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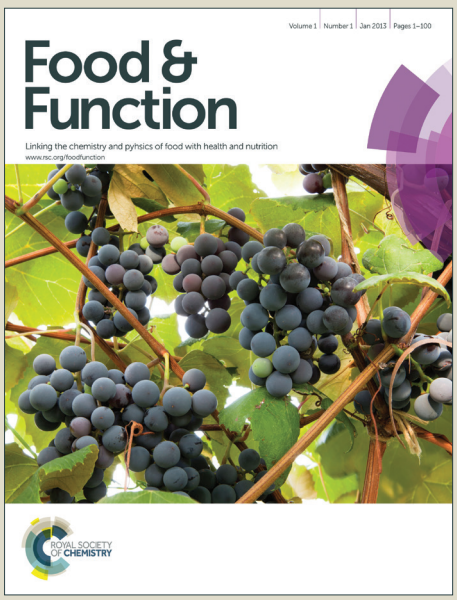

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A Low Calorie Morning Meal Prevents the Decline of Hepatic Glycogen Stores: A Pilot *in vivo* ^{13}C Magnetic Resonance Study

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Abstract

Previous studies have reported a meal-induced rise in hepatic glycogen stores from baseline levels following a fast and it is generally assumed that glycogen levels rise steadily following meals throughout the day. However, measurements are normally taken in conditions that are not typical of the Western breakfast, which is relatively carbohydrate rich with a lower calorific content than most experimental test meals. As such, little is known about the normal metabolic response to a realistic, low calorie morning meal. Therefore, the aim of this pilot study was to evaluate the effects of a low dose oral glucose intake on hepatic glycogen levels following an overnight fast in healthy subjects. Glycogen levels were monitored in vivo using ^{13}C Magnetic Resonance Spectroscopy at baseline and hourly for 4 hours following either a 50g glucose drink (773 kJ) or a control drink (0 kJ) given over two different visits. During the control visit hepatic glycogen levels decreased throughout the experiment with statistically significant decreases from baseline at 190 minutes ($P<0.05$) and 250 minutes ($P<0.05$). By contrast, the low dose glucose intake maintained glycogen concentrations with no significant decrease from baseline over 4 hours. A comparison between visits revealed that mean glycogen concentrations were significantly greater during the glucose visit (control visit, AUC=218 ± 39 mol/l min; glucose visit, AUC = 305 ± 49 mol/l min; $P < 0.05$). Liver volume decreased significantly from baseline at 180 minutes ($P<0.05$) post consumption in both groups, with no significant

difference found between visits. Gastric content volumes were significantly higher for the glucose visit immediately following consumption (P<0.001) and at 60 minutes (P=0.007) indicating slower gastric emptying for the glucose compared with the control. In conclusion, following an overnight fast, a low dose oral glucose challenge prevents a reduction in hepatic glycogen content but does not increase it above fasted levels.

Introduction

After feeding, consumed carbohydrates are broken down (predominantly to glucose) and absorbed into the blood stream before being transported to cells to provide energy. Excess glucose in the meal is taken into the liver and muscle and stored as glycogen or triglycerides. Although triglycerides are the primary long term energy reserve, hepatic glycogen is quickly mobilised in the early stages of fasting and hence functions as a secondary, smaller energy store.

Natural abundance Carbon-13 Magnetic Resonance Spectroscopy (^{13}C MRS) is a well validated technique that has been used in many studies to monitor non-invasively the storage and breakdown of glycogen in hepatocytes and muscle cells following carbohydrate consumption *in vivo* [1]. However, the vast majority of these studies have used acute testing conditions such as large calorie intakes [2], feeds after long periods of fasting [3] or high levels of exercise [4]. As such, little is known about the physiological response following a low calorie, high carbohydrate morning meal that is similar in carbohydrate content to the commonly consumed Western breakfast.

This pilot study was designed to explore the effects of a low calorie carbohydrate morning intake following an overnight fast on hepatic glycogen levels in vivo using ^{13}C MRS. This will lay a foundation for further research exploring the fundamental principles of metabolism in relation to dietary intakes with different nutritional compositions.

