

University of Nottingham
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Mechanism of Constipation and Response to Diet: Novel Insights from Manometry and Magnetic Resonance Imaging

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Thesis submitted to the University of Nottingham

for the degree of Doctor of Philosophy

April 2020

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Acknowledgements

To begin I wish to thank my supervisors Robin Spiller, Maura Corsetti and Luca Marciani for their incredible support and guidance throughout the entire process. They managed to make the experience both educational and enjoyable. I am also grateful to the Medical Research Council, who funded the RECLAIM study and my three years in research.

I wish to acknowledge the hard work and skills, as well as practical help from the team at the Sir Peter Mansfield Imaging Centre particularly Caroline Hoad, Penny Gowland and Chris Bradley. I also wish to thank Mark Scott and Phil Dinning for their valuable contributions to the manometry studies.

I would like to thank all our nursing support from the Nottingham Biomedical Research Centre, in particular Helen Handford and Jill Garrett.

I must also express my gratitude for all the administrative support provided by Emma, Beth, Natalie and Lesley.

A very large thank you goes to Chris, for all his support through the ups and downs and providing many a cup of tea.

And finally, I am very grateful to all the participants who have kindly taken part in these studies, and without whom there would be nothing to say.

Candidate Statement

I confirm that the work described here is my own with the following exceptions:

Dr Caroline Hoad designed and created the MRI sequences and analysis programs used throughout the studies.

Dr Neele Dellschaft and Lucy Ashleigh (BMedSci student) assisted with the analysis of a proportion of the MRI data in the KIWI and LETIS studies, respectively.

Dr Phil Dinning and Dr Lukasz Wiklendt devised the programs for and subsequently performed the analysis of the manometry data for the RECLAIM Study. We were also supported by the endoscopy and radiology departments at Nottingham and QMUL to perform the manometry tests.

Helen Handford, Jill Garrett, Mark Scott and Laura Brockway were all invaluable in the recruitment and running of RECLAIM study days at the Nottingham and London sites.

Presentations and Publications arising from the work contained in the thesis:

Publications:

- Wilkinson-Smith V, Menys A, Bradley C, et al. The MRI Colonic Function Test: Reproducibility of the Macrogol Stimulus Challenge. Neurogastroenterol. Motil. (in-press, accepted 18 June 2020) DOI:10.1111/nmo.13942
- Wilkinson-Smith V, Dellschaft, N, Ansell, J, et al. Mechanisms underlying effects of kiwifruit on intestinal function shown by MRI in healthy volunteers. Aliment Pharmacol Ther. 2019; 49: 759– 768. https://doi.org/10.1111/apt.15127
- Wilkinson-Smith, V. C., Major, G., Ashleigh, L., Murray, K., Hoad, C. L., Marciani, L., Gowland, P. A., Spiller, R. C. (2018), Insights into the Different Effects of Food on Intestinal Secretion Using Magnetic Resonance Imaging. Journal of Parenteral and Enteral Nutrition, 42: 1342-1348. doi:10.1002/jpen.1157

Oral Presentations:

- FNM, Adelaide, 2020. *Young Investigator Award* High-resolution colonic manometry reveals postprandial

motility differences between constipation subtypes. (Delayed until 2021 due to COVID-19)

- BSG, Liverpool, 2020 (Delayed until 2021 due to COVID-19) MRI methods to define colonic function in health and constipation.
- FNM, Amsterdam, 2018 (PA-4C-002)
  Increase in small bowel water content and fluidity of ascending colon contents induced by kiwifruit is associated with more frequent looser stools in healthy volunteers: A mechanistic MRI study

Poster Presentations:

- FNM Adelaide, 2020
  The RECLAIM study: symptoms, bowel movements, psychological comorbidity and the balloon expulsion test confirm differences between IBS-C, FC and HV as defined by Rome IV criteria (Delayed due to COVID-19)
- FNM Adelaide, 2020
  Quantifying abnormal colonic function in constipation using MRI (Delayed due to COVID-19)
- FNM, Amsterdam, 2018 (P-09-10)
  Varying effects of bran, psyllium and prickly pear on small intestine water content and colonic volume demonstrated by MRI

- DDW, Washington DC, 2018 (Mo1053)

  Effect of kiwifruit on gastrointestinal symptoms: a mechanistic MRI study in healthy volunteers.
- DDW, Washington DC, 2018 (Tu1971) and ISMRM, Paris, 2018 (692)

  Assessment of colonic motility using magnetic resonance imaging: reproducibility of a macrogol challenge
- BSG, London, 2017 (2017-276) and NeuroGastro, Cork, 2017 (P-04-007)

  Title: In vivo assessment of foods that stimulate intestinal secretions using magnetic resonance imaging: Implications for dietary advice in ileostomy care

ABSTRACT

Constipation is a common condition in which an individual suffers with hard stools which are infrequent and difficult to pass. It has an estimated prevalence of 15-20% however the pathophysiology of symptoms remains poorly understood and treatment is often unsatisfactory for patients. In approximately 60% of patients their constipation is associated with abdominal pain, whilst in the remaining this appears in absence of pain.

This thesis is split into three sections, in the first I will review the background and current investigation and treatment strategies in constipation. The second will look at two healthy volunteer studies performed looking at the effect of different foods on gut function and how these could be relevant to treatment of constipation. In the third I will describe our patient study into the pathophysiology of constipation that take advantage of two new techniques. These are Magnetic Resonance Imaging (MRI), which allows us to see the contractions of the colon and High-Resolution Manometry (HRM) which measures the contraction using pressure sensing catheters placed into the bowel. Using these techniques, we will identify patterns of contractions in each patient and compare to healthy controls to determine whether these could be used to predict different treatment response

SECTION 1: INTRODUCTION AND BACKGROUND

Chapter 1: Overview of Constipation

Introduction

Constipation is a common gastrointestinal condition in which a person's bowel movements occur less frequently than usual and/or consist of hard, dry stools that are difficult to pass. It can be acute or chronic and is associated with significant morbidity worldwide[1].

Definition

Over time the definition of constipation has varied and clinically there has never been a single widely accepted definition. This is because there is often disagreement between the clinicians perspective and that of the patient, with up to 50% of the patient population defining constipation differently from accepted medical views[2]. 11% of people in a recent survey were unable to identify or did not know the symptoms of constipation[3].

It is traditionally defined by physicians based on stool frequency, most commonly quoted as fewer than 3 bowel movements per week[4].

Studies looking into patient perspective however show that the patient experience often encompasses a wider range of

symptoms which goes beyond frequency and includes stool consistency, straining, bloating, abdominal pain or discomfort and feelings of incomplete evacuation[5].

Epidemiology

Prevalence

A systematic review by Suares in 2011[6] found the global prevalence of chronic constipation was 14%, though the precise figure depends on whether this is by self-report where the prevalence is higher compared to whether specific criteria were used. There was slight variation by region with the highest at 18% in South America and lowest in South East Asia at 11%. This is in keeping with previous reviews such as Peppas et al, which found between 5 to 35% of the population in Europe are affected with a mean value of 17.1 % and median value 16.6%[7], and previous data from the US which quoted prevalence between 1.9% and 27.2% with an average of 14.8%[8].

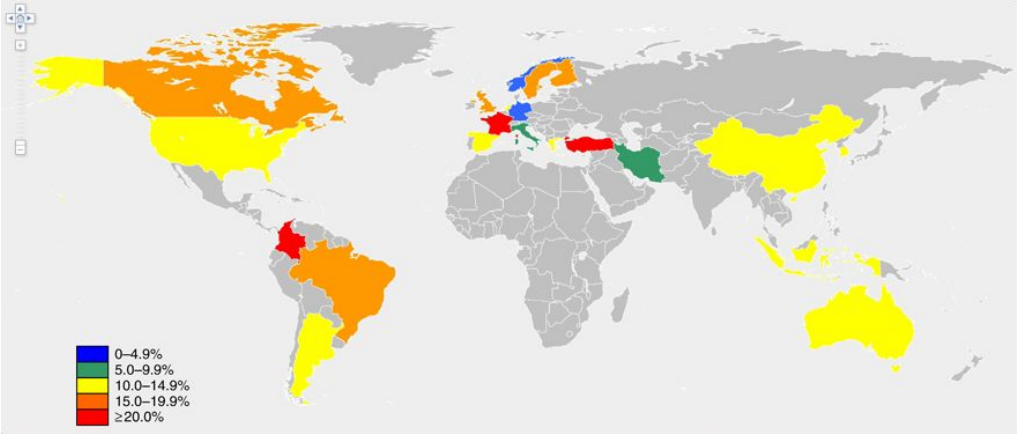


Figure 1: Map showing worldwide prevalence of constipation (from Peppas et al[7])

Risk factors

These reviews also looked at the various factors that had been identified as being associated with constipation. Prevalence is higher in women with a median male: female ratio of 1.58. Lower socio-economic class, increasing age and psychological factors such as anxiety and depression have also all been associated with higher levels of constipation[6,7]. The Nurse's health study in 2003[9] found that low exercise and low fibre intake were also risk factors for constipation in women.

Quality of life

Constipation has also been shown in multiple studies to have a significant negative impact on quality of life comparable to that seen with other chronic conditions such as diabetes, arthritis and chronic allergy[10].

Economic impact

A report published by Coloplast in 2019[11] found an estimated £162million was spent in 2017/18 on unplanned hospital admissions due to constipation in the UK. The total cost is likely to be much more as this does not consider GP consultations. However, it needs to also be noted that many admissions with patients having abdominal pain of unknown cause may be labelled as "constipation" without much evidence which will inflate the estimated cost of the condition. The prescription cost of laxatives was estimated at £91 million in the same period of time and again this is likely to be an under-representation as many people will also self-medicate with over the counter agents.

Gender Differences

Prevalence of constipation has been reported widely as being greater in women than in men. This is particularly in women of reproductive age, and is not an imbalance seen in the paediatric population, where male dominance is seen[12]. However, in young males this is more associated with stool withholding and is considered a behavioural/psychological problem rather than a functional colonic one.

Within irritable bowel syndrome subtypes, female predominance significantly increases with the severity of constipation relative to the severity of diarrhoea[13].

There are a number of different theories as to why women seem to be more affected by constipation than their male counterparts including prolonged transit, greater colonic capacity, and hormonal effects on motility.

Evidence that colonic transit times vary between men and women is conflicting, with some studies finding it is longer in women than men[14–20], however others find no difference between the genders[21–24]. Within these studies – both Jung[21] and Hinds[22] found transit time prolonged in women, but did not reach significance. Madsen[23] and Evans[24] both included post-menopausal women in their study population, which might diminish any effect of the menstrual cycle on transit.

There is also variable evidence as to whether colonic transit time varies within different stages of the menstrual cycle. Some studies have found colonic transit time is significantly prolonged in the luteal phase compared to the follicular stage[15,21,25], however others have found no statistically significant difference[14,26].

Considering the high prevalence of constipation in the later stages of pregnancy it has also been hypothesised that hormonal changes, such as increased progesterone, may have an impact. This is also supported by evidence that increased abdominal pain and GI disturbance (constipation/diarrhoea) occurs in some women during the late luteal and menses phases of the cycle relative to other phases.

It has been hypothesised that progesterone may affect gut wall motility. The precise mechanism is not fully understood however, following work on animals[27] and on colonic muscle cells in vitro[28,29] it is thought that the progesterone decreased muscle contractility by down-regulating G proteins which promote contractility and up-regulating inhibitory G proteins.

One study in the Mayo clinic[30] gave progesterone to post-menopausal women to attempt to study its effect, their results are somewhat contradictory, in that following progesterone transit time was shortened. However, their study had a small sample size and was likely under-powered and the study population does not include the group that suffers most from constipation, namely pre-menopausal woman.

A study looking at fasted colonic volumes using magnetic resonance imaging (MRI) methods found that it is larger in women than men, in both healthy and diseased bowel

(diarrhoea predominant irritable bowel syndrome)[31]. This factored in the participants height into a height-standardized regional colonic volume index (HSCV), found by dividing the colonic volumes by height in meters cubed. This appeared to be mostly due to greater ascending colon volumes in women. Studies of the ascending colon volume in constipation show it to be above the normal range in 48% of patients with functional constipation most of whom were female[32] but assessing any gender effect is difficult as most series of constipated patients include very few males.

Why this might be the case is unclear – a larger colon would allow increased water absorption – and historically, this may have been more favourable in women for childbearing, as a major cause of death worldwide would have been diarrhoeal illness. This is undoubtedly an area which requires further study.

Exercise

There is also evidence that decreased mobility/lack of exercise contributes to constipation, a study[33] looking at nursing home residents found that lack of mobility increased risk for constipation and faecal impaction, however such studies are often confounded often by other comorbidities and often painful conditions (e.g. arthritis/cancers) which are treated with

opioids. This is discussed later as one of the first lifestyle changes recommended in treatment.

Structure and Function of the Colon

In order to start to understand constipation, we must understand the normal structure and function of the colon and rectum.

Anatomy

The Colon

The colon is the largest section of the large intestine, at around 1.5m long it begins from the caecum at the ileocecal valve and ends at the rectum. It is divided into four sections: ascending, transverse, descending and sigmoid colon.

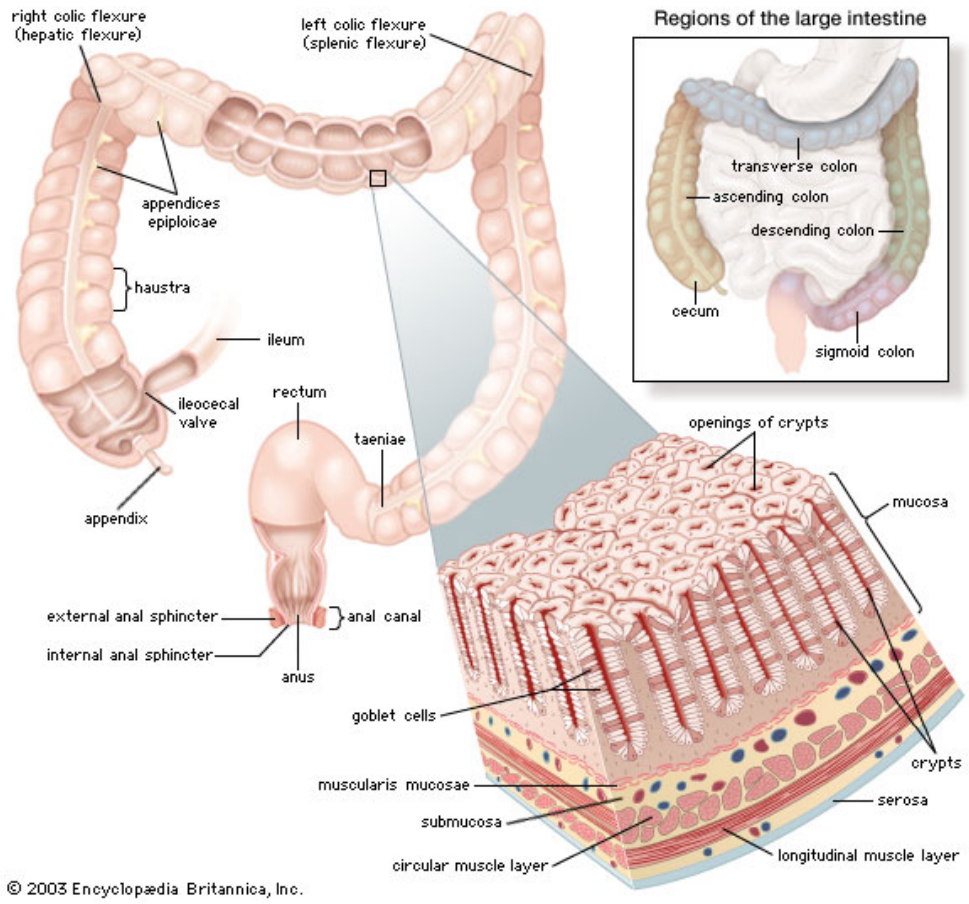


Figure 2: Large intestine: mucosa and musculature in humans. [Illustration]. Encyclopædia Britannica Online. Retrieved 02 May 2017, from https://www.britannica.com/science/large-intestine?oasmId=68639

The blood supply to the colon is by the superior and inferior mesenteric arteries through the right, middle and left colic branches and multiple sigmoid branches. The terminal branches of these arteries entering the colonic wall are called the vasa recta.

The nerve supply to the colon is mostly via the superior mesenteric plexus which provides the sympathetic, parasympathetic, and sensory supply to the ascending colon

and proximal 2/3 of the transverse colon, with some Vagus input to the parasympathetic action in the AC. The distal 1/3 of the transverse colon, descending colon and sigmoid colon receive their sympathetic, parasympathetic and sensory supply via nerves from the inferior mesenteric plexus.

The colon, much like the rest of the gastrointestinal system, consists of four layers: the mucosa, submucosa, muscularis propria, and serosa.

The mucosa contains a columnar epithelium with mucous-secreting goblet and enteroendocrine cells, lamina propria containing numerous immunocytes and mast cells together with the muscularis mucosa. The submucosa contains the blood vessels and nerve plexus. The muscularis propria contains the inner circular and outer longitudinal muscles and myenteric nerve plexus; teniae coli are formed by outer longitudinal muscles – these muscles contract to form the haustra. The taenia coli extend until sigmoid colon, at which point it fuses with the longitudinal layer. The serosa of the colon is visceral peritoneum.

Pelvic Floor

The pelvic floor is a funnel-shaped structure. It attaches to the walls of the lesser pelvis, separating the pelvic cavity from the

perineum inferiorly, extending from the pubic symphysis anteriorly to the coccyx within the pelvic sidewalls. It encloses and supports the pelvic viscera (bladder, uterus, and rectum) with two hiatuses: the urogenital hiatus – an anteriorly situated gap, which allows passage of the urethra (and the vagina in females) and the rectal hiatus – a centrally positioned gap, which allows passage of the anal canal. Between the urogenital hiatus and the anal canal lies a fibrous node known as the perineal body, which joins the pelvic floor to the perineum.

