DETAILED STUDY PROTOCOL
V3.0 12/06/2017

Title:
Effect of kiwifruit on Gastrointestinal Fluid Distribution and transit in Healthy volunteers

Background:
Chronic constipation affects approximately 17% of the population worldwide[1] and remains an important unmet need since patients are currently often dissatisfied with treatment[2]. Current treatments which stimulate propulsive colonic motility or osmotic laxatives are successful in increasing stool frequency but are often associated with bloating, cramps and abdominal discomfort. Furthermore, such powerful treatments taken intermittently often create abnormal colonic contents and may result in alternation between diarrhoea and no stools. Many patients would benefit from a less powerful treatment which could be taken daily.

Kiwifruit offer such an alternative and have been shown to increase stool volume and frequency[3]. The mode of action however is unclear. Kiwi is 3% fibre (approximately 1/3 insoluble) and contains around 3% nonstarch polysaccharide including pectins, hemicellulose and cellulose, with high water holding capacity 1.5 times that of ispaghula, a commonly used laxative. Two kiwifruit (150g) contain approximately 6.2gm of glucose and 6.5g fructose. The predominant protein is actinidin a cysteine protease. There are also a number of amines including serotonin and its precursor tryptophan, though these are present in only small amounts (serotonin 0.6g/100g). An unusual component is microcrystalline calcium oxalate monohydrate called rafides. While these dissolve in an acid pH it is unknown to what extent these would escape intact into the small intestine where their action might well excite a protective secretory response in enterocytes. Pig studies indicate that while the soluble component of kiwi fibre is well digested in the small bowel the insoluble component passes largely intact into the colon. Fermentation by microbiota lead to increases in ileal and faecal SCFAs[4].

Soluble fibre is a proven laxative[5] softening stool and increasing stool frequency. Early studies in ileostomy patients showed that ispaghula increases ileal flow[6] but until recently its mode of action in the intact colon was unclear. We have recently shown using novel non-invasive MRI techniques that ispaghula 3.5gm taken three times daily (t.d.s.) substantially increases small bowel water content in healthy volunteers and this is associated with an increase in colonic volumes and water content and an increase in the gastroileal response to eating, propelling larger amounts of ileal fluid into the ascending colon immediately after eating a 1000kcal meal[7].

Insoluble fibre in the form of 15g of bran has also been demonstrated to increase postprandial small bowel water content[8] and accelerate small bowel transit[9].

It seems likely that kiwifruit soluble and insoluble fibre will lead to similar increases in small bowel water but whether the other components of kiwifruit such as actinidin and / or rafides will further enhance this is unclear nor what it effects is on colonic water and faecal viscosity.

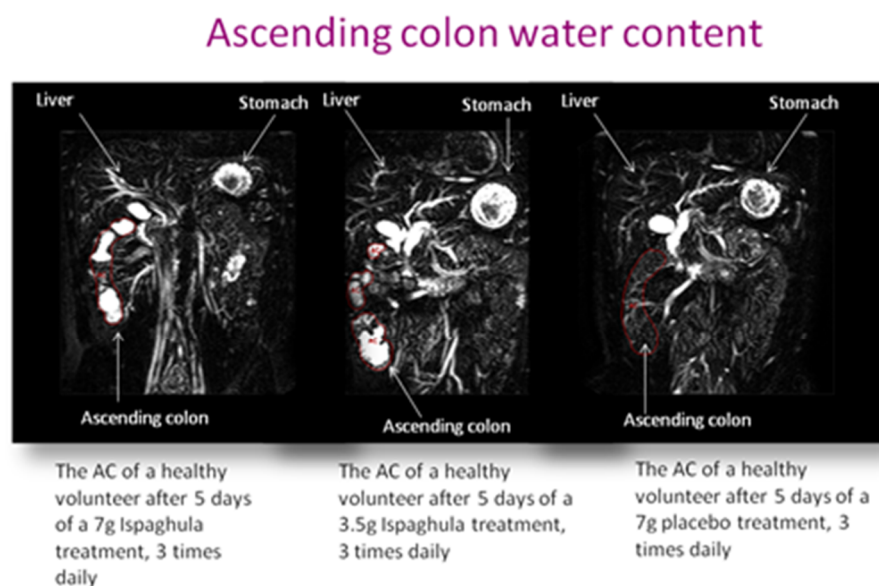*Value of MRI in assessing small bowel and colonic physiology*
Until recently defining the site of action of kiwifruit at the level of the small bowel and colon would require either invasive intubation studies or the study of ileostomists whose physiology may well differ significantly from the target population of healthy individuals. Using our recently developed and

