The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

DETAILED STUDY PROTOCOL

TITLE

A pilot study to assess the effect of Lettuce on intestinal water content through Magnetic Resonance Imaging of the Small Bowel: LETIS

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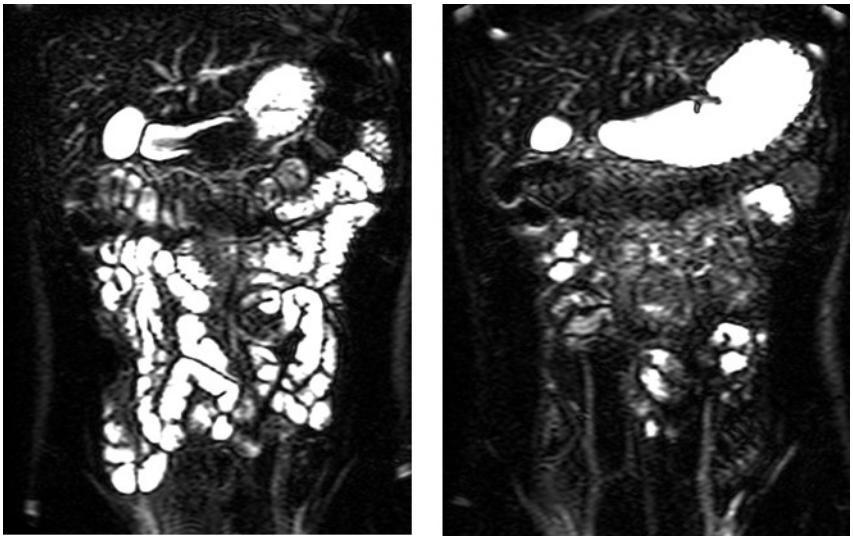
BACKGROUND AND RATIONALE

In recent years certain poorly digested carbohydrates, grouped together by the term FODMAP (fermentable oligo-, di-, mono-saccharides and polyols), have been proposed to exacerbate symptoms of irritable bowel syndrome (IBS) such as abdominal discomfort and bloating. This phenomenon has also been observed in patients with an ileostomy, where certain foods have been associated with increased fluid output from the stoma(1). This is in accordance with past work surveying ileostomy patients on foods that altered stoma function(2). However, there may be other factors that drive fluid output from a stoma. Rhubarb, a food listed by 1 in 3 patients as exacerbating watery diarrhoea, also contains anthraquinones that are thought to have laxative effects(3), such as in senna.

A food less commonly associated with laxative effects is lettuce but 1 in 6 patients reported that eating lettuce led to an increase in watery stoma output. Certain lettuce varieties such as Romaine (Cos) exude a milk-like latex material when cut, giving rise to the latin name *Lactuca sativa*. While the methylcellulose is insoluble and would not be expected to hold water in the lumen of the small bowel, latex could be expected to stimulate intestinal secretion. This may contribute to post-prandial sensations of bloating by a different mechanism to the osmotic effects and colonic fermentation seen with FODMAPs(4).

The Nottingham GI MRI group has been at the forefront of elucidating the effects of poorly digested carbohydrates on gastrointestinal (GI) physiology. We have published techniques to measure free water in the small bowel(5) and assessment of the physical form of chyme in the colon using MR relaxometry(6). This includes the demonstration that fructose ingestion on its own leads to increased free water in the small bowel compared to co-ingestion with glucose(4) – see panel. We want to apply these techniques to compare the effect of different foods: white bread, lettuce and rhubarb. We have previously shown that bread lead to a reduction in small bowel water and so can active as a negative control(7). Rhubarb should serve as a positive control. This will allow us to assess the effect of lettuce.



Images from a coronal single-short turbo spin-echo MRI sequence used to quantify free water in the lumen of the small bowel one hour after (a) 40g fructose (b) 40g fructose + 40g glucose(4)

TRIAL OBJECTIVES AND PURPOSE

Purpose

The purpose of the study is gather pilot data on the effect of different foods on intestinal physiology.

Objective

The primary objective is to gather pilot data on the change from baseline in small bowel water content in healthy volunteers after a meal of lettuce in comparison to rhubarb, a stimulant food, and white bread, a food known to promote intestinal water absorption.

Secondary Objectives

Secondary objectives will be to gather pilot data on the effects of food on the physical form of colonic chyme, colonic fermentation and bloating symptoms.

Hypotheses

- The test meals will lead to different amounts of post-prandial water in the small bowel
- The test meals will lead to differences in the post-prandial physical form of chyme in the colon
- Meals with higher small bowel water will also induce greater symptoms of bloating, irrespective of markers of fermentation

DETAILS OF PRODUCTS

The products to be used will be food products on general sale to the public in the UK through major supermarkets:

Description

Meal A. Tesco Rhubarb in light syrup, drained and washed. One tin, drained and washed, contains 245g rhubarb and has an estimated calorie content of 25kcal (fat 0g). Served with 60g Lactofree cream (210kcal, 22.2g fat) will provide 235kcal.

Meal B. Tesco White medium sliced bread. Two slices provide 164 kcal (fat 1.3g). Served with 10g butter (75kcal, 8.2g fat) will provide 239kcal.

Meal C. Tesco Romaine (Cos) lettuce. 250g will provide 40kcal (fat 1.25g). Served with mayonnaise 30g (207kcal, 22.7g fat) will provide 247kcal.

In order to avoid contamination of rhubarb effects by sucrose the rhubarb in syrup will be drained, soaked in water, re-drained and then prepared with fresh water.

STUDY DESIGN

Trial Configuration

This will be a three-period, three-treatment crossover trial with blinding of data analysis.

Primary endpoint

- Small bowel water content (mL), measured by MRI

Secondary endpoints

- T1 relaxation time (ms) of the chyme in the ascending colon, measured by MRI
- Breath hydrogen and methane (ppm), measured using GastroCHeck
- Bloating and satiety score, measured using 100mm visual analogue scale

We will also measure gastric volume to confirm passage of the meal into the small bowel.

Stopping rules and discontinuation

Given the short time frame of the study there will be no planned stopping rules.

Randomisation and Blinding

All participants will consume all three study meals. The order of consumption will be determined by random sequence generated using the online program www.randomization.com. Consumption of each meal will be separated by at least 5 days in order to minimize carryover effects.

It will not be possible to blind participants or researchers to the meal intervention on each

day. MRI images will be coded, anonymized and separated from data on study day intervention. This will allow blind analysis of small bowel water and colon metrics.

Trial Management

The trial is not sufficiently big to require a trial Steering Committee or Data Monitoring Committee. The Chief Investigator has overall responsibility for the study and shall oversee all study management.

The data custodian will be the Chief Investigator.

Duration of the Trial and Participant Involvement

End of the trial

The trial is expected to last up to 4 months from initiation to the last visit of the last patient.

The last visit of the last patient will be considered to be the end of the trial. Data analysis will continue after this time.

Selection and withdrawal of participants

Recruitment

Healthy volunteers who meet eligibility criteria will be recruited by general advertisement on Nottingham University campuses and through the Sir Peter Mansfield Imaging Centre (SPMIC) and Nottingham Digestive Diseases Centre (NDDC) databases of healthy volunteers who have expressed a wish to be contacted about further studies. We will also advertise through the divisional and group social media presence on Facebook and Twitter e.g. www.facebook.com/UoN.Gastrointestinal.MRI.

Prospective participants will have the study fully explained and will be given an information sheet to read and retain. It will be explained to the potential participant that entry into the trial is entirely voluntary and that they can withdraw at any time but attempts will be made to avoid this occurrence. In the event of their withdrawal it will be explained that their data collected so far cannot be erased and we will seek consent to use the data in the final analyses where appropriate.

The limited resources of the trial mean that we will not recruit participants who do not understand English. Other exclusions will include those for whom consumption of the study meals would prove difficult because of medical issues e.g. diabetes.

Eligibility Criteria

Inclusion

- Aged 18 or older
- Able to give informed consent

Exclusion

- Pregnancy declared by candidate
- History declared by the candidate of pre-existing gastrointestinal disorder that may affect bowel function
- A positive diagnosis of irritable bowel syndrome based on the Rome III criteria questionnaire
- Reported history of previous resection of the oesophagus, stomach or intestine (excluding appendix)
- Intestinal stoma
- Any medical condition making participation potentially compromising participation in the study e.g. diabetes mellitus, respiratory disease limiting ability to lie in the scanner, known allergy to one of the food products
- Contraindications for MRI scanning i.e. metallic implants, pacemakers, history of metallic foreign body in eye(s) and penetrating eye injury
- Will not agree to dietary restrictions required in 24 hours before each study day
- Unable to stop drugs known to alter GI motility including mebeverine, opiates, monoamine oxidase inhibitors, phenothiazines, benzodiazepines, calcium channel antagonists for the duration of the study (Selective serotonin reuptake inhibitors and low dose tricyclic antidepressants will be recorded but will not be an exclusion criteria)
- Inability to lie flat or exceed scanner limits of weight <120kg
- Poor understanding of English language
- Participation in night shift work the week prior to the study day. Night work is defined as working between midnight and 6.00 AM
- Participation in any medical trials for the past 3 months
- Anyone who in the opinion of the investigator is unlikely to be able to comply with the protocol e.g. cognitive dysfunction, chaotic lifestyle related to substance abuse

Expected duration of participant participation

Each participant will be involved for a minimum of 12 days from enrolment. This may be extended, either for patient convenience or to accommodate bookings on the MRI scanner, as long as each study day is separated by at least 5 days.

Removal of participants from therapy or assessments

Participants may be withdrawn from the trial either at their own request or at the discretion of the Investigator. The participants will be made aware that this will not affect their future care. Participants will be made aware (via the information sheet and consent form) that should they withdraw the data collected to date cannot be erased and may still be used in the final analysis.

Informed consent

All participants will provide written informed consent. The Informed Consent Form will be signed and dated by the participant before they enter the trial. The Investigator will explain the details of the trial and provide a Participant Information Sheet, ensuring that the participant has sufficient time to consider participating or not. The Investigator will answer any questions that the participant has concerning study participation. Where consent is obtained by a student investigator they will be directly supervised in the process until the PI is assured of their competence, and then indirectly supervised with help available.

Informed consent will be collected from each participant before they undergo any interventions (including physical examination and history taking) related to the study. One copy of this will be kept by the participant, one by the Investigator, and a third by the SPMIC.

Should there be any subsequent amendment to the final protocol, which might affect a participant's participation in the trial, continuing consent will be obtained using an amended Consent form which will be signed by the participant.

TRIAL TREATMENT AND REGIMEN

Study sites

Nottingham Digestive Diseases Centre (NDDC), Queen's Medical Centre
Sir Peter Mansfield Imaging Centre (SPMIC), University Park Campus

Feasibility testing

On a single day two volunteers will undergo testing with the rhubarb meal with scanning every 30 minutes. The aim of this will be to confirm the time course of small bowel water after rhubarb, which is intended to be the positive control. This work will be undertaken under a pre-existing ethics approval G19062014_SoPA_SPMMRC: "MR Imaging to evaluate the gastrointestinal fate of foods and beverages". This is for MRI development work in healthy volunteers.

This may lead to modifications of the study day timetable set out below, but will not increase the number of MRI scan sessions undergone by participants. Other assessments, breath tests and symptom scores, are considered a sufficiently light burden that it would be reasonable to add up to 4 additional assessments per day if a longer study day is required.

Study Schematic

Consent	1 day dietary restriction	**Study Day 1**	5 day break	1 day dietary restriction	**Study Day 2**	5 day break	1 day dietary restriction	**Study Day 3**

Study Day

Time (AM)	0830	0900	0945	1000	1030	1100	1130	1200	1230	1300
MRIs	Arrive fasted	Scan	Meal	Scan		Scan		Scan		
Breath H_2, CH_4		*		*	*	*	*	*	*	*
Bloat & Satiety Scores		*		*	*	*	*	*	*	*

On each study day four participants will attend, taking turns to receive scans, so timing are indicative.

Details of Trial Regimen

This study will use a three-period, three treatment design with 10 participants.

Visit 1 (Consent) – Day 0
Visit 1 will take place at the NDDC and last 20-30 minutes. After consent has been obtained participants will be screened against eligibility criteria will be enrolled if they are eligible. An MRI safety screening questionnaire will be completed as part of this process. A record will be taken of height, weight, current medication, any medical conditions and smoking history.

Visits 2 - 4 (Study Days)

On the day before a study day participants will be asked to avoid caffeine (tea, coffee, cola), alcohol, dietary or sports supplements including those containing trace elements. They should also avoid apples, beans, peas, pulses (e.g. lentils) and sweetcorn. They should not eat after 8pm.

On each study day participants should remain nil by mouth apart from essential medicines. They will attend the 1.5T Phillips scanner of the SPMIC as directed by the study team. Once consent and safety of MRI scanning has been confirmed, they will change into surgical scrubs (provided) and undergo fasted assessment: 1) MRI scan; 2) measurement of breath hydrogen (H_2) and methane (CH_4) by breathing into a gas analyser (GastroCHECK, Bedfont, UK); assessment of bloating and satiety symptoms by 100mm visual analogue scale. Further assessments will be staggered between participants so that no two participants have the same assessment timetabled at the same time.

Participants will then consume the test meal assigned for the day. 200mL still water will be provided to drink with the meal. Participants will be given up to 20 minutes to consume each meal. The first post-prandial assessments will be taken immediately after the meal is fully consumed. Further assessments will be made at 30-minute or 60-minute intervals as set out in the figure.

MRI Scanning Schedule

Subjects will be scanned on a research dedicated 1.5T Philips Achieva MRI scanner, using a parallel imaging SENSE 16-element torso coil. A range of MRI sequences will be used to image the abdomen including:

1) A balanced gradient echo (called balanced turbo field echo, bTFE or trueFISP) sequence (TR = 3.0 ms, TE = 1.5 ms, FA 80^{o}, SENSE factor 2.0) to acquire 50 transverse images each with an in-plane resolution of 2.00 mm x 1.77 mm and slice thickness of 5 mm, with no gap between slices. This will be used to measure gastric volumes.

2) A single shot, fast spin echo sequence (rapid acquisition with relaxation enhancement, RARE) to acquire in a single breath-hold 24 coronal images with in-plane resolution interpolated to 0.78 mm x 0.78 mm and a slice thickness of 7 mm, with no gap between slices (TR = 8000 ms, TE_{eff} = 320 ms, AQR = 1.56 mm x 2.90 mm). This sequence yields high intensity signals from areas with fluid and little signal from body tissues and will be used to measure small bowel water content.

3) A high resolution bTFE sequence to acquire images of the contents of the ascending colon (TR = 3.1 ms, TE = 1.56 ms, acquired resolution = 1.50 mm x 1.50 mm, reconstructed resolution= 0.86 x 0.86, 8 slices 5 mm thick, up to 5.0 mm gap to cover whole ascending colon, flip angle 45^{o}).

4) We will assess T_1 of colonic contents using previously published methods(6, 8, 9).

Images will be acquired with an expiration breath-hold between 13 and 24 seconds. Participants will spend approximately 10 minutes inside the magnet at any one time.

STATISTICS

Sample Size and justification

This is a pilot study as no data are available on the effect of ingested rhubarb on MRI parameters. We will recruit up to 16 participants: in order to gather 2 data sets for feasibility testing and 10 complete data sets for the study itself.

Two-way analysis of variance will be used to assess for any difference between the test meals. Any difference identified will then be explored by direct comparison between meals. The Wilcoxon Signed Rank test for non-parametric paired data will be used.

Assessment of Efficacy

Primary endpoint	**Outcome Measure**
Change from baseline in small bowel water content (0 - 2 hours)	Difference between treatment means
Secondary endpoints	**Outcome Measure**
Difference in T1 relaxation time between fasting baseline and 2 hours postprandial	Average difference between treatment means
Difference in breath hydrogen and methane between fasting baseline and 3 hours postprandial	Average difference between treatment means
Difference in bloating and satiety scores between fasting baseline and 3 hours postprandial	Average difference between treatment means

GOOD CLINICAL PRACTICE

The study will be performed according to Good Clinical Practice principles. The volunteer study will undergo submission through the University of Nottingham Research Medical School Research Ethics Committee. All volunteers will give written informed consent. Their names will be entered into a subject log in the Trial Master File and a unique alpha numeric study code assigned. A simple Case Report Form will be kept for each volunteer.

ADVERSE EVENTS

We do not expect any adverse events following this intervention with food products on general sale. Should a Serious Adverse Event occur it will be noted, dealt with according to University SOPs and reported to the Ethics Committee.

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