There is some controversy over the naming and arrangement of the pelvic floor that is mainly composed by the four components of the levator ani (the puborectalis, pubococcygeus, iliococcygeus and coccygeus) and by the fascia coverings/ surrounding connective tissue of the muscles. The puborectalis is a U-shaped sling muscle that is responsible for maintenance of the anorectal angle which helps preserve continence.

Anorectum

The rectum is a continuation of the sigmoid colon at the level of the sacrum. It ends in the anal canal, a 2.5-5cm long canal that is surrounded by the internal and external anal sphincters (IAS and EAS). The IAS is a smooth muscle structure, considered as a continuation of the circular layer of the rectal muscularis

propria, innervated by the autonomic nervous system. The EAS is a striated muscle that is innervated voluntarily via the pudendal nerve and it (as well as some of the puborectalis) is an extension of the longitudinal muscles of the rectum.

Function

The main function of the colon is to maximise extraction of water, salts and nutrients from food components which resist digestion in the small bowel along with desquamated cells and mucus. The colon reabsorbs salts and water while bacteria and fungi in the colon ferment undigested substances, producing various substances including short chain fatty acids – which stimulate sodium and water absorption as they are absorbed[34–36] as well as provide nutritional support for colonocytes. Strict anaerobic microenvironments are created for some of the microbes by poorly stirred layers compartmentalised by the haustra. Microbiota also deconjugate and dehydrogenate primary bile salts to produce secondary bile acids thereby promoting their reabsorption, about 15% of which occurs in the proximal colon. This creates faecal matter with a low water and salt content which then travels on to the rectum from where it is excreted.

The epithelium of the colon is well adapted to this, being able to both absorb and secrete. The net effect is the absorption of

water, electrolytes and it is thought to reabsorb around 1.5 litres of water each day[37]. The colon can accommodate a wide range of input and up to 5L of isotonic saline can be infused into the caecum per 24 hours without inducing diarrhoea[38]

Stool water content varies widely being normally around 70% but increasing up to 90% when diarrhoea is experienced, and as low as 30% in constipation. The water content is important for continence and ease of defecation and an imbalance here can cause either diarrhoea (too much water) or constipation (too dry). The relationship between stool water and viscosity is non-linear with a critical water content (around 80%) at which point further increases in water content lead to rapidly falling viscosity[39]. The impact that the motility in the colon has on water content is discussed in more detail later but as movement of anything across the epithelium can only occur with direct contact, the time contents spend in the colon, and the amount of mixing that occurs are likely to influence the amount of water that can be absorbed and therefore the consistency of faecal matter produced.

Maintenance of normal defecation

Normal defecation and maintenance of continence is a complex process requiring rectal distention by colonic load, the perception of the distension and the discrimination of the nature

of the rectal contents and the ability to subsequently propel the stool by relaxing the pelvic floor muscles and anal sphincters in a coordinated sequence of events or to postpone defecation if socially inconvenient[40].

Stool is propelled into rectum from the sigmoid colon by colonic propulsive contractions. Once there, the rectal distension evokes a rectal contraction and the IAS relaxes. This allows rectal contents to contact the epithelial nerve endings in the anal mucosa and lets us know what type of contents are present. Then a conscious decision occurs about how and whether to evacuate the rectal contents. It is at this point that voluntary actions come into play with control of the EAS, so if defecation is inconvenient it can be postponed. On the other hand, if defecation can occur, voluntary initiation of defecation involves both relaxation of pelvic floor muscles (puborectalis and pubococcygeus) and of the EAS so that the pelvic floor descends, and anal canal opens. On top of this, abdominal contractions aid expulsion from rectum and further colonic contractions will push more stool down into the rectum so the entire left colon may be emptied. At the end of this complex process, the subject is more or less satisfied that rectal emptying has occurred.

Constipation Classification

Primary vs secondary

Constipation can be split into that caused by an underlying or co-existing condition (secondary) or that for which no organic cause can be found (primary).

Common causes of secondary constipation include:

- primary diseases of the colon such as a stricture, cancer, anal fissure, inflammatory bowel disease
- neurological disorders (parkinsonism, multiple sclerosis, spinal cord lesion)
- metabolic disturbances (hypercalcaemia, hypothyroidism, diabetes mellitus)
- medications (opiates, antispasmodics, tricyclic antidepressants and many more)

Primary constipation is further divided into Functional Constipation (FC) and Irritable Bowel Syndrome Constipation Predominant (IBS-C). These are the disorders that this thesis will focus on.

Functional constipation

According to the Rome IV criteria FC is defined as the presence of two or more of the following during the previous 3 months:

a) defecatory straining (≥ 25% bowel movements); b) hard or lumpy stools (≥ 25% bowel movements); c) a feeling of incomplete evacuation (≥ 25% bowel movements); d) defecatory obstruction (≥ 25% bowel movements); e) manual manoeuvres to facilitate defecation (≥ 25% bowel movements); and f) fewer than 3 spontaneous complete bowel movements per week. Symptoms must be present for at least 6 months before the diagnosis, diarrhoea must not be present except after using a laxative, and IBS criteria must not be met[41].

IBS – C

According to the Rome IV criteria, IBS is diagnosed based on the presence of recurrent abdominal pain, which must be present for at least one day weekly, with two or more of the following characteristics: a) it is associated with defecation; b) it relates to a change in bowel movement frequency; and c) it is linked to a change in stool consistency as assessed by Bristol Stool Form. This change in consistency is what is then used to subtype the condition (those with predominantly a change towards harder stools or lack of bowel motions are constipation type, those with a change towards watery and more frequent stools are diarrhoea typed). These criteria must have been met

for the past three months, and symptoms must have had their onset at least six months prior to the diagnosis[41].

Separate conditions or spectrum?

Functional Constipation (FC) and Irritable Bowel Syndrome with Constipation (IBS-C) are both considered as functional bowel disorders (FBDs) and the most commonly used definitions are found in the Rome Criteria as described above. The key difference between them is whether the constipation is associated with pain. The two conditions often overlap in both presentation and treatment. For this reason, the Rome IV committee described the two conditions as lying on a spectrum. There are patients suffering constipation and severe abdominal pain at one end (IBS-C), and patients with constipation and no pain at the other end (FC) because in practice, most patients fall somewhere in between[42–44].

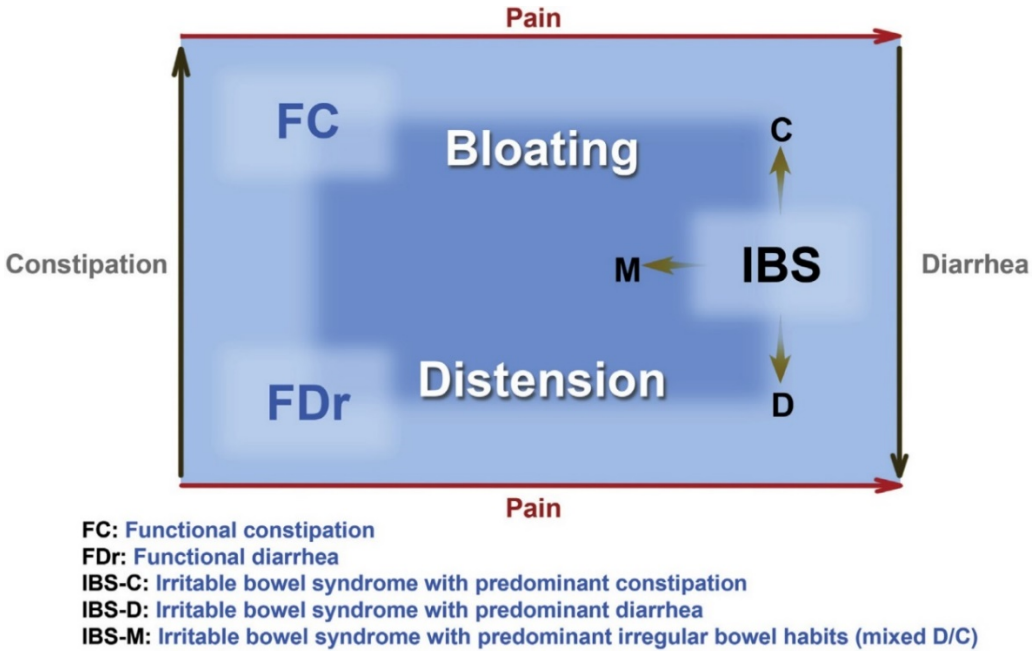


Figure 3: Conceptual framework to explain Functional Bowel Disorders. From Lacy et al[41]

This primary constipation (whether FC or IBS-C) has traditionally been further classified into three categories based on elements of the pathophysiology. These are those with normal transit, prolonged transit and those with a defecatory disorder, or obstructed defecation as it is sometimes referred. The methods used to differentiate normal and prolonged/slow transit will be discussed later.

Where does obstructed defecation fit in?

Obstructed defecation or defecation disorder describes a patient group who may or may not have other motility issues but do have some form of impairment of rectal emptying. This may be from inadequate rectal propulsion or abnormal relaxation of the muscles responsible for opening the anal canal.

This can then be associated with and may result in diminished rectal sensitivity and structural pelvic floor defects such as rectocele, enterocele or intussusception.

It is defined within the Rome IV criteria as: features of impaired evacuation during repeated attempts to defecate as demonstrated by 2 of the following 3 tests: a. Abnormal balloon expulsion test (BET), b. Abnormal anorectal evacuation pattern with manometry or anal surface EMG, c. Impaired rectal evacuation by imaging. The patient must also satisfy diagnostic criteria for functional constipation and/or irritable bowel syndrome with constipation. These criteria need to be fulfilled for the last 3 months, with symptom onset at least 6 months before diagnosis[45].

The current problem with defining this disorder is how we measure it. As there is no current gold standard, the Rome criteria require two out of three from the balloon test, manometry, and pelvic imaging as part of the diagnostic work-up. However, a study in 2004 found that 30% of patients who reported symptoms suggestive of difficult defecation had normal findings on balloon test and manometry[46]. In other studies, around 25% of healthy subjects have been found to have results in these tests that could be considered abnormal[47,48] and one study found up to 90%[49]. A review in

2016 of 13 studies looking at the BET found that it had a sensitivity of 67% and a specificity of 80%[50]. Equally the agreement between the three tests is poor – with the best agreement being "fair" (kappa of 0.27) between BET and proctography[51].

A recent consensus paper[52] has been published looking at current diagnostic tests of the anorectum and their utility in clinical practice and has put forward some standardisation procedures and interpretation guidance. However, this also highlights the current variability in both the performance and interpretation. It is also not possible at present to determine whether the condition is caused by co-existing underlying colonic dysfunction or just defecatory disorder alone, and equally if some defecatory disorders are simply present because the stool is far too hard. It has also been shown that the smaller the stool the more challenging it is, for even healthy people, to evacuate and so severe constipation with small, hard stools is likely to also be associated with difficulty in expulsion anyway[53].

The Role of the Microbiota

Background

The microbiota refers to the community of microbes that inhabit the human body. It is complex and thought to consist of

approximately the same order of bacterial cells as the number of human cells at around with estimates for the number of human and bacterial cells at $3.0x10^{13}$ and $3.8x10^{13}$ respecitvely[54]. The greatest proportion of these are found within the GI tract. Previously it had been described as a commensal relationship however it is now being recognised as more mutual whereby there are benefits to both the host cells and the bacteria[55].

The microbiota is described in traditional taxonomic nomenclature (phylum, class, order, family, genus, and species) and there are more than 2000 bacterial species classified into 12 phyla described within the GI tract[56]. The composition has been seen to vary at different sites along the GI tract with three predominant phyla in the colon: Bacteroidetes, Firmicutes and Actinobacteria, with each comprising a number of orders, families, genera, and species.

Proposed mechanisms of action

With such a large population of bacteria a diverse ecosystem is present. Like any other ecosystem a vast number of factors determine its makeup and a change in these can have an impact. Equally changes in the microbiota have the potential to effect change in the gut physiology. The directionality of this is

uncertain, most human studies that have been done are descriptive and cannot distinguish cause from effect.

Microbiota affecting the Physiology?

The exact mechanisms by which the microbiota may affect the gut physiology are not yet fully understood, however there are a number of proposed mechanisms.

Barbara et al[57] proposed three of these. First the effects of bacterial substances which are released and/or the end products of bacterial fermentation, second the neuroendocrine factors in the intestine and thirdly the effects of immune mediators released within the GI tract.

Physiology affecting the Microbiota?

There is clearly a complex interplay between the diet, GI transit and the microbiota. The diagram below from Kashyap et al[58] shows a model outlining these interactions. Their study published in 2014 looked at mice, both germ-free and humanised and exposed them to a number of different environments, changing dietary factors and gut transit and recording the effect on the microbiota.

There is also a relationship observed in humans between stool consistency and microbiota diversity. A 2016 study found a negative correlation (Spearman's $r=-0.45$, $p=0.0007$) between

higher Bristol stool form (looser stool suggesting faster transit) and the species richness observed in healthy female controls[59].

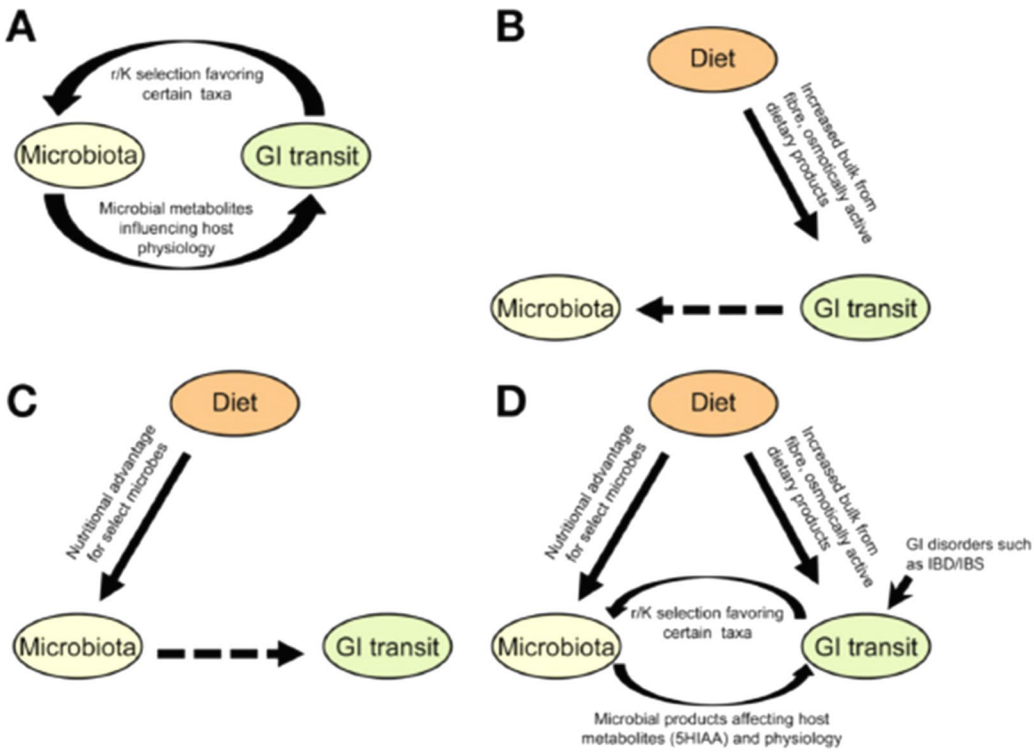


Figure 4: Model for interactions between diet, gut microbiota and GI transit time. From Kashyap et al[58]

Methane

Methane is a gas produced by anaerobic bacteria in the colon which has been linked to constipation. A meta-analysis by Kunkel in 2011[60] reviewed 9 studies that had investigated methane production in IBS and FC. They found that there is

significant association between constipation (both FC and IBS-C) and increased methane production.

They also looked at studies assessing colonic transit time and methane production, again found a significant relationship in those patients who produced methane having delayed transit. It is unclear however if the link is causative (methane directly or indirectly causes the slow transit) or whether methanogens are slower to grow and need a slow transit to flourish. In the 2016 consistency and microbiota study[59], more methanogens were seen in the healthy participants with harder stools.

Current Findings in Constipation

Studies in patients with functional constipation and IBS-C have shown that the microbial composition of the colon is different compared to both healthy controls and other IBS subtypes[61–64].

These studies primarily looked at the faecal microbiota, whereas more recent works have also started to examine the mucosal microbiota as well.

Parthasarathy et al[65] conducted a study in 2016 looking at both the mucosal and faecal microbiota, as well as other measures such as diet and transit time in patients with constipation (mixed FC/IBS-C) and healthy controls. They found that the mucosal microbiota could discriminate between constipation

and healthy control with an accuracy of 94%. This was independent of diet and colonic transit. In contrast, the faecal microbiota was associated with colonic transit and breath methane production but not constipation.

With this in mind there has been work looking into whether altering the microbiota, via changes to diet, probiotics or faecal microbiota transplantation, might be used as treatment in constipation. This has found some success – recently summarised in a review by Zhao and Yu[64] and briefly discussed in the next chapter on treatment strategies.

Behaviour and Psychology

It is established that some psychological factors are associated with constipation such as anxiety and depression. One review study found that 27% of patients with major depression suffered with constipation[66]. The reasons behind this are less well established, however.

There are also other associations. For example, in a few studies, links between anorexia nervosa and constipation have been seen. In one such study 69% of patients eventually diagnosed with anorexia in one centre had initially presented with symptoms of constipation[67] and in another around a quarter of

patients who presented with anorexia suffered with functional constipation[68].

Within the groups of slow and normal transit constipation (discussed in more detail later) those with normal transit have been found to have higher scores for psychological distress than those with slow transit and control subjects[69].

There has also been a lot of work in paediatrics as faecal incontinence and constipation in children is common and the majority of cases are thought to be functional in nature. Faecal incontinence is often associated with constipation[70]. One behaviour that can develop is stool withholding. Whilst this is well described in paediatrics, its role in adults is anecdotal. It may not be deliberate stool withholding but certainly factors such as ease of access to toileting facilities and hectic work schedules may influence an individual's stool habit.

