Impact of isocaloric fructose and glucose diets on lipid metabolism studied *in vivo* by multinuclear MR spectroscopy

Patricia M Nunes[1], Alan J Wright[1], Andor Veltien[1], Jack JA van Asten[1], Cees J Tack[2], John G Jones[3], and Arend Heerschap[1]

[1]*Radiology, Radboud University Nijmegen Medical Centre, Nijmegen, Gelderland, Netherlands,* [2]*Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, Gelderland, Netherlands,* [3]*Center for Neurosciences and Cell Biology, Coimbra, Coimbra, Portugal*

Target audience: General audience and those dealing with metabolism and MR spectroscopy.

Purpose: The excessive intake of processed fat and soft drinks rich in fructose contribute to increased numbers of obesity worldwide. Consumption of fructose sweetened drinks among patients with non-alcoholic fatty liver is 2-3 times higher compared to patients without steatosis [1]. Fructose is associated with increased plasma triglycerides (TG), visceral adipose tissue accumulation, hypersecretion of apoliprotein (Apo)B48 and impairment of insulin responses particularly in postprandial state [2]. However, these effects of fructose are not clearly disassociated from an excessive caloric intake. Thus, we aimed to investigate *in vivo* the impact of isocaloric diets of fructose and glucose in their ability to induce lipid deposition in hepatic, muscle and adipose tissues using ^{1}H MRS, MRI, and test whether the intake of these sugars promotes changes in hepatic energy (^{31}P MRS). Furthermore, we resolved the sources of hepatic triglycerides (HTG) into dietary and hepatic synthesis with ^{1}H{^{13}C}MRS and ^{2}H NMR.

Methods: Adult C57Bl6J mice were fed over 8 weeks with 60% glucose or 60% fructose diets. Whole-body glycemic control was assessed throughout the diets with glucose tolerance test and insulin measurements. MR was performed on mice under anaesthesia (isoflurane 1-2% in gas mixture with 50%O_2/50%N_2O). Abdominal adipose tissue volume was assessed by MRI at 11.7T (Biospec, Bruker Biospin) at 0, 4 and 8 weeks of diets. Mice were placed in a ^{1}H quad. volume coil and 25 continuous axial slices from the abdominal region were acquired using a spin-echo sequence: matrix 256x256, TR/TE of 1000/13ms and 14 av. T1-weighted images were processed with ImageJ (NIH, USA). Hepatic and intramyocellular lipids were assessed at the same diet times with ^{1}H MRS at 11.7T and 7T (Clinscan, Bruker Biospin). HTG were assessed with a ^{1}H quad. volume coil, from an 8µL voxel, positioned in the right lobe of the liver. ^{1}H MR spectra were acquired with PRESS:TR/TE of 1500/16 ms and 1024 av. Intramyocellular lipids (IMCL) were assessed from a 3.1µL voxel in the tibialis anterior using a ^{1}H surface quad. coil. ^{1}H MR spectra were acquired with a PRESS: TR/TE of 1500/16ms, 1024 av. and VAPOR water suppression. HTG and IMCL levels were quantified by NUTS pro-software (Acorn NMR, USA). FIDs were apodized, Fourier transformed and the peaks of interest were fitted. HTG content was expressed as the ratio between the lipid methylene peak and the H_2O peak; IMCL level was expressed as the ratio of the lipid methylene peak and creatine methyl peak after correction for T_1 and T_2 relaxation. Hepatic energy status was assessed at 6 weeks of diet with ^{31}P 2D-MRSI (32x32x4mm^3) at 7T. The liver was positioned on top of a ^{1}H/^{31}P surface coil, scout images were taken with a gradient echo sequence (TR/TE of 269/4ms) and ^{31}P MRSI acquired with a pulse acquire sequence using adiabatic excitation: BIR-4, 45º flip angle [3], 96 av, and TR 3s. ^{31}P MR spectra were processed with jMRUI (AMARES) using prior-knowledge. Amplitudes of ATP, Pi, NADPH, UDP,PME, PDE were corrected for partial saturation[4], and concentrations determined using the phantom replacement method. To discern the sources of HTG, an i.p. bolus of $^{2}H_2O$ was given to trace *de novo* lipogenesis (DNL). Additionally, an oral bolus of [U-^{13}C]algal lipids (5g/kg) was given to trace the dietary contribution to HTG pool *in vivo* with ^{1}H{^{13}C}MRS. For this mice were positioned with the liver on top of a ^{1}H surface coil Tx/Rx surrounded by an Aldermant-Grant ^{13}C Tx coil. Localized proton observed carbon edited (POCE) MRS was done in an 8µL voxel in the right lobe of the liver by ACED-STEAM[5] with TR/TE of 2000/7.9ms. HTG ^{13}C enrichment was determined by dividing the integral of ^{13}C labeled lipid methylene peak with that of total lipid. ^{13}C and ^{2}H NMR of HTG extracts allowed to resolve the absolute contributions from "dietary" lipids and DNL to the HTG pool.

