Real-time assessment of *in vivo* postprandial lipid storage in rat liver using ^{1}H-[^{13}C] MRS

R. Jonkers[1], T. Geraedts[1], L. van Loon[2], K. Nicolay[1], and J. Prompers[1]
[1]Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands, [2]Department of Human Movement Sciences, Maastricht University Medical Centre+, Maastricht

Introduction - Insulin resistance and type 2 diabetes are associated with elevated liver lipid (intrahepatocellular lipid, IHCL) content [1]. It remains to be established whether this is attributed to an increase in lipid uptake, a decrease in lipid oxidation, or an imbalance between lipid uptake and mobilisation. In current research, ^{13}C labeled lipids are often used in arterio-venous balance experiments and *in vitro* studies to elucidate the dynamics of lipid uptake and oxidation. These measurements provide rather indirect measures of lipid handling. Moreover, *in vitro* setups are not adequately reflecting the *in vivo* situation. To identify the exact origin of abnormal lipid handling in insulin resistant tissue, direct *in vivo* measurements of lipid dynamics are essential. In this study, we measured for the first time postprandial lipid storage in rat liver *in vivo* using ^{1}H-[^{13}C] magnetic resonance spectroscopy (MRS) and ^{13}C labeled lipids as tracers.

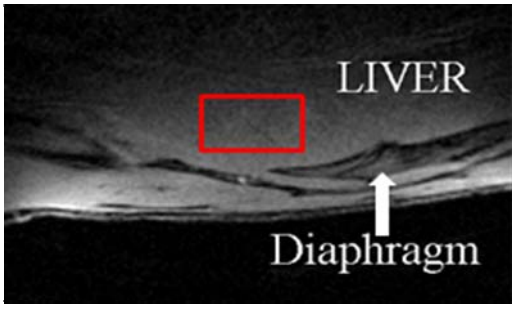

Figure 1: T_1-weighted transversal image of rat abdomen with a 4x2x4 mm^3 voxel (red) in liver.

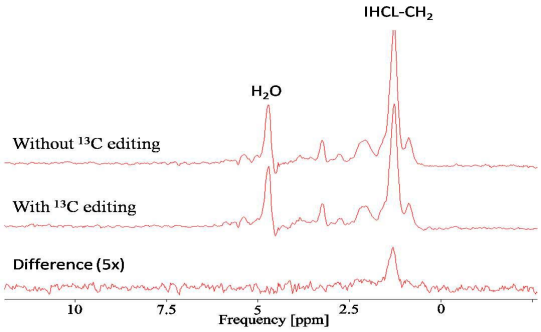

Figure 2: LASER-POCE spectra of a rat liver without ^{13}C editing (top), with ^{13}C editing (middle), and the calculated difference spectrum (5 x magnification, bottom). Peak annotations: IHCL: intrahepatocellular lipids; H_2O: residual water.

Methods - Six Wistar rats (16 weeks) were measured at baseline, 5, and 24 h after oral administration of 400 mg [U-^{13}C] Algal lipid mixture. During MR measurements, animals were anesthetized using 1-2% isoflurane administered through a face mask with medical air (0.6 L·min^{-1}). Experiments were performed on a 6.3T horizontal Bruker MR system using a circular single turn ^{1}H surface coil (20 mm) in combination with a single turn ^{13}C butterfly coil (40/100 mm). Rats were positioned in a prone position with the diaphragm above the ^{1}H coil. No respiratory gating was needed, because T_1-weighted images showed breathing motion was negligible at the site of interest (Figure 1). Localized ^{1}H MRS was performed on a 4x2x4 mm^3 voxel placed in the liver (Figure 1) using the LASER sequence which was combined with a POCE element [2] for ^{13}C editing (TR = 2 s, TE = 27 ms, TE_{POCE} = 7.9 ms, SWAMP water suppression, ^{13}C WALTZ decoupling, 16 averages, 64 sequential experiments, 34 min). LASER-POCE spectra were acquired in an interleaved fashion with an adiabatic full passage ^{13}C editing pulse turned on every other experiment. Spectra with and without the ^{13}C editing pulse were added separately and the difference spectrum was calculated (Figure 2). In the difference spectrum all uncoupled resonances are eliminated, making resonances of spins that are directly bonded to ^{13}C nuclei observable in the ^{1}H MR spectrum. ^{13}C enrichment of the IHCL-CH_2 signal at 1.3 ppm was calculated from the difference spectra using a nonlinear least squares algorithm (AMARES) in the jMRUI software package. Spectra were fitted using prior knowledge for the line shapes and the line widths of the IHCL-CH_2 peak. Data are expressed as means ± SD. Data were analyzed with mixed-model repeated-measures analysis of variance with time point (baseline, 5 h post, and 24 h post) as the within-subjects factor. Bonferroni corrections were applied when appropriate. Statistical analyses were performed using SPSS 17.0. The level of significance was set at $p < 0.05$.