Material and Methods

Subjects

Ethical permission was obtained from the local Medical School Research Ethics Committee and all subjects provided informed written consent before participation. Five healthy subjects were recruited and studied over two visits. All subjects were self-reported healthy male non-smokers with no known cardiovascular, metabolic or liver related diseases (mean age 21 ± 3 years; BMI 24 ± 2 kg/m3).

Study Design

Subjects were studied on two separate days in random order with a 7 day wash out period between study days. Prior to study days subjects were asked to refrain from alcohol and vigorous activity for three days. They were asked to eat the same evening meal on the nights prior to each scan day and then to refrain from consuming any food or drink except water from 10pm until they arrived at the test centre the following morning. On the morning of the study subjects were asked to refrain from vigorous activity on the way to the test centre and to arrive at 8:00am.

Protocol

Initially, natural abundance ^{13}C MR spectra were acquired from the liver to determine hepatic glycogen levels at baseline, after which subject consumed a 250ml drink of either 50g glucose (Thornton & Ross, Huddersfield, UK) solution (773 kJ) or water (0 kJ). Immediately after ingestion and at hourly intervals thereafter for 4 hours following ingestion, further ^{13}C MR spectra were acquired to monitor any changes in glycogen levels. MRI images were also obtained before each spectral acquisition to measure the volume of the liver and gastric contents.

Data Acquisition

All measurements were performed on a Philips Achieva 3T system (Philips, Best, The Netherlands) using the built in ^{1}H transmit / receive body coil for image acquisition and a PulseTeq (Surrey, UK) ^{13}C surface coil with quadrature proton decoupling for spectral acquisition (Figure 1). A T_1 weighted gradient echo MR image was acquired during a single breath hold to measure liver volume (flip angle = 10 TE = 1.5 ms, TR = 3.1 ms, voxel size = 2 x 2 x 7 mm^3, 36 slices) and a T_2 weighted Turbo Spin Echo MR image was acquired during a single breath hold to measure the volume of gastric contents (TE= 83 ms, TR= 1210 ms, TSE factor = 92, voxel size = 0.8 x 0.8 x 10 mm^3, 20 slices) using the inbuilt scanner body coil. ^{13}C spectra were acquired by placing the surface coil over the liver and marking its position on the subject's skin to give a consistent sensitive volume across subsequent time points. The acquisition involved a $\pi/2$ pulse-acquire sequence with an adiabatic half passage pulse to minimise the effects of B_1 field inhomogeneity within the volume of interest, along with narrow band proton decoupling (7000 Hz bandwidth, 512 samples, TR = 2150 ms, number of averages = 576, total scan duration ~20 mins) as previously described [3].

MRI and MRS Quantification

The liver volumes and gastric content were measured by drawing around regions of interest in Analyze9 software (Mayo Foundation, Rochester, MN, USA). Gastric emptying half-life $T_{1/2}$ was estimated from the change in gastric content with time. ^{13}C spectra were zero filled and line broadened using in house software, Lorentzian curves were fitted to the spectra and the areas of the C1-glycogen peak (100.4 ppm) and an external reference peak were calculated. Distance from the coil was calculated from the ^{1}H MR images and quantification achieved by comparison with a cylindrical phantom using the following formula:

$$[Glyc]_{liver} = \frac{R_{liver}}{R_{phantom}}[Glyc]_{phantom}$$

where $[Glyc]_{liver}$ and $[Glyc]_{phantom}$ are the total glycogen content of the liver and phantom respectively; and R_{liver} and $R_{phantom}$are the corrected ratios of integrals of glycogen-to-reference peaks in the liver and phantom respectively [2].

Statistical Analysis

All statistical analysis was done using a matched pair Student's t test. The study was powered *a priori* at (1-β) = 0.8 to reach statistical significance for a 55 mmol/l rise (20% of 50g glucose to liver) from basal hepatic glycogen levels based on values and standard deviations from previous studies [5, 6]. Statistical significance was attributed to an alpha < 0.05. For changes from baseline, mean difference from baseline and deviation at each time point was used to determine respective effect size and p values calculated using G power software (University of Dusseldorf, Dusseldorf, Germany). Similarly, mean differences and deviations were used to calculate p values for changes between group time-points and area under curves (AUC). Coefficients of variation (%CV) were calculated using standard equations (inter and intra subject variability). All values are quoted as mean values ± standard error (SEM) unless

otherwise stated. Glycogen data time-points are offset by 10 minutes from the time the acquisition started to represent the centre of the 20 minute Carbon-13 scan duration.