validated novel non-invasive MRI techniques, the University can assess transit and fluid distribution in the small intestine[10]. The University has also developed methods to assess colonic fluid, volumes and motility.

The University shall use these technique to measure fasting and postprandial small and large bowel water content and volumes to assess the relative significance of small intestinal and colonic secretion after kiwifruit.

The University has recently published data showing that IBS-D is associated with a constricted small bowel and accelerated transit[11], while constipation is associated with dilated small bowel and colon with slower transit[12], a novel insights which could only be obtained using the University's technique. Since this work, the University has further developed the technique so it can now assess not only colonic volumes but also the apparent water content from the relaxation time T1 parameter, which the University has recently showed correlates well with % water as assessed by freeze drying. The relaxation time T1 is simply the time constant with which the sample magnetization returns to baseline after excitation, a parameter well known since chemists started using it in the 1950s. The University can also assess whole gut transit time using its validated MRI transit markers, inert plastic capsules filled with a MRI-visible fluid.

The University has already performed an RCT of ispaghula and shown its ability to alter the freely mobile water content of the ascending, transverse and descending colon as assessed by our MRI techniques (See Figure 1).

Ascending colon water content

The AC of a healthy volunteer after 5 days of a 7g Ispaghula treatment, 3 times daily

The AC of a healthy volunteer after 5 days of a 3.5g Ispaghula treatment, 3 times daily

The AC of a healthy volunteer after 5 days of a 7g placebo treatment, 3 times daily

Figure 1: *images of bowel water content showing bright signal (e.g. fluid) in the colon of a subject after ispaghula treatment but no bright signal ion the colon of the same subject after placebo*

Key questions to be addressed:
Impact of kiwifruit on small and large bowel water content

Aims:
The aim is to evaluate the impact of kiwifruit on small and large bowel water content in order to explain the mechanism of action for kiwifruit on digestive comfort.

Methodology:
We will compare the effect of 3 days of 2 kiwifriut b.d vs placebo (28 gm maltodextrin drink providing 120kcal equal to that of 2 kiwifruit) daily.

Study Design:

(a) Study design

This will be a randomised, placebo controlled, 2-way cross-over study in 15 healthy volunteers. The University will allow 2 weeks between studies to ensure return to baseline. In the unlikely event that these healthy subjects' bowel habit has not returned to normal the University will extend this by 1 or 2 weeks as required.
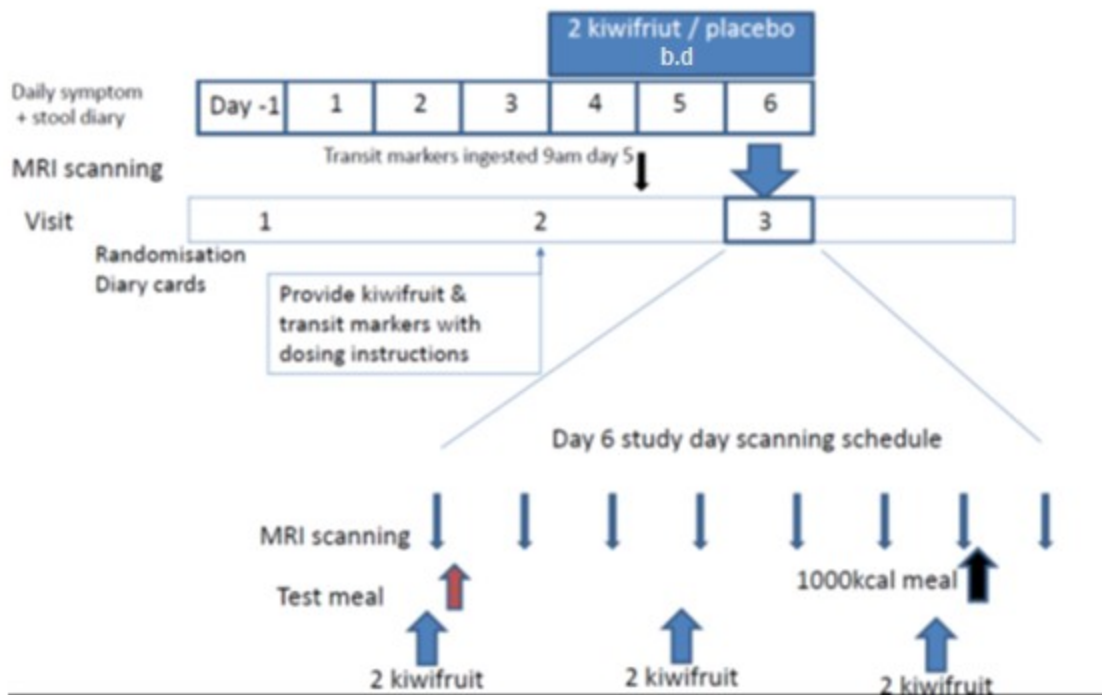
(b) Study visits

After a screening visit to confirm eligibility, study subjects will be randomised as to the test product schedule and be given the transit markers and instructions as to consume the test product two times daily for 2 days prior to the MRI study day on which they will also consume the kiwifruit as for the previous 2 days (see Figure 2). They will attend for the main imaging study on day 6 (see Figure 2).

Subjects will complete a daily stool diary documenting stool frequency and consistency using the Bristol Stool Form Scale during study days 1-6. They will visit the study site on the morning of Day 4 to receive their MRI marker capsules and kiwifruit or maltodextrin placebo. They will consume 2 kiwifruit b.d. starting on Day 4. At 9 am of the morning of Day 5 they will ingest 5 MRI transit markers as described below and note the time of ingestion. The University will contact subjects to remind them to take the pills and bring their test product packs with them as they will be required to take the same ~~3~~ doses on the study day 6. The markers will be imaged at 24 hours (baseline scan of Day 6).

On the main study day (day 6), the subjects will arrive fasted and undergo fasting scans before consuming that day's allocated test product with 250ml of water. After 30 minutes considered to be Time =0 they will consume a standard rice pudding meal as used in previous studies [8]. They will then undergo serial scanning hourly for 8 hours taking the second dose of test product plus 250ml water at Time 180 minutes. At 380 minutes they will consume a second, larger test meal consisting of 400 g microwaveable macaroni cheese ready meal (Sainsbury), 100 g cheesecake slice (Sainsbury), and 250 mL bottled still water. The second test meal provides 1007 kcal, 43.4% from carbohydrate and 45.8% fat. The final scan will at time 420 minutes to assess the effect of ileal emptying on ascending colon water content. They will then be allowed home.

Figure 2

Study time lines

(c) Subjects

Inclusion criteria: Healthy volunteers scoring within the normal range for bowel symptoms as assessed using the GSRS, aged 18-65 years.

Exclusions:
1. Inability to discontinue medication likely to alter GI transit.
2. Previous gastrointestinal surgery (excluding cholecystectomy and appendectomy).
3. Known inflammatory bowel disease, coeliac disease.
4. Known intolerance of kiwifruit.
5. Inability to discontinue drugs likely to alter gut transit.
6. Subjects considered by the investigator unlikely to comply with study protocol.

(d) Test meal

220 grams of Sainsbury's creamed rice pudding, 34 grams of Sainsbury's seedless raspberry jam and 100 ml water. Total calorie content 290 kcal.

