Introduction: Increased intake of refined sugars over the last few decades, particularly fructose, has led to rises in obesity, type II diabetes, kidney disease and fatty liver disease. In rodents a high sucrose diet has been shown to increase intraperitoneal lipid (IMCL) and muscle IR. 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 isocaloric diet, in which 25% of the diet was provided in the form of either fructose or glucose, on hepatic and muscle lipid stores, and 31P 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 IMCL, EMCL 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 isocaloric 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. 31P MRS: All MR data were acquired on a Philips Achieva 3T system using the Q-Body coil for H\(^1\) transmission and reception and a 140mm transmit/receive loop coil for 31P MRS. IHCL and IMCL: 31P MR spectra were acquired from a PRESS localized region with the following parameters: 128 repetitions, VOI=30x30x30mm\(^3\), 24 spectra were acquired without water suppression for each subject, and 8 spectra were corrected for coil sensitivity. Spectra were individually realigned and phase corrected in JMRI 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\(_3\) T\(_2\) values were calculated from the peak areas with TE, and the lipid lipid content was calculated as described by Szczeniak et al., applying a T\(_2\) correction based on the average measured T\(_2\) value for each subject. IMCL and EMCL: 31P 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/MT/TR=13/17/7000ms. No. averages = 16. Spectra were phase corrected in JMRI before the peak areas of IMCL and extra-muscular lipid (EMCL) (CH\(_2\), CH\(_3\)) were fitted using the AMARES algorithm. 2 spectra were acquired without water suppression for each subject (one spectrum to absolute quantification of CH\(_3\)) and 12 spectra were acquired with water suppression for each subject (AIM). No. averages = 48. 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 line broadening before phase correction in JMRI. Peak areas for phosphocreatine (PCr), phosphomonoesters (PDE), phosphoaminoesters (PME), ATP (\(\gamma\), \(\alpha\) and \(\beta\)), and inorganic phosphate (P\(_i\)) were fitted using the AMARES algorithm in JMRI. An non-localized 31P spectrum was acquired, using identical parameters, for quantification 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=2.0x2.0x2.0mm\(^3\), 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 means\(\pm\)SD.

Results and Discussion: A summary of the results are shown in Table 1. No significant change in BMI was seen during the isocaloric 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 isocaloric diet in either groups, or during hyperenergetic feeding with fructose or glucose. In contrast, liver volume increased significantly following feeding 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, and not 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 isocaloric 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 period, 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. 31P 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 diet. This study shows that 2 weeks of a high fructose isocaloric diet does not 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.