Results

Absolute Hepatic Glycogen Content

No significant difference in basal glycogen content was measured between the control (72 ± 19 g) and glucose (89 ± 18 g) visits.

On the control day, absolute glycogen content decreased steadily from baseline and reached statistical significance at 190 minutes and 250 minutes with reductions of 17 ± 10 g ($P < 0.05$) and 31 ± 23 g ($P < 0.05$) respectively (Figure 2). In contrast, on the glucose visit no statistically significance differences compared to baseline were measured at any time point.

Mean glycogen content was higher during the glucose visit compared with the control visit with the greatest difference seen in the AUC across the final two time points (glucose visit: 11 ± 3 g min, control visit: 21 ± 5 g min, $P < 0.05$).

Liver Volume

There was no difference in the subjects' overnight fasted liver volumes between visits (control: 1.8 ± 0.2 l, glucose: 1.9 ± 0.2 l, $P = 0.41$). The intra-subject coefficient of variation (CV% = 5.6) was much lower than the inter-subject variation (CV% = 23.2) across visits. During both visits changes in liver volumes from baseline were small but significant with a low standard deviation on the mean differences. The maximum change from baseline for both visits was found at t = 180 min (glucose visit: 1.8 ± 0.2 l, control visit: 1.7 ± 0.2 l).

No statistically significant difference in liver volume was found between visits at any time point (Figure 3), with a maximum difference in % of baseline at t = 60 min (Control = 96 ± 1

$\%_{Baseline}$; Glucose = 99 ± 1 $\%_{Baseline}$; P = 0.47). At this same time point there was a significant change from baseline in the control arm (liver volume at t = 60 was 1.7 ± 0.2 l, P ≤ 0.05) whereas no significant change from baseline was found in the glucose arm (P = 0.54).

Glycogen Concentrations

Baseline hepatic glycogen concentrations were 217 ± 38 mmol/l for the control visit and 247 ± 32 mmol/l (P = 0.55) for the glucose visit with an inter-subject CV% = 17.5 and 13.0 respectively, and an intra-subject CV% = 32.0 across both visits (Figure 4). During the control visit there was a trend for a reduction from baseline at 190 minutes and 250 minutes to 173 ± 41 mmol/l (P < 0.1) and 120 ± 23 mmol/l (P < 0.1) respectively, whereas there was no change from baseline during the glucose visit.

Glycogen concentrations were higher during the glucose visit compared with the control visit. The post meal AUC across the time course was significantly lower during the control visit (AUC = 218 ± 39 mol/l min) compared with glucose visit (AUC = 305 ± 49 mol/l min, P < 0.05).

Gastric Content

The subjects' overnight fasted gastric content volumes remained consistent across visits (23 ± 11 ml and 20 ± 11 ml for the control and glucose groups respectively, P = 0.73). Gastric content was significantly greater than baseline immediately following consumption for both visits, with increases of 41 ± 21 ml (P < 0.01) during the control visit and 204 ± 36 ml (P < 0.001) during the glucose visit (Figure 5). This volume returned back to baseline at 60 minutes for the control visit, but remained significantly higher than baseline for the glucose visit (42 ± 17 ml, P = 0.007).

Gastric content volumes were significantly higher immediately following consumption for the glucose visit compared with the control visit, with a difference of 160 ± 51 ml ($P < 0.005$). Estimates of gastric emptying half-life were $T_{1/2} = 17 \pm 3$ min for the control visit and $T_{1/2} = 44 \pm 9$ min for the glucose visit, with a significant difference between groups of 27 ± 8 min ($P < 0.005$).

Discussion

This preliminary study has shown for the first time that a low carbohydrate morning meal alleviates the decline in hepatic glycogen stores, which generally results from prolonged fasting. At baseline there was no significant difference in hepatic glycogen levels between visits, followed by a subsequent decline in mean values across the time course during the control visit which was not observed during the glucose visit.