In the past functional constipation in children was attributed to underlying psychological cause however studies have not shown this to be the case. Nevertheless it is clear that having problems with defecation can cause subsequent behavioural difficulty, which resolves on treatment of faecal incontinence[12].

It has been observed that between a quarter to one third of children who suffer will continue to experience symptoms in

adulthood[71,72] and this poor outcome was significantly associated with older age at onset and delay to specialist care[71]. Therefore, in initial adult assessment it may be useful to ascertain duration and onset of symptoms and ask about any issues in childhood.

Another aspect of childhood that needs to be considered is abuse. Childhood abuse has been associated with onset of IBS in adulthood. A review by Sansone[73] in 2015 looked at the studies examining relationships between different types of abuse and onset of IBS. They found a relationship between childhood sexual abuse and IBS but results for emotional or physical abuse were inconsistent.

With regard to constipation (diagnosed by Rome III) on its own, there is evidence that again, childhood abuse shows a significant association, and this is for all types of abuse. Equally children with constipation who have a history of abuse have more somatic symptoms and more severe bowel symptoms[74].

There is therefore a strong argument for the inclusion of psychological assessment in all patients who present and need for consideration of different therapeutic approach if it is thought there is a psychological element present.

Chapter 2: Current Treatment Strategies in Constipation

A review article was published based in part on some of this chapter in 2018[75]

Aims and Endpoints

Considering there is such an overlap and lack of clarity in definitions, determining an appropriate endpoint for treatment also proves challenging. Some patients may be focussed on number of bowel movements, some on consistency and some on pain, or a mixture of these and/or other personal parameters. It is therefore a necessity that this be discussed between the patient and clinician and expectations laid out from the start. Equally, research study endpoints may not necessarily correlate with what the patient might consider to be a satisfactory response, and this should be considered when evaluating the efficacy data of different laxatives in order to choose an appropriate therapy for the individual.

Lifestyle Measures

For patients at first presentation, lifestyle changes may be recommended as the first step. These are mainly changes to diet with advice to increase fibre and fluids and eat more regular

meals as patients with FGIDs are more likely to skip breakfast and lunch[76]. Patients can also be recommended where practicable to increase exercise as low levels of exercise have been shown in the Nurses' Health Study to be associated with constipation[9] and has been shown to benefit unselected IBS in clinical trials[77]. Possible causes of the constipation such as other prescribed or OTC medications should also be reviewed. Toileting habits can also be reviewed, and patients given advice about this.

In patients with chronic constipation (both FC and IBS-C), evidence supports the recommendation of increasing fibre intake but there is conflicting evidence regarding whether increased fluid intake and/or exercise do have a significant benefit[78]. They are still both generally recommended for their wider health benefits and the risk of harm is low.

Psychological Measures

There is likely a role for some psychological interventions, particularly with the IBS-type patients. CBT has been shown to be effective for improvement of GI symptoms[79,80] and is currently recommended by NICE for refractory IBS[81]. A recent study also found that telephone/web-based CBT was effective and this would be much more practical than face-to-face sessions[80]. Other psychotherapies that have been assessed and

shown moderate improvements are hypnotherapy and relaxation therapy, however they show greater improvement in mental health and daily functioning than GI symptoms[79,82].

Medication

If lifestyle measures have proved unsuccessful then the next step is laxative use. There are several different types of laxative that work in different ways but the end aim being to promote the bowel movement. Several types of laxatives exist however each works somewhat differently. Examples of laxatives include: Fibre supplements/Bulk forming laxatives, Stimulants, Osmotic, Lubricants and Stool softeners. According to NICE[83], following lifestyle modification, initial treatment should be with a bulk-forming laxative, if stools remain hard or difficult to pass one should add or switch to an osmotic laxative, such as a macrogol and if stools are soft but difficult to pass or there is a sensation of inadequate emptying, add a stimulant laxative. Dosage and combinations however are very much led by the patient's responses.

Fibre and Bulk-Forming Laxatives

A diet rich in fibre is recommended to relieve constipation and supplements can assist with achieving recommended amounts. Fibre is most often considered as water-soluble or insoluble.

Dietary sources of soluble fibre include fruits and beans whereas insoluble is found more in wheat bran, green vegetables, brown rice, and cereals. Soluble fibres are thought to help by trapping water in the stool whereas insoluble fibres increase faecal mass and stimulate peristalsis. There is also a possible effect on the microbiota which may in turn influence transit and stool characteristics. Fibre amounts should be increased slowly, as a sudden increase, particularly in insoluble fibre, can worsen symptoms such as abdominal pain and distention despite improving defecation.

Soluble fibre has a stronger evidence base than insoluble. A systematic review and meta-analysis published in 2016[8484] found 7 RCTs using fibre supplementation in chronic constipation with 6 looking at soluble fibre. They found that overall, in the 4 papers that looked at response to therapy by symptomatic improvement, 77% of patients responded to fibre vs 44% on placebo, with a suggested number needed to treat (NNT) of 3 (however this was under-powered). Fibre supplementation also increased stool frequency (measured in 6 papers) when compared to placebo with a standardised mean difference of 0.39 (95% CI 0.03–0.76; P = 0.03) and softened stool consistency (measured in 5 studies) compared with placebo with a SMD of 0.35 (95% CI 0.04–0.65; P = 0.02). The strongest effects were seen in psyllium supplementation, a

soluble fibre. Side effects however were also moderately increased with the fibre arm, with flatulence and bloating significantly increased.

A systematic review in 2015[85] also looked at increased fibre (both by supplements and whole food dietary interventions) in both chronic constipation and IBS-C with 8 studies in CC and 3 in IBS-C. This study also concluded that soluble and mixed fibres had benefits in both cases, despite significant limitations due to the heterogeneity of studies as well as short durations and lack of consistent endpoints.

Osmotic

Osmotic laxatives, such as lactulose and polyethylene glycol, contain non-absorbable ions or molecules that retain water in the bowel lumen, thus increasing the water content of faeces and making them easier to pass. However, they should be accompanied with plenty of fluid as otherwise these can cause dehydration. A systematic review with meta-analysis in 2010[86] of polyethylene glycol in chronic constipation found it resulted in significant increases in defaecations per week over placebo (all studies: additional 1.98 stools/week; p = 0.0003, high-quality studies: additional 2.34 stools/week; p=0.0001) and was also superior to lactulose (all studies: additional 1 stool/week; p=0.0017, high-quality studies: additional 1.65

stools/week; p=0.021). A Cochrane review[87] in the same year also came the same conclusion that polyethylene glycol was superior to lactulose with increased stool frequency per week (mean difference 0.65 [95% CI 0.15 to 1.15]). It was also found to be more cost effective compared to lactulose[88]. This may reflect the fact that lactulose is fermented to SCFAs which are absorbed and thus removed from the colon whereas PEG remains unaltered in the colon.

Stimulants

Stimulant laxatives such as senna, bisacodyl and sodium picosulfate act by inducing colonic peristalsis or by causing water or electrolyte secretion in the colon. Bisacodyl and picosulfate are both metabolised to the active agent BHPM

(Bis-(p-hydroxyphenyl)-pyridyl-2-methane) however picosulfate is thought to require intestinal bacteria to assist with metabolisation whereas endogenous enzymes metabolise bisacodyl[89].

They are more effective in treating patients with constipation than those with IBS-C as they can worsen abdominal pains. A review in 2017[90] looked at the evidence for bisacodyl and sodium picosulfate and found that both were effective with a good quality of evidence. They determined a pooled relative risk

of 2.46 (95% CI 1.14 to 5.31) for bisacodyl and 2.83 (95% CI 1.27 to 6.31) for picosulfate when considering responders who had ≥3 CSBMs/week with the drug.

Lubricants & Softeners

These substances, such as docusate, act by decreasing surface tension and increasing penetration of intestinal fluid into the faecal mass. These are less commonly prescribed alone, and are often in combination with other classes, and are more widely used in opioid-induced constipation[83]. A review of the evidence around docusate in 2014[91] found it was not effective versus placebo in the hospital or long term care setting, and there was no high quality evidence to support its use.

Relief of associated symptoms

Pain relief proves challenging as opioid analgesics have a constipating effect, and so should be avoided, as there is risk of increasing constipation and pain leading to increased consumption of analgesics in a vicious cycle which will be difficult to break. If a patient's main concern has been abdominal pain, then antispasmodics or non-constipating doses of tricyclics are better options.

Other symptoms such as cramping and bloating are difficult to treat by themselves and they would be expected to resolve with

regular bowel opening. Often, due to the overlap with IBS-C, drugs used in this condition (such as anti-spasmodics and low dose antidepressants[81]) may be of some benefit.

New Agents

Newer medications are available for patients with constipation who have tried and failed on laxatives (NICE recommends at least two laxatives from different classes have been tried at the highest tolerated recommended doses for at least 6 months and failed to relieve symptoms). These are Lubiprostone and Linaclotide in IBS-C[9293] and Prucalopride in FC [94].

Lubiprostone is a member of a novel class of bicyclic fatty acids called prostones. It is derived from prostaglandin E1 and is a chloride channel 2 activator. It works by increasing fluid secretion in the intestine, which increases intestinal muscle movement and helps passage of stool. Scintigraphy studies show it accelerates both small and large bowel transit suggesting it stimulates both small and a large bowel secretion[95]. It also inhibits colonic tone in man and in experimental animals it causes circular muscle relaxation which may allow faster transit. Unfortunately, due to marketing issues, it is currently not available in Europe.

Prucalopride is a selective serotonin (5-HT4) receptor agonist that predominantly stimulates propulsive colonic contractions by acting pre-synaptically to increase acetyl choline release.

Linaclotide is a guanylate cyclase-C receptor agonist (GCCA), an analogue of the heat stable secretory toxin of *E coli* that activates the CFTR chloride channel increasing intestinal fluid secretion and accelerated intestinal transit and also causes extracellular release of GC which has been suggested to mediate the decreased visceral pain in some animal models of colonic distension[96].

In clinical trials, all these agents were better than placebo. In chronic constipation, NNT ranged from 4 (95% CI 3-7) for Lubiprostone to 6 (5-8), for linaclotide and 6 (5-9) for prucalopride[97]. As expected for an endpoint including pain in IBS-C, the NNT was much higher being 13 for Lubiprostone and 7 for linaclotide (5-8). This likely reflects the higher placebo response and greater variability for such a subjective and complex endpoint as pain.

Prucalopride has not been studied in IBS-C but being a stimulant might possibly increase pain given the visceral hypersensitivity which is such a key feature of IBS. Of note, the NNT for polyethylene glycol in chronic constipation is 3 (2-4) for an endpoint of increased bowel movement though this does not

necessarily translate into patient satisfaction especially if it increases bloating and distension. Since placebo, not over-the-counter laxatives, were the comparator in all studies, head-to-head comparisons of these drugs versus simple laxatives are unavailable. Finally, failure to respond to over-the-counter laxatives was an eligibility criterion for studies with prucalopride but not for studies with linaclotide. Hence, the incremental utility of these agents over traditional laxatives is unknown.

It is also important to note that there are currently no standard agreed endpoints that IBS-C or constipation trials use, due to the heterogeneity of the conditions. The US Food and Drug Administration and European Medicines Agency both developed separate guidance which have differing ideas of what the most important symptoms are to consider (between overall degree-of relief vs abdominal pain vs stool frequency) and therefore what a clinically significant difference may be, and they are all based on subjective patient rated symptom severity scores[98]. This presents a challenge both for clinicians designing trials as well as those examining evidence to best treat their patients.

Microbiota manipulation

Recently there has been a surge of interest in how the microbiota could impact patient symptoms, as briefly addressed in the first chapter. It has therefore been proposed that

alteration to this could provide another treatment option, either in the form of probiotic type therapy or FMT (faecal microbiota transplant). Data here are still limited as many of the trials are inadequately powered and or poor quality however there are some promising results with probiotics in both FC and IBS-C. There are important differences between different probiotics and exactly which strains/species are most effective in which combinations remains unclear, however *Lactobacillus* or *Bifidobacterium* appear somewhat beneficial in some studies[99,100].

The evidence for FMT is more limited still, with a handful of small studies reporting improvement in IBS symptoms[101], however other studies showing that despite a change in the microbiota, symptoms were not improved (and in fact the placebo group did better)[102]. Also, a majority of placebo controlled studies currently ongoing (including those currently registered on clinicaltrials.gov) include only patients with diarrhoea predominant IBS, or do not specifically stratify[101]. A recent European consensus[103] concluded that there is currently insufficient evidence to support FMT use in IBS/constipation outside of research.

Patient Satisfaction

There is limited data available on general patient satisfaction to treatment, however that which is available suggests that despite all these options, optimal therapeutic outcome is not guaranteed. A study in 2007[5] found that in FC (Rome III) patients 47% were not completely satisfied with their current treatment. Most (82%) of this was efficacy related: including "does not work well" (39%), "inconsistent results" (25%), "continuous dosage" (7%), "still requires laxative use" (3%), "not fast acting" (1%) and "does not treat infrequent bowel movements" (2%). It is conceivable that some of this could be improved by better communication (for example managing the expectation that this will potentially be a long term therapy not a quick fix from the start, and double checking compliance) however it simply just not seeming to work is relatively common. This is supported by another more recent study in 2012[104], which reported 28% of patients on laxative were "very satisfied" or "satisfied" with their treatment, 44% were "neutral" and 28% were "dissatisfied" or "very dissatisfied" with their treatment. This study however used patients self-reported symptoms of constipation rather than a physician/questionnaire-based diagnosis such as ROME.

In the IBS subgroup this is also seen, with a recent survey in the US of Rome IV confirmed IBS-C[105] finding that 66% and 63% were not satisfied with over-the-counter or prescription treatment, respectively. And primarily in this case cited "inadequate efficacy" (55%) and "side effects" (39%) as most common reasons.

Defecatory Disorders

Defecatory disorders (DD), which are diagnosed by symptoms of CC or IBS and abnormal anorectal tests, are appropriately managed with pelvic floor biofeedback therapy. It can be challenging to diagnose defecatory disorders in some patients because the results of anorectal tests may be equivocal. The proportions of patients with slow colon transit and DD varies among series[106,107] and they frequently overlap. Some constipated patients also have visceral hypersensitivity, which is more common in IBS-C than CC[108], however hyposensitivity may also occur[109].

While pelvic floor biofeedback therapy is beneficial for defecatory disorders, the technique is operator dependent and not widely available. Indeed, even in a clinical trial from an experienced centre, the response rate was only 61%[110].

Options for refractory constipation

Refractory constipation is an inadequate improvement in constipation symptoms evaluated with an objective scale, despite adequate therapy (i.e., pharmacological or behavioural), which is guided by the underlying pathophysiology, also including combinations of different drugs. A practical definition of adequate duration is a minimum of 4 weeks for each drug and 3 months for pelvic floor biofeedback therapy. The clinical decision-making tool proposed by an international consensus statement provides a simple and effective approach for evaluating the response to therapy[111]. The 4 week criterion is underpinned by the recognition that most patients who response to medications for constipation generally do so within 4 weeks[112] and this is also reflected in some of the NICE guidance[81].

Whilst the pharmacological options for chronic constipation have expanded over the past few years, a substantial proportion of constipated patients do not respond to these medications. Minimally invasive (i.e., rectal irrigation and percutaneous endoscopic colostomy) and surgical therapies are used to manage refractory constipation.

Rectal irrigation

Rectal irrigation involves the instillation of water into the rectum to facilitate contraction and therefore emptying of the rectum and sigmoid colon. The volume of water used varies (up to 1L) and there are a variety of devices that can be used to achieve this[113]. The majority of its use so far has been in the management of neurogenic bowel dysfunction[114–116], however it has also been studied in patients with non-neurological chronic constipation (CC) and faecal incontinence[117]. It is increasingly used in units that manage patients with CC.

A recent systematic review and meta-analysis of TAI in CC[118] identified seven studies including a total of 254 CC patients. All studies were uncontrolled and all but two were retrospective. The proportion of patients reporting a positive outcome of therapy varied from 30% to 65%.

Since that review, another large retrospective report[119] in which outcome questionnaires were available in 102 of 148 consecutive patients has been published. The patients reported 21,476 irrigations over 119 patient years, with a mean duration of therapy use of 60.5 weeks. The proportion of patients in whom symptoms improved were as follows: general well-being (65%), rectal clearance (63%), bloating (49%), abdominal pain (48%), and bowel frequency (42%). When asked about overall

satisfaction, 67% showed improvement, with 39% reporting they were "moderately better" and 28% "very much better". However, baseline characteristics (i.e., age, duration of constipation, proctographic findings of obstructive defaecation, and colonic transit time) did not predict the response to TAI. These studies however used different types of equipment, which may affect the response.

TAI is contra-indicated in early pregnancy and where there is an increased risk of perforation (e.g., colitis, cancer, recent resection). Rectal perforation is uncommon (i.e., approximately 1 in 500,000 irrigations) and has been mostly reported in in bedridden patients[120,121]. Over 50% of patients who discontinue therapy do so because they find it too inconvenient or because they experience technical difficulties (e.g., water leakage, catheter expulsion, a burst balloon, and peri-anal discomfort)[122].

While TAI is relatively expensive per procedure (a "Peristeen" system for example costs around £80 with a consumable pack of 15 catheters at £130), it reduces the overall cost of care and is cost-effective in patients with spinal cord injury and constipation[123], with a recent model showing savings of £2,867 per patient over nearly 40 years[124]. It has recently been approved for use in the UK in patients with bowel dysfunction

(including non-neurogenic causes) by NICE with recent guidance issued in 2018. At present this is only available with specialist support, as it needs initial prescription by specialist teams and nurse-led training in use of the system prior to commencement, as well as a support system whilst use is ongoing[124].

Surgery and sacral nerve stimulation

Subtotal colectomy with ileorectal anastomosis is an option for the rare patient with slow transit constipation that is unresponsive to pharmacological and behavioural approaches.

