Investigating Alterations in Hepatic ATP levels following Fructose and Fructose+Glucose Ingestion: A Simple Non-invasive Technique to Assess Liver Function Using ^{31}P MRS

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Background: Fructose metabolism in the liver differs from glucose metabolism in that the primary enzyme for phosphorylation (fructokinase) is not regulated through negative feedback[1]. A large intake of fructose can therefore induce rapid ATP depletion which can be monitored using ^{31}P magnetic resonance spectroscopy (MRS). Non alcoholic fatty liver disease (NAFLD) has been associated with an impaired rate of ATP synthesis in hepatic mitochondria[2] and it has been suggested that the subsequent rate of recovery of ATP, measured using MRS, can be used as a biomarker for NAFLD[3]. In addition, slower ATP recovery following fructose infusion has been linked with increased BMI in healthy subjects[3]. This pilot study aims to investigate the effects of oral fructose intake on ATP levels in the liver, and to relate recovery rates to BMI and basal liver lipid and glycogen levels. Since the intestinal absorption rate of fructose has been reported to vary greatly between healthy subjects, but is fully absorbed when given in an equal ratio with glucose[4], this investigation also examines differences in ATP depletion following 100% oral fructose dose (which may be absorbed at different rates across subjects) and a 1:1 fructose+glucose dose.

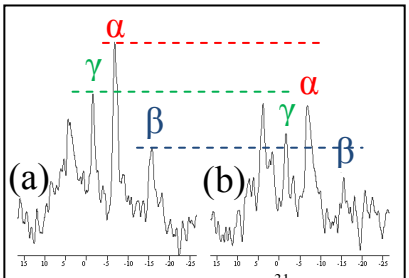

Figure. 1: Example ^{31}P spectra acquired from the liver at (a) t=15m and (b) t=50m after oral fructose intake showing drop in ATP peaks

Methods: 4 healthy males were studied on 2 visits (wash out period=7d) following an overnight fast. Basal ^{1}H and ^{13}C MRS measurements were taken to determine liver lipid and liver glycogen levels respectively. Subjects were then orally given 500ml solution containing fructose (75g) or 500ml 50% fructose (39.2g) + 50% glucose (39.2g) solution (calorie and volume matched to equalize gastric emptying rates between visits). All measurements were performed on a Philips Achieva 3T system using transmit/receive body coil for ^{1}H MRS, ^{13}C surface probe with quadrature proton decouple coil for ^{13}C MRS and ^{31}P surface coil for ^{31}P MRS. Liver lipid Measurements: ^{1}H MR spectra were acquired from the right lobe of the liver using a respiratory triggered, water suppressed STEAM sequence: TE/TR=13/5000ms, VOI = 30x30x30mm^3, N=40, BW=2000Hz, 1024 samples. 2 spectra were collected without water suppression for correction to absolute lipid levels. Water suppressed spectra were realigned and phase corrected using in-house software and peak areas were calculated using the AMARES algorithm in jMRUI, fitting to Lorentzian line shapes. Glycogen measurement: ^{13}C spectra were acquired using a proton-decoupled pulse acquire sequence with BW=7000Hz, samples=512, TR=2150ms, 576 averages. Total acquisition time ~20 minutes. Spectra were zero-filled to 4096 points and 50Hz Lorentzian line broadening was applied before the peak areas of glycogen at 100.4ppm and the external reference peak were calculated using in-house software. ^{31}P Measurement: ^{31}P MR spectra were acquired from the right lobe of the liver using a respiratory triggered ISIS sequence with NOE and proton decoupling: TR=5000ms, VOI=60x60x60mm, BW=3000Hz, 2048 samples. Spectra were acquired continuously for 90 minutes. Due to respiratory triggering, the number of scans in a given time window was variable. Data were averaged into 5 minute blocks before processing in jMRUI (Line broadening=32Hz, 1024 sample truncation, 3072 zero filling with manual 1ST order phase correction). Data were then averaged together to a 15min rolling average and the area of β ATP peak was calculated using the AMARES algorithm, fitting to a triplet with Lorentzian line shapes.

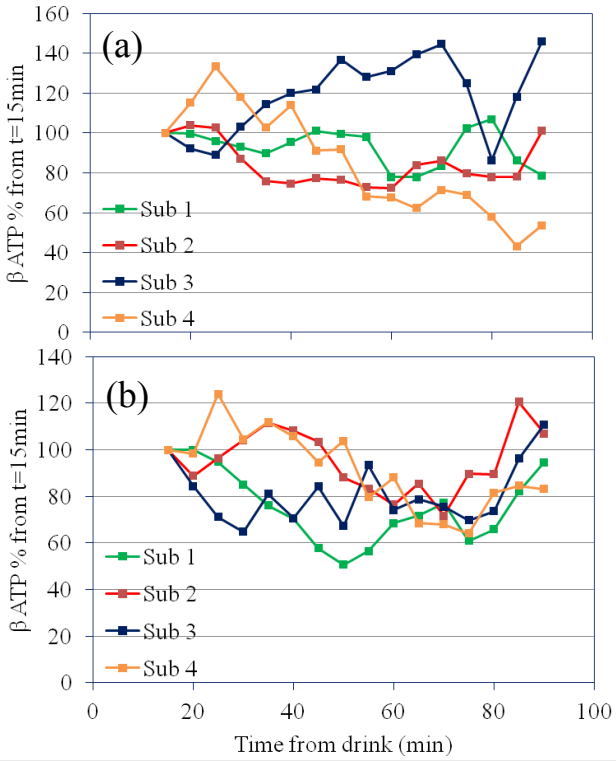

Figure 2: Time course showing % change in ATP β signal across subjects for (a) 100% fructose and (b) 1:1 fructose+ glucose

Results and Discussion: Malabsorption of Fructose: Figure 2 shows depletion of ATP following ingestion of a 100% fructose solution (a) versus an isocaloric, isovolumic 1:1 fructose+glucose solution (b). Despite the fact that the fructose dose was almost double for the 100% fructose solution compared with the fructose+glucose solution , depletion of ATP was slower. The variation in the timecourses between subjects also tended to be larger for the 100% fructose dose. This can be explained by the intrasubject variation in absorption rates and efficiency of pure fructose reported previously[4]. Recovery Rates: Following ingestion of the mixed solution, hepatic ATP levels fell and then begin to recover rapidly after around 60min. Although only small numbers have been studied so far, the rate of recovery (determined from linear gradient of the recovery period) shows a moderate inverse correlation with liver lipids (R^2=0.55) and a modest correlation with BMI (R^2=0.33) whilst showing no relationship with basal glycogen levels.

Conclusions: This pilot study has shown that it is possible to measure depletion of ATP in the liver due to fructose ingestion, and that this depletion is more pronounced, as well as more consistent across subjects, when the fructose load is given in 1:1 ratio with glucose, despite the overall lower dose of fructose. This method offers a promising and completely non-invasive technique to assess hepatic mitochondria function, although further investigation is needed to fully validate correlation between recovery rate and liver lipid levels.

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