The resting rate of endogenous glucose production in healthy individuals is approximately 2 mg kg^{-1} min^{-1} [7] which decreases when a meal is consumed due to an increased insulin release and suppression of glucagon. In the postprandial state absorbed carbohydrates raise blood sugar levels, resulting in an increased rate of cellular glucose uptake and glycogen synthesis and storage in the liver [8]. Hepatic glycogen stores become particularly important in the fasting state as glycogenolysis and gluconeogenesis provide the energy required for the brain, renal medulla, and erythrocytes [9]. Our data show that a 50g carbohydrate intake following an overnight fast is not sufficient to replenish liver glycogen levels but does prevent a further decline. This suggests that hepatic glycogen levels will only increase if the amount of glucose absorbed exceeds the glucose utilisation rate. In order to fully understand this relationship, further investigations should vary the amount of glucose intake and measure the hepatic glycogen storage response. In addition, glycogen storage in muscle tissue should be considered for protocols including exercise.

Breakfast is often thought as the most important meal of the day, and research has shown that there are many associated health benefits [10]. Although nutritionists advise for individuals to consume a substantial breakfast amounting to 20% or 30% daily requirements, it is often the case in Western culture that breakfast consists of a low energy and carbohydrate rich meal, such as cereal or toast and orange juice (often less than 1000 kJ) and there is reported to be an increasing rate of breakfast skipping in children and adolescents [11-13]. This study suggests that following an overnight fast, such low calorie meals halt further reduction in the glycogen content of the liver for up to 4 hours after consumption and provide carbohydrates directly for cells' metabolic needs. Our data show that a larger energy intake is required in order to increase the glycogen content, which may have an effect on subsequent meals throughout the day.

A recent study using ^{13}C MRS observed that liver glycogen levels decline rapidly during fasting with changes from baseline at 12h and 24h after feeding [3] and a subsequent rise on refeeding. Hepatic glycogen reserves provide the main source of energy for the nervous system during this prolonged fasting period. It is generally assumed that ingested carbohydrates are distributed via the portal vein to the liver for hepatic glycogenesis and then to the rest of the body via the wider circulatory system following all feeds in a similar way [14] and current computational models assume blood glucose levels to be the only or main contributing factor [15]. In contrast, this present study shows that below a certain level of carbohydrate intake there is no net increase in liver glycogen (i.e. no storage), suggesting a suppression of glycogenolysis and utilisation of the ingested carbohydrate by the cells and tissues. Along with other recent work [16] our data suggest that hepatic glucose metabolism is more complex than the simple model allows for.

Our data showed that gastric emptying was greatly reduced during the glucose visit compared with control, with the emptying half-life being more than double following glucose intake

compared with control feed. This is consistent with previous studies and is in keeping with current knowledge that the amount of energy consumed relates to the gastric throughput in the postprandial state [17]. We suggest this has a direct impact on hepatic metabolism as slower gastric emptying results in a delayed absorption of nutrients in the small intestines and therefore a delayed transfer into the portal vein. Whilst in this study there was a convergence in gastric content volume between groups by 120 minutes, other studies may have longer periods of significantly different gastric content volume. As such, we believe this is an important feature in such investigations that is often overlooked and MR technology provides a simple additional measure that is easily incorporated into experimental protocol.

Previous studies have shown that liver volume increases following test meals due to increased glycogen and lipid storage and blood flow, and whilst this present study did not show a statistically significant difference in liver volume between groups, postprandial liver volumes tended to be greater in the glucose visit compared to the control visit. At 60 minutes post consumption there was a significant decrease from baseline in the control visit which was not observed during the glucose visit, suggesting between visit volumetric differences that may be observed in further studies.