A recent systematic review of the main procedures for constipation was performed in 2017[125–131]. They concluded that the evidence itself is very poor. In 113/156 (72.4%) studies, the evidence was rated as level IV; only 4 level I RCTs were included[125,131]. However in keeping with the experience of many now abandoned surgical procedures the 40 observational studies provide an unduly positive view, with a global satisfaction rate of 86% after colectomy for slow-transit constipation[131]. In contrast a recent, large, US retrospective cohort study of over 2000 patients[132] reported high complication rates and greater long-term post-procedural health utilization (ambulatory care, hospital admissions, radiology etc.) after surgery.

This evidence was used by an expert consensus panel to create a series of graded practice recommendations which address patient selection, procedural considerations and patient counselling[127].

There is therefore need for caution when recommending surgery for chronic constipation and careful patient selection and counselling beforehand.

The beneficial outcomes of sacral neuromodulation for CC, previously based on observational data, have been refuted by 2 subsequent RCTs that both show no benefit of this procedure over sham stimulation[133,134].

The role of Percutaneous Endoscopic Colostomy

Percutaneous Endoscopic Colostomy (PEC) is a variation of the percutaneous endoscopic gastrostomy technique. First proposed in 1986 by Ponsky et al.[135], it is currently used as a less invasive alternative to surgery for patients suffering with sigmoid volvulus (SV) or chronic intestinal pseudo-obstruction (CIPO). More recently, is also used to manage patients with chronic constipation.

The procedure itself involves the insertion of a tube through the abdominal wall into the colon, assisted by colonoscopy. The tube is used to provide antegrade colonic irrigation and lavage.

This is similar in theory to the Malone antegrade continence enema (MACE) procedure, commonly performed in patients with faecal incontinence.

The evidence for PEC is limited to retrospective reports and, like the surgical literature, data on the efficacy of PEC as well as long term outcomes are limited.

While newer pharmacological options and pelvic floor biofeedback therapy are effective for chronic constipation, a substantial proportion of patients are refractory to these therapies. A majority of patients report high global satisfaction rates after colectomy for slow transit constipation. However, few studies of colectomy, and other options, including PEC and rectal irrigation, used rigorous validated outcomes. Indeed, the evidence for PEC and rectal irrigation is mostly based on retrospective case reports.

Conclusions

There are a wide range of options currently available for treatment of constipation, however a significant proportion of patients remain dissatisfied with treatment, whether this is due to ineffective therapy or ineffective management of expectations is unclear. However, the fact remains that work needs to be done to improve patient care in this field, and this

is likely by a combination of better diagnosis and potentially treatment stratification as well as communication.

Chapter 3: Measuring colonic physiology

The work in this thesis uses relatively novel modalities of MRI and high-resolution manometry to assess intestinal and colonic physiology. In this chapter I will summarise the technological basis and methodology for these and review current findings in constipation.

Magnetic Resonance Imaging

The scientific basis behind MRI comes down to the fact that within the human body there are many billions of hydrogen atoms –found within water and fat molecules. These hydrogen atoms contain a single proton and a single electron. These protons are able to receive and transmit back radiofrequency (RF) signals. These protons are constantly 'spinning' and can be thought of like spinning tops, with all the protons spinning at random directions at any one time. They all possess a magnetic moment, which means that on application of a magnetic field they will all line up with this field, either in the direction of the field or at 180 degrees. It can be thought that each of those two scenarios require slightly different amounts of energy – it requires slightly more energy to align at 180 degrees to the field , compared to in the direction of the field. Within the billions of atoms within the body there will be a large proportion which

cancel each other out (paired atoms) however due to the mis-match of energy states, there will be more protons aligning with the field (at the lower state) which are 'unpaired' or 'unmatched'.

At 1.5T strength field, there will be approximately 9 of these excess protons per 2 million. This is equal to 6 $x10^{15}$ excess protons per voxel of water (2mm x 2mm x 5mm). It is these unmatched protons that are 'seen' on MRI.

When a radiofrequency pulse is applied to the protons within the magnetic field these low energy protons will gain energy and move to a higher energy state. When the RF pulse is lost the protons return to their original position and they release this energy as electromagnetic waves. This energy signal can then be detected.

To image in the 3 dimensions of space, there are 3 gradient coils around the field which can slightly vary the strength of field in any one spot. Using this combination of changing the magnetic gradients and applying radiofrequency waves you can select certain slices to image and based on the sequence parameters the computer can use this information to generate the 3D image.

The signal that comes back from different tissues is also different, this is due to what we call T1 and T2. T1 is the longitudinal relaxation time and reflects the speed at which the protons return to their original state following the RF pulse. T2 is the transverse relaxation time and refers to the time that the excited protons take to de-phase and decay. If the T1 and T2 values of a tissue are known, then a sequence can be devised to highlight that tissue in contrast to its surroundings.

Manometry

Similar to the MRI, a complete review of the manometry system is not appropriate for this thesis, however an understanding of the principles helps when understanding what is measured.

Traditionally there are two types of manometry catheter – water perfused and solid state.

In recent years, a fibre optic model (being developed in Australia) has also been used, however is not currently on the market.

Water perfused manometry catheters are made of a flexible material with recording ports (between 4 and 16) at distances between 1 and 15cm. These ports are open and connect to a pneumohydraulic infusion pump which gives a flow of water. When the wall of the colon contracts, these ports are occluded

and the flow of water interrupted, causing a rise in pressure within the tube. This pressure change is detected by external transducers and this is recorded. The nature of the contraction (duration, amplitude) affects the level of resistance measured.

Solid state catheters use strain gauges within a flexible PVC catheter. These are wired to a recording system. Traditionally these catheters had up to 10 sensors at around 10cm spacing, but they are now becoming longer with smaller spacing (high resolution). The significance of this will be discussed further later on.

MRI assessment of intestinal physiology

Over the last 10-15 years there has been extensive work looking at how we can use MRI to assess the physiology of the GI tract. This includes measures of small bowel and colonic water content, and colonic volumes both at fasting and after various stimuli. These relatively novel techniques are all used in the studies reported in the following chapters, so I will give a brief overview of their development and validation.

Small Bowel and Colonic Water Content

Assessment of small bowel water content was first described and validated in 2007[136]. This technique involves the evaluation of heavily T2 weighted images captured on MRI (this highlights

differences in the T2 relaxation time of tissues meaning water shows up very brightly and surrounding tissues as dark). Originally this was developed using an adapted MRCP sequence to measure a known amount of saline and mannitol solution (which is neither absorbed nor promotes secretion) which was infused in boluses via a naso-duodenal tube into the small bowel (Figure 5). It is now performed using a sequence called RARE (rapid acquisition with relaxation enhancement; a single shot, fast spin echo sequence)[137]. These are captured in a single breath hold.

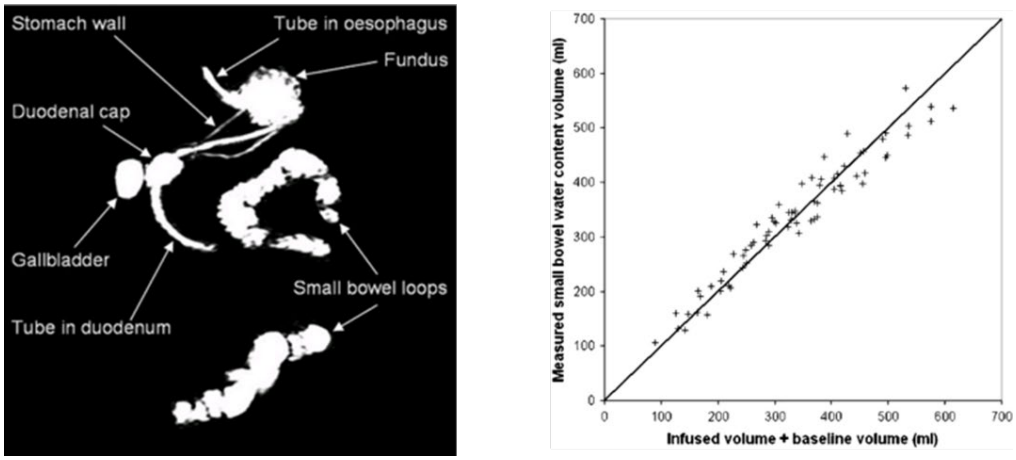


Figure 5: Projection of an MRCP dataset showing the NG tube and small bowel loops. Validation data with line of identity (from Hoad et al. Phys Med Biol 2007)

A threshold level for the free fluid is then set, based upon the signal intensity (brightness) of the spinal cord fluid. A region of interest (ROI) is then manually drawn on the slices containing small bowel, excluding other fluid containing structures such as the gallbladder and urinary bladder (Figure 6).

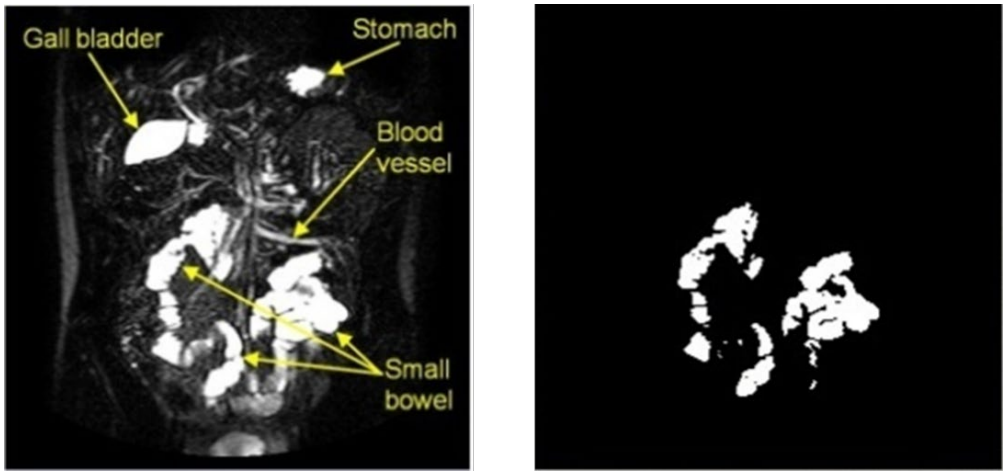


Figure 6: Heavily T2 image with anatomical labels and then after ROI drawn

This technique can also be applied to the colonic regions, however, there tends to be much less free fluid here. This could be due to both its absorptive properties and the effect of the microbiota potentially altering the signal produced. As an alternative, assessment of the T1 relaxation time of the chyme is used as a surrogate measure.

The T1 of a substance is dependent on the molecules' freedom to move relative to each other and therefore a less viscous fluid will have a longer T1. This hasn't been directly validated however a recent study which also measured the T1 of the descending colon found a reasonable correlation with the % faecal water content (assessed by weighing before and after freeze drying) of the next stool which was passed after the scan (Figure 7)[138].

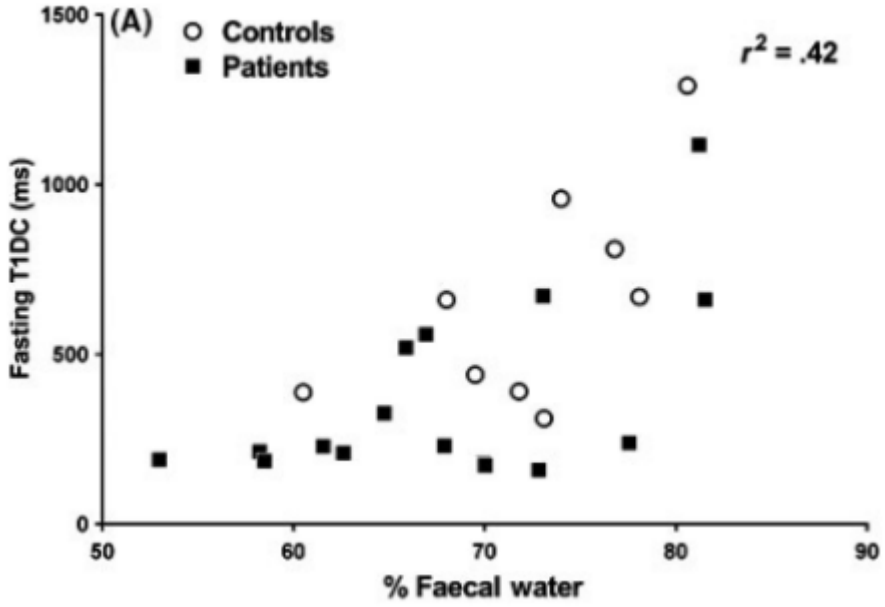


Figure 7: T1 plotted against faecal water from Major G, Murray K, Singh G, et al.

Colonic Volume

This was first assessed using MRI in 2013[31] and involves using a coronal anatomical image, with water and fat imaged in and out of phase. This highlights the bowel wall by altering the contrast between the fat frequently found around the colon wall and colonic contents, making it easier to draw around. The ROIs for each desired segment are then manually drawn and are then used to build a 3D representation using commercially available software and a volume calculated (Figure 8).

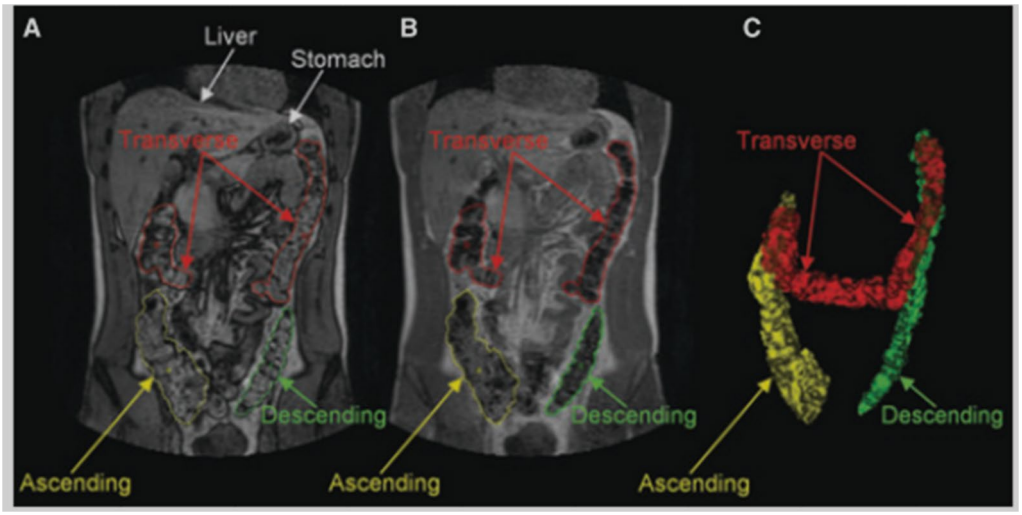


Figure 8: Representative example of the anatomical segmentation of the colon on coronal magnetic resonance imaging (MRI) images. Image A shows imaging with fat and water out of phase, and B in phase. From Pritchard et al 2014

In this study, fasted data were obtained from 75 healthy volunteers which was then used to determine a normal range.

How to measure colonic motility

Colonic motility is complex and only partially understood. Detailed investigation is challenging; the colon is a relatively inaccessible area and due to technical limitations, it is under-studied. Its function is distinctly different from any other part of the bowel and as a result there are many gaps in our knowledge. One of the significant issues is that many of our measurement methods require evacuation of the colonic contents which inherently alters that which we are trying to measure.

Measurement of colonic motility can traditionally focus on either the transit time or intra-luminal pressures/contractions. Transit time can be measured in a number of ways however colonic manometry is currently the only technique available which records intra-luminal pressures over multiple sensors. There is now a smart pill which can measure intraluminal pressure but with only one recording point and no control over where the pill is[139]. An experimental tool which uses magnetic moment imaging to track the movement of a small magnet has also been developed[140] . MRI techniques have been developed which look at transit time and a new measure of colonic wall motility is being developed and will be discussed later in the thesis.

Measurement of Colonic Transit Time

Radiopaque pills

This method was first described by Hinton *et al.* in 1969[141] it involves ingestion of radio-opaque markers (ROM) and subsequent assessment of their movement through the GI tract by use of plain abdominal X-rays. There are several published methods involving administration of both single sets of markers and multiple sets, but no standardisation of the test exists.

There are two widely accepted techniques in routine clinical practice at present[142]. First is the 'simple' radio-opaque marker

test. This involves swallowing a single capsule containing 20–50 markers on day 0, and taking an abdominal X-ray on day 4 or 5[143] which will determine the number of markers remaining in the colon. The second is the 'multiple marker' method, which is modified from that initially described by Metcalf *et al.* at the Mayo Clinic[18]. In this method, three capsules are administered on three consecutive days and each contains differently shaped markers. Abdominal X-rays are then taken at days 4 and 7. Transit can be estimated in hours from the formula 1.2 X number of pills retained at day 4. Slow transit has been defined as retention of greater than 80% of markers ingested on day 1 on the abdominal x-ray on day 4[144] More recently a detailed evaluation suggests accuracy can be improved by variation in the number and timing of pill ingestion[20]. These studies showed that increasing the number of pellets improves reproducibility and also that splitting the final days dosing gets round the problem that in individuals with fast transit the final X-ray can show zero pills which raises questions about whether they took the pills at all.

Scintigraphy

This technique was first published in 1986 by Krevsky et al[145]. This first study instilled indium ^{111}diethylene-triamine-pentaacetic acid (^{111}In) into the cecum via a 2-mm tube, which

was passed orally, and serial images were obtained over 48 h. Techniques have since evolved and there are now two commonly used methods involving oral ingestion of a radionuclide (usually ^{111}In) which is then monitored via gamma camera as it travels through the GI tract[146]. One is the ingestion of a solid-liquid meal and the alternative is the use of a resin coated capsule containing the radionuclide which is designed to dissolve at the ileum pH of 7.2-7.4[147]. The Mayo clinic group used the encapsulated isotope to deliver a bolus of isotope to the caecum to measure colonic transit but whether this refinement is really necessary remains unclear.

Bristol stool chart

In 1997 a standard measure of describing stool form was published by Lewis and Heaton, based in Bristol – so named the Bristol Stool Form Scale. This comprises of seven items which are described in everyday language and has been widely adopted since. Correlations are found between stool score and transit time measurements[148–150], however some IBS patients can have hard stools with normal transit time, a paradox which needs further exploration.

