In vivo magnetic resonance spectroscopy of lipid handling in steatotic rat liver

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Purpose: Hepatic steatosis is the abnormal and excessive accumulation of triglycerides in the liver and is a histological hallmark of non-alcoholic fatty liver disease (NAFLD). NAFLD is regarded as the hepatic manifestation of the metabolic syndrome and has emerged as the most important cause of chronic liver disease [1-4]. Hepatic steatosis occurs when the rate of import or synthesis of fatty acids by hepatocytes exceeds the rate of export or oxidation. While ^{1}H MRS is very powerful to detect abnormalities in lipid storage, it cannot discriminate between disturbances in fatty acid uptake and/or synthesis on one hand and fatty acid export and/or oxidation on the other. However, knowledge about the relative contributions of these different mechanisms in the pathogenesis of NAFLD is essential for the development of effective treatment strategies. The aim of this study was to determine differences in *in vivo* lipid metabolism in livers of rats on different high-fat diets using ^{1}H MRS with ^{13}C editing together with the oral administration of ^{13}C-labeled lipids.

Methods: For this study 27 male Wistar rats (11 weeks of age; 348 ± 2 g) were used, which were divided into 3 groups. The control group received a low-fat diet (10% of calories from fat; CON). The second group received a high-fat palm oil diet (45% of calories from fat; HFP), while the third group received a high-fat lard diet (45% of calories from fat; HFL). After 10 weeks of diet, ^{1}H-[^{13}C] MRS experiments were performed at baseline, and 4 and 24 h after the oral administration of 1.5 g [U-^{13}C] Algal lipid mixture per kg body weight. All experiments were executed on a 6.3 T horizontal Bruker MR system with a ^{1}H surface coil (20 mm) combined with a ^{13}C butterfly coil (40/100 mm). A voxel of 4x2x4 mm^3 was localized in the median lobe of the liver using the LASER sequence, which was combined with a POCE element for ^{13}C editing [5]. The ^{13}C-editing pulse (AFP, pulse length=2 ms, bandwidth=5 kHz) was centered on the lipid methylene resonance and the POCE echo time was tuned to $1/^{1}J_{1H\text{-}13C}$ for lipid methylene protons (7.9 ms). Other LASER-POCE parameters were as follows: TR=2 s, TE=26.8 ms, SWAMP water suppression, ^{13}C WALTZ decoupling, 16 averages, 64 sequential experiments, scan time = 34 min. Spectra obtained with and without the POCE ^{13}C-editing pulse were subtracted to give a difference ^{1}H spectrum. Spectra were analyzed using AMARES in jMRUI. The CH_2 signal from intrahepatocellular lipids (IHCL) at 1.3 ppm (Figure 1) was used to calculate the total IHCL content (from the spectra without ^{13}C editing) and the ^{13}C-labeled IHCL content (from the difference spectra), which were both expressed as a percentage of the unsuppressed water signal. The average relative ^{13}C enrichment determined at baseline was used to correct the ^{13}C-labeled IHCL levels for natural abundance of ^{13}C. All data are expressed as means ± SEM. Statistical analysis was performed using paired ANOVA with repeated measures (SPSS).

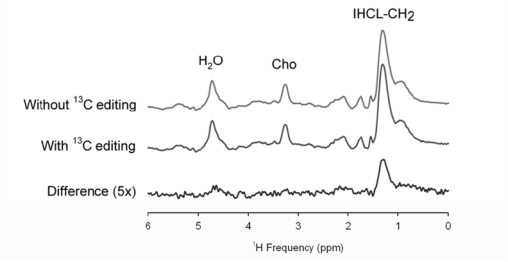

Figure 1. Example of LASER-POCE spectra of the liver at 4 h after the administration of ^{13}C-labeled lipids without ^{13}C editing (top), with ^{13}C editing (middle), and the calculated difference spectrum (bottom). H_2O, water; Cho, choline; IHCL, intrahepatocellular lipids.

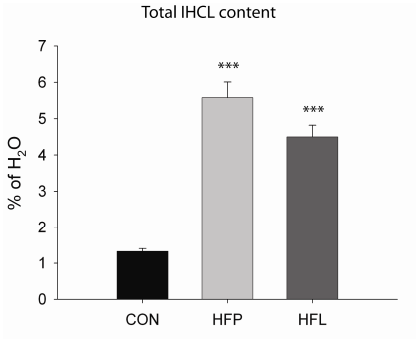

Figure 2. Total IHCL content expressed as percentage of unsuppressed water signal.*** $p < 0.001$ vs CON.

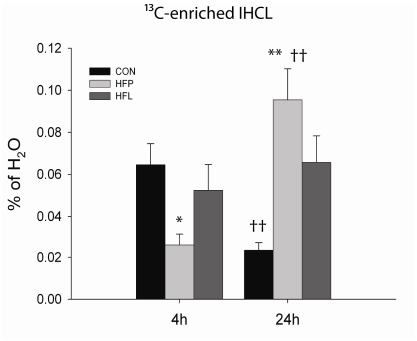

Figure 3. Absolute ^{13}C enrichment of IHCL expressed as percentage of the unsuppressed water signal and corrected for natural abundance ^{13}C enrichment. * $p < 0.05$ vs CON, †† $p < 0.01$ vs 4h, ** $p < 0.01$ vs CON.

Results: At baseline, total IHCL content was 3 to 4 fold higher in the two high-fat diet groups compared with the CON group ($p < 0.001$, Figure 2). Four hours after administration of ^{13}C-labeled lipids, ^{13}C enrichment of IHCL was lower in the HFP group compared with CON ($p < 0.05$), while there was no difference between CON and HFL (Figure 3). Between 4 and 24 h, the ^{13}C enrichment of IHCL decreased in CON ($p < 0.01$). In contrast, the content of ^{13}C-enriched IHCL did not change between 4 and 24 h in the HFL group, while it even increased in the HFP group ($p < 0.01$).

Discussion and Conclusion: Animals on both high-fat diets developed steatotic livers. This was however not accompanied by increased lipid uptake in the liver during the early postprandial phase. In the HFP group, liver lipid uptake in the early postprandial phase was even lower than in CON. In control rats, the ^{13}C enrichment of IHCL rapidly decreased between 4 and 24 h after ^{13}C-labeled lipid administration, which is in accordance with our previous results [5] and reflects the rapid turnover of lipids in the liver by means of export and/or oxidation. This decrease in ^{13}C-enriched IHCL between 4 and 24 h was however not observed in the high-fat diet groups, which indicates that the turnover of IHCL was significantly hampered. In the HFP group, the ^{13}C enrichment of IHCL even increased between these time points, suggesting a prolonged postprandial lipid uptake. In conclusion, it was shown that high-fat diets induced liver steatosis in rats, which was accompanied by a significantly reduced lipid turnover.

References: [1] Fon Tacer K and Rozman D, J Lipids 201: 783976, 2011; [2] Adams LA and Feldstein AE, J Dig Dis 12: 10-6, 2011; [3] Lebovics E and Rubin J, Diabetes Metab Res Rev 27: 419-424, 2011; [4] Feldstein AE, Semin Liver Dis 30: 391-401, 2010; [5] Jonkers RAM, et al., Magn Reson Med 68: 997-1006, 2012