This study has further reinforced the potential value of combined MRS and MRI studies as tools in investigating the metabolic effects of diet and its composition. Natural abundance ^{13}C MRS provides a powerful non-invasive *in vivo* method of monitoring glycogen levels in the liver, and can be combined with liver volume and gastric content MRI to provide a full picture of the physiological and metabolic effects of low calorie meal intake. In addition to the measurements undertaken in this study, muscle glycogen levels can also be monitored [1, 18] and both hepatic and muscle lipid levels can be obtained using ^{1}H MRS [19, 20]. Whilst these techniques have been used for many years, current advances in magnet strength, processing software, pulse sequencing and coil design have improved signal-to-noise ratios

and acquisition times, allowing more in depth data to be acquired with a higher temporal resolution.

Future experiments should explore the basic effects of increasing doses of carbohydrate consumption under normal physiological conditions *in vivo*, which would provide better models of hepatic response to meal intake and be the foundation for further work. Given the increasing prevalence of metabolic disorders and liver related diseases, there is a current need for investigations into physiological responses to differing meal compositions or lifestyle choices, such as glycaemic index, interval between meals or types and regularity of exercise in healthy or obese subjects. In addition, these MRS techniques can be used to observe the effects of various feeding regimes on patients with metabolic disorders such as Type 2 diabetes or non-alcoholic fatty liver disease (NAFLD). Alongside blood sampling for glucose and hormone measurements these experiments provide a more detailed understanding of the metabolic effects of life-style changes.

Conclusion

In conclusion, this pilot study has shown that a low energy morning carbohydrate intake following an overnight fast inhibits reduction in liver glycogen and may reduce or abolish hepatic glucose output. This suggests that carbohydrate demands are sustained through an alternative pathway (presumably utilisation of the ingested carbohydrate) for up to 4 hours after consumption and lays the foundation for further investigations.

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Notes and references

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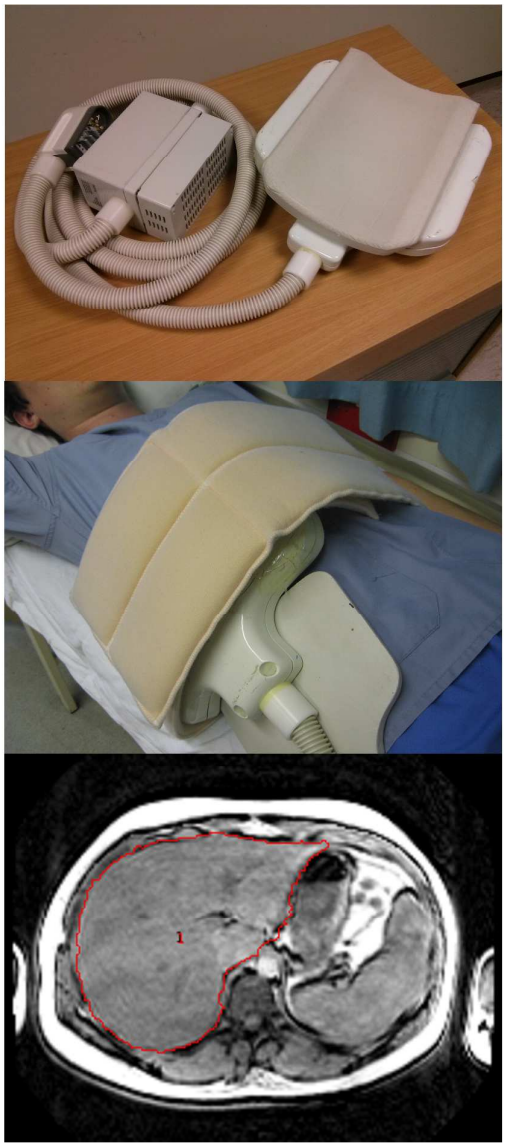

Figure 1. Picture showing Pulseteq ^{13}C surface coil with quadrature proton decoupling coil used for glycogen signal detection (top), position of subject for scanning routine (middle), and T_1 weighted gradient echo sequence MR image of liver (bottom) showing region of interest (red outline). The gastric content of the stomach is also visible.