Wireless motility capsule

Recently an ingestible wireless motility capsule (WMC), similar to the capsule video endoscopy, has been developed. This gives

us another, less-invasive alternative with which we can measure gut transit. It is able to measure pH, pressure and temperature. It has shown good correlation to ROM method and has been validated[151,152]. A significant advantage is that it assesses motility under real physiological conditions though its large size may alter transit. Previous studies comparing isotopically labelled resin with a single large 22X8mm pill showed the larger unit was more rapidly propelled through the colon[141]. Studies using the radio pill give an upper limit of normal of whole gut transit around 50 hours[152]. It also has the advantage of being able to simultaneously measure pressure and has been used to show regional variation in motility though compared to manometry the localisation is poor, and it cannot assess the directionality of any propagating pressure waves[153].

Magnet Tracking System

This is similar to the WMC, in that it is a small capsule that is ingested, this time a magnet which can be tracked by magnetic moment imaging using magnetic field sensors strapped to the body which allows movement within the intestine to be mapped[154]. This is complementary to other techniques and gives important insights as to the variable movement of contents through the colon, particularly highlighting the role of mass movements, which can move contents rapidly from one

region of the colon to another[140]. A recent study in Denmark[155] with this system has demonstrated that this technique is also able to detect a variety of motor patterns in healthy volunteers, similar to those seen in High Resolution Manometry : long fast antegrade movements, fast antegrade, slow antegrade, slow retrograde, and fast retrograde movements.

MRI methods of Transit

At Nottingham, a novel MRI technique for assessing Whole Gut Transit Time (WGTT) has been developed (Figure 9). This is based on the morphological distribution of five MR visible markers 24 hours after ingestion. The markers are capsules made of polyoxymethylene which each contain 0.4mL of 15μM Gadolinium, which is a recognised MRI contrast agent, dissolved in water. This method was validated against the standard radiopaque marker method (Metcalfe) and showed good correlation (r_s = 0.85 ($p < 0.0001$)) and repeatability (Intraclass correlation coefficient was 0.61 for the MRI marker capsules ($p = 0.001$) and 0.69 for the ROM method ($p < 0.001$))[156]. Work is ongoing to further improve this with studies looking at using a larger number of pills, as there is evidence that this accuracy is best at around 10-12 pills (using the ROM technique)[20], and making the pills themselves smaller and easier to swallow.

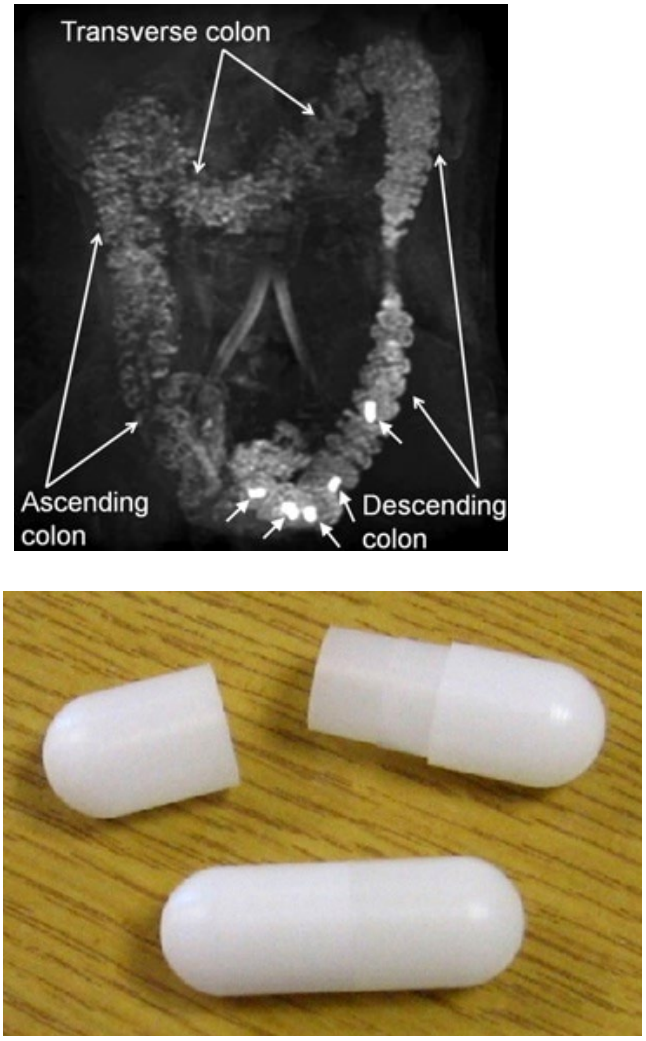


Figure 9 - MRI Marker Pills seen in the Colon and example capsule. From Chaddock et al[156]

Measuring Colonic Contractions

Conventional Manometry

Manometry is the process of placing a probe with pressure sensors placed along it into the large bowel by colonoscopy. This then allows data to be collected about the varying pressures inside the lumen over a period of time.

High Resolution Manometry

High resolution manometers are catheters with many more sensors than the conventional catheters. These are placed at 1cm intervals. Initially these were either solid-state or water perfused sensors and since 2009 a team in Australia have used fibre optic technology[157]. These have further revolutionised our ability to study colonic motility and studies performed with these new manometers show colonic activity to a new level of detail never seen before.

The initial study in Australia[158] looked at comparing the sensor spacing of the older style manometer with their new fibre optic catheter which can accommodate sensors 1cm apart. They studied 11 patients with proven slow transit constipation, 11 patients with neurogenic faecal incontinence and nine healthy subjects and used the 1 cm spaced data and subsampled data sets simulating sensor spacing of 10, 7, 5, 3, and 2 cm.

This paper showed that accuracy of data recording dramatically decreases with increased sensor spacing. They found that in all groups, the frequency of propagating sequences detected at 1 cm spacing was significantly greater than their frequencies detected at 5, 7, and 10 cm spacing ($P < 0.05$) as many only propagate short distances. The frequency of detected events at 1 cm spacing did not differ significantly from the propagating

events detected at 2 and 3 cm spacing. They determined that less than 5% of the propagating activity observed at 1 cm spacing can still be seen at 10 cm spacing. It was found that these wider spaced measuring sites can lead to misinterpretation of retrograde contractions as being antegrade (Figure 10).

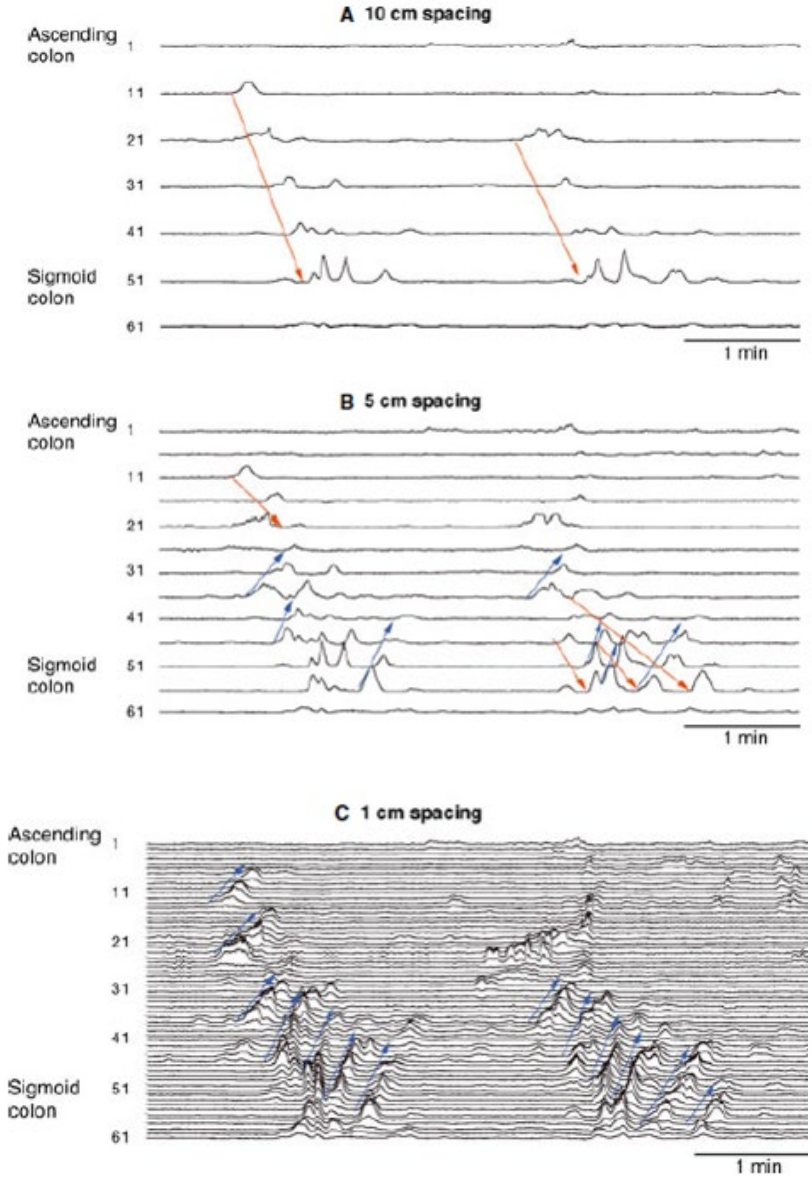


Figure 10 - Colonic Motility Recording Showing Impact of Different Sensor Spacing, from Dinning et al[158]

In light of this it is clear that many of the older studies may need to be carried out with these new HRM catheters as results could be very different particularly the role of cyclical retrograde propagated contractions.

New MRI methods

A method of assessing colonic motility via the quantification of wall movement has recently been developed by teams in Nottingham and London. This automated registration software has been validated as surrogate measure of gut motility in the small bowel[159] and colon[160]. In this method, non-rigid image registration techniques[161] developed at University College London are used to correct for respiratory motion and subsequently a series of lines are drawn at right angles to the colonic axis connecting points on the wall across the MRI image of the colon. Changes in the length of these lines are analysed to create a "motility index", defined as the percentage of the time the line had a change in length (i.e., wall velocity) between consecutive time points of greater than 0.25, 0.5, and 0.75 mm/s[160].

MRI tagging sequences, in which superimposed horizontal stripes (tags) are superimposed onto the images that distort if contents are moving, can also be used to assess the flow of contents within the colon and has shown differences between healthy and constipated individuals[162]. These newer methods will be discussed in greater detail later in the thesis (chapters 6 & 7).

Observed Patterns of Contractility

Healthy controls

Use of the conventional manometry technique as a physiologic tool was first published in the late 1980s. This study looked at 24h colonic motility recordings in healthy adult volunteers. 14 volunteers were studied using a probe that took readings from four recording sites, 12cm apart in the transverse and descending colon They found that normal colonic motility shows diurnal variation with rest during sleep and increased activity on awakening and postprandially[163].

Since then multiple studies have been performed on both healthy subjects and those suffering from constipation, and a number of different colonic motor patterns were identified: antegrade high amplitude propagating contractions, low amplitude propagating contractions, non-propagating contractions, and rarely episodes of retrograde propagating contractions[142].

These studies have found that 90% of activity appeared to cause mixing with limited overall propulsion which supports other observations made using different techniques which assess movement of colonic contents e.g. the magnetic tracking system. Propulsion occurs episodically as “mass movements”

when the entire contents of a colonic segment move by means of distinctive motor patterns characterised by the high amplitude propagating contractions (HAPCs).

The new high-resolution techniques have subsequently been used and have attempted to re-define the different types of motor patterns seen in healthy control subjects. These are now thought to consist of: (i) cyclic motor patterns (at 2–6/ min); (ii) short single motor patterns; (iii) long single motor patterns; and (iv) occasional retrograde, slow motor patterns (v) high-amplitude propagating sequences (HAPS)[164] as well as pan-colonic pressurizations (which appear to be simultaneous along the length of the bowel and associate with anal sphincter relaxation)[165].These are all evident in differing levels before and after a meal stimulus[164].

In this 2014 study of 10 healthy subjects[164], observed motor activity prior to the meal mainly consisted of non-propagating pressure events and no HAPS were recorded in this time. After the meal there was significant increase in the motor activity recorded in all regions of the colon with a clear proximal to distal gradient, with activity increasing distally. This was rapid in onset and occurred within minutes of eating. It was evident for around 30 minutes in the proximal and descending colon before

return to baseline. However, in the sigmoid colon it persisted for up to 2 hours.

Of the cyclic propagating motor patterns, retrograde movements accounted for 97% of the increase. There was no change in anterograde cyclic propagating patterns. HAPS were demonstrated in 5 out of 10 subjects after the meal and more originated on the right side than the left. There was also no significant increase in short or long single motor patterns.

Interestingly some older scintigraphy studies[166,167] saw similar patterns in healthy subjects with some post-prandial retrograde movements of materials in the sigmoid colon, and lack of this retrograde movement in a constipated group[168]. This was particularly striking in patients with diarrhoea, perhaps because in such patients, colonic contents are less viscous due to high water content This likely represented the cyclical movements we now see however at the time the significance of this was not recognised.

It is also worth noting however that these manometry techniques do require colonic lavage, either by oral bowel preparation or enema and this has been seen to alter motility in itself by increasing high amplitude propagated contractions(8 unprepared , 22/ 24h after preparation)[169] and so attempts

should be made to control for the bowel preparation in all motility studies.

This shows that as well as similarities to what was previously seen with conventional techniques, these new advances give new insights, especially regarding the retrograde movements which were largely missed in previous studies using widely spaced measuring ports.

More work needs to be done to create data from which patterns and thresholds for disease can be set. A consensus is currently under review which attempts to bring some consistency to the terminology and should help with comparing and compiling future manometric data[170].

Patient Group – Constipation

Studies looking into colonic motility in patient groups show a wide amount of variability. This is in part due to the variety of methods used to measure motility and also to different criteria used to recruit different patient groups.

Early studies in the 1980s looked at transit time in constipation. Cann et al[171] looked at IBS (diarrhoea and constipation sub-groups) vs healthy controls and found the transit time significantly increased in constipation. Lanfranchi et al[172] separated constipation patients into those with pain and those

without. They found that transit was significantly increased in those without pain, however the group with pain had variable transit, and the mean was in the normal range. Törnblom et al[150] in 2012 looked at transit times in a very large group of IBS patients (359) and found that within the IBS-C subgroup, 86% had normal transit times compared to 12% with delayed and 2% with increased. Such very large studies are likely to be more representative of a typical patient than the earlier smaller studies. The finding of normal transit in IBS-C suggests that there is only an indirect connection between transit and stool consistency and as such one can get hard stool with normal transit if one has over-efficient absorption perhaps because of different motor patterns which cause more mixing allowing faster fluid absorption.

There is also variability in measures of motility via manometry. Some studies have shown an overall decrease in the frequency of HAPCs in constipated patients[168,173] and even a decrease in the velocity of the contractions when they did happen[174]. This is not always the case however, as Bassotti et al[175] saw in IBS-C patients no difference in the HAPCs frequency but the 24hr overall motility score was decreased.

A study using the WMC, which was able to look at both transit time and motility combined, found increased motility in

constipated subjects with normal transit, compared with those who have slow transit[153]. The increases were most obvious in the 4th quarter of the colonic passage (presumed to cover the time the recording capsule passed through the descending and sigmoid colon). This is also consistent with recent MRI study that found an increased motility in IBS-C compared to FC[32].

This creates the hypothesis that within the group of constipated patients who complain of abdominal pain, yet has normal transit, there is abnormal, and possibly increased, motility. The theory being that they have increased motility however the motor patterns are potentially un-coordinated or possibly retrograde, contributing to their constipation. If some patients do have increased, but un-coordinated, motor activity, then this would have important implications for treatment and may also explain why current treatments are unsatisfactory to so many at present.

The rest of this thesis is therefore split into two sections; chapters 6 and 7 will begin to address the question above using MRI and manometry, but before that chapters 4 and 5 will look at how we have applied these novel MRI methods to assess the impact of different foods on the normal GI tract, and how this may influence management of patients with dietary interventions.

SECTION 2: USING MRI TO STUDY THE EFFECTS OF FOOD

The next two chapters describe two mechanistic studies performed on healthy volunteers to investigate the effects of different foods on the intestine. It has been well established that foods impact bowel habit and we wanted to further determine the mechanism behind this using MRI and whether the effects were on the small or large bowel or both.

Chapter 4: Insights into the different effects of food on intestinal secretion using magnetic resonance imaging.

A version of this work has been published in the Journal of Parenteral and Enteral Nutrition (JPEN)[176]

Abstract

Background

Plant foods may stimulate intestinal secretion through chemicals designed to deter herbivores, including lactucins in lettuce and rhein in rhubarb. This may increase ileostomy output in short bowel syndrome patients and induce diarrhoea in people with intact bowels.

Objective

Determine the effect of different foods on intestinal water content in healthy volunteers using Magnetic Resonance Imaging (MRI).

Method

Crossover trial of three isocaloric meals in healthy adults. Subjects underwent MRI scans fasting and hourly to 180min postprandial. Meals: 2 slices white bread with 10g butter; 300g

rhubarb with 60mL lactose-free cream; 300g lettuce with 30mL mayonnaise. Visits one week apart. Primary outcome: small bowel water content (SBWC) using MRI. Secondary outcomes: ascending colon water content (ACWC); T1 relaxation time of ascending colon (T1AC); gastric volume; visual analogue scales of bloating and satiety (0-100) scored half-hourly.

Results

15 subjects completed the study. 9 female, 6 male, median age 21(IQR 20,22). Bread induced a fall in SBWC compared to a rise after lettuce and greater rise after rhubarb, with significant differences in the area under the curve (0-3 hours) of the change from baseline ($P<0.01$, paired t-test). Rhubarb increased T1AC but differences at 3 hours were not significant ($P=0.06$). Symptom scores were higher for lettuce >rhubarb >bread.

Conclusion

Lettuce and rhubarb meals increased intestinal water content, demonstrating how different foods can alter small bowel secretion and stool consistency.

