^{1}H MRS methodology, dietary effects and impact of surgical stress on hepatic lipids in NAFLD animal models

J-B. Hövener[1], U. Dahmen[2], B. Merkel[3], O. Dirsch[4], J. Hennig[1], and D. von Elverfeldt[1]
[1]Department of Radiology, Medical Physics, University Medical Center, Freiburg, Germany, [2]Experimentelle Transplantationschirurgie, Klinik für Allgemein-, Viszeral- und Gefäßchirurgie, Jena, Germany, [3]Fraunhofer MEVIS, Germany, [4]Institut für Pathologie, Jena, Germany

1. Introduction

The incidence of non-alcoholic fatty liver disease (NAFLD) is rising and a burden to patients and healthcare. ***(a) The methodology to diagnose NAFLD*** nowadays is liver biopsy and histology, posing a considerable risk for the patient. The only means to quantify the lipid content of the liver *non-invasively* is magnetic resonance spectroscopy [1]. Furthermore, ***(b) the efficiency of NAFLD animal models*** is largely unknown. This is of serious concern, as these models are key to investigate potential therapeutic strategies and to understand the molecular mechanics. ***(c) The impact of surgical stress*** on the viability of liver transplants is of vital importance: While the supply of donor livers is limited, only few quantitative parameters exist for a transplant surgeon to decide whether to use an organ or to reject it. None of these include hepatic lipids, and the impact of surgical stress on the lipid content of NAFLD grafts is not known. These questions are investigated here using an animal model: (a) we evaluate the reproducibility of the method to absolutely quantify liver lipids, (b) monitor dietary effects on liver lipids and (c) evaluate the effect of surgical stress by partial hepatectomy (PH) on liver lipid content.

2 Methods

Samples: Cohorts of lew-rats (n = 12) were fed with NAFLD-inducing diets: low methionine - low choline diet (LMC)**,** methionine - choline deficient diet (MCD) and methiodine-choline deficient diet combined with high fat (MCD HF). For each diet, two liver samples were collected from each rat by partial hepatectomy (70 %) and harvest 24 hrs later (rat 1: day 7 and 8, rat 2: day 14 and 15, etc, 24 total). All samples were stored in formalin solution (varying sizes, approx. 6 x 6 x 12 mm^3). A model solution containing peanut oil of similar shape and volume was prepared. ***MR-methodology:*** A dedicated small-animal MRI system equipped with a mouse quadrature coil was employed (FA Bruker, Germany, B_0 = 9.4 T). ^{1}H single voxel MRS was prescribed centrally on the samples on FLASH images (PRESS, TE = 30 ms, TR = 3 s, V = (3 mm)^3, 64 transients, outer volume suppression with 1 mm gap). To minimize chemical shift displacement, strong r.f. pulses were applied and the excitation order chosen to assure voxel placement with the sample (Fig. 1c, hermite localization pulses: band width ($bw_{90°}$) = 12 kHz, pulse width ($pw_{90°}$) = 410 µs and $bw_{180°}$ = 6.33 kHz, $pw_{180°}$ = 540 µs, corresponding to a chemical shift displacement between H_2O and the lipid resonance at 1.3 ppm of 312 µm and 664 µm, respectively). Three methods to improve the B_0 field homogeneity of the excited volume where evaluated: iterative adjustments of first order shims proved superior regarding speed and outcome (line width) to more sophisticated methods (FASTMAP [2], MAPSHIM [3]). ***Evaluation:*** lipid (l_r) and water resonances were quantified using LC model (liver-6 package, (4)). Total lipid content (l_c) of the voxel was estimated by comparison to the spectrum of a peanut oil model solution acquired under identical conditions (density ρ_{oil} = 0.91 g / ml).

3 Results

Nine lipid resonances (l_r) were detected in the spectra and quantified (Fig. 1a, 1b). To estimate ***the reproducibility of the method*** (Fig. 1c), a representative sample was measured (i) serially at constant conditions, (ii) after repositioning of the sample and readjustments of the MR system and (iii) on different days (sample resected at d 7 of MCD HF diet, Fig. 1a). For each resonance, the coefficient of variance (c_v) was calculated. The inter-day variations (c) most closely estimate the error of the latter measurements (for different samples, e.g. c_v = 8.5 % for the $lr_{Lip1.3}$, c_v = 1.2 % for $lr_{Lip1.3}$ of peanut oil). ***The dietary effect*** on the lipid content was evaluated using the samples gained from partial resection (Fig. 1d, solid symbols). Rats exposed to MCD and MCD HF diets exhibited strong, non-linear increase of liver lipid signals during the feeding period. In the extreme, $lr_{Lip1.3}$ exceeded the water signal three-fold, corresponding to an estimated lipid content of l_c = (522 ± 50) µg / ml (MCD at d 28, compared to the model solution of peanut oil). In contrast, samples of LMC diet did not exhibit lipid resonances at d 7 and only moderate, linear increase to max. l_c = (8 ± 1) µg / ml at d 28 (in contrast to MCD and MCD HF at d 7: l_c = (50 ± 5 and 90 ± 9) µg / µl, respectively). ***The effect of surgical stress*** was evaluated comparing samples gained from PH (Fig. 1d, solid symbols) and harvest 1 d later (Fig. 1d, hollow symbols). Each diet had a different effect: $lr_{Lip1.3}$ of all LMC samples increased significantly after PH (p = 0.02). For all MCD samples, the $lr_{Lip1.3}$ decreased significantly, while $lr_{Lip1.3}$ of MCD HF samples was not significantly different (p = 0.6).