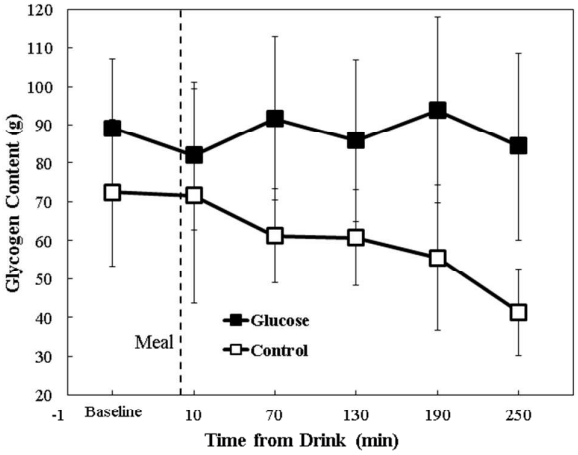

Figure 2. Mean (±SEM) absolute glycogen content at baseline and hourly following an 250ml oral ingestion of either 50g glucose (■) or water (□) in healthy volunteers (n = 5)

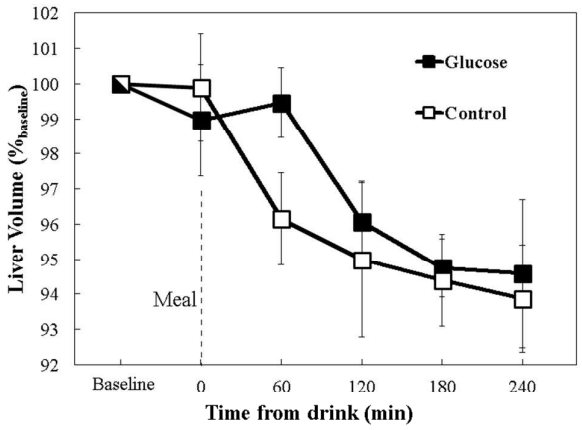

Figure 3. Mean (±SEM) absolute liver volume as % of baseline at baseline and hourly following an 250ml oral ingestion of either 50g glucose (■) or water (□) in healthy volunteers (n = 5)

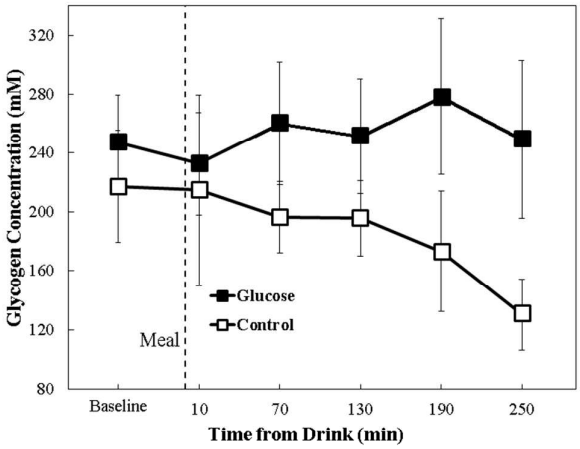

Figure 4. Mean (±SEM) glycogen concentration at baseline and hourly following an 250ml oral ingestion of either 50g glucose (■) or water (□) in healthy volunteers (n = 5).

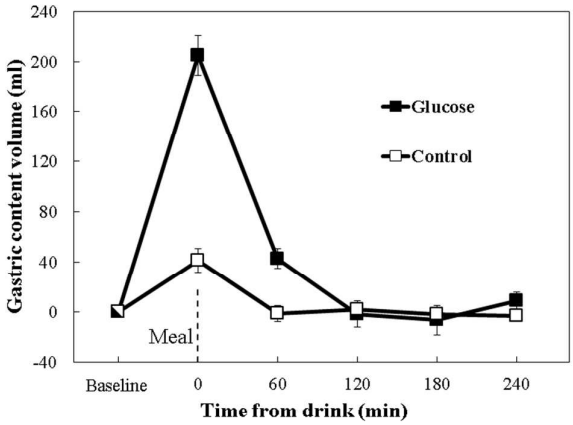

Figure 5. Mean (±SEM) gastric content volume at baseline and hourly following an 250ml oral ingestion of either 50g glucose (■) or water (□) in healthy volunteers (n = 5) showing gastric emptying across the time course

Contents abstract

A low dose oral glucose challenge following an overnight fast inhibits further reduction in hepatic glycogen reserves without raising levels above baseline