Introduction

In patients with a small bowel stoma, high stoma output impairs quality of life and leads to dehydration and in extreme cases induces acute kidney injury. High stoma output is commonly caused by an inadequate length of small bowel remaining after resection to maintain absorptive function (short bowel) although partial small bowel obstruction, infection and inflammation can also contribute. Chronic high output is the most significant factor in determining the need for parenteral nutritional support. There is a limited evidence base for interventions to reduce stoma output. Agents such as loperamide, codeine which slow transit and proton pump inhibitors to reduce gastric secretions are recommended. A glucagon-like peptide 2 analogue (teduglutide) has also shown some benefit, possibly by stimulating mucosal growth and enhancing nutrient absorption[177]. Modifying dietary intake also plays an important role particularly sodium intake. Patients are currently advised to restrict or avoid drinking oral hypotonic fluids, as these can cause further loss of water and sodium, and instead drink an oral sodium and glucose solution.

Composition of dietary intake is also important; diets that are high in fermentable oligo-, di-, mono-saccharides and polyols (FODMAPs) have been shown to increase ileostomy output by

22%[178], demonstrating that the osmotic effects of food can alter the balance between secretion and absorption in the small bowel. Other mechanisms including stimulation of secretion may also operate. A survey of ileostomy patients looking at a large variety of different foodstuffs found that both rhubarb and lettuce were perceived to increase stoma output[179] though this was not directly measured. Objective experimental quantification of such effects is problematic because of patient heterogeneity and also because such studies imposes a burden on frail patients who have significant health problems. This limits its suitability for early phase assessment of therapeutic interventions.

We have validated techniques to measure free water in the intestine[136] and assess the physico-chemical composition of the colonic chyme[180,181] and have previously shown that bread, which is rapidly digested to free monomeric sugars and absorbed, leads to a reduction in small bowel water[182]. Our aim was to apply these techniques to investigate the effects of equicaloric lettuce and rhubarb meals on intestinal fluid distribution, in comparison with a standard bread meal. We hypothesised that rhubarb and lettuce would both significantly increase small bowel water when compared with bread, and that, in healthy subjects with an intact colon, we would be able

to detect a consequent increased fluidity of the ascending colon content.

Methods

Study design

The study was a three-period, three-treatment crossover design with randomisation of treatment order. The protocol was approved by the Ethics Committee of the University of Nottingham and volunteers gave written informed consent. The study was carried out according to Good Clinical Practice as defined by the Declaration of Helsinki. The study was registered on clinicaltrials.gov, reference: NCT02939716.

Study participants

Subjects were aged ≥18 and able to give consent. Subjects were excluded if they had a) history of GI surgery (other than appendicectomy), GI disorder, or a positive diagnosis of irritable bowel syndrome by Rome III criteria b) contra-indication to MRI or any medical condition compromising participation, such as an inability to lie in the MRI scanner c) inability to cease use of medication affecting gut function or follow the dietary restrictions required for the study d) lifestyle factors likely to disrupt gut function or compliance with the

protocol such as night shift working. All subjects gave written consent.

Interventions and Procedures

Consent, screening, and enrolment took place at the Nottingham Digestive Diseases Biomedical Research Unit, Nottingham. All subsequent study visits took place at the 1.5T scanner annex of the Sir Peter Mansfield Imaging Centre, Nottingham. Subjects visited the Centre on three separate occasions, with at least one week between each visit to minimise any carryover effect. Subjects were asked to eat similar foods in the 24 hours before each visit and to avoid apples, beans, peas, pulses, sweetcorn, dietary supplements, and alcohol. They abstained from caffeine and strenuous exercise for 18 hours before the visit and did not eat after 8pm on the evening beforehand.

On the day of the visit subjects fasted before attendance, other than sips of water with essential medicines. On arrival they completed two 0-100 visual analogue scales to assess bloating and satiety and underwent an MRI scan. They were then given 15 minutes to consume the test meal before repeat assessment of symptoms and further MRIs (t=0 min). Symptom scores were repeated at 30-minute intervals for 180 minutes on the same

0-100mm visual analogue scales. MRIs were repeated every 60 minutes for 180 minutes.

Test meals

Three isocaloric test meals (Figure 11) were used: 1) 2 slices white bread (Tesco Stores Ltd., Welwyn Garden City, U.K.) with 10g butter (Lurpak, Arla Foods Ltd, Leeds, UK) (10g fat, 248 kcal); 2) 300g organically grown rhubarb, cooked by microwave, with 60mL lactose-free cream (Arla Foods, Leeds, UK) and 2 sweetener tablets (Hermesetas, Hermes Sweeteners Ltd, Zurich.) (22.5g fat, 243 kcal); 3) 300g Romaine lettuce (Tesco Stores Ltd., Welwyn Garden City, U.K.) with 30mL mayonnaise (Hellman's, Unilever, London, UK) (23.5g fat, 246 kcal). Each of these was consumed with 200ml water. Subjects were required to consume all of the meal and water.



Figure 11: Test meals: bread, rhubarb and lettuce

Outcomes

The primary outcome was AUC [0-180 minutes] of small bowel water content (SBWC, mL.minutes). The technique measures

the volume of freely mobile water using a previously validated MRI technique.

Secondary MRI outcomes were: ascending colon water content (ACWC, mL); This is measured in the same way as the small bowel water content and reflects only freely mobile water with long transverse relaxation time, and is only a small fraction of the total water[181]. We therefore also measured ascending colon longitudinal relaxation time (T1AC, ms) which in this context can provide a more useful assessment of colonic contents. T1 is a fundamental MRI parameter that reflects the time constant for the water's hydrogen protons to return to their equilibrium state following a radiofrequency excitation pulse. In the context of this paper, more watery chyme has a longer T1 relaxation time. Symptom scores for bloating and satiety were also compared.

MRI protocol

MR scanning was performed on a whole-body 1.5T scanner (Achieva, Philips Medical System, Best, The Netherlands). Volunteers were positioned feet first and supine in the scanner with a 16-element SENSE (Philips Medical System) receive torso coil around their abdomen.

The SBWC and ACWC were measured using 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, TEeff = 320 ms, AQR = 1.56 mm x 2.90 mm) as previously described[136]. This sequence yields high intensity signals from areas with fluid and little signal from body tissues. Each slice was analysed in turn and the total summed to yield total volume using a program built in-house.

T1AC was measured using a single slice balanced turbo field echo (bTFE) sequence with a preparatory 180 degrees inversion pulse applied before acquiring the imaging data. Data were acquired from 8 different inversion times (TI) (time between inversion pulse and imaging pulses) ranging from 25 - 4925 ms.

Images were acquired during an expiration breath hold lasting between 13-24 seconds. Each period of imaging lasted between 10 and 15 min, after which volunteers were removed from the scanner and allowed to sit upright.

Randomisation and Blinding

Randomisation of treatment order was achieved using the remote, online programme www.randomization.com. Neither subjects nor researchers were blind to the meal consumed on study days. Scans were coded and pseudo-anonymised through a shell programme so that MRI analysis was conducted blind to the intervention.

Power and statistical analysis

This was a pilot study as no data are available on the effect of ingested lettuce or rhubarb on MRI parameters. However, using n=16 we had previously detected significant differences in small bowel water content induced by the mild laxative bran[7] which were less than the changes anticipated in this study.

Statistical tests were performed using GraphPad Prism version 7.03 for Windows, (GraphPad Software, La Jolla California USA). Data normality was assessed using Shapiro–Wilk test. Two-way analysis of variance was used to assess for any difference between the test meals during the postprandial period. Any difference identified was then explored by direct comparison between meals. The Wilcoxon Signed Rank test for non-parametric paired data was used. Tukey's multiple comparisons test was performed to adjust for multiple comparisons.

Statistical differences were considered significant at $P<0.05$.

Results

Recruitment took place from September 2016 to January 2017. 18 subjects were enrolled, of whom 3 were unable to complete the study due to scanner technical problems (Figure 12). 15 subjects completed the study: 9 female, 6 males; median age 21 (IQR 20, 22) and mean body mass index 21.4 $kg.m^{-2}$ (SD ±2.2).

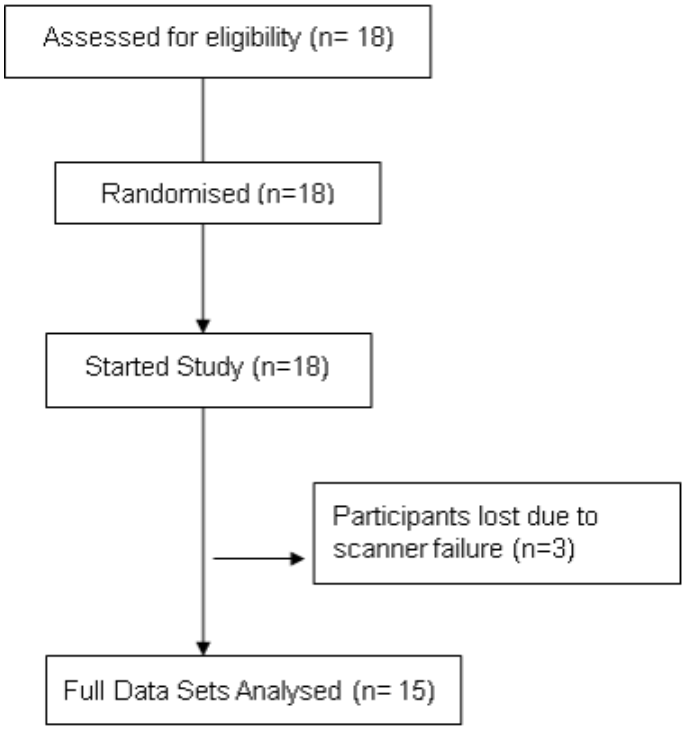


Figure 12: Consort Flow Diagram

Small bowel water content (SBWC)

There was no difference in mean fasting SBWC between intervention days but responses to the meal varied considerably (Table 1, Figure 13).

The primary outcome of AUC [0-180 mins] of the change from baseline was significantly different between all interventions ($P < 0.01$ for each paired t-test). Bread induced a fall in SBWC similar to that seen in previous studies (14), from fasting mean 47±28 mL to 4±4 mL at 60 mins ($P < 0.001$). No such decline was seen with lettuce or rhubarb. Mean SBWC with the lettuce meal was 60±55 mL at baseline and 65±53 mL at 60 minutes, rising significantly at 180 mins to 108±50 mL ($P < 0.005$). With the rhubarb meal, mean SBWC increased after 60 mins, from 55±33 mL at fasting to 103±44 mL ($P < 0.001$), then 141±61 mL and 142 ±45mL at 120 and 180 mins respectively, a peak that was significantly higher than for lettuce ($P < 0.05$).

Table 1: Fasting and postprandial Small Bowel Water Content (SBWC) in mL for the 3 test meals

Mean (SEM)	Fasting	Time postprandial (min)				AUC (mL.min) [change from baseline]
		0	60	120	180	
Bread	47 (7)	24 (6)	4 (1)	13 (4)	32 (7)	-5662 (1209)
Lettuce	60 (14)	69 (13)	65 (14)	78 (16)	108 (13)	3194* (1574)
Rhubarb	55 (9)	51 (8)	103 (11)	141 (16)	142 (12)	10586*# (1629)
P <0.01 versus bread; # P <0.01 vs. lettuce						

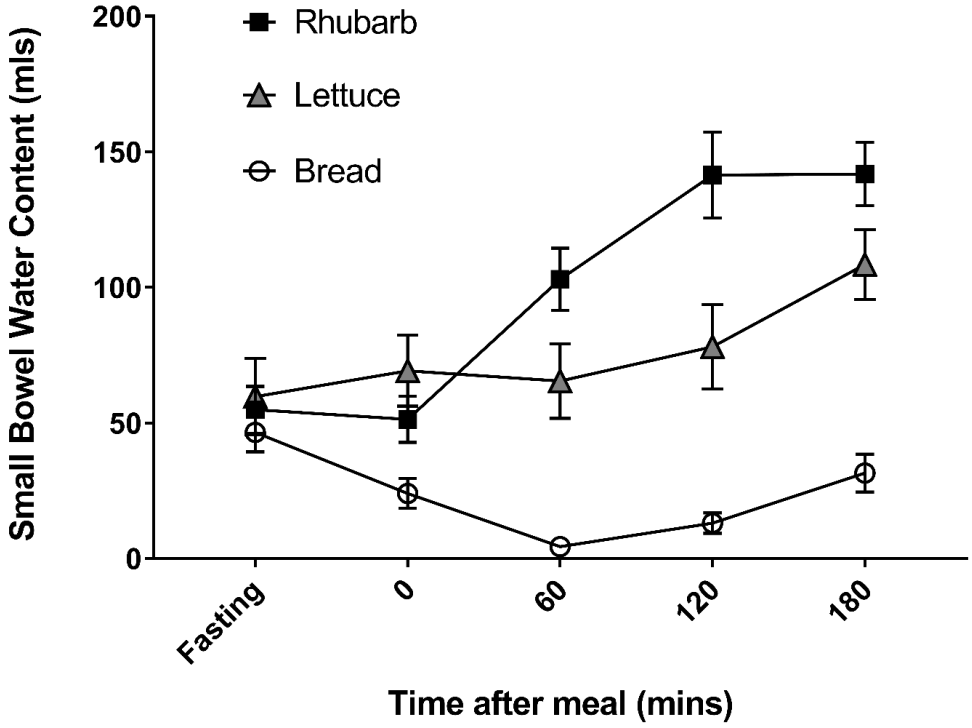


Figure 13: Time course data for MRI measurements of small bowel water content (SBWC, mean ±SEM) when fasting and at intervals after isocaloric test meals of bread and butter, lettuce and mayonnaise or rhubarb and cream in 15 healthy adult subjects.

Ascending colon free water content and relaxation times

Mean values for ascending colon (freely mobile) water content (ACWC) are shown in Figure 14, with AUC and peak shown in Table 3. As previously reported with standard rice meals, volumes were low; during the bread intervention only one subject had >1 mL detectable at any point. After lettuce, four subjects had detectable ACWC on the immediate post-prandial scan, with volumes of 3, 12, 12, and 45 mL but by 180 mins it was only detectable in two subjects with 2 and 4 mL, respectively. Significantly more ACWC was detectable after rhubarb than bread ($P < 0.01$ Wilcoxon) as by 180 mins seven subjects had >1 mL detectable.

The fact than many subjects had no free water detectable is in keeping with previous work and reflects the fact that freely mobile water entering the colon from the ileum is rapidly absorbed or mixed with the chyme thereby losing its MR signal. In contrast, relaxation times (T1), which are a marker of the fluidity of the colonic chyme that does not depend exclusively on free water, could be readily measured in all subjects and rose after all meals. Absolute values are shown in Table 2 with change from baseline shown in Figure 15. The changes after bread and lettuce were similar with mean rise of 111±206 ms and 143±262ms, respectively. Rhubarb induced a larger rise of

313±318 ms but the difference from bread just failed to reach conventional statistical significance (mean difference 202 ms, 95% CI -12 to 416, P =0.06).

Table 2: T1 in milliseconds of Ascending Colon (AC) contents for the 3 test meals

Mean (SEM)	Fasting	Time postprandial (min)				AUC [change from baseline]
		0	60	120	180	
Bread	674 56)	726 (58)	771 (60)	750 (48)	785 (64)	15188 (6717)
Lettuce	763 (65)	851 (75)	874 (54)	854 (61)	906 (83)	18990 (7884)
Rhubarb	602 (73)	781 (71)	919 (56)	957 (70)	837 (66)	35744 (9013)

Table 3: AUCs and peak for ACWC, Bloating and Satiety Scores

	ACWC (mls)		Bloating (VAS score)		Satiety (VAS Score)	
Meal	AUC	Peak	AUC	Peak	AUC	Peak
Bread	78 (43)	1 (T=0)	4196 (637)	27.6 (T=0)	7943 (783)	49.4 (T=0)
Lettuce	409 (231)	5 (T=0)	7071 (905)	44.7 (T=0)	9498 (941)	64.8 (T=0)
Rhubarb	291 (89)	3 (T=180)	5113 (927)	33.2 (T=0)	7904 (835)	47.5 (T=0)

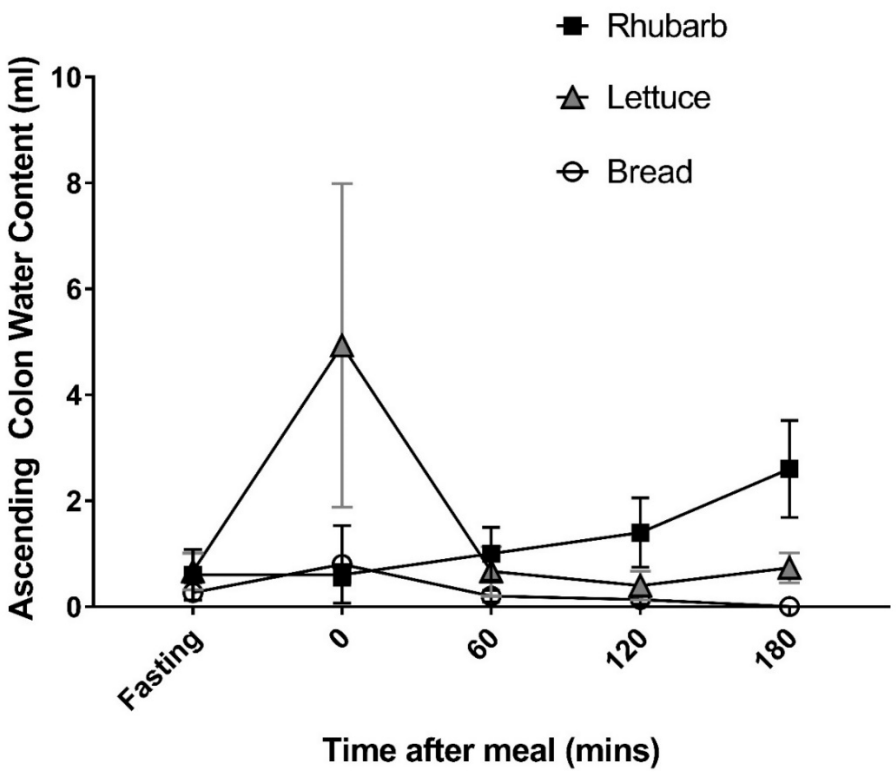


Figure 14: Time course data for MRI measurements of ascending colon water content (ACWC, mean ± SEM) when fasting and at intervals after isocaloric test meals in 15 healthy adult subjects.

