Fructose increases *de novo* lipogenesis in the liver of rats: an *in vivo* ^{1}H-[^{13}C] MRS study

Sharon Janssens[1], Klaas Nicolay[1], and Jeanine J. Prompers[1]
[1]*Biomedical NMR, Eindhoven University of Technology, Eindhoven, Noord-Brabant, Netherlands*

Target audience: This work is relevant to the field of liver lipid metabolism in general and in particular dietary factors affecting liver lipid metabolism.

Purpose: Non-alcoholic fatty liver disease (NAFLD) is the most common liver disorder in Western countries and is characterized by excessive accumulation of fat in the liver (hepatic steatosis). An important contributing factor to the rapid rise in the prevalence of NAFLD is the increased consumption of carbohydrates such as glucose and fructose present in caloric sweetened beverages. This can be explained by a process termed *de novo* lipogenesis, in which excess carbohydrates are being converted to fat in the liver[1-4]. Of great value in the fight against obesity are non-caloric sweeteners such as aspartame, but their effects on liver lipid metabolism, and especially *de novo* lipogenesis, remain unknown[5]. In this study we implemented 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). Using this method we then studied the effects of caloric and non-caloric sweeteners on *de novo* lipogenesis as well as total IHCL content in rats.

Methods: Twenty-four male Wistar rats (10-11 weeks of age; 350 ± 3 g) were divided into 4 diet groups receiving normal chow and (1) water (CON), (2) a 13% glucose solution (GLU), (3) a 13% fructose solution (FRUC), or (4) a 0.4% aspartame solution (ASP), for 7 weeks. After this diet period, baseline ^{1}H-[^{13}C] MRS measurements were performed, after which the diets were continued and the rats were administered 3.33 g/kg bodyweight [U-$^{13}C_6$] glucose, 2 times a day for 5 days by oral gavage. For all diet groups this amounted to 5.16±0.13 % of their total carbohydrate consumption. The next day, ^{1}H-[^{13}C] MRS measurements were repeated. All experiments were executed on a 7 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 5x2x4 mm^3 was localized in the median lobe of the liver and ^{1}H-[^{13}C] MRS was performed using the LASER sequence combined with a POCE element for ^{13}C editing[6]. 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/TE=2000/26.8 ms, SWAMP water suppression, ^{13}C WALTZ decoupling, 16 averages, 64 sequential experiments, and scan time = 34 min. Spectra obtained with and without the POCE ^{13}C-editing pulse were subtracted to give a difference ^{1}H spectrum (Figure 2). Spectra were analyzed using AMARES in jMRUI. The CH_2 signal from IHCL at 1.3 ppm (Figure 2) was used to calculate total and ^{13}C-enriched IHCL contents from the spectra without ^{13}C editing and the difference spectra, respectively, and are expressed as a percentage of the unsuppressed water signal. The relative ^{13}C enrichment determined at baseline was used to correct the ^{13}C-labeled IHCL level after 5 days of [U-$^{13}C_6$] glucose administration for ^{13}C natural abundance. All data are expressed as means ± SEM. Statistical analysis was performed using ANOVA (SPSS).

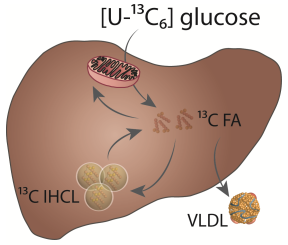

Figure 1. Overview of liver metabolism. Orally administered [U-$^{13}C_6$] glucose is oxidized or converted to ^{13}C fatty acids, that can be oxidized in mitochondria, secreted as very-low density lipoproteins (VLDL), or stored as ^{13}C IHCL.

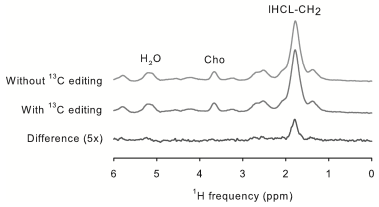

Figure 2. Example of LASER-POCE spectra of rat liver without ^{13}C editing (top), with ^{13}C editing (middle), and the calculated difference (bottom). H_2O, water; Cho, choline; IHCL, intrahepatocellular lipids.

