

## **MRI assessment of the postprandial gastrointestinal motility and peptide response in healthy humans**

**RUNNING HEAD:** GI motility response to a meal by MRI

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**KEY MESSAGES:**

- The clinical relevance of measuring intestinal motility using MRI has been recently shown. However most investigative MRI paradigms are undertaken in the fasting state using bowel preparation. Here we develop pan-intestinal motility assessment in a single session using MRI in a fed and physiological state and compare imaging findings to postprandial GI peptide responses and symptoms in healthy volunteers.
- The test meal challenge was effective in inducing a change in multiple physiological quantified end-points and in monitoring markers of GI motility in a single MRI study session which was acceptable to the subjects.
- Mapping out post-prandial physiological changes in disease groups will allow us to better understand patient symptoms and perhaps identify GI peptides as possible biomarkers of dysmotility and patient symptoms. This work will serve as a platform to enhance our diagnostic capabilities in relevant disease groups such as Crohn's.

## **Abstract**

**Background:** Feeding triggers inter-related gastrointestinal (GI) motor, peptide and appetite responses. These are rarely studied together due to methodological limitations. Recent MRI advances allow pan-intestinal, non-invasive assessment of motility in the undisturbed gut.

This study aimed to develop a methodology to assess pan-intestinal motility and transit in a single session using MRI and compare imaging findings to GI peptide responses to a test meal and symptoms in a healthy volunteer cohort.

**Methods:** Fifteen healthy volunteers ( $29.3 \pm 2.7$  years and BMI  $20.1 \pm 1.2$  Kg/m<sup>2</sup>) underwent baseline and postprandial MRI scans, symptom questionnaires and blood sampling (for subsequent GI peptide analysis, Glucagon-like peptide-1 (GLP-1), Polypeptide YY (PYY), Cholecystokinin (CCK)) at intervals for 270min following a 400g soup meal (204kcal, Heinz, UK). Gastric volume, gall bladder volume, small bowel water content, small bowel motility and whole gut transit were measured from the MRI scans.

**Key Results:** (mean $\pm$ SEM) Small bowel motility index increased from fasting  $39 \pm 3$  arbitrary units (a.u.) to a maximum of  $87 \pm 7$  a.u. immediately after feeding. PYY increased from fasting  $98 \pm 10$  pg/ml to  $149 \pm 14$  pg/ml at 30min and GLP-1 from fasting  $15 \pm 3$   $\mu$ g/ml to  $22 \pm 4$   $\mu$ g/ml. CCK increased from fasting  $0.40 \pm 0.06$  pmol/ml to  $0.94 \pm 0.1$  pmol/ml. Gastric volumes declined with a  $T_{1/2}$  of  $46 \pm 5$  min and the gallbladder contracted from a fasting volume of  $19 \pm 2$  ml to  $12 \pm 2$  ml. Small bowel water content increased from  $39 \pm 2$  ml to  $51 \pm 2$  ml postprandial. Fullness VAS score increased from  $9 \pm 5$  mm to  $41 \pm 6$  mm at 30min postprandial.

**Conclusions and Inferences:** The test meal challenge was effective in inducing a

change in MRI motility end-points which will improve understanding of the pathophysiological postprandial GI response.

## **Introduction**

The clinical relevance of measuring intestinal motility has been recently shown through its correlation to intestinal inflammation in Crohn's disease <sup>1, 2</sup>.

MRI methodology has recently come to the fore offering repeatable means to measure global and segmental motility<sup>3</sup>. Further, MR does not expose the subject to ionising radiation, permitting serial scanning <sup>4-6</sup> which is commonly indicated in chronic intestinal disorders.

Abdominal pain represents a predominant symptom both in functional and organic intestinal diseases and commonly occurs in the post-prandial state rather than after a prolonged fast. Feeding triggers a range of gastrointestinal (GI) responses such as motility and secretion of fluid and hormones. Postprandial GI motility is stimulated by luminal distention and is modulated by luminal nutrients. Enteroendocrine cells react to the luminal contents by releasing a variety of gastrointestinal peptides like Cholecystinin (CCK), Peptide YY (PYY) and Glucagon-like peptide 1 (GLP-1)<sup>7</sup>. CCK delays gastric emptying and stimulates gall bladder contraction <sup>8</sup>, PYY slows meal transit through a delay in gastric emptying and increasing the GI transit time (known as ileal brake) <sup>9</sup>, while GLP-1 delays gastric emptying <sup>10</sup> and decreases small bowel motility separately <sup>11, 12</sup>.

It thus comes as rather a major diagnostic limitation that most investigative MR paradigms are undertaken in the fasting patient and not in the physiological state with an abnormally distended bowel with contrast agent. Advances in the availability of MRI scanners, increases in the speed of acquisition and growing acceptance of this method for the investigation gastrointestinal disease has driven its role in the GI physiology. A number of techniques to assess fasting motility in ta fasted and prepared during an MR enterography (MRE) are now available <sup>13</sup> enabling rapid, reproducible and sensitive

assessment of global and segmental motility to complement morphological changes seen through structural imaging <sup>14, 15</sup>.

In this study we aim to develop a methodology to assess pan-intestinal motility assessment in a single session using MRI in a fed and physiological state and to compare imaging findings to postprandial GI peptide responses and key patient symptoms in a healthy volunteer's cohort. This work will serve as a platform to enhance our understanding of GI physiology and diagnostic capabilities in relevant disease groups.

## **Materials and methods**

### *Subjects*

This study was approved by the local Ethics Committee of the University of Nottingham (H19062014). This study is registered on clinical trials.gov with identifier NCT02717117. All participants gave informed written consent.

Healthy volunteers (median age 26 (range 19-50) years, BMI mean  $20 \pm 1$  kg/m<sup>2</sup>) were recruited from the local campus population. Participants with a history of inflammatory bowel disease, smokers <sup>16</sup>, a history of bowel resections or any gastrointestinal surgery, history of pancreatic insufficiency, thyroid disease, diabetes, proton-pump inhibitor usage or any medication that affects gastric emptying or small bowel transit and any potential participants scoring very highly on the depression scale questionnaire were excluded. Standard MRI exclusion criteria were applied.

### *Test meal*

The test meal consisted of: cream of chicken soup (400g) (or mushroom for vegetarians) (Heinz, Wigan, UK), chosen respectively by 6 and 9 participants. The

nutrient content of this meal/100g was: energy (kcal) 51, protein 1.5 g (1.5%), carbohydrate 4.7 g (4.5%), fat 2.9 g (2.9%). This meal has been previously shown to reliably induce a GI peptide response<sup>17, 18</sup>.

### *Study design*

This study was open label. MRI marker capsules were used to measure whole gut transit time<sup>19</sup>. Participants were given 5 MRI marker capsules (20 mm x 7 mm) filled with 0.4mL 15 $\mu$ M Gadoteric acid, an MRI contrast agent, to take home. They were instructed to swallow these 24 h before attending the unit for their study day and undergoing the baseline MRI scans as previously described<sup>19</sup>. They were asked to fill in a questionnaire to ensure adherence to the study day restrictions.