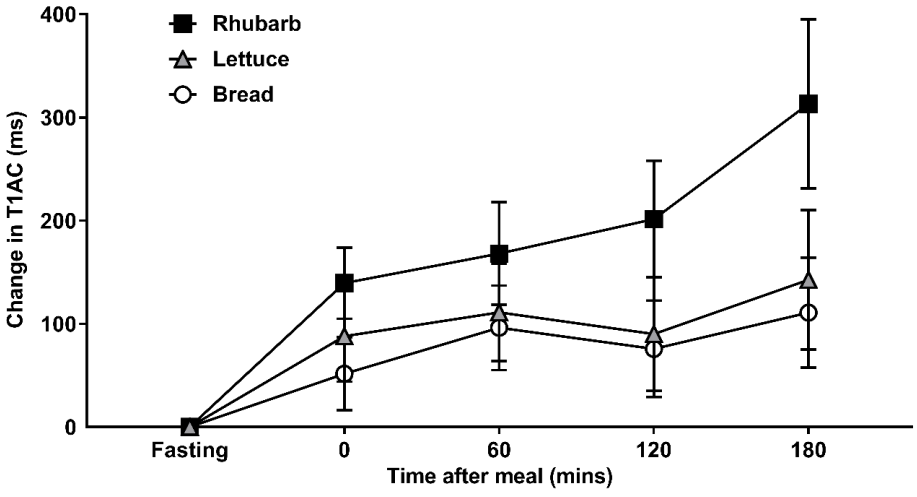


Figure 15: Time course data for T1 MRI longitudinal relaxation time of the chyme in the ascending colon (T1AC). Values (mean ± SEM) show change from fasting baseline after isocaloric test meals in 15 healthy adult subjects.

Bloating and Satiety

Both bloating and satiety scores (0 - 100 on VAS) peaked immediately after the meal (t= 0mins) for all three meals. For bloating, scores were highest for lettuce (45±23) then rhubarb (33±20) then bread (28±14). Scores for bread vs. lettuce remained significantly different up to t= 90mins (p= 0.036). Peak satiety scores for lettuce (64±19) were significantly higher than for both rhubarb (47±13, p= 0.01) and bread 49±20 (p = 0.03). AUC and peaks for these symptoms are shown in Table 3.

Discussion

The aim of the study was to use non-invasive methods to characterise and quantify intestinal fluid distribution after different meals. This is the first study to use MRI to address the effect of foods specifically implicated in affecting stoma output. Our hypothesis, that lettuce and rhubarb would increase SBWC compared to bread, was confirmed, with differential effects also found between the two test meals. This objective demonstration of a physiological effect supports the subjective reports of ileostomy patients that these foods stimulate increased stoma outflow.

The fall in SBWC after the bread meal is similar to our previous studies[182] and likely reflects the very rapid hydrolysis of wheat starch by superabundant amylase in the proximal duodenum. The liberated glucose is rapidly absorbed, and with it water, so that both the blood glucose profile, which in other studies peaks within 30 minutes[183], and SBWC profile, mimic those seen with a pure glucose solution[137].

In contrast, the striking rise in SBWC after the rhubarb and the less marked, but still significant, rise after lettuce may reflect several different processes. Both meals had considerable fat content and some of the increase in SBWC will be due to pancreatico-biliary secretions, stimulated by a cholecystokinin response to hydrolysed fat in the duodenum during the intestinal phase of digestion. Previous studies in Nottingham have shown that high fat meals induce marked increases in SBWC, an effect enhanced by using a stable emulsion[184] such as mayonnaise.

The rhubarb and lettuce meals were matched for fat content so the difference between these meals is likely to reflect other factors present in those foods.

Rhubarb has been used for many years as a laxative agent in the practice of traditional Chinese/herbal medicine[185]. This laxative effect is caused by the compounds rheinanthrone and

rhein which account for 2% by weight of rhubarb and appears to act similarly to bisacodyl by activating macrophages, increasing PGE2 and thereby decreasing colonic expression of AQPs[186].

Lettuce is another food that was reported to increase stoma output by 1 in 6 patients[179] but is less commonly associated with laxative effects in an intact bowel. Some lettuce varieties exude a milk-like latex material (Lactucarium) when cut[187]. This is a mild cytotoxin and so could be expected to stimulate intestinal secretion.

Having considered direct chemical stimulation by food components, it should also be noted that the fibrous nature of rhubarb and lettuce may lead to partial obstruction in ileostomy patients (there can be constriction of the lumen due to scaring and fibrosis), stimulating increased secretion. Such an effect cannot be excluded in our volunteers although abdominal pain, nausea and vomiting were not reported during the study.

Although the exact mechanism of such effects remains uncertain, the observed alterations in SBWC will not necessarily alter bowel function in a subject with an intact colon; the colon can normally compensate for the wide variation in fluid entering from the ileum that can be expected from a normal mixed diet. As such, little free water is normally found in the resting colon.

However, we did find some evidence of increased ascending colon water content 180 minutes after rhubarb. The timing of the increase in water content would fit with the first arrival of meal residue in the caecum.

The T1 longitudinal relaxation time reflects the freedom of molecules to move relative to each other in a material. We have previously shown that in a study with ispaghula it correlates with stool water content[138]. The T1AC results for rhubarb, whilst not reaching significance in this study, also support the arrival of more water/watery contents into the ascending colon. The use of such techniques is a relatively new approach but provides a useful platform to demonstrate proof of principle for therapeutic agents before their application in patients with functional bowel disorders such as constipation as well as those with high stoma output.

We have previously found that, when small bowel water content of volunteers with an intact bowel was increased 400% by 40g of fructose, small bowel diameter only increased by 20%[188] suggesting that the small bowel copes with increased volumes by utilising more of the normally collapsed distal bowel rather than by increasing its diameter. This reserve capacity will be limited in patients who have had small bowel resected and will be proportionate to the remaining bowel. This phenomenon

would also tend to exacerbate the effect of foods promoting secretion over absorption.

Limitation of the study include the small sample size and the 60-minute interval between MRI scans, which limited our ability to accurately define the time course of the changes we observed. Although the meals were equicaloric comparison between the bread meal and other meals is somewhat limited by differences in meal size, energy density and fat content. Future studies would need to control for these aspects or else use the possible active ingredients in purified form such as Rhein and Lactucarium. Finally, we studied volunteers with intact bowels, with small bowel and colon in continuity. Whilst we might interpret changes in the consistency of chyme in the ascending colon as corresponding to changes in stoma output, this has never been shown. Patients with short bowel syndrome and stomas have different physiology and are likely to have different secretions and transit due to reduced levels of gut hormones such as GLP-1, GLP-2 and PPY. Our study represents a model for investigation but extrapolation to patients' responses must be qualified and requires further studies in patients.

In conclusion, the study has shown that these different foods can alter intestinal fluid balance, which may be relevant to

dietary management of patients with small bowel stomas as well as functional bowel disorders. The methods demonstrated are readily applicable to other dietary or pharmaceutical interventions. Future work should explore the effect of other therapies, including food choices, on intestinal fluid balance, and should include correlation between MRI findings and stoma output/bowel habit in patients.

Chapter 5: Effect of Kiwifruit on Gastrointestinal Fluid Distribution and Transit in Healthy volunteers

The second of the food studies looked at another food, kiwifruit, and its effect on the fluid distribution in the small and large intestine. This study was funded by Zespri group Ltd and published in 2019[189].

Abstract

Background

Chronic constipation affects approximately 17% of the population worldwide and remains an important unmet need since patients are often dissatisfied with treatment. Kiwifruit may offer an alternative to traditional laxatives and have been shown to increase stool volume, frequency and improve consistency.

Aims

Using non-invasive MRI techniques, we assessed the effect of ingestion of kiwifruit on fluid distribution in the intestines and bowel function.

Methods

Two period crossover trial of kiwifruit versus control in healthy adults. Intervention: two kiwifruits twice daily vs isocaloric control (maltodextrin) twice daily, consumed for a total of three days. Subjects underwent MRI scanning fasted and at hourly intervals for 7 hours on the third day. Primary outcome: T1 relaxation time of ascending colon (T1AC) using MRI. Secondary outcomes: Small bowel water content (SBWC), colonic volume, gut transit time, T1 of descending colon, stool frequency and form.

Results

14 volunteers completed the study. T1AC was higher after kiwifruit ingestion (P=0.029) during the second half of the day (when meal residue would be expected to reach the AC, T240-420 mins. SBWC (P<0.001), colon volumes (P=0.004), as well as stool frequency (P=0.034) and stool form (P=0.011) were increased in participants consuming kiwifruit compared to control. .

Conclusion

Consumption of kiwifruit in healthy volunteers increases water retention in the small bowel and ascending colon and increases total colonic volume. The data may explain the observed increase in stool frequency and looser stool consistencies,

suggesting that kiwifruit could be used as a dietary alternative to laxatives in mild constipation.

Introduction

Chronic constipation affects approximately 17% of the population worldwide[7] and its management remains an important unmet need since patients are often dissatisfied with treatment[104]. Current stimulant or osmotic laxatives are successful in increasing stool frequency[90] but are often associated with diarrhoea, bloating, cramps and abdominal discomfort[112]. Furthermore, such powerful treatments taken intermittently may result in alternation between diarrhoea and no stools, this is both inconvenient for the patient and potentially disturbs the microbiota with uncertain consequences[59]. Many patients including those with mild symptoms who do not seek health care would benefit from a less powerful treatment which could be taken daily producing more regular stools of normal consistency.

Kiwifruit offer such an alternative and, in two open label and one randomised placebo controlled trials, have been shown to increase stool volume and frequency in patients with constipation[190–192]. The mode of action remains unclear as kiwifruit have many potentially active ingredients[193].

Kiwifruit are the berry of the Actinidia vine and there are two varieties that are widely commercially available. These are the 'Hayward' or green variety (*Actinidia deliciosa*) (marketed as Zespri® Green) and 'Zesy002' or gold variety (*Actinidia chinensis*) (marketed as Zespri® SunGold).

The fruit are nutrient-dense and are high in vitamins C, E, K, and in micronutrients potassium, folate, phytochemicals and carotenoids, as well as in fibre[194].

A dose of 2 Kiwifruit weighing 300g contains approximately 12g fructose, 12g glucose and 9g of dietary fibre. The fibre comes primarily from the plant cell walls and is a mixture of soluble (one third) and insoluble fibre (two thirds). The soluble fibre is pectic polysaccharides and the insoluble fibre is cellulose and hemicellulose[195]. Pig studies showed that while the soluble component of kiwifruit fibre is well digested in the small bowel, the insoluble component passes largely intact into the colon[196].

Kiwifruit cell walls have a large swelling and water retention capacity both before and after digestion and in vitro tests (which involve suspension in 250ml of water and allowing to settle overnight) found that it swells to over three times its original volume for both green and gold kiwifruit[195]. This swelling is around 1.5 times that of psyllium, a commonly used laxative, and more than 6 times that of apple fibre. The water

retention capacity of the fibre, defined as the amount of water which remains after centrifugation of kiwifruit pulp suspended in water, was around 12-13 g of water per gram of insoluble fibre. Both this swelling and water retention are high compared to other readily available forms of dietary fibre such as apple, orange, wheat bran and sugar beet fibre[195].

Soluble fibre is a proven laxative[197], softening stool and increasing stool frequency. Early studies in ileostomy patients showed that psyllium increases ileal flow[198] but until recently its mode of action in the intact colon was unclear. However recently we have shown using novel non-invasive MRI techniques that psyllium 3.5 g taken three times daily substantially increases small bowel water content in healthy volunteers, retaining fluid in the small bowel. This increases the effect of the gastro-ileal response to eating which propels the retained fluid from the ileum to the ascending colon immediately after eating a 1000 kcal meal[138]. This is associated with an increase in colonic volume and water content. Kiwifruit appear clinically to be as effective as ispaghula as shown in a recent multi-centre trial in Japan, Italy and New Zealand of 178 participants (n= 61 healthy, n= 57 FC and n = 60 IBS-C). The effects of 4 weeks of kiwifruit (2/day) and psyllium (7.5g/day) were comparable, with an increase of 2.1 complete spontaneous bowel movements per week in the kiwifruit arm

compared to 0.92 per week with psyllium compared to baseline[199].

Kiwifruit also contains insoluble fibre, which in the form of 15 g bran has also been demonstrated to increase postprandial small bowel water content[137] and accelerate small bowel transit[200].

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 recently developed and validated novel non-invasive MRI techniques, we have assessed transit and fluid distribution in the small intestine as well as colonic fluid and volume[31,136,156,181] in order to define the mechanisms behind the known laxative effects of kiwifruit consumption. We hypothesised that consuming kiwifruit would combine the effects of both soluble and insoluble fibre, increasing the water content of small and large intestine contents in healthy volunteers.

Methods

Study Design

This study was a two period, two treatment crossover study with randomisation of treatment order. The study protocol was

approved by the University of Nottingham Medical School Ethics Committee (Ref A200317) and all volunteers gave written informed consent. The study was carried out according to Good Clinical Practice as defined by the Declaration of Helsinki. The study was registered on clinicaltrials.gov, reference: NCT03303417.

Study Participants

Adult participants (aged 18-65), able to give consent and scoring less than 2 on the 15 individual items (i.e. no more than occasional symptoms of short duration <15/45 total) of the Gastrointestinal Symptom Rating Scale[201,202] were recruited. Exclusion criteria were existing GI disorder or major GI surgery, inability to cease medication affecting GI transit or motility, known intolerance of kiwifruit, contraindication to MRI scanning or lifestyle factors likely to disrupt gut function or compliance with the protocol such as night shift working.

Test Products and Meals

Test product was either two large and ready to eat green variety kiwifruits without skins (approximate weight 150g per kiwifruit) eaten twice per day or calorie matched control drink (28 g maltodextrin, average degree of polymerisation = 5, in 250ml water providing 120kcal and estimated osmolality of 124

mOsmol/l). These were consumed for 48 hours prior to the study day and on the scanning day (12 kiwifruit/6 drinks total).

On the study day (see Figure 16 for an overview), participants were fed two standard meals that we have used in previous studies[137]. Meal A consisted of 220 g creamed rice pudding (Sainsbury's, London, United Kingdom), 34 g seedless raspberry jam (Sainsbury's) and a glass of 100 ml pure orange juice from concentrate (Sainsbury's), providing 331 kcal. Meal B consisted of 400 g microwaveable macaroni cheese ready meal (Sainsbury's), 100 g cheesecake slice (Sainsbury's), and 250 ml bottled still water, providing 1007 kcal.

Study Day Protocol

Consent, screening and enrolment took place at the Nottingham Digestive Diseases Biomedical Research Unit, Nottingham. All subsequent study visits took place at the Sir Peter Mansfield Imaging Centre, University of Nottingham. Subjects visited the Imaging Centre on two separate occasions, with at least two weeks between each visit to minimise any carryover effect. Subjects were asked to abstain from alcohol, caffeine and strenuous exercise for 18 hours before the visit and did not eat after 8pm on the evening beforehand. The day before the scan they ingested five MRI marker pills at 9 AM, which were imaged 24 h later at the beginning of the study day. Ingestion was

confirmed by a time- stamped video. The marker pills, which were developed in-house, are inert capsules containing the MRI contrast agent gadolinium, making the pills clearly visible on the MR scan. Each pill is allocated a score based on its position in the colon and a weighted average position score calculated as previously described[156]. The same publication showed that this score correlates well with transit time in hours as measured using the well-established radio-opaque marker test[18].

On the day of the visit subjects fasted before attendance, with the exception of sips of water with essential medicines. On arrival they underwent a baseline MRI scan. They were then given 10 minutes to consume the test product. 30 mins later they consumed Test Meal A followed by another MRI Scan (Time=0). They then underwent hourly scanning for 7 hours (T= 0-420). They had a second dose of test product immediately before the scan at T180 to simulate the effect of 2 doses daily giving time to see any effect before the final Meal B between T=360 and T=420 (Figure 16).

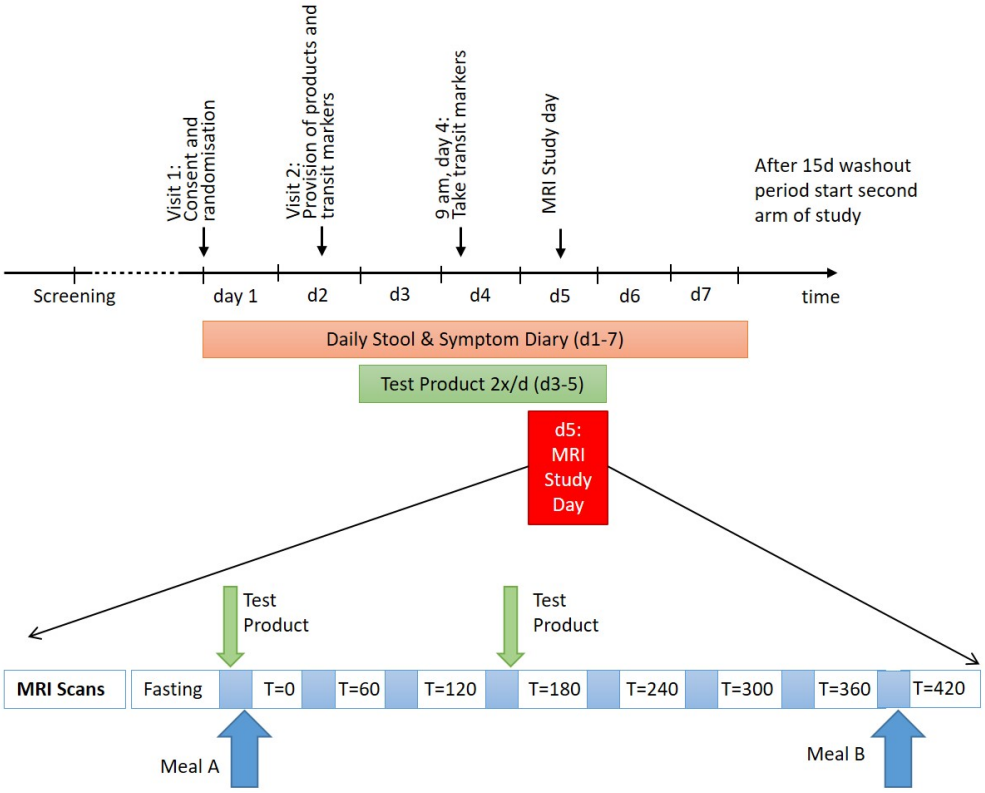


Figure 16: Schematic Diagram of Study

Background diet was not controlled other than the instructions given for the day before and during the MRI day, however participants were asked to remain on their normal diet and not eat any other kiwifruit during the study period (except those provided by the study team).