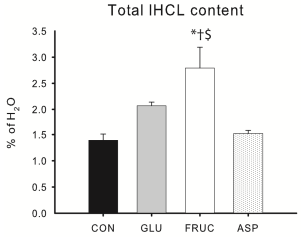

Figure 3. Total IHCL content expressed as percentage of unsuppressed water signal. * p < 0.05 vs CON, † p < 0.05 vs ASP, $ p < 0.05 vs GLU.

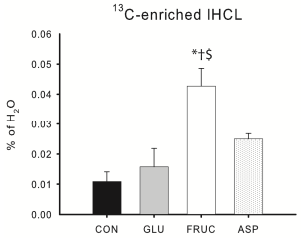

Figure 4. ^{13}C-enriched IHCL content expressed as percentage of unsuppressed water signal. * p < 0.05 vs CON, † p < 0.05 vs ASP, $ p < 0.05 vs GLU.

Table 1. Animal characteristics

	CON	GLU	FRUC	ASP
Weight gain (g)	88 ± 7	84 ± 9	104 ± 7	82 ± 3
Food intake (kJ/wk)	2322 ± 6	1184 ± 41 *†	1569 ± 34 *†$	2351 ± 4
Fluid intake (ml/wk)	181.6 ± 0.1	802.3 ± 28.3 *†	507.2 ± 24.2 *†$	219.7 ± 7.2
Energy intake (kJ/wk)	2322 ± 6	2930 ± 21 *†	2672 ± 19 *†$	2366 ± 4
Liver weight (g)	12.9 ± 0.2	13.1 ± 0.6	16.3 ± 0.6 *†$	13.2 ± 0.2
Liver glycogen content (µg/mg ww)	96.4 ± 2.8	117.7 ± 8	116.8 ± 8.2	115.2 ± 8.0

* p < 0.05 vs CON; † p < 0.05 vs ASP; $ p < 0.05 vs GLU

Results: After 7 weeks of diet, there were no differences in body weight gain among diet groups (Table 1). Although animals in the GLU and FRUC groups consumed less food compared to CON and ASP, their total energy consumption was higher due to the intake of calories from sugar in their drinking water. Liver weight of animals receiving FRUC was higher compared with the other groups. Liver glycogen content did not differ among groups. Total IHCL content was higher in the FRUC animals compared with the other groups (Figure 3). At baseline, the average relative ^{13}C enrichment of IHCL for all groups was 1.28±0.08 %, which was used to correct the level of ^{13}C-enriched IHCL after 5 days of [U-$^{13}C_6$] glucose administration for natural abundance of ^{13}C. ^{13}C-enriched IHCL content was significantly higher in FRUC animals as compared to the other groups (Figure 4), indicating an increased contribution of *de novo* lipogenesis to IHCL storage.

Discussion and Conclusion: In contrast to glucose and aspartame, fructose had marked effects on liver lipid metabolism, i.e. 7 weeks of fructose consumption increased total liver lipid content which was accompanied by an increased conversion of ^{13}C-labeled glucose to lipids in the liver. Therefore, *de novo* lipogenesis seems to be an important contributor to fructose-induced liver steatosis, which is likely explained by the activating effect of fructose on sterol regulatory element binding protein 1c[1].

References: 1. Samuel VT, Trends Endocrinol Metab 22: 60-5, 2011; 2. Schaalan M, et al., J Diabetes Complications 23: 199-208, 2009; 3. Montonen J, et al., J Nutr 137: 1447-54, 2007; 4. Schutz Y, et al., Int J Obes Relat Metab Disord 28: S3-11, 2004; 5. Wiebe N and Padwal R, BMC Med 9: 123, 2011; 6. Jonkers RAM, et al., Magn Reson Med 68: 997-1006, 2012