The subjects were asked to fast from 2000 h the previous evening and to avoid alcohol, caffeine, strenuous exercise and any medication that could affect gut function for 18 h before the experiment (S1).

On the day of the scan, Participants attended the 1.5T Philips Achieva MRI scanner (Philips Healthcare, Best, the Netherlands) at the Sir Peter Mansfield Imaging Centre (SPMIC) at the University Park Campus, University of Nottingham. Participants were cannulated at arrival (0800) and underwent a baseline fasting scan at 0900 hours (defined at  $t = -20$  min time point), together with a fasting baseline blood sample. The 60 minute interval between cannulation and acquisition of baseline measurements was introduced to allow the subjects to normalise to a baseline calm physiological state. At 0925 hours, they were asked to consume all their test meal in a quiet lounge next to the scanner within a maximum time of 20 min so that at 0945 hours the subjects underwent a first immediate postprandial scan (defined as  $t = 0$  min).

This was followed with data collection (MRI, questionnaire data and 10 ml blood

samples) time points every 15 min for the first 60 min and then every 30 min up to 270 min. Blood samples were collected by a research nurse. The first 2 mL of each sample was discarded to avoid contamination with the saline used to maintain patency. Blood was collected into vacutainer tubes containing 0.325 mL of aprotinin, (Trasylol, Bayer) (Dorset, England).

At each time point, the MRI procedures took approximately 15 minutes, including subject positioning on the scanner bed, setup of the MRI scans and data collection. For the first 60 minutes the subjects were kept inside the scanner. After this the subjects were kept sitting upright in a quiet lounge next to the scanner for the rest of the study day except for the subsequent MRI acquisitions. At each time point, they filled a 100mm Visual analogue scale (VAS) symptoms questionnaire scoring their feeling of fullness, bloating, distension, abdominal pain/discomfort and nausea<sup>17</sup>. The VAS anchors are from 'not' to 'extremely'. At the end of the 270 min healthy volunteers were allowed to leave.

### *Magnetic Resonance Imaging*

MRI scanning was carried out supine. Participants were scanned using a range of sequences. At each time point scans were acquired to assess gastric volume<sup>20</sup>, gall bladder volume<sup>21</sup>, small bowel water content<sup>22</sup> and small bowel motility<sup>15</sup>. In addition at baseline the position of the MRI marker capsules were determined to measure whole gut transit time<sup>19</sup>.

Gastric emptying was assessed using a balanced gradient echo sequence (bTFE) acquiring 50 contiguous axial slices with reconstructed in-plane resolution 2.0x 1.77 mm<sup>2</sup>, slice thickness 5 mm and 0 mm slice gap, echo time (TE) =1.5 ms, repetition time (TR) =3.0ms, flip angle 80°, SENSE 2.0 within one breath hold of ~16 s. This



imaging sequence yields good contrast between the stomach contents and other abdominal organs. Samples containing mobile water appear brighter than viscous samples with reduced water content and mobility.

The content of apparent freely mobile water in the small bowel was assessed as previously described<sup>22</sup> using a single-shot fast spin echo sequence acquiring 24 contiguous coronal slices with reconstructed in-plane resolution  $0.78 \times 0.78 \text{ mm}^2$ , slice thickness 7mm and slice gap 0mm, TE =320 ms, TR =8000 ms, SPIR fat saturation within one breath hold of 24 s. This sequence yields high-intensity signals from areas with freely mobile fluid and dark signals from poorly mobile or bound water and all other body tissues.

Small bowel motility was assessed using a single slice cine-MRI acquisition set at six contiguous parallel coronal planes through the small bowel. Data was acquired using a balanced gradient echo sequence (bTFE) with reconstructed in-plane resolution  $1.49 \times 1.7 \text{ mm}^2$ , echo time (TE) =1.52 ms, repetition time (TR) =3.0 ms, flip angle  $80^\circ$ , SENSE 2.0 at a repetition time of 1 image per second for a duration of 1 minute for each plane with gentle free breathing throughout the acquisition.

Whole gut transit was assessed using two different Coronal 3D T1 weighted turbo field echo (TFE) sequences. Each comprised two separate stacks with a 30 mm overlap, acquired on two separate breath holds. Firstly a T1 weighted 3D TFE sequence with SPAIR fat saturation. Sequence parameters for each station were as follows: TE = 1.9 ms, TR = 4.0 ms, FA =  $10^\circ$ , FOV =  $250 \times 398 \times 160 \text{ mm}^3$ . Acquired resolution  $2.3 \times 2.3 \times 5 \text{ mm}^3$ , reconstructed to  $1.4 \times 1.4 \times 2.5 \text{ mm}^3$ , with a  $288 \times 288$  reconstructed matrix. 64 slices were acquired with a half-scan factor of 0.7 in the phase direction and 0.85 in the slice direction in a 23 sec breath hold. The second sequence, another T1-weighted 3D TFE sequence with a 2-echo readout and mDIXON reconstruction of

the water only images<sup>23</sup> was acquired with increased resolution using SENSE and 36 maximum intensity projection (MIP) images were generated from this data to create a 3D view of the colon to aid in defining the position of the capsules. Imaging parameters at each station were as follows: TE1/TE2 = 1.4/2.5 ms, TR = 3.8 ms, FA = 10°, FOV = 250 x 371 x 200 mm<sup>3</sup>. Acquired resolution 1.8 x 1.8 x 3.6 mm<sup>3</sup>, reconstructed to 1.0 x 1.0 x 1.8 mm<sup>3</sup>, with a 384 x 384 reconstructed matrix. 111 slices were acquired with a SENSE factor of 2.0 in the phase direction in a 22 s breath holds.

Gall bladder volume was measured pre- and post-prandial, at every acquisition time point up to 60 min postprandially<sup>21</sup>. This was carried out using the same images as for the gastric volumes as previously shown.

#### *Plasma collection and peptides assays analysis*

On the morning of the test, 0.325 ml of aprotinin was added to vacutainer tubes (BD-361017, BD Diagnostics, Oxford) for collection for each time point aiming for a final volume of 6.5 ml. Fasting 10 ml blood sample was drawn and collected in the tubes. After the test meal, data were acquired every 15 min for the first 60 min and every 30 min thereafter to 270 min. Twelve samples were taken totalling 120 ml. Samples were centrifuged at 3000 rpm for 10 min and stored on ice<sup>17</sup>. Plasma peptides (total GLP-1, total PYY) were analysed through enzyme-linked immunosorbent assay (ELISA) techniques (Millipore, UK) as previously shown<sup>17</sup>. The concentrations of serum CCK were measured by radioimmunoassay (RIA) (Euro Diagnostic Products, Sweden) as previously shown<sup>24</sup>.

