FEEDING WITH LARGE AMOUNTS OF FRUCTOSE OR GLUCOSE INCREASES HEPATIC LIPID CONTENT ONLY WHEN POSITIVE ENERGY BALANCE IS ACHIEVED

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Introduction: Increased intake of refined sugars over the last few decades, particularly fructose, has been linked to rises in obesity[1], type II diabetes, kidney disease[2] and fatty liver disease[3]. In rodents a high sucrose diet has been shown to increase intrahepatocellular lipid (IHCL) levels and insulin resistance (IR) within 1 week[4]. A prolonged high sucrose diet leads to increases in intramyocellular lipid (IMCL) and muscle IR[4]. Results from human studies are less clear. Since many studies investigating the effects of fructose on liver and muscle lipid levels involve hyperenergetic feeding, it is unclear whether changes are related to fructose, or are a consequence of energy overfeeding. This study aims to observe the effects of an isoenergetic diet, in which 25% of the diet was provided in the form of either fructose or glucose, on hepatic and muscle lipid stores, and ^{31}P metabolite levels, and to compare these with a hyperenergetic diet in which fructose or glucose (25% of the daily energy intake) was provided in addition to their normal diet.

Methods: 32 healthy male volunteers gave informed written consent to participate in the study, and were randomly assigned to two groups; fructose (FI, N=15, age=35±11 years, BMI=29±2 kg m^{-2}) and glucose (GI, N=17, age=33±9 years, BMI=30±3 kg m^{-2}). Subjects attended an initial scanning visit (V1) to assess baseline measurements of IHCL, IMCL, ^{31}P metabolite levels, and liver volume. They were then provided all foodstuffs for 2 weeks in which 25% of their predicted energy intake (assessed by a 3 day food diary) was replaced by either fructose or glucose. Subjects attended the repeat MR scanning visit (V2) at the end of this period, prior to resumption of normal diet. Following the isoenergetic intervention, normal diet was resumed and, after a minimum of 6 weeks, subjects returned for a third MR scanning visit (V3). During the following two weeks (the hyperenergetic phase) subjects were requested to eat their normal diet and an additional 25% of their energy intake was provided as fructose/glucose. At the end of this period the final MR scanning visit (V4) was carried out. MR Measurements: All MR data were acquired on a Philips Achieva 3T system using the Q-Body coil for ^{1}H transmission and reception and a 140mm transmit/receive loop coil for ^{31}P MRS. IHCL: ^{1}H MR spectra were acquired from a PRESS localized region with the following parameters: VOI=30x30x30mm^3, BW=2000Hz, samples=1024, TR=5000ms. 24 spectra were acquired with TE=40ms and 8 at TE=50ms,60ms and 70ms. Spectra were individually realigned and phase corrected in jMRUI before averaging across each TE. Peak areas of water and CH_2 lipid peak were calculated using an in-house built Matlab script. Water and lipid CH_2 T_2 values were calculated from the variation of peak areas with TE, and the liver lipid content was calculated as described by Sczcpaniek *et al*[6], applying a T_2 correction based on the average measured T_2 value for each subject. IMCL:^{1}H MR spectra were acquired from a STEAM localized region with water suppression applied, and the following parameters: VOI=20x20x50mm^3, BW=2000Hz, samples=1024, TE/TM/TR=13/17/7000ms, No. averages = 16. Spectra were phase corrected in jMRUI before the peak areas of IMCL and extra-myocellular lipid (EMCL) (CH_2 and CH_3) were fitted using the AMARES algorithm. 2 spectra were acquired without water suppression for correction to absolute concentrations, using previously acquired T_2 values (T_2 water=31ms, T_2 IMCLCH_2=89ms, T_2 EMCL CH_2=78ms). 31P MRS: Spectra were acquired from an ISIS localized region with decoupling and NOE applied and the following parameters: VOI=60x60x60mm^3, BW=3000Hz, samples=2048, TR=5000ms, No. averages = 96. Spectra were processed by adding 12Hz Lorentzian linebroadening before phase correction in jMRUI. Peak areas for phosphocreatine (PCr), phosphodiesters (PDE), phosphomonoesters (PME), ATP (γ, α and β), and inorganic phosphate (P_i) were fitted using the AMARES algorithm in jMRUI. An non-localized ^{31}P spectrum was acquired, using identical parameters, for quantitation of metabolite levels relative to a reference marker. Signals were corrected for coil sensitivity based on distance to the marker. Liver volume (LV): T_1-weighted 3D-TFE: resolution=2.08x2.08x7.00mm^3, no. slices=36, no. voxels in-plane=180x182, TR=3.11ms with total scan time (equal to breath-hold time)=14.4s. Images were analyzed by region drawing in Analyze9 to calculate liver volume. Statistics: All values are given as mean±SD. Significant differences between visits and between FI and GI groups were assessed using t-tests in SPSS 17.

