In vivo determination of *de novo* lipogenesis in rat liver using localized ^{1}H-[^{13}C] magnetic resonance spectroscopy

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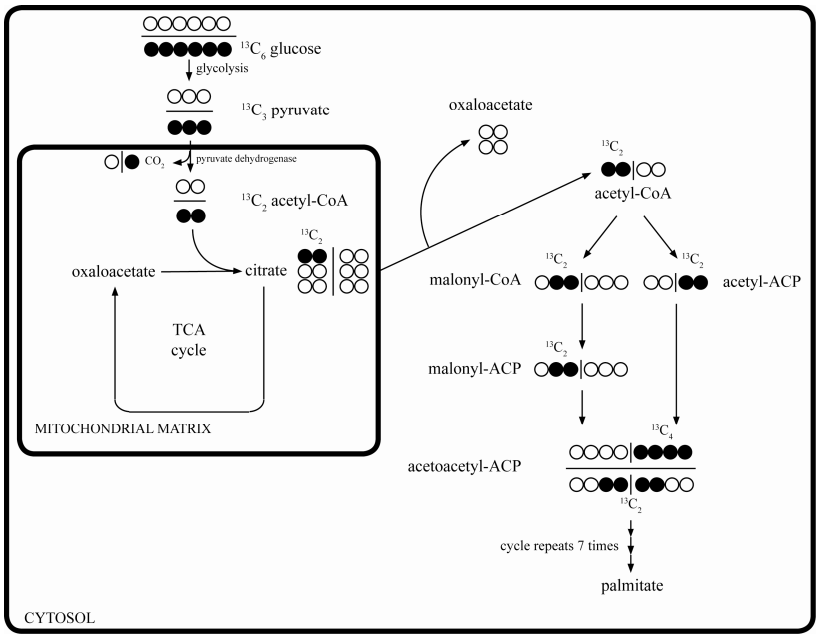

Figure 1. Major metabolites involved in *de novo* lipogenesis. The carbon atoms indicated by black circles become ^{13}C labeled when [U-$^{13}C_6$] glucose is used as a substrate. For clarity only labeling through the first cycle is shown.

Purpose: *De novo* lipogenesis is the primary pathway in which excess dietary carbohydrates are being converted to fat in liver and adipose tissue, with liver being the main site in humans [1, 2]. Increased *de novo* lipogenesis has been associated with hepatic steatosis in non-alcoholic fatty liver disease (NAFLD), insulin resistance and type 2 diabetes [3, 4], but the exact contribution of *de novo* lipogenesis to the accumulation of lipids in the liver in these disorders is unknown. Indirect calorimetry and stable isotope techniques are commonly used to assess the hepatic output of *de novo* synthesized lipids in the plasma or in the expired air after oxidation. However, for the measurement of the contribution of *de novo* lipogenesis to the accumulation of lipids in the liver a biopsy is necessary. The aim of this study was to develop a non-invasive method for the direct *in vivo* measurement of *de novo* lipogenesis in the liver using localized ^{1}H MRS with ^{13}C editing to detect ^{13}C-labeled intrahepatocellular lipids (IHCL) after oral administration of [U-$^{13}C_6$] glucose. Figure 1 shows the conversion of [U-$^{13}C_6$] glucose to ^{13}C-labeled palmitate through the *de novo* lipogenesis pathway.

Methods: For this study 6 male Wistar rats (10 weeks of age) were used. First a baseline ^{1}H-[^{13}C] MRS measurement was performed after which the rats received 1.6 g/kg body weight glucose (50% glucose and 50% [U-$^{13}C_6$] glucose), 2 times a day for 5 days by oral gavage. The next day, another ^{1}H-[^{13}C] MRS experiment was performed. 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 (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 IHCL-CH_2 signal at 1.3 ppm was used to calculate the total IHCL content (from the spectra without ^{13}C editing; expressed as a percentage of the unsuppressed water signal), and the fractional ^{13}C enrichment of IHCL (from the difference spectra) (figure 2). All data are expressed as means ± SEM. Statistical analysis was performed using a paired student's t-test (SPSS).

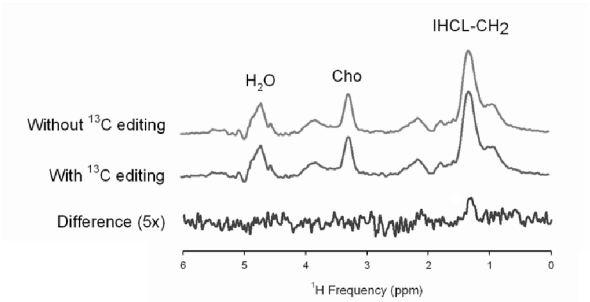

Figure 2. Example of LASER-POCE spectra of the liver after 5 days of [U-$^{13}C_6$] glucose administration without ^{13}C editing (top), with ^{13}C editing (middle), and the calculated difference spectrum (bottom). H_2O, water; Cho, choline; IHCL, intrahepatocellular lipids.

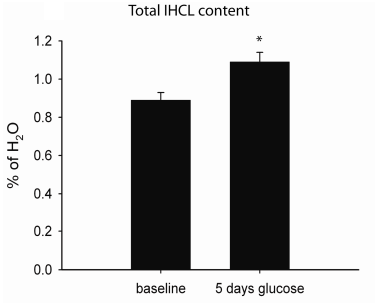

Figure 3. Total IHCL content at baseline and after 5 days of [U-$^{13}C_6$] glucose administration.* $p < 0.05$ vs baseline.

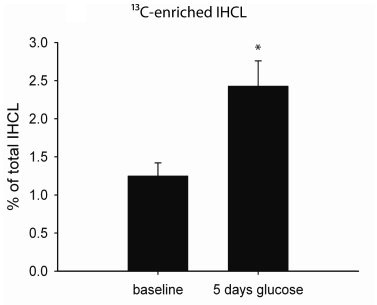

Figure 4. Relative ^{13}C enrichment of IHCL at baseline and after 5 days of [U-$^{13}C_6$] glucose administration.* $p < 0.05$ vs baseline.

Results and Discussion: After 5 days of glucose administration, total IHCL content was 24 ± 8 % increased compared with baseline ($p < 0.05$, Figure 3). At baseline, the fractional ^{13}C enrichment of IHCL was 1.25 ± 0.17 % (Figure 4), which is in accordance with the 1.1% natural abundance of ^{13}C. After 5 days of [U-$^{13}C_6$] glucose administration, the fractional ^{13}C enrichment of IHCL had risen to 2.43 ± 0.33 %, which is the result of *de novo* synthesis of fat from ^{13}C-labeled glucose in the liver, i.e. *de novo* lipogenesis.

Conclusion: The application of localized ^{1}H-[^{13}C] MRS in combination with the administration of [U-$^{13}C_6$] glucose allows for the *in vivo* assessment of the conversion of glucose to fat in the liver through *de novo* lipogenesis. In future research, this method will contribute to a better understanding of the contribution of *de novo* lipogenesis to liver lipid metabolism and, in particular, to the development of liver steatosis.

References: [1] Jequier E, et al., Am J Clin Nutr 59: 682S-685, 1994; [2] Schutz Y, et al., Int J Obes Relat Metab Disord, 28: S3-11, 2004; [3] Hellerstein MK, et al., Eur J Clin Nutr 53: S53-65, 1999; [4] Fon Tacer L, et al., J Lipids 2011: 783976, 2011; [5] Jonkers RAM, et al., Magn Reson Med 68: 997-1006, 2012