#### **Data analysis**

##### *Motility assessment*

All dynamic data was processed with Dual Registration of Abdominal Motion (DRAM)

(Motilent, Ford, UK). DRAM first removes respiratory motion<sup>25</sup> before applying the optic flow registration as previously described by Odille et al<sup>14</sup> to correct local deformation caused by bowel wall motion and model intensity changes caused by luminal flow. Registration results were further analysed using a customised graphical user interface written in MATLAB (MathWorks, Natick, MA, USA).

Published methods of assessing small bowel motility have been applied in Magnetic Resonance Enterography (MRE)<sup>14, 15</sup>. MRE involves distending the bowel lumen with a large volume of luminal contrast medium, which stimulates wall movement and facilitates at the same time the registration process. The aim of our project was to measure motility in a physiological state after a nutrient meal rather than an artificially distended state. Since postprandial small bowel motility does not produce the same motion or appearance of the lumen as in MRE, a different approach to assessing the small bowel motility was required. The technique used in this study quantified the motility of the bowel using the pixel signal changes through the time series, within a defined region of interest (ROI) placed over the small bowel loops. The metric was calculated as follows:

For each pixel in the registered dataset, a power spectrum of the intensity changes across the time series (smoothed using a running average of 5 pixels to reduce noise) was calculated and then summed across all frequencies. This metric was termed the total power, and reflected bowel motility, both in terms of segmental oscillations and bolus movement of contents, typically seen postprandially<sup>26</sup>.

Two independent observers (AK, CH) drew ROIs manually over all the loops of the small bowel in all the slices for each imaging datasets (Figure 1). From these ROIs the mean total power across all small bowel pixels was calculated. A larger total power motility index represents higher small bowel motility.

### *Small bowel water content (SBWC)*

SBWC was measured as previously validated<sup>22</sup>, using in house software written in IDL (Research Systems Inc. Boulder, Colorado, USA). Briefly, this method assumed that any pixel with signal intensity above the calculated threshold in the heavily T2-weighted coronal images is filled with free water. Structures such as blood vessels, bladder and gall bladder are manually excluded.

### *Gall bladder and gastric volumes*

Gall bladder and gastric volumes were quantified using in house software written in IDL (Research Systems Inc. Boulder, Colorado, USA). This method uses a semi-automatic previously validated thresholding-region growing technique to define the content of the stomach and gas within the stomach on each image slice<sup>20</sup>. For total gastric volume was calculated as the sum of the stomach contents and any gas. Postprandial gastric content volumes were fitted to a 5 parameter equation<sup>27</sup> to model the emptying process and allow the calculation of the gastric half-emptying time ( $T_{1/2}$ )

### *Whole gut transit (WGT)*

Whole gut transit was assessed as previously described<sup>19</sup>. From the two sets of MRI images a transit score was calculated by sub-dividing the bowel into eight sections and each capsule was scored according to its position in the colon at 24 h. A weighting factor was calculated for each capsule depending on the difference of the capsule score from the median capsule score.

### *Visual analogue scale (VAS)*

Symptoms regarding appetite, satiety and abdominal pain were scored at each time point using previously validated questionnaire<sup>17</sup>.

## **Statistical analyses**

Due to the pilot nature of this study, it was not possible to power it but similar studies done by our group have used similar-sized cohorts<sup>17, 28, 29</sup>. The data are expressed as mean±standard error of the mean (SEM). Normality of the data was assessed using Shapiro–Wilk’s test. One-way analysis of variance (ANOVA) was used to assess the significance of differences. When the analysis of variance was significant, *post hoc* test assessments of the individual time points were performed using the Dunnett’s for parametric data or Dunn’s test for non-parametric data, to account for multiple comparisons. All statistical analyses were performed using GraphPad Prism 7.01 (La Jolla, USA). A p-value less than 0.05 was considered statistically significant.

## **Results**

All fifteen healthy volunteers (9 female, 6 male, age 29.3±2.7 years and BMI 20.1±1.2 Kg/m<sup>2</sup>) completed the study and tolerated the experimental procedures well without any adverse event.

### *Gallbladder volumes*

The changes in gallbladder volumes with time are shown in supplementary material 2 (S2). This demonstrated a moderate and significant postprandial decrease in the volume from 19±2ml (mean ± SEM) at baseline to 12±2ml immediately postprandial t=0 min (p≤0.0001).

### *Gastric volumes*

The baseline gastric volumes showed small amount of resting gastric juices of 26±7ml. Gastric content volumes rose significantly upon feeding to 418±17ml at t=0 (p≤0.0001) after which the volume of the stomach declined and went back to baseline (37±7ml) at 150min with an average time to empty half of the stomach contents (T<sub>1/2</sub>) of 46±5min

(Fig 2).

#### *Small bowel motility*

From figure 3 it can be seen that the motility index increased significantly from fasting  $39\pm 3$  arbitrary units (a.u.) to a maximum of  $87\pm 7$  a.u. immediately postprandial ( $t=0$ min) ( $p\leq 0.001$ ) after feeding and then gradually decreased back to around baseline ( $44\pm 4$  a.u.) in 90 minutes. Motility index rose slightly again at 120 min to  $55\pm 4$  a.u. and decreased back again to  $38\pm 4$  a.u. at 240 min.

#### *Small bowel water content*

The data in figure 4 shows a small amount of fasting ( $t= -20$ min) small bowel water content of  $39\pm 2$ ml. The test meal induced a significant change in small bowel water content. Immediately, after the all soup meal was ingested, this increases to a maximum of  $51\pm 2$ ml ( $p\leq 0.05$ ) at 15 min. The volume decreased towards baseline ( $38\pm 2$ ml) at 60 min after which a second peak at 180 min is clearly seen with a volume of  $65\pm 3$ ml.

#### *Whole gut transit (WGT)*

The median average weighted position score (WAPS) of the MRI capsules was 1.0 (0-3.8). As described previously<sup>19</sup>, the WAPS at 24 hr was converted to WGT in hours, giving a median of 33 hr.

#### *Total GLP-1*

The meal induced significant postprandial changes in plasma measures of total GLP-1 ( $p\leq 0.01$ ). The mean GLP-1 data (Fig 5) from healthy volunteers showed a postprandial peak from fasting volume of  $15\pm 3$  $\mu$ g/ml to  $22\pm 4$  $\mu$ g/ml ( $t=0$  min). The GLP-

1 levels dropped ( $12\pm 3\mu\text{g/ml}$ ) after 30 minutes and remained around baseline levels for the rest of the study day.

#### *Total PYY*

PYY (Fig 6) increased significantly ( $p\leq 0.001$ ) from fasting  $98\pm 10\text{pg/ml}$  to  $149\pm 14\text{pg/ml}$  at 30min postprandially in healthy volunteers. PYY returned to baseline after 2 hours postprandially and later dropped even further to reach  $71\pm 13\text{pg/ml}$  at 270 min.