4. Discussion and Outlook ***Reproducibility of methodology*****:** ^{1}H MRS has proved a stable tool to quantify the lipid content in liver samples. The error for the absolute signal (in arbitrary units) of $lr_{1.3ppm}$ acquired on different days was measured to c_v = 8.5 %. For the absolute quantification the error amounted to c_v (abs) = c_v (liver) + c_v (oil) = 9.6 %. This c_v does not take into account the difference between model solution and liver lipid or regional differences in lipid content. The ratios of the individual *lr* of liver and oil, however, were not significantly different (p = 7 · 10^{-4}, e.g. $lr_{1.3ppm}$ (liver) vs. $lr_{1.3ppm}$ (oil), *etc.* for all resonances). $lr_{Lip2.8}$, though, was three times larger in the liver (relatively, corresponding to the C**H$_2$** resonance between two unsaturated bonds: -CH=CH-C**H$_2$**-CH=CH- (resonance in bold) (5)). ***Dietary effects:*** The method allowed monitoring the effect of diets on the lipid content, as well as the impact of partial hepatectomy. Surprisingly, LMC diet did not exhibit increased lipids and therefore is of limited use for NAFDL studies. ***Surgical stress*** was answered differently by each model; – a very interesting fact which will be closely observed in an upcoming study. Future work will focus on translation *in vivo,* both rodents and humans, and correlation with other methods. The ability of the method to evaluate transplant organs and quantify surgical stress is very promising and will be investigated in an upcoming study.

References: (1) SR Mehta *et al.* World J Gastroenterol 2008;14(22):3476-3483. (2) R Gruetter. Magn Reson Med 1993;29(6):804-811. (3) A Manabe, Proc. ISMRM 1994. p 696. (4) SW Provencher. Magn Reson Med 1993;30(6):672-679. (5) K Strobel, *et al.* Journal of Lipid Reseach 2008;49:473 - 480.

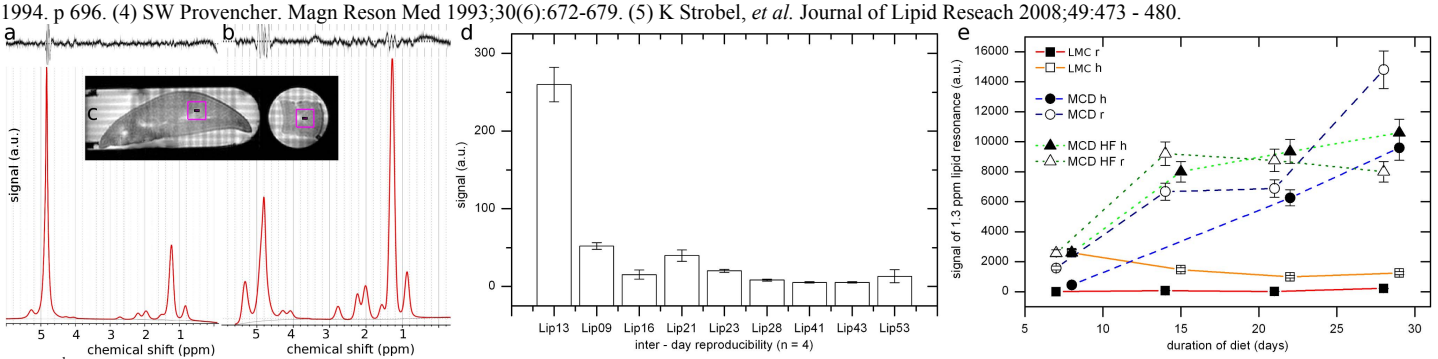

Fig. 1: ^{1}H MRS (black), fit (red) and residual (top) of a liver sample after exposure to MCD HF diet for (a) 7 d and (b) 28 d. Prescription of the voxel (c). Inter-day reproducibility (d) of quantification of individual resonances of spectrum 1b (lip09 = resonance at 0.9 ppm). (e) Development of the signal of 1.3 ppm lipid resonance of liver samples from rats exposed to LMC, MCD and MCD HF diets for 7 – 29 d. r: partial hepatectomy, h: harvest.