Questionnaires

Participants were asked to keep a diary of symptoms (abdominal pain, nausea, bloating, gas estimated on a scale of 0-3: none, mild, moderate, severe) and bowel habit (frequency and form according to Bristol stool form chart) during the week of the study day – this included the 2 days prior to the

consumption of the test product, the 3 days consuming (including study day) and 2 days after this. Data were analysed for kiwifruit or control on days 3-6 of the respective study arm.

MRI Protocol

Imaging was carried out on a fully research-dedicated 3.0T Philips Achieva scanner. (Best, The Netherlands). Subjects were positioned supine in the scanner with a parallel imaging SENSE XL-Torso receiver coil wrapped around the abdomen. Different imaging sequences were used to optimally image the different regions of the gut as follows.

1) A high resolution balanced gradient echo sequence to acquire sagittal images of the contents of the ascending colon, 8 slices 5 mm thick, 0.58 mm gap was acquired to include the entire ascending colon. This was repeated for the descending colon.
2) The longitudinal relaxation time T1 was measured in the ascending colon and separately in the descending colon, using a single slice inversion recovery balanced turbo field echo sequence with a preparatory 180 degrees inversion pulse applied before acquiring the imaging data. The slice for the T1 is chosen from the high-resolution images and is chosen as the slice which provides the best cross-section through the colonic segment maximising the colonic content

to sample. The parameters for this sequence were a field of view of 400 (HF) x 280 (AP) mm; 7 mm slice thickness; half Fourier acquisition; 256 x 256 matrix; repetition time/echo time 2.4/1.2 ms; flip angle 45°. Data were acquired from 8 different inversion times (TI; time between inversion pulse and imaging pulses) ranging from 0.05 – 4.95 s[181]. There was a 15 second gap between each acquisition to allow the system to return to equilibrium.

3) A single shot, fast spin echo sequence (rapid acquisition with relaxation enhancement; effective echo time TE = 400 ms) to acquire in a single breath-hold, 20 coronal images with in-plane resolution interpolated to 0.78 mm × 0.78 mm and a slice thickness of 7 mm, with no gap between slices (acquired voxel size = 1.4 × 1.76 × 7 mm^3), SENSE = 2.0. This sequence yields high-intensity signals from areas with freely mobile fluid and little signal from body tissues and was used to measure small bowel water content[136,137].
4) Colonic volumes were assessed using a coronal 3D dual-gradient echo sequence (TE1 = 1.07 ms, TE2 = 1.9 ms, repetition time =3.0 ms) and mDIXON readout[203]. The field of view at each station was 250 mm (HF) x 375 mm (LR) by 200 mm (AP) with acquired slice thickness 3.6 mm, interpolated to 1.8 mm with a reconstruction matrix of 432. These 2 sets of images were combined into a single image

of matrix size 543 (HF) x 432 (LR) and 37 slices of thickness 5.4 mm and were used to visualize the colonic anatomy. Four different images types are produced from this acquisition, water only, fat only, fat and water in-phase and fat and water out-of-phase data. Slices were acquired in two overlapping coronal stacks (30 mm overlap), each acquired in a breath hold. Colonic volumes were measured manually as previously described[31]. The water-only images from this sequence were also used to locate the position of the transit pills within the GI tract[156].

Data were all 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 spent approximately 20 minutes inside the magnet for every time point and the rest of the time sitting upright in an adjacent room.

Outcomes

The primary outcome was longitudinal relaxation time (T1, an MRI constant reflecting water mobility) of the chyme in the ascending colon, this reflects the fluidity of the contents, with more fluid contents having a longer T1 time e.g. liver has a T1 of 750 ms while cerebrospinal fluid has a T1 of 4000ms at 3T field strength. Secondary MRI outcomes were: T1 of descending

colon, small bowel water content, colonic volume, gut transit time as assessed by WAPS at 24 h (where a higher score means longer transit time), and questionnaire-based scores for symptoms and bowel habit. Stool consistency was measured using the Bristol stool form scale (ranges from 1= hard lumpy stools to 7=watery stools).

Randomisation and Blinding

The order of the test products was randomised using www.randomisation.com. Neither subjects nor researchers were blind to the meal consumed on study days. All study data and images carried an anonymising study ID number and MRI analysis was conducted blind to the intervention.

Power Calculation

Our data[138] with a standard laxative dose of psyllium 7g three times per day showed a change of the area under the curve of T1 versus time from time 0 to time 360 minutes (T1 AUC 0-360) of mean (SD) 88 (55) sec*min. Using this data, we calculated that n=15 healthy volunteers will give us >90% power to detect a similar difference.

Data Analysis

The image data set from each subject and each time point was stored on a password-protected server and processed as

previously described using either software written in house in IDL (Version 6.4 Research Systems Inc., Boulder, Colorado, USA; for analysis of SBWC and T1 relaxation times) by Dr Caroline Hoad at the Sir Peter Mansfield Imaging Centre (SPMIC), or commercial software (Analyze v.9, Biomedical Imaging Resources, Mayo Clinic, Rochester, MN, USA; for analysis of colon volumes)[31,136,181].

Statistical analysis

Graphpad Prism (v.7; San Diego, CA, USA) and SPSS (v.24; IBM; Armonk, NY, USA) were used for statistical analysis of the data. We assessed the changes over time and between treatments using a two-way analysis of variance (ANOVA) test. Normal distribution of data was determined using the Shapiro-Wilk test. Within each scanning time point, data were then compared with a paired t-test, for parametric data, or with a paired Wilcoxon test, for non-parametric data. P=0.05 was used as the threshold for statistically significant outcomes. When data were ordinal (stool frequency, stool form score), a Wilcoxon test (kiwifruit compared to control) or Friedman ANOVA (kiwifruit compared to control and baseline) was carried out. For the outcomes recorded in the questionnaires over several days (stool frequency, stool form, symptoms) the data within each period (kiwifruit and control) were summarised by

their median for each participant, before applying the appropriate tests. Data are displayed as median and interquartile range.

Results

Sixteen participants were recruited but two dropped out after a single visit due to unexpected work commitments so 14 volunteers completed the study. Of these 14, 6 were female, 8 were male with a median age of 26±4 (range 21-33) years and a body mass index of 23±4 (range 18-30) kg/m^2. Median score 0.5 (range 0-4) on Gastrointestinal Symptom Rating Scale. The study procedures were well tolerated by the subjects.

T1 relaxation time of chyme in the ascending colon

The primary outcome was defined as the area under the curve for T1 relaxation time measured in the ascending colon over the time from baseline until the end of the study day (T420), comprising of eight hourly scans, and was increased in the kiwifruit arm however did not reach significance (P=0.056, **Table 4**). As individual data points measured at each scan, T1 relaxation time is not different as analysed by two-way ANOVA, P=0.75 (**Appendix A**).

Analysing the T1 relaxation time data further, when the area under the curve was measured from the time point when the

first meal and treatment products start reaching the colon, T240-T420, this showed a statistically significant increase of T1 relaxation time from the control to the kiwifruit arm of the study (+27%, *P*=0.029, Table 4 and Figure 17).

Table 4: Data for MRI variables measured as AUC of hourly scans. AUC expressed as a function of time (SBWC and colon volume ml.minutes or T1 second.minutes). Data are expressed as mean ± SD.

MRI Endpoint	Kiwifruit	Control	p-value
AUC T1 AC (sec.min) Baseline-420	356 ± 109	291 ± 110	P=0.056
AUC T1 AC (sec.min) T240-420	137 ± 39	108 ± 40	P=0.029 *
AUC T1 DC (sec.min) Baseline-420	216 ± 120	203 ± 114	P=0.3
AUC T1 DC (sec.min) T240-420	96 ± 50	87 ± 52	P=0.4
AUC SBWC (ml.min) Baseline-420	85,530 ± 34,923	35,023 ± 15,557	$P=6x10^{-6}$ *
AUC Colon Volume (ml.min) Baseline-420	283,583 ± 59,402	243,182 ± 46,682	P=0.004 *

AUC – area under the curve; T1 – T1 relaxation time; AC – ascending colon; DC – descending colon; SBWC – small bowel water content

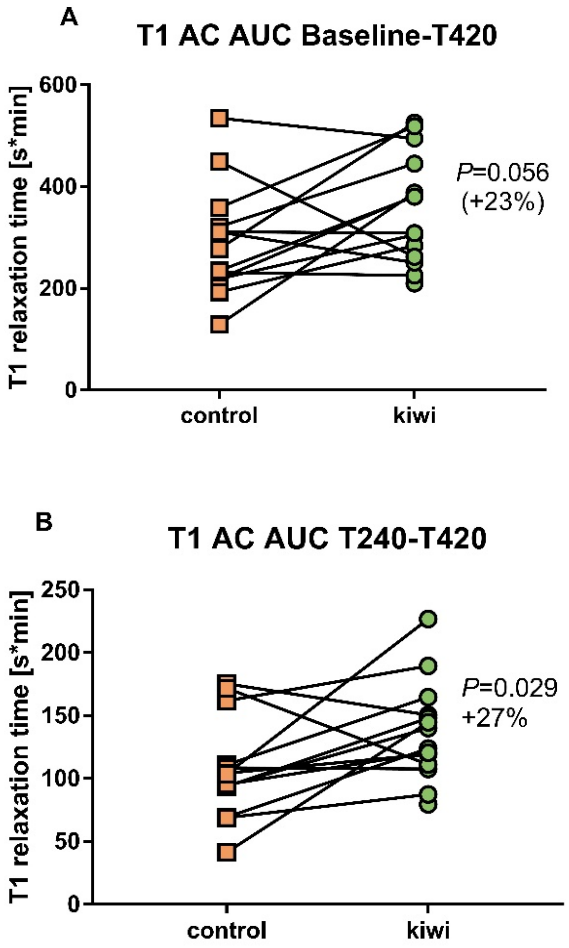


Figure 17: T1 relaxation time in the ascending colon expressed as area under the curve from baseline until the end of the scan day (Baseline-T420, A); and from the time that the first meal is assumed to have reached the ascending colon until the end of the scan day (T240-T420, B).

T1 relaxation time in the descending colon

In the descending colon measuring T1 relaxation time was challenging, as the descending colon was sometimes empty, therefore some data sets were excluded from the analysis (n= 9 for control and n=12 for kiwifruit). There was no difference between the kiwifruit and the control arms of the study, regardless whether the area under the curves was considered

over the whole day (P=0.3, **Table 4**), over the later time points (T240-T420, P=0.4, **Table 4**) or when each time point was considered individually (two-way ANOVA P=1.0, **Appendix A**).

Small bowel water content

Kiwifruit markedly increased small bowel water content, both when expressed as area under the curve (P=$6*10^{-6}$) (**Table 4**), and when measured throughout the day (two-way ANOVA, overall P=$3*10^{-32}$, difference between treatment products P=$2*10^{-22}$, difference between time points P=$2*10^{-18}$, interaction P=$8*10^{-7}$). Small bowel water content was increased at all time points except for baseline, when participants arrived fasted (paired tests, P<0.05 indicated by asterisks, Figure 18, Appendix A).

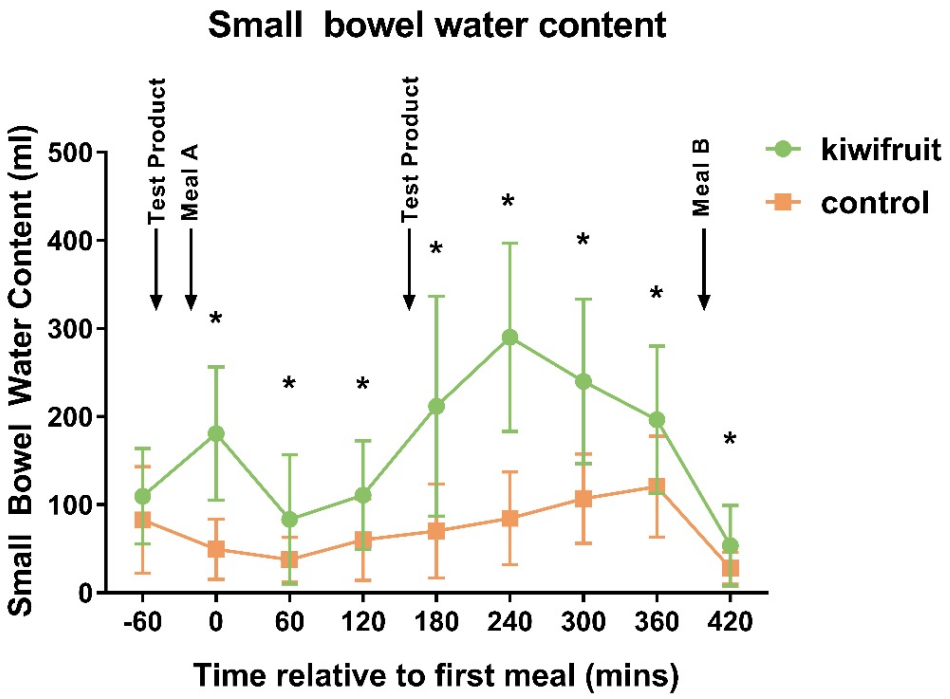


Figure 18: Small bowel water content shown over the course of the study day. Points represent mean ± SD and asterisks indicate P<0.05

Regional and total colon volumes

As Table 4 shows, area under the curve from baseline-T420 for the total colon volume is increased in the kiwifruit arm of the study (*P*=0.004, see Figure 19 and Table 4), due to an increase in ascending colon volume (*P*=0.003). Both transverse and descending colon volume AUCs were similar between the groups.

Two-way ANOVA showed significant effects of treatment but not time for total colon volumes and ascending colon volumes, $AUC_{base\text{-}420\ min}$ of total colon volumes (overall *P*=0.011, difference between treatment products $P=2\times10^{-7}$, difference

between hourly time points P=0.8, interaction P=1.0) and $AUC_{base\text{-}420\ min}$ of ascending colon volumes (overall P=0.035, difference between treatment products $P=2x10^{-6}$, difference between time points P=0.8, interaction P=1.0). However, ANOVA for transverse and descending colon volumes showed no significant differences (P=0.9 and P=0.148, respectively).

Total colon volume was increased at all time points except baseline (paired tests, $P<0.05$). Similarly, volumes of the ascending colon were increased at most time points (except baseline and T180), whereas transverse and descending colon volumes were only significantly increased at a few time points, which for the descending colon tended to be towards the end of the scanning session (**Appendix A**).

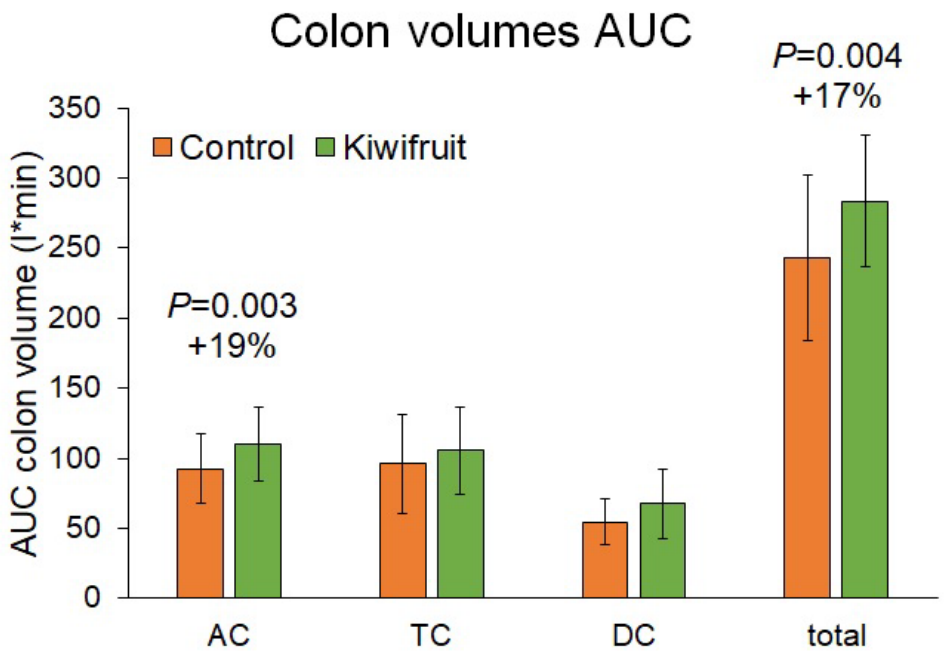


Figure 19: Total and sectional colon volumes expressed as the area under the curve (AUC) throughout the study day (baseline to T420)

Whole gut transit

Whole gut transit was similar on both arms of the study with weighted average position score (kiwifruit median score 0.8 (0-1.4 IQR), control median score 1.0 (0.5-3.1 IQR), *P*=0.11).

Stool diary and symptom questionnaire

Daily stool frequency was significantly increased during the kiwifruit arm (defined as days when the treatment product is consumed plus the following day, i.e. days 3-5, indicated by the green shaded area in Figure 16) compared to the control arm (1.46 ± 0.66 vs 1.14 ± 0.46 stools per day; *P*=0.034 Wilcoxon; Figure 20a).

The consistency scores of stools were different between groups (*P*=0.049, Figure 20b), with higher scores (softer/more watery stools) in the kiwifruit arm compared to control (*P