#### *CCK*

Plasma CCK levels (Fig 7) increased significantly ( $p\leq 0.01$ ) from fasting  $0.40\pm 0.06\text{pmol/ml}$  to  $0.94\pm 0.1\text{pmol/ml}$  at  $t=30\text{min}$  and then steadily decreased after 60 min to reach baseline ( $0.4\pm 0.01\text{pmol/ml}$ ) for the rest of the study day.

#### *Symptom VAS data*

The meal induced a significant increase in fullness. VAS increased from  $9\pm 5$  to  $44\pm 5$  at 0 min and  $41\pm 6$  at 30 min postprandial ( $p< 0.001$ ). The feeling returned to baseline thereafter. Bloating, distention, pain and nausea did not change significantly. (Table1).

### **Discussion**

The test meal challenge was effective in inducing a change in multiple physiological quantified end-points and in monitoring markers of GI motility in a single MRI study session which was acceptable to the subjects. To our knowledge this is the first study measuring gut motility in an undisturbed bowel after a nutrient meal without artificially distending the bowel.

The physiological parameters measured in this study were in the expected range for a normal healthy cohort as we have shown repeatedly in our previous studies<sup>28-30</sup>.

The test meal was ingested within a maximum of 20 min by the 15 subjects and hence acted as a food bolus in the stomach. The meal itself was richer in carbohydrate content (4.5%) and fat (2.9%) rather than protein (1.5%) and hence would be a good stimulus for GLP-1, PYY and CCK secretion by the enteroendocrine L cells and I cells respectively.

The meal used for this experimental work was a homogenous soup rather than a solid meal.  $T_{1/2}$  for the gastric emptying was  $57 \pm 5$  min starting off with a maximal volume of  $458 \pm 20$  ml. The gastric emptying was approximately linear from 0 min to 90 min postprandial. This finding is similar to our previous observations whereby following a solid meal with a drink, there is an initial rapid gastric emptying of the liquid drink in the first 0 to 75 min postprandial, followed by slower phase of emptying of the solid food. The mean reported fullness VAS increased from 9.0 in the fasting state to 44 immediately postprandial with a decline to baseline within  $\sim 180$  min. This rapid gastric emptying had an effect on the GI peptide responses to the test meal, changes in small bowel water content and the small bowel motility. In this work we once again observed a bimodal peak in small bowel water content in the postprandial state up to 270 min. The subjects' mean fasting baseline small bowel water content was  $39 \pm 2$  ml, increasing to a maximum of  $51 \pm 2$  ml. The volume decreased towards baseline ( $38 \pm 2$  ml) at 60 min in an early 'gastric phase' after which a second peak at 180 min was clearly seen with a volume of  $65 \pm 3$  ml, otherwise known as a later 'intestinal phase'<sup>29</sup>. The early drop observed is probably representative of absorption of water and available nutrients (sucrose and glucose) which are co-transported with sodium and water following the resulting osmotic gradient across the intestinal mucosa. This net absorption together with an activated post-prandial gastro-ileal reflex causing emptying of the contents of the distal ileum into the ascending colon, lead to a decrease in small bowel water



content. The second peak or intestinal phase is due to an increase in pancreatico-biliary and enterocyte secretion<sup>31</sup>.

The plasma levels of GLP-1 increased rapidly from  $15\pm 3$  ug/ml to  $22\pm 4$  ug/ml in response to the carbohydrate load within the meal. It is known that one of the principal factors governing gastric emptying is the total carbohydrate load<sup>32</sup>, which through proximal duodenal absorption of simple digestible carbohydrates leads to an increase in GLP-1 secretion and a delay in gastric emptying<sup>10</sup>. The level of CCK usually increases 10-30 minutes after food intake<sup>8</sup> as was confirmed in this study. In a parallel, gall bladder contraction occurred within 20 min of meal ingestion which would explain the 50% decrease in gall bladder volume.

In this work we measured motility of the unprepared small bowel in response to a nutrient test meal for the first time. Mean motility peaked at  $87\pm 7$  a.u. immediately postprandial. The early increase may be due to the fact that large boluses of meal move rapidly through the duodenum and upper jejunum increasing the volume amount of small bowel associated with the motion per unit of intestinal fluid volume present. Moreover, a prompt gastro-ileal reflex would facilitate emptying of the distal small bowel content into the ascending colon. The small bowel motility decreased back to baseline at approximately 90 minutes, when the emptying of the stomach has almost completed. This decrease coincided with maximal plasma levels of GLP-1 and PYY<sup>33</sup>, which have previously been associated with delayed small bowel transit<sup>34</sup>. Mean small bowel motility once again peaked at 120 min. There could be multiple possible explanations for this change. Between 90 and 120 min, mean plasma levels of PYY and GLP-1 dropped back to pre-prandial levels hence minimising their negative effect on small bowel motility. At a similar time period small bowel water content increased due to an increased in intestinal and pancreatico-biliary secretions. Such a small bowel

distention could make small bowel motility more apparent and easier to measure or otherwise be a stimulus itself for smooth muscle contraction<sup>35</sup>. To our knowledge, this is the first time that such detailed and non-invasive assessment of small bowel physiology has ever been described in the post-prandial state with MRI.

The whole gut transit time reported in this study was a median of 33 hours. This is comparable to our previous reports of 31 hours using the same methodology<sup>19</sup>.

There are limitations in the study. The meal used was small in both volume and caloric intake, hence providing a submaximal stimulus to the GI tract. We chose this meal, due to its known effects on GI peptide response and because it could be tolerated in an otherwise sick patient population and could be administered safely and in a timely fashion in a clinical setting. Despite this postprandial physiological changes have been observed both in MR measures, patient symptoms and GI peptides. The age range 19-50 years was broad though we would not expect major differences in GI motility within this range.

In this study we have established an unprecedented platform for measuring post-prandial small bowel motility in human subjects using MRI, and correlated this to known physiological GI peptide responses and symptoms. This work shows that such methodology exists and is feasible. The length of our study is such that it may be difficult to implement in a busy clinical setting as is, though one could choose to scan only during the early postprandial time where the response is maximal. Presently intestinal motility had only been measured in the fasting state or on a bowel prepared with large volumes of luminal contrast, which may be less relevant to some of the post prandial symptoms experienced by patients. Mapping out such post-prandial physiological changes in disease groups will allow us to understand patient symptoms better and perhaps identify GI peptides as possible biomarkers of dysmotility and patient

symptoms attributing to a poor quality of life. Further future work may be able to normalise such dysmotility through commercially available GI peptide inhibitors

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AK, CLH, LM and GWM designed the research.

AK, AN recruited the patients.

AK collected the data. AK, AM, CLH, ML, YF, GS and SP analysed the data.

AK, CLH, LM and GWM wrote the manuscript draft.

All authors revised the final manuscript.