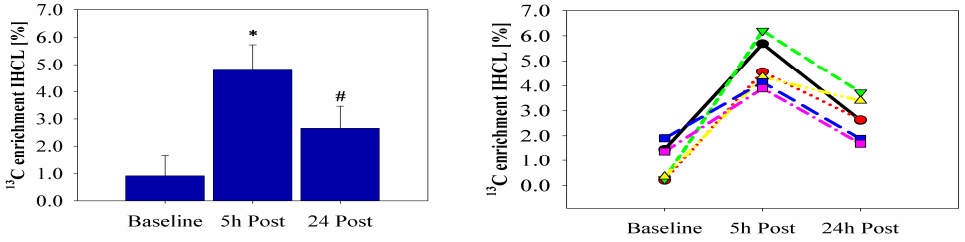

Figure 3: ^{13}C enrichment of IHCL-CH_2 in rat liver. Data are expressed as percentage of total IHCL-CH_2 signal; mean ± SD (left); data per individual rat (right). Significantly higher than baseline ($p < 0.005$); # Significantly lower than 5 h post ($p < 0.005$).*

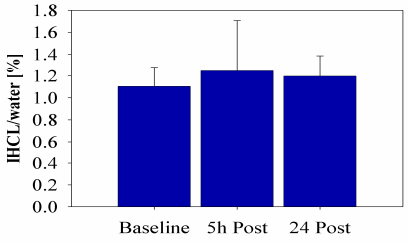

Figure 4: IHCL/H_2O ratio. Data are expressed as a percentage of the IHCL-CH_2 signal with respect to the unsuppressed water signal ± SD.

Results - A typical example of 2 LASER-POCE spectra with and without ^{13}C editing and the corresponding difference spectrum is displayed in Figure 2. Figure 3 shows the ^{13}C enrichment of IHCL in rat liver at baseline, 5, and 24 h post tracer administration. At baseline, 0.9 ± 0.7% ^{13}C enrichment of IHCL was determined. At 5 h post ingestion of the ^{13}C labeled lipid, a significant increase in ^{13}C enrichment of IHCL to 4.8 ± 0.9% was observed ($p < 0.005$). ^{13}C enrichment of IHCL significantly decreased to 2.7 ± 0.8% 24 h after administration ($p < 0.005$). The total IHCL/H_2O ratio remained constant throughout the study (Figure 4).

Discussion - Baseline levels of ^{13}C enrichment were in accordance with the natural abundance of ^{13}C (1.1%). 5 h after ingestion of ^{13}C labeled lipid, ^{13}C enrichment of the IHCL pool increased 5.2-fold due to the postprandial uptake of ^{13}C labeled lipids. 24 h after ingestion a decrease in ^{13}C enriched IHCL was observed, which implies that ^{13}C labeled fatty acids have been liberated from the IHCL pool. In conclusion, the application of LASER-POCE in combination with ^{13}C enriched lipid administration allows for successful real-time *in vivo* assessment of changes in ^{13}C enriched lipid content in rat liver. In future research, this method will be used with an increased temporal sampling to detect abnormalities in lipid handling in the insulin resistant state and, as such, will contribute to a better understanding of the etiology of type 2 diabetes.

References - [1] Kelley DE, et al, AJP Endo Metab, 285: E906 - E916, 2003; [2] De Graaf RA, et al, NMR Biomed, 16: 339 – 357, 2003