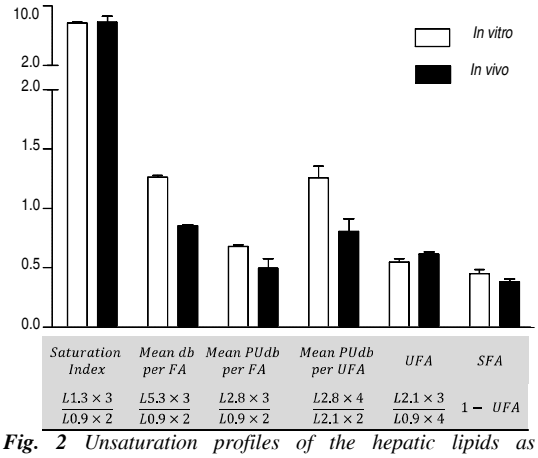

Fig. 2 *Unsaturation profiles of the hepatic lipids as determined from the* in vivo *and* in vitro *spectra of adult female mice. The equations used for the different parametrs are shown. db, double bound; PUdb, poly-unsaturated db; UFA, unsaturated fatty acyl chain; SFA, saturated fatty acyl chain; FA, fatty acyl chain.*

DISCUSSION AND CONCLUSION: In this study, we clearly showed that during adulthood there is an increased of HLC that is aggravated in obese mice; this event occurs in parallel with an unbalance of the PUFA/MUFA ratio in favor of the latter. The differences between the *in vivo*- and *in vitro*-determined unsaturation profiles suggest a potential contribution from highly poly-unsaturated membrane lipids in the tissue extracts [3]. On the other hand, only the mobile lipids from cytosolic droplets are visible by *in vivo* MRS. By employing high field strength, both HLC and the lipid profile of cytosolic lipids can be non-invasively assessed from the same volume in short experiment settings, opening the possibility to study liver disease models longitudinally.

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