## **Disclosures**

GW Moran has received: Consultancy fees from AbbVie, Takeda Pharmaceuticals, Janssen and Dr Falk; Speaker fees from Merck Sharp, Dohme Ltd, AbbVie, Ferring, Janssen and Takeda Pharmaceuticals and Financial support for educational activities from AbbVie, Merck Sharp, Dohme Ltd, Ferring, NAPP pharmaceuticals and Dr Falk. A Menys is CEO of Motilent Ltd. SA Taylor is an NIHR senior investigator.

## **References**

1. Menys A, Atkinson D, Odille F, Ahmed A, Novelli M, Rodriguez-Justo M, Proctor I, Punwani S, Halligan S, and Taylor SA. Quantified terminal ileal

- motility during MR enterography as a potential biomarker of Crohn's disease activity: a preliminary study. *Eur Radiol* 2012. **22**: p. 2494-2501.
2. Menys A, Helbren E, Makanyanga J, Emmanuel A, Forbes A, Windsor A, Punwani S, Halligan S, Atkinson D, and Taylor SA. Small bowel strictures in Crohn's disease: a quantitative investigation of intestinal motility using MR enterography. *Neurogastroent Motil* 2013. **25**: p. 967-+.
  3. Plumb AA, Menys A, Russo E, Prezzi D, Bhatnagar G, Vega R, Halligan S, Orchard TR, and Taylor SA. Magnetic resonance imaging-quantified small bowel motility is a sensitive marker of response to medical therapy in Crohn's disease. *Aliment Phar Therap* 2015. **42**: p. 343-355.
  4. Knuesel PR, Kubik RA, Crook DW, Eigenmann F, and Froehlich JM. Assessment of dynamic contrast enhancement of the small bowel in active Crohn's disease using 3D MR enterography. *Eur J Radiol* 2010. **73**: p. 607-13.
  5. Cronin CG, Lohan DG, Browne AM, Alhajeri AN, Roche C, and Murphy JM. MR enterography in the evaluation of small bowel dilation. *Clin Radiol* 2009. **64**: p. 1026-1034.
  6. Fidler JL, Guimaraes L, and Einstein DM. MR Imaging of the Small Bowel. *Radiographics* 2009. **29**: p. 1811-1826.
  7. Bruen CM, O'Halloran F, Cashman KD, and Giblin L. The effects of food components on hormonal signalling in gastrointestinal enteroendocrine cells. *Food Funct* 2012. **3**: p. 1131-1143.
  8. Huda MSB, Wilding JPH, and Pinkney JH. Gut peptides and the

- regulation of appetite. *Obes Rev* 2006. **7**: p. 163-182.
9. Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, Ghatei MA, and Bloom SR. Inhibition of food intake in obese subjects by peptide YY3-36. *New Engl J Med* 2003. **349**: p. 941-948.
  10. Little TJ, Pilichiewicz AN, Russo A, Phillips L, Jones KL, Nauck MA, Wishart J, Horowitz M, and Feinle-Bisset C. Effects of intravenous glucagon-like peptide-1 on gastric emptying and intragastric distribution in healthy subjects: Relationships with postprandial glycemic and insulinemic responses. *J Clin Endocr Metab* 2006. **91**: p. 1916-1923.
  11. Schirra J, Nicolaus M, Roggel R, Katschinski M, Storr M, Woerle HJ, and Goke B. Endogenous glucagon-like peptide 1 controls endocrine pancreatic secretion and antro-pyloro-duodenal motility in humans. *Gut* 2006. **55**: p. 243-51.
  12. Horsthuis K, Bipat S, Bennink RJ, and Stoker J. Inflammatory bowel disease diagnosed with US, MR, scintigraphy, and CT: Meta-analysis of prospective studies. *Radiology* 2008. **247**: p. 64-79.
  13. Bickelhaupt S, Pazahr S, Chuck N, Blume I, Froehlich JM, Cattin R, Raible S, Bouquet H, Bill U, Rogler G, Frei P, Boss A, and Patak MA. Crohn's disease: small bowel motility impairment correlates with inflammatory-related markers C-reactive protein and calprotectin. *Neurogastroent Motil* 2013. **25**.
  14. Odille F, Menys A, Ahmed A, Punwani S, Taylor SA, and Atkinson D. Quantitative assessment of small bowel motility by nonrigid registration of

- dynamic MR images. *Magnet Reson Med* 2012. **68**: p. 783-793.
15. Menys A, Taylor SA, Emmanuel A, Ahmed A, Plumb AA, Odille F, Alam A, Halligan S, and Atkinson D. Global Small Bowel Motility: Assessment with Dynamic MR Imaging. *Radiology* 2013. **269**: p. 442-449.
  16. Kadota K, Takeshima F, Inoue K, Takamori K, Yoshioka S, Nakayama S, Abe K, Mizuta Y, Kohno S, and Ozono Y. Effects of smoking cessation on gastric emptying in smokers. *J Clin Gastroenterol* 2010. **44**: p. e71-5.
  17. Moran GW, Leslie FC, and McLaughlin JT. Crohn's disease affecting the small bowel is associated with reduced appetite and elevated levels of circulating gut peptides. *Clin Nutr* 2013. **32**: p. 404-11.
  18. Leslie FC, Thompson DG, McLaughlin JT, Varro A, Dockray GJ, and Mandal BK. Plasma cholecystokinin concentrations are elevated in acute upper gastrointestinal infections. *QJM Int J Med* 2003. **96**: p. 870-871.
  19. Chaddock G, Lam C, Hoad CL, Costigan C, Cox EF, Placidi E, Thexton I, Wright J, Blackshaw PE, Perkins AC, Marciani L, Gowland PA, and Spiller RC. Novel MRI tests of orocecal transit time and whole gut transit time: studies in normal subjects. *Neurogastroent Motil* 2014. **26**: p. 205-214.
  20. Hoad CL, Parker H, Hudders N, Costigan C, Cox EF, Perkins AC, Blackshaw PE, Marciani L, Spiller RC, Fox MR, and Gowland PA. Measurement of gastric meal and secretion volumes using magnetic resonance imaging. *Phys Med Biol* 2015. **60**: p. 1367-1383.
  21. Marciani L, Cox EF, Hoad CL, Totman JJ, Costigan C, Singh G, Shepherd V, Chalkley L, Robinson M, Ison R, Gowland PA, and Spiller RC. Effects