	V1	V2	V3	V4
FI BMI (kgm^{-2})	29.2±2.3	29.1±2.3	29.4±2.5	29.6±2.4 (##)
GI BMI (kgm^{-2})	29.7±3.4	29.7±3.5	29.7±3.5	30.0±3.5 (##)
FI LV (L)	2.09±0.38	2.11±0.31	2.12±0.36	2.28±0.37 (##)
GI LV (L)	2.02±0.27	2.04±0.25	2.10±0.26	2.15±0.25
FI IHCL (%)	7.2±5.6	7.6±4.1	7.8±5.4	9.1±5.7 (##)
GI IHCL (%)	8.9±5.1	8.4±4.5	8.1±4.6	10.0±4.8 (##)
FI IMCL (%)	8.6±3.2	8.1±3.1	9.0±2.4	9.7±3.1
GI IMCL (%)	8.5±3.3	9.4±3.0	8.2±3.7	10.0±2.3
FI EMCL (%)	9.9±4.3	10.1±3.2	10.2±4.6	9.8±4.6
GI EMCL (%)	11.2±4.9	10.0±3.8	9.9±4.6	10.4±4.6
FI ATP (AU)	10.1±4.7	9.6±3.6	10.1±3.0	11.7±3.7
GI ATP (AU)	11.0±3.5	11.0±3.3	12.5±8.3	12.6±5.1
FI P_i (AU)	4.6±1.3	5.3±1.6	4.6±1.1	5.8±1.8 (##)
GI P_i (AU)	4.7±1.6	4.8±1.6	4.1±2.0	5.2±1.2 (##)
FI PDE (AU)	10.9±2.0	12.7±3.1(#)	13.8±2.3(&)	14.2±4.5
GI PDE (AU)	12.0±2.7	14.3±4.5	12.0±5.6	15.3±5.2
FI PME (AU)	6.0±1.3	6.3±2.3	6.8±2.8	6.7±1.7
GI PME (AU)	5.8±1.8	5.8±1.7	5.0±2.6	6.4±2.8

Table 1: A summary of the results. Significant differences (p<0.05) are shown for V1-V2 (#), V1-V3 (&) and V3-V4 (##). Data are mean ± SD.

Results and Discussion: A summary of the results are shown in Table 1. BMI: No significant change in BMI was seen during the isoenergetic diet in either the FI or GI group indicating that diets were well controlled. BMI significantly increased during the hyperenergetic phase for both groups (FI=+0.8±1.1%, GI=0.8±1.3%, relative to V3). Changes in BMI were not different between the FI and GI group. Liver Volumes: Liver volumes were not altered during the isoenergetic diet in either groups, or during hyperenergetic feeding with glucose. In contrast, liver volumes were increased following overfeeding with fructose. Increases in liver volume could be due to a number of factors including increased energy stores (lipid and glycogen) and increased perfusion. T_2: No difference in T_2 was measured across visits. However, there was a significant correlation between water T_2 values between visits ($p<0.001$) and lipid T_2 values between visits ($p<0.001$). This implies that variation in T_2 between subjects was due to biological variation, as opposed to measurement error. No correlation was seen between water T_2 and lipid T_2 values indicating that factors affecting the T_2's of water and lipid are not the same. IHCL and IMCL: Hepatic lipid stores were not altered following the isoenergetic diet with either glucose or fructose. In the hyperenergetic period, increases in hepatic lipid content were seen in both FI and GI, to a similar extent. This implies that increases in hepatic lipid due to fructose ingestion, measured in previous studies, are likely due to increase in energy intake rather than perturbed metabolism following fructose consumption. There was a tendency for IMCL levels to increase during the hyperenergetic phase, but this did not reach significance. No changes were measured for EMCL. Increases in IMCL tend to occur later than increases in IHCL and so it is possible that the duration of the study periods (2 weeks) was too short to see changes in myocellular lipid. ^{31}P metabolite levels: Levels of ATP and PME, were not altered at any timepoint. Similarly, no changes were seen in pH. Levels of P_i were not significantly different following the isocaloric diet, but were significantly increase following the hypercaloric diet with both fructose and glucose overfeeding. Increases in P_i may be due to an upregulation of hepatic energy consumption.

Conclusions: Previous studies, implicating increased fructose consumption in the rise of obesity and liver disease, have tended to provide fructose in excess of the normal diet and without a control group. This study shows that 2 weeks of a high fructose isoenergetic diet does not to increase lipid deposition in hepatic or muscle tissue. Increasing energy intake by supplementing fructose or glucose on top of a normal diet for two weeks leads to increases in hepatic lipid levels over only 2 weeks. In addition, increases in P_i following the hyperenergetic diet, may be an indication of altered hepatic energy metabolism. However, these changes occur following hyperenergetic fructose and glucose diets, indicating that the effects are more likely the result of energy overfeeding, and are not a consequence of perturbed metabolism following fructose ingestion.

References: **1.** Bray GA, Nielsen SJ, Popkin BM, *Am. J. Clin. Nutr.* (2004) 79(4), pp537-543. **2.** Johnson RJ *et al*, *Am J Clin Nutr*, (2007) 86(4), 899-906. **3.** Ouyang, X *et al. Hepatology,* (2008), 48(6), pp993-999. **4.** Pagliassotti MJ *et al*. *Am J Physiol Regul Integr Comp Physiol* (1996) 271: R1319–R1326.