Hepatic energy levels are not altered by fructose feeding in mice

Patricia Maria Nunes[1], Andreas Boss[2], Alan Wright[1], Andor Veltien[1], Cees Tack[3], John Jones[4], and Arend Heeschap[1]

[1]Radiology, Radboud University Nijmegen Medical Centre, Nijmegen, Gelderland, Netherlands, [2]Radiology, Radboud University Nijmegen Medical Centre, Gelderland, Netherlands, [3]Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, Gelderland, Netherlands, [4]Life sciences, Centre for Neurosciences and Cell Biology, Coimbra, Portugal

Introduction: Hepatic fructose metabolism, unlike that of glucose, bypasses phosphofructokinase key regulation point leading to an "uncontrolled" carbon flow through glycolysis and making avid use of ATP for sequential phosphorylations. Therefore, in acute doses, hepatic fructose metabolism results in a transient depletion of ATP while increasing phosphomonoesters (PME) species. A long-term excess consumption of fructose has been associated with hepatic energy synthesis disturbances. For example, among non-alcoholic fatty liver disease patients, those with a greater consumption of fructose sweetened drinks (>15 g/day) have compromised hepatic ATP synthesis upon an acute load of fructose (250mg/Kg) [1]. However, it is likely that excess consumption of fructose co-exists with a greater caloric intake, leading to confounding results.

Thus, the *aim of this study* was to test whether a chronic, but isocaloric supply of fructose compromises hepatic energy balance. We assessed longitudinally, the hepatic ATP levels during 8 weeks fructose feeding in mice with ^{31}P MRS. The effects of fructose were compared to those of glucose, which was supplied to another group of mice in isocaloric conditions.

Materials and methods: Twenty male C57Bl6j mice with 8 weeks old were fed *ad libitum* with a 60% fructose diet (TD 89247, n=10) or a 60% glucose diet (TD 05256, n=10) for 8 weeks. These diets are calorically equivalent to the standard rodent chow. Caloric intake was assessed at week 5 of diets by housing the mice in metabolic cages for 24h *plus* 3-6 h of habituation period. Hepatic high-energy phosphate (HEP) levels were assessed at baseline, after which mice were randomly divided into the two groups: fructose and glucose fed mice. Thereafter, HEP levels were assessed at 2 and 10 days, 4 and 6 weeks of fructose and glucose diets, with 3D ^{31}P MRSI in a 7T MR system (Clinscan, Bruker Biospin). For this, mice were anaesthetized with isoflurane 1-2% in gas mixture with 50%O_2/50% medical air and their abdomen was positioned on top of a double Tx/Rx ^{1}H/^{31}P surface coils. Breath-gated scout images for the MRSI were taken with gradient echo sequence (TR/TE of 269/4ms) and breath-gated ^{31}P MRSI was acquired with a pulse acquire sequence using adiabatic excitation BIR-4, 45º flip angle, TR of 1500 ms and 96 averages (see Fig. 1). Hepatic ^{31}P MR spectra were processed using jMRUI (AMARES) routine and prior-knowledge. Amplitudes of ATP, inorganic phosphate (Pi), nicotineamide dinucleotide (NAD) species, phosphomonoesters (PME) and phosphodiesters (PDE) were corrected for partial saturation [2], and metabolite concentrations were determined by the phantom replacement method for each voxel analyzed. Intracellular pH was estimated using a modified equation [3]: pH=6.75+log((S-10.82)/(13.24-S)), where S is the chemical-shift difference between Pi and α-ATP peaks.

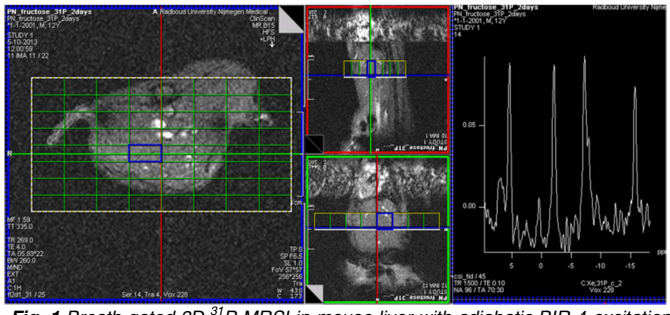

Fig. 1 *Breath-gated 3D ^{31}P MRSI in mouse liver with adiabatic BIR-4 excitation, 45°flip angle, TR of 1500ms. Matrix 16x32x32 and voxel size 2x4x4 mm^3.*

Results & Discussion: During the diet period, fructose and glucose fed mice gained similar body weight concurrent with an identical caloric intake (0.30±0.02 vs. 0.36±0.05 Kcal/g/24h, p=0.6). We have recently shown that even under these isocaloric conditions, fructose has a stronger negative impact on hepatic lipid content of mice, compared to glucose. This effect is mainly attributed to fructose stimulated *de novo* lipogenesis [4]. Despite this stimulation of ATP demanding pathways (glycolysis and *de novo* lipogenesis), fructose feeding does not seem to disturb hepatic energy levels in mice. Hepatic ATP concentration was maintained throughout the dietary period at levels of 2.62±0.11mM for fructose fed mice and 2.74±0.10 mM for glucose fed mice. Intracellular pH was also not disturbed by the diets and was within the values observed at baseline [7.17-7.23]. At day 2 of diets, fructose fed mice had a mild elevation in Pi/ATP ratio compared to glucose fed mice (1.38±0.14 vs. 0.99±0.10, p=0.08), a difference that vanished with dietary time. Also at day 2, we observed a resonance, in PDE spectral region in almost all glucose fed mice (see example of Fig. 2). From our estimates this PDE metabolite has a concentration of ~3 mM at day 2 of feeding and about 1.7 mM after 4-6 weeks of glucose diet. For fructose fed mice, we estimate that this PDE metabolite has a stable concentration of about 1.5 mM. Further *ex vivo* analyses are being performed to assign this PDE ^{31}P resonance.

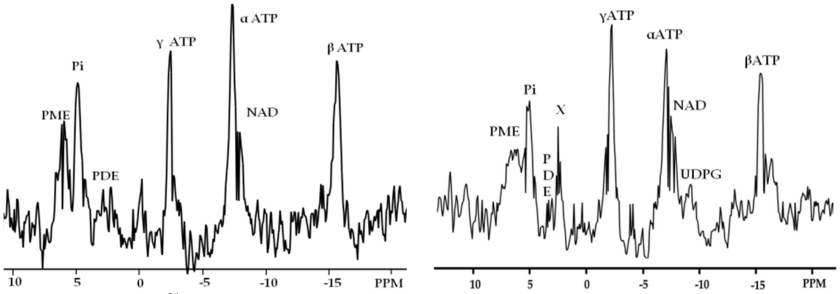

Fig. 2 *Example of ^{31}P MR spectra acquired from a 2-day fructose fed mouse (left) or glucose fed mouse (right). Note the existence of a PDE (x) resonance at ~2.4 pmm in the liver of glucose fed mouse.*

Conclusion: Our data demonstrate that fructose feeding in mice, does not alter hepatic ATP levels differently from glucose, as shown by the longitudinal data. This suggests that the mice liver is perfectly able to respond to any energetic demand inherent to fructose metabolism, e.g. due to stimulation of *de novo* lipogenesis or high glycolytic flux.

References[1] Abdelmalek MF *et al.*, Hepatology, 2012. [2] Chemlik M *et al.*, ISMRM 2012.[3]Moon RB and Richards JH. J Biol Chem 1973. [4] Nunes PM *et al.*, ISMRM, 2013.

Acknowledgments: This work was supported by CTMM project PREDICCT 2010.