- of various food ingredients on gall bladder emptying. *Eur J Clin Nutr* 2013. **67**: p. 1182-1187.
22. Hoad CL, Marciani L, Foley S, Totman JJ, Wright J, Bush D, Cox EF, Campbell E, Spiller RC, and Gowland PA. Non-invasive quantification of small bowel water content by MRI: a validation study. *Phys Med Biol* 2007. **52**: p. 6909-22.
  23. Eggers H, Brendel B, Duijndam A, and Herigault G. Dual-Echo Dixon Imaging with Flexible Choice of Echo Times. *Magnet Reson Med* 2011. **65**: p. 96-107.
  24. Astbury NM, Taylor MA, and Macdonald IA. Breakfast consumption affects appetite, energy intake, and the metabolic and endocrine responses to foods consumed later in the day in male habitual breakfast eaters. *J Nutr* 2011. **141**: p. 1381-9.
  25. Hamy V, Dikaios N, Punwani S, Melbourne A, Latifoltojar A, Makanyanga J, Chouhan M, Helbren E, Menys A, Taylor S, and Atkinson D. Respiratory motion correction in dynamic MRI using robust data decomposition registration - Application to DCE-MRI. *Med Image Anal* 2014. **18**: p. 301-313.
  26. Silverthorn DU. *Human Physiology: an integrated Approach*. London: Pearson; 2012.
  27. Parker HL, Tucker E, Hoad CL, Pal A, Costigan C, Hudders N, Perkins A, Blackshaw E, Gowland P, Marciani L, and Fox MR. Development and validation of a large, modular test meal with liquid and solid components for assessment of gastric motor and sensory function by non-invasive imaging. *Neurogastroenterol Motil* 2016. **28**: p. 554-68.

28. Marciani L, Cox EF, Hoad CL, Pritchard S, Totman JJ, Foley S, Mistry A, Evans S, Gowland PA, and Spiller RC. Postprandial changes in small bowel water content in healthy subjects and patients with irritable bowel syndrome. *Gastroenterology* 2010. **138**: p. 469-77, 477 e1.
29. Marciani L, Pritchard SE, Hellier-Woods C, Costigan C, Hoad CL, Gowland PA, and Spiller RC. Delayed gastric emptying and reduced postprandial small bowel water content of equicaloric whole meal bread versus rice meals in healthy subjects: novel MRI insights. *Eur J Clin Nutr* 2013. **67**: p. 754-8.
30. Marciani L, Hall N, Pritchard SE, Cox EF, Totman JJ, Lad M, Hoad CL, Foster TJ, Gowland PA, and Spiller RC. Preventing Gastric Sieving by Blending a Solid/Water Meal Enhances Satiation in Healthy Humans. *J Nutr* 2012. **142**: p. 1253-1258.
31. Kellum JM, Albuquerque FC, Stoner MC, and Harris RP. Stroking human jejunal mucosa induces 5-HT release and Cl<sup>-</sup> secretion via afferent neurons and 5-HT<sub>4</sub> receptors. *Am J Physiol* 1999. **277**: p. G515-20.
32. Gentilcore D, Nair NS, Vanis L, Rayner CK, Meyer JH, Hausken T, Horowitz M, and Jones KL. Comparative effects of oral and intraduodenal glucose on blood pressure, heart rate, and splanchnic blood flow in healthy older subjects. *Am J Physiol Regul Integr Comp Physiol* 2009. **297**: p. R716-22.
33. Pironi L, Stanghellini V, Miglioli M, Corinaldesi R, Degiorgio R, Ruggeri E, Tosetti C, Poggioli G, Labate AMM, Monetti N, Gozzetti G, Barbara L, and



Go VLW. Fat-Induced Ileal Brake in Humans - a Dose-Dependent Phenomenon Correlated to the Plasma-Levels of Peptide-Yy. *Gastroenterology* 1993. **105**: p. 733-739.

34. Marathe CS, Rayner CK, Jones KL, and Horowitz M. Effects of GLP-1 and Incretin-Based Therapies on Gastrointestinal Motor Function. *Exp Diabetes Res* 2011.
35. Edelbroek M, Horowitz M, Dent J, Sun WM, Malbert C, Smout A, and Akkermans L. Effects of duodenal distention on fasting and postprandial antropyloroduodenal motility in humans. *Gastroenterology* 1994. **106**: p. 583-92.

## Tables

Table 1

Visual analogue scale (100 mm VAS scores)

Time point	Fullness	Bloating	Distention	Pain/discomfort	Nausea/sickness
-20	9±5	7±3	4±2	1±1	6±4
0	44±5**	9±3	4±2	0±0	2±2
15	41±6*	10±3	5±3	0±0	2±2
30	41±6**	6±3	3±2	0±0	1±1
45	37±7	7±3	3±2	0±0	1±1
60	31±7	5±3	2±2	0±0	1±1
90	26±6	5±3	3±3	0±0	0±0
120	20±6	7±4	3±2	0±0	1±1
150	13±4	4±2	1±1	0±0	0±0
180	12±5	2±1	1±1	0±0	0±0
210	7±4	1±1	1±1	0±0	0±0
240	5±2	0±0	1±1	0±0	0±0
270	3±1	0±0	0±0	0±0	0±0

\*\* p≤0.001 and \*p<0.05 versus baseline value

## Table Captions

Table 1: Visual analogue scale (100 mm VAS scores).

## Figure Captions

Figure 1: Motility assessment. Reference image (A) and a motility map (B) for a healthy volunteer. ROI's were placed on the reference image to include small bowel loops only.

Figure 2: Changes in stomach content volumes. Data are mean  $\pm$  SEM from n=15 healthy volunteers. \*\*\*\*  $p \leq 0.0001$  and \* $p < 0.05$

Figure 3: Changes in small bowel motility. Data are mean  $\pm$  SEM from n=15 healthy volunteers. \*\*\*  $p \leq 0.001$ , \*\*  $p \leq 0.01$  and \* $p < 0.05$

Figure 4: Changes in small bowel water contents. Data are mean  $\pm$  SEM from n=15 healthy volunteers.

Figure 5: Changes in GLP-1 levels with study time. Data are mean  $\pm$  SEM from n=15 healthy volunteers.

Figure 6: Changes in PYY levels with study time. Data are mean  $\pm$  SEM from n=15 healthy volunteers. \*\*\*  $p \leq 0.001$ , \*\*  $p \leq 0.01$  and \* $p < 0.05$

Figure 7: Changes in CCK plasma levels with study time. Data are mean  $\pm$  SEM from n=15 healthy volunteers. \*\*  $p \leq 0.01$  and \* $p < 0.05$

## Supporting information

Figure S1: Diagram summarizing the study protocol.

