

Can ^{13}C MRS Be Used to Monitor Changes in Hepatic Glycogen Levels Following a Low Dose Oral Glucose Challenge?

S Bawden¹, M Stephenson¹, E Ciampi², J Lane², L Marciari³, P Morris¹, I MacDonald⁴, G Aithal⁴, K Hunter², and P Gowland¹

¹SPMMRC, University of Nottingham, Nottingham, Notts, United Kingdom, ²Unilever Discover, Bedford, Beds, United Kingdom, ³NDDC Biomedical Research Unit, University of Nottingham, Nottingham, Notts, United Kingdom, ⁴Medical School, University of Nottingham, Nottingham, Notts, United Kingdom

Background: After an overnight fast, hepatic levels of glycogen fall as it breaks down to provide energy. Previous studies have shown that after a subsequent mixed meal ~20% of carbohydrate consumption reaches the liver¹ leading to a postprandial increase in hepatic glycogen levels. ^{13}C MRS provides a validated tool^{2,3} which has been widely used in research to determine these levels in both animal and human studies^{4,5}. However, in previous studies volunteers tend to consume large amounts of glucose (e.g. 75g or more) and as far as we are aware there is no published work using natural abundance ^{13}C MRS, investigating liver glycogen response to lower doses of glucose, more relevant to common diets. The sensitivity of this technique for measuring low levels of consumed carbohydrate is therefore yet to be established. This is particularly important since it is possible that the proportion of the meal entering the liver and being stored as glycogen depends non-linearly of the quantity of carbohydrate consumed. In this study we administered 50g of glucose (equivalent carbohydrate content in 2 small cheese and ham sandwiches) and determined change in liver glycogen levels over 4 hours.

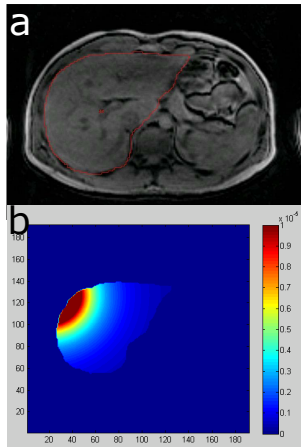


Figure 1: Liver volume scan ROI (a) and Matlab results indicating distance from the coil centre. (b)

Method: 6 healthy males (age=21±3y, BMI=24±2kg/m²) were studied on 2 visits following an overnight fast. Basal gastric content and liver volume scans were acquired and basal liver glycogen levels were measured using natural abundance ^{13}C MRS. Subjects were then given orally 300ml of either 50g glucose solution or water and were scanned hourly for 4 hours to monitor gastric content, liver volume and liver glycogen levels. All measurements were performed on a Philips Achieva 3T system using a body coil for MRI and a ^{13}C surface probe with quadrature proton decoupling. **MRI measurements:** A T₁ weighted TFE sequence (TE=1.5ms, TR=3.1ms, voxel=2x2x7mm, size=192x192x36) was used to assess liver volume; a T₂ weighted TSE sequence (TE=83ms, TR=1210ms, voxel=0.8x0.8x10mm³, size=512x512x20) was used to measure gastric content since it enhanced signal from the liquid content (e.g. fig 2). Volumes were calculated by manually drawing regions of interest using Analyze9 (fig 1a).

Glycogen measurements: ^{13}C spectra were acquired using a proton-decoupled, adiabatic half passage, pulse acquire sequence (BW=7000Hz, n=512points, TR=2150ms, 576 averages, total time ~20 min). Spectra were zero-filled (4096 points) and line broadened (30Hz) before peak areas of the C1-glycogen peak (100.4ppm) and an external reference peak were calculated using in-house software. Resulting ratios were then scaled based on voxel distance from coil centre (fig 1b).

Results: Gastric Content: Fig 3a shows the emptying profiles

of glucose and water. After 20min there was a significant difference between meals in the gastric content (p<0.01, 230±50cm³ and 60±30cm³ for glucose and control). At later times, the data from both meals converged and by 140min the stomach had fully emptied.

Liver volume: Whilst there was no significant difference in liver volume between groups there was a significant decrease relative to baseline for both meals at 200min (94±3%, p<0.001) and 240min (93±5%, p<0.01) with a downward trend with time, consistent with liver glycogen stores being metabolized after the fast and even after the small meal.