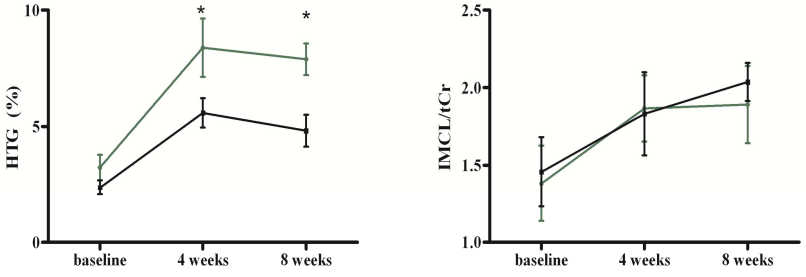

Fig.1 ***Hepatic triglyceride (HTG) and intramyocellular lipid (IMCL) contents of fructose (green) and glucose (black) fed mice.***

Results: Glucose and fructose mice had similar body-weight gain over 8 weeks and similar caloric intake. Glucose tolerance was impaired in both groups at 8 weeks, although insulin levels were higher in fasted fructose mice (0.54±0.19 vs.0.28±0.08ng/mL, p<0.05). After 8 weeks of diet, abdominal adipose tissue was mildly increased, 21±18% vs. 24±15% in fructose and glucose mice. IMCL content was maintained throughout the diet, as shown by the IMCL/tCr ratios at baseline and 8 weeks, of fructose (1.4±0.7 vs. 1.9±0.7) and glucose (1.5±0.6 vs. 2.0±0.3) mice. Levels of HTG increased within 4 weeks in both groups, however more significantly in fructose than in glucose mice (8.4±4.6% vs. 5.5±2.4%, p<0.05). During the last 4 weeks, HTG content in fructose and glucose mice was similar to that of week 4 (7.9±2.4% vs. 4.8±2.5%, Fig.1). Hepatic energy levels were found similar between fructose and glucose mice: ATP (1.9±1.1mM vs. 1.6±1.2mM), Pi (2.9±0.7mM vs. 3.3±1.0mM), NADPH (1.6±1.1mM vs. 2.2±1.3mM) and PME/ATP (2.5±1.1 vs. 2.7±2.1). To determine if fructose promoted absorption of dietary lipids, for instance through the hypersecretion of ApoB48, we investigate *in vivo* whether after an oral load of [U-^{13}C]lipids, fructose fed mice had increased ^{13}C enrichment in the HTG pool. Using ^{1}H{^{13}C}MRS we determined that, 5h after the bolus, HTG pool of fructose and glucose mice had a ^{13}C enrichment of 3.2±1.6% vs. 4.3±2.3%, which corresponds to a "dietary" absorption of 2.6±1.7% vs. 3.9±2.3% (p=0.15). *De novo* lipogenesis, investigated by ^{2}H NMR, was found significantly increased in fructose fed mice compared to those fed with glucose (5.3±3.0 µmol/g vs. 2.4±1.1 µmol/g, p=0.01, Fig.2).

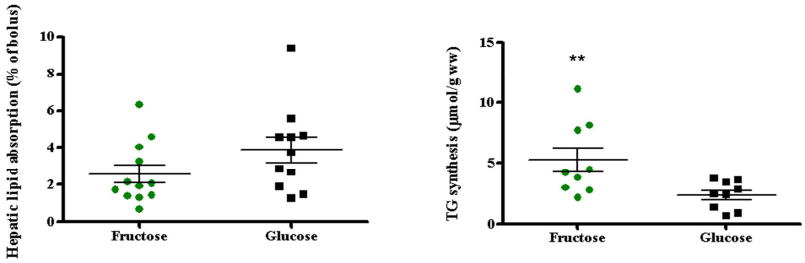

Fig.2 ***Hepatic absorption of oral lipids and de novo lipogenesis in fructose and glucose mice***

Discussion: Our data demonstrate that isocaloric fructose and glucose feeding over 8 weeks causes similar changes in abdominal adipose tissue and intramyocellular lipid content. Both diets promote HTG accumulation, however in fructose fed mice this ectopic lipid accumulation is significantly higher. This seems to be solely a result of increased hepatic *de novo* lipogenesis, and not increased dietary fat uptake. Unlike acute doses of fructose, a continuous dietary supply of this sugar does not modify hepatic energy levels.

Conclusion: Fructose consumption represents a risk factor for the development of hepatic steatosis and whole-body insulin resistance.

References: [1]Ouyang X *et al*., Fructose consumption as a risk factor for non-alcoholic fatty liver disease. *J Hepatol*. 2008;48:993-9. [2]Stanhope KL *et al*., Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest*.2009; 119:1322-34.[3]Kobus T *et al*., *In Vivo* ^{31}PMR spectroscopic imaging of the human prostate at 7T: Safety and feasibility. *Magn Reson Med*. 2012,in press. [4]Chemlik M, *et al*., T1 relaxation times of ^{31}P metabolites in human liver at 7T ISMRM; 2012.[5]Pfeuffer *et al*., Localized *in vivo*^{1}H NMR detection of neurotransmitter labeling in rat brain during infusion of [1-^{13}C]glucose. *Magn Reson Med*. 1999;41:1077-83.

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