Figure S2: Changes in gallbladder volume. Data are mean  $\pm$  SEM from n=15 healthy volunteers. \*\*\*\*  $p \leq 0.0001$

**Figures**

Figure 1

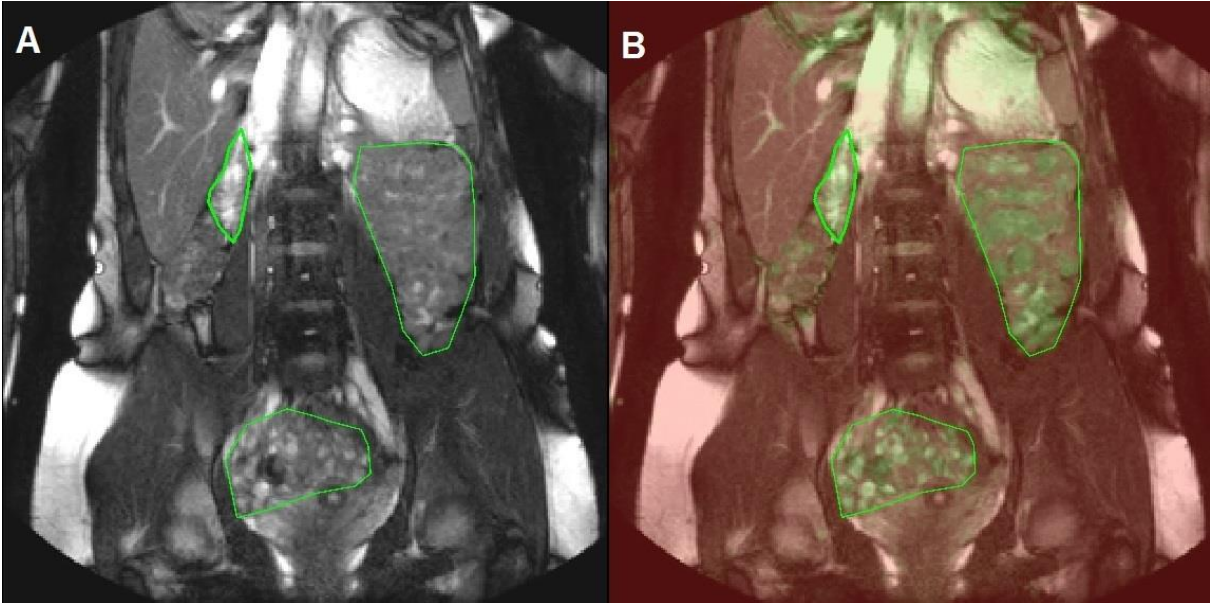


Figure 2

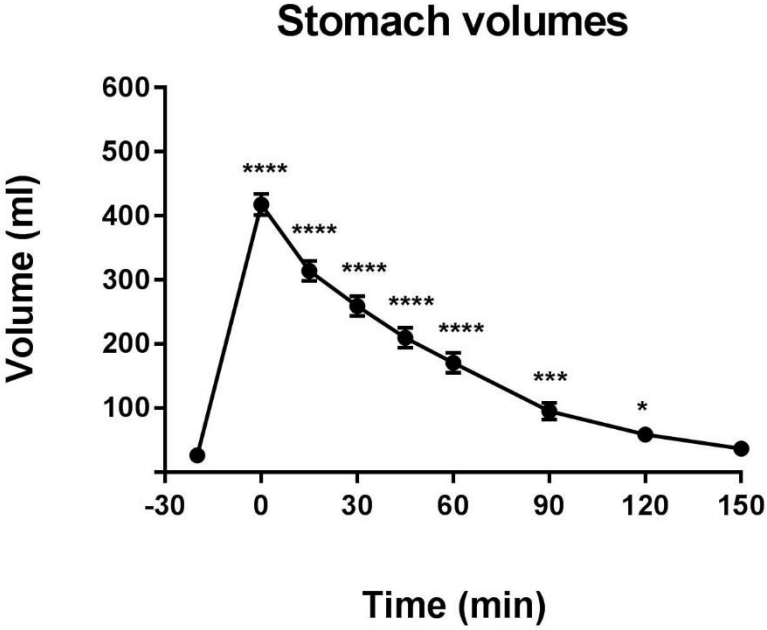


Figure 3

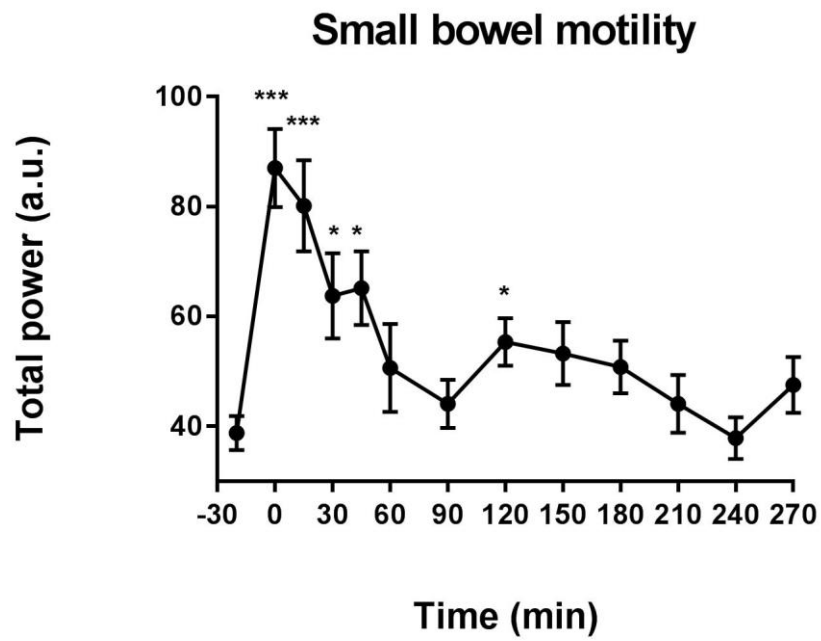


Figure 4

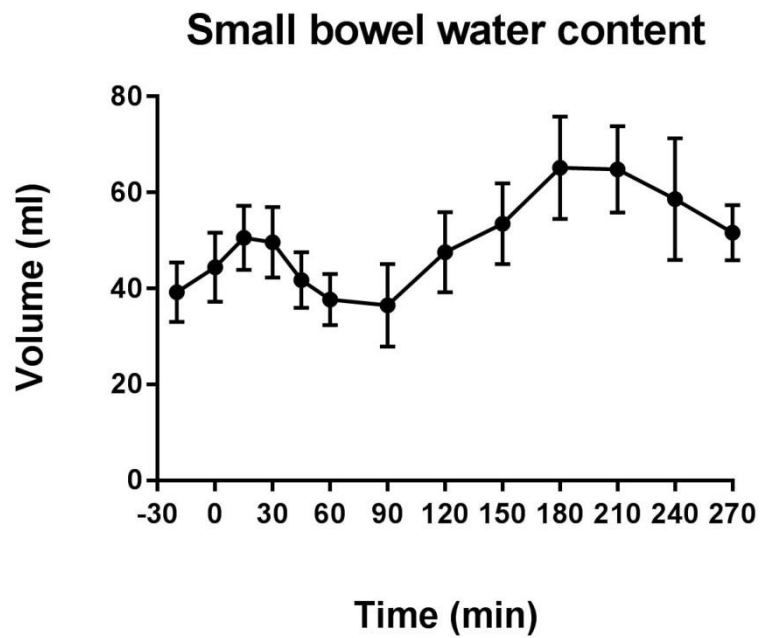


Figure 5