Hepatic Glycogen levels: As expected the area of the ^{13}C glycogen peak tended to be smaller after the water meal compared to glucose meal (fig 3c) and there was a significant difference in the area under curves (power>0.8, p<0.05, 300±30min and 260±40min for glucose and water).

Discussion: Whilst there was no significant change in glycogen spectral peak area from baseline at any individual time point, the overall AUC of glycogen levels across the four hour timecourse were significantly higher for the 50g glucose challenge compared with a control meal. The control arm highlighted the underlying drop in basal liver glycogen due to continuing glycogen breakdown for energy production which was halted by the glucose meal. This shows the importance of comparison with a control meal as well as baseline when small changes are expected. The large significant difference in gastric emptying between meals at t=20min shows that glucose emptying was delayed due to its higher calorie content and explains why hepatic glycogen levels of both meals were still decreasing at t=10min as little glucose emptying had occurred at this time. More frequent sampling would allow better modelling of this. Finally, whilst previous studies have observed that glucose intake slows the decrease in liver volume compared to control⁴, no such change was seen in this study presumably because the concentration of glucose administered was too low or maybe because of the small sample size.

Conclusion: This study has shown that natural abundance ^{13}C MR can successfully monitor changes in hepatic glycogen levels, even after low 50g oral glucose intake.

References: 1.Taylor R *et al*, J Clin Invest(1996)97pp126-132 544 2.Taylor R *et al*, Mag Res in Med(1992)27pp13-20 3.Guetter R *et al*, Mag Res in Med(1994)31(6)pp583-588 4.Casey A *et al*, Am J Phys End Metab(2000) 278ppE65-E75 5.Awad S *et al*, Clin Nut(2010)29p538

Acknowledgments: We thank Unilever and BBSRC for funding the first author in his industrial CASE studentship

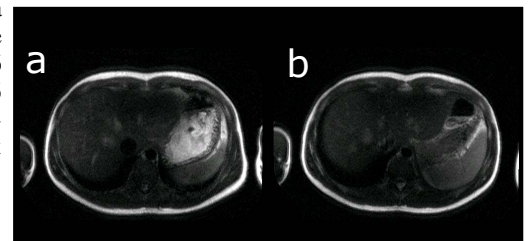


Figure 2: Stomach content volume at t=20min of a single subject for glucose solution (a) and water (b)

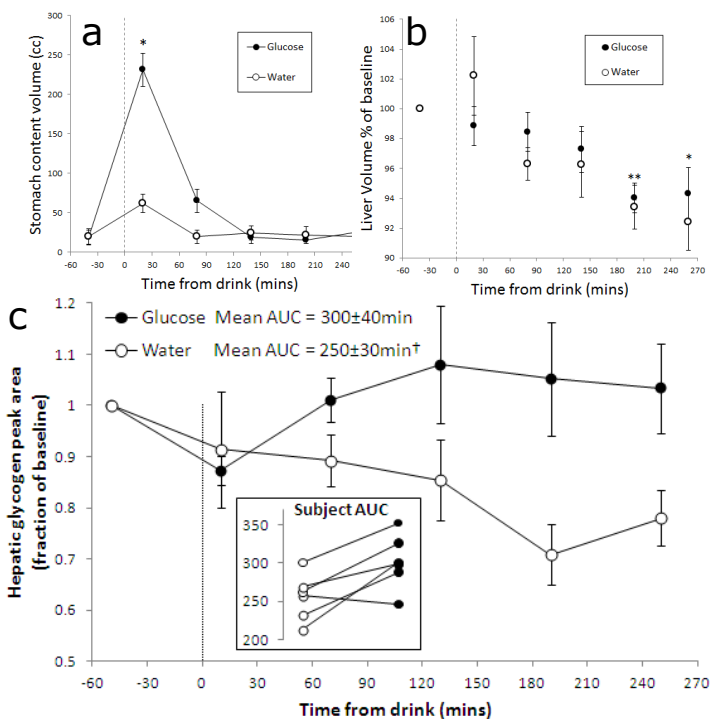


Figure 3: Mean Differences in stomach content(a), liver volume (b) and liver glycogen (c) ** p < 0.001, *p < 0.01, †p < 0.05, Standard error shown