(e) Test product allocation

This cannot be blinded but since the outcomes are objective and mechanistic this is less important than it would be if symptoms were the primary outcome. Subjects will be given either the kiwifruit or

unlabelled

sachets of maltodextrin which subjects will be told are dietary supplements. Analysis of the MRI images will be done by an investigator blinded as to the test product.

(g) *Scanning Schedule*

Imaging will be carried out on a state-of-the-art, fully research dedicated 3.0T Scanner with a parallel imaging SENSE abdominal body receiver coil. A range of MRI sequences will be used to image the abdomen including:
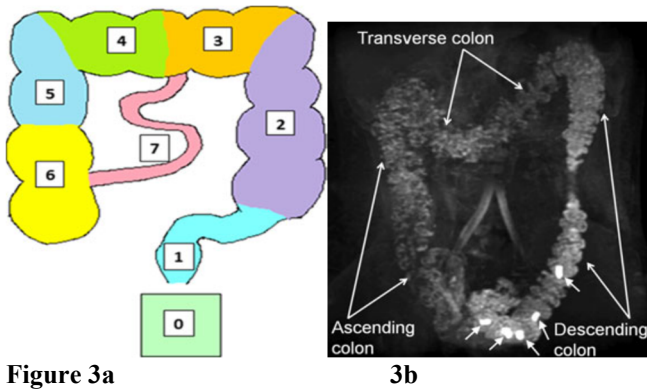1) A HASTE (half-fourier single shot turbo spin echo) sequence will be used to measure gastric volumes and hence assess gastric emptying. Slice thickness will be 10 mm with 24-30 axial slices acquired to cover the full stomach anatomy.
2) A high resolution A balanced gradient echo (called balanced turbo field echo, bTFE or trueFISP) sequence to acquire sagittal images of the contents of the ascending colon 8 slices 5 mm thick, 0.58 mm gap will be acquired to cover the AC anatomy.
3) A single shot, fast spin echo sequence (rapid acquisition with relaxation enhancement, RARE) to acquire 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 This sequence yields high intensity signals from areas with fluid and little signal from body tissues and is used to measure bowel water content.
4) Colonic volumes are assessed using a coronal dual echo fast field echo sequence. Slices are acquired in two overlapping coronal stacks, each acquired in a breathhold. Colonic volumes are measured manually as previously described. [13]. This sequence is also used to locate the position of the transit pills within the GI tract.
5) The University will assess T1 of colonic contents using previously published methods [8;14].

Data will be acquired on an expiration breath-hold with duration between 13 and 24 seconds depending on the sequence, monitored using a respiratory belt. Including set-up and imaging, the volunteers spend approximately 20 minutes inside the magnet for every time point and the rest of the time sitting upright in an adjacent room.

(f) *Colonic transit*

The subjects will swallow 5 inert MRI transit marker capsules under the supervision of one of the study investigators with the study test meal. The marker capsules are made of Polyoxymethylene, the shape of a size 0 gelatine capsule, filled with 0.2 ml of 15μM Gadoteridol solution and sealed. Transit will be assessed from a scan performed 24 and 48 hours after marker ingestion. The transit will be assessed by scoring the position of the capsules according to the scoring system represented below in Figure 3A.
The weighted geometric centre of the 5 capsules at 24 and 48 hours will be calculated to give a measure of colonic transit time. To reduce the effect of outliers a weighting factor is calculated for each capsule depending on the difference of the capsule score from the median capsule score. For a difference of 0 and 1 the weighting factor was 1, for all differences larger than 1 the weighting factor is the inverse of the difference.

Figure 3: *(A) Scoring system (B) an examples of maximum intensity projection from a volunteer's T1-weighted TFE sequence scan 24 hours after swallowing 5 transit capsules showing the position of the capsules (indicated by the small arrows) within the colon. On these sequences the capsules appear bright.*

Dietary guidelines and miscellaneous instructions:

The day before each test day subjects will be asked to minimise changes in their usual lifestyle and to refrain from using alcohol, and doing strenuous exercise. They will be asked to keep a daily diary logging bowel symptoms, stool frequency and consistency for 1 week prior to the first study and during each subsequent study week up to day 7

Eating after 20.00 hour in the evening before the test day will not be allowed. Drinking water after waking on the study day and before reaching the study unit is not allowed. The volunteers will arrive at the test facility 30 minutes before starting the test.

During the test day, the volunteers will not be allowed to eat and drink anything else apart from the study test meals and drinks.

Lower Dose Test

At the end of the study we will scan a further 3 participants in exactly the same protocol however at a lower dose of kiwifruit (2 fruit once daily) to determine if the effect is still visible.

Study site:

Sir Peter Mansfield Magnetic Resonance Centre, School of Physics and Astronomy, University of Nottingham, and Nottingham Digestive Diseases Centre, University of Nottingham, UK.

Primary endpoint:

Effect of Kiwifruit on Ascending colon T1 area under curve from time 0-420.

Secondary endpoints:

Effect of Kiwifruit on the following measures both fasting and postprandial AUC 0-420 minutes:

1) small bowel water content
2) ascending (AC), transverse (TC) and descending (DC) colonic volumes
3) Transit of markers through gut as assessed by the weighted position score at 24 h (WAPS24)
4) Colonic motility
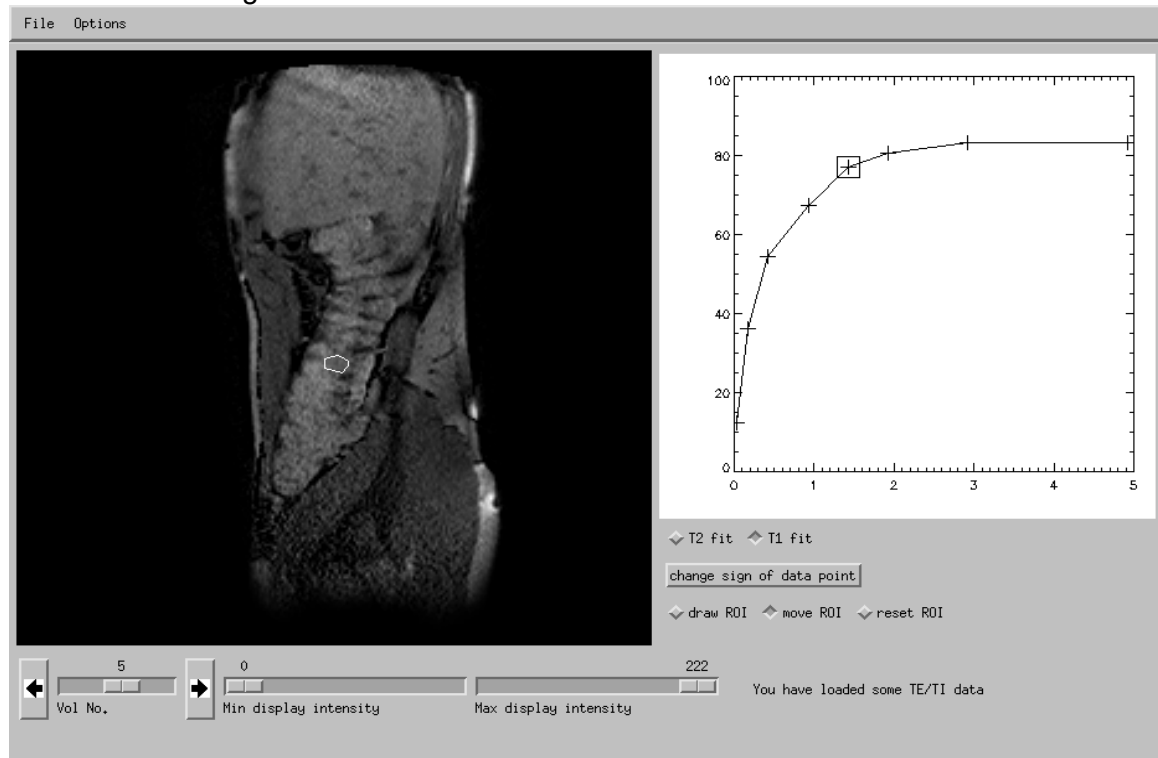5) Gastric emptying of test meal
6) T1 of AC and DC

Data analysis:

Data will be assessed for normality and expressed as means ± SE or median(range) if not normally distributed. Images will be coded and analysed blind as to allocation to avoid any bias in analysis. Measurements of the gastric emptying will be performed by manually drawing the region of interest on each slice using Analyze9 software (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN, USA) and summing across all the slices to determine the total volume at each time point. In particular the gastric volumes will be analysed distinguishing the contents and the gas. The total gastric volume is the sum of the contents and the gas.

The University will assess small bowel water content from images acquired using the RARE sequences as previously validated[10]. Colonic volumes will be assessed coronal dual echo fast field echo sequence.

The longitudinal relaxation time T1 in the ascending colon and, separately, in the descending colon will be measured using a single slice balanced Turbo Field Echo (bTFE) sequence with a preparatory 180 degrees inversion pulse applied before acquiring the imaging data [15]. Data will be acquired from 8 different inversion times (TI) (time between inversion pulse and imaging pulses) ranging from 25 - 4925 ms. There will be a 10 second gap between each acquisition to allow the system to return to equilibrium. Small regions of interest will be drawn on the resulting images to calculate the mean signal intensity for the region at each different TI or TE. Care will be taken to exclude areas of gas or bowel wall. The data will then be fitted to a model of the signal evolution of the tissue after application of all the preparation and imaging pulses to determine the relaxation times.

The figure below shows the same example but for a T1 recovery curve with the fit to the data

shown as well on the right.

T1 colon data sets will be processed using custom written software in IDL® (Research systems Inc, Boulder, CO) to generate a region of interest in the ascending colon chyme avoiding small 'pockets' of free water (high intensity signal) if present. Then using the mean of this region at all the different echo times to calculate the T1 of the tissue using an in-house program.

Transit will be expressed as WAPS at 24hours (WAPS24) of the 5 markers and converted to transit time in hours using a nomogram (Figure 4) based on previous validation studies.

MRI motility measurement:
Cine bTFE data will be acquired over 5-10 minutes. An observer will draw a line along the main axis of the AC which will be used to create a number of lines at 90^0 to this axis. These lines will be automatically propagated through the time series using the deformation fields from the non-rigid registration of the images performed by Motilent. The resulting time series of line lengths will be smoothed to enable measurement of a Motility Index.

Power and statistical analysis:
Our pilot data with a standard laxative dose of ispaghula 7g t.d.s. showed a change of T1 AUC 0-360 of mean (SD) 88 (55) sec.min . Using this data we calculate that n=15 healthy volunteers will give us >90% power to detect such a difference. Statistical analysis will be carried out using Prism 5 (GraphPad Software Inc.). All data will be tested for normality using the Shapiro-Wilk's Test. Two-way analysis of variance (ANOVA) will be used to assess the significance of differences over time, followed by post-hoc tests corrected for multiple-comparison. Paired comparisons will be performed using two-tailed Student's t test for normal, and two-tailed Wilcoxon's test for non-normal data. A p-value < 0.05 will be considered to be statistically significant.

Withdrawals
Any volunteer who wishes to withdraw from the study at any time is entitled to do so without giving any reason.

Good Clinical Practice:

The study will be performed according to Good Clinical Practice. As this is a non-invasive study in healthy volunteers, it will undergo submission through the University of Nottingham Research Ethics Committee, which meets monthly. All volunteers will give written informed consent. Their names will be entered into a subject log and a unique alpha numeric study code assigned. A Case Report Form will be kept for each volunteer on each study day.

Adverse events:

These are likely to be rare but will be noted and reported to the local Ethics Committee.

Significance of Findings

This study will show how kiwifriut alters the water distribution within the small and large intestine in healthy volunteers. It may point to the relative importance of large and small bowel secretion in the laxative effect observed. Comparison with the University's previous ispaghula data may provide information as to whether the additional components other than soluble fibre play a significant role.

Reference List

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(12)   Lam C, Chaddock G, Marciani L, Costigan C, Paul J, Cox E et al. Colonic response to laxative ingestion as assessed by MRI differs in constipated irritable bowel syndrome compared to functional constipation. Neurogastroenterol Motil 2016.

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Effect of kiwifruit on Gastrointestinal Fluid Distribution and transit in Healthy volunteers

Investigators: Dr. Luca Marciani, Dr Victoria Wilkinson-Smith, Dr. Giles Major, Dr. Caroline Hoad, Prof. Robin Spiller, Prof. Penny Gowland

Healthy Volunteer's Written Consent Form

Please initial the box for each statement

- I voluntarily agree to take part in this study.

- I confirm that I have been given a full explanation by the above named and that I have read and understand the information sheet given to me which is attached.

- I have been given the opportunity to ask questions and discuss the study with one of the above investigators or their deputies on all aspects of the study and have understood the advice and information given as a result.

- I agree to comply with the reasonable instructions of the supervising investigator and will notify him immediately of any unexpected unusual symptoms or deterioration of health.

- I authorise the investigators to disclose the results of my participation in the study but not my name.

- I understand that information about me recorded during the study will be kept in a secure database. If data is transferred to others it will be made anonymous. Data will be kept for at least 10 years after the end of the study.

- I understand that the image of my body may be used in future studies (approved by an Ethics Committee) beyond the scope of the study explained here. Any images used will be made anonymous, and I will not be identifiable.

- I understand that I can ask for further instructions or explanations at any time.

- I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing.

- I confirm that I have disclosed relevant medical information before the study.

- **Optional: Consent for storage and possible use in future research**
I agree that the data gathered about me can be stored by Prof Spiller's research team at the University of Nottingham for possible use in future studies. I understand that some of these studies may be carried out by researchers other than current team of Prof Spiller, who ran the first study, including researchers working for commercial companies. Any samples or data used will be anonymised, and I will not be identified in any way.

Please Turn Over   Please Turn Over   Please Turn Over

- I shall receive an inconvenience allowance of £100 for each study period, totalling £200 for the 2 study periods, plus a bonus of £50 on completion (grand total on completion £250). If I withdraw from the study for medical reasons not associated with the study I will receive an inconvenience allowance proportional to the length of the period of participation, but if I withdraw for any other reason, the inconvenience allowance to be received, if any, shall be at the discretion of the investigator.

- I have not been a subject in any other research study in the last three months which involved: taking a drug; being paid a disturbance allowance; having an invasive procedure (eg venepuncture >50ml, endoscopy) or exposure to ionising radiation.

Abnormal Findings in Scans
- I understand that the SPMIC is not a clinical diagnostic facility and so does not routinely inspect images for abnormalities. I understand that my MR scans will NOT routinely be reviewed by a radiologist (or any other medically qualified person) to look for any signs of disease, and it is unlikely that any abnormalities that may be present will be detected.

- On the other hand I understand that if one of the investigators should happen to notice something on my scan which they think is abnormal then they will show my scans to a medically qualified doctor who will contact me if further action is required.

- I understand that if an abnormality were detected on my scan it may affect my ability to get life insurance.

- I agree to my GP being informed of any medically relevant information gained from the study.

GP Name: _____ Telephone number: _____

Surgery Address: _____

Full Name: ………………………………………   **Telephone number:** …………………………..

Address: ……….

Signature: …………………………………….   **Date:** …………………………..

To be filled in by an Investigator
I confirm that I have fully explained the purpose of the study and what is involved to:

…………………………………………………………………………………………………
I have given the above named a copy of this form together with the information sheet.

Investigators Signature :   ……………………………   **Date**………………….

Name :   ……………………………………………………….

Ethics Code : ………...............   **Volunteer Number :** ………………..................….

Please return this form to the MR Centre receptionist for storage.