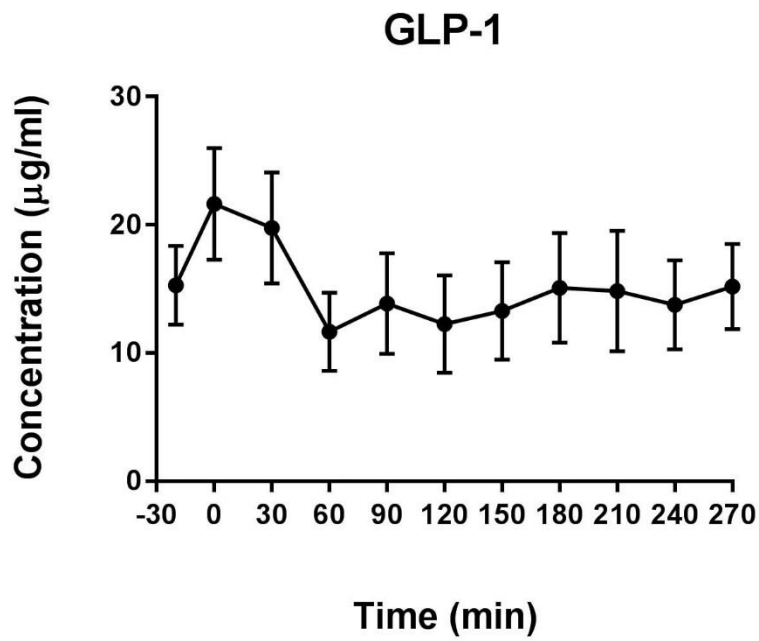


Figure 6

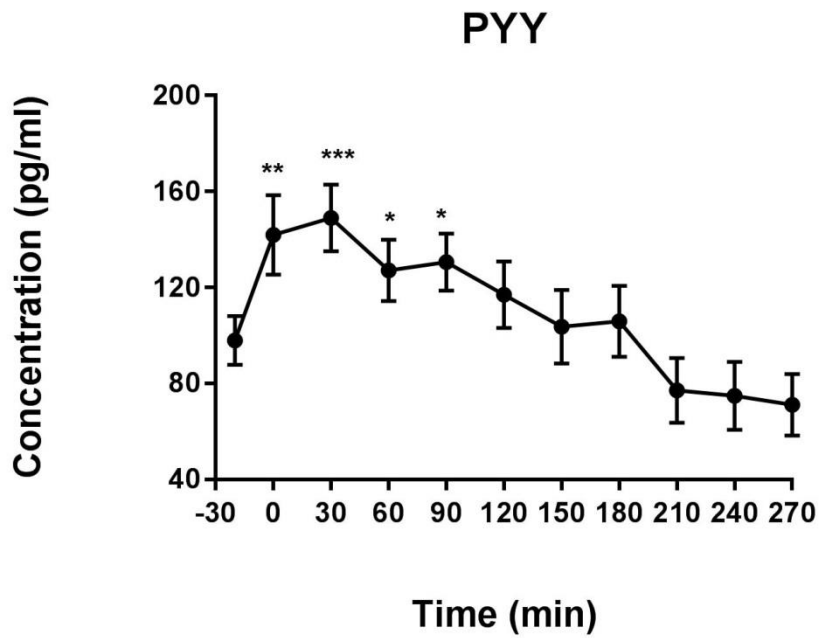
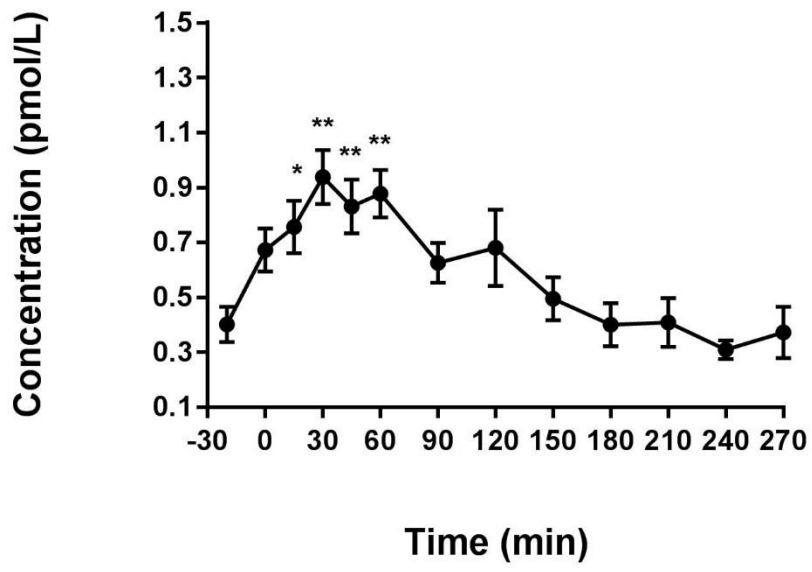


Figure 7

# CCK



## Supporting information

Figure S1: Diagram summarizing the study protocol.

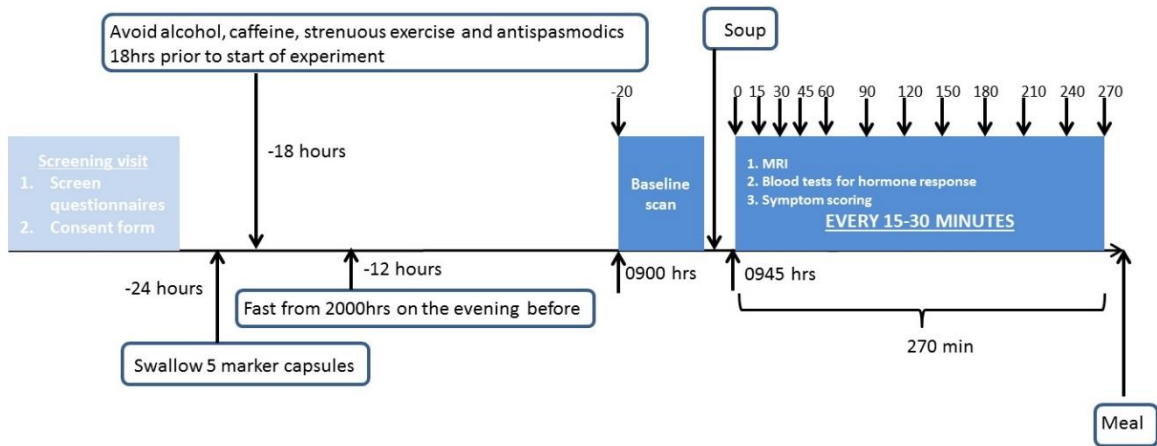


Figure S2: Changes in gallbladder volume. Data are mean  $\pm$  SEM from n=15 healthy volunteers. \*\*\*\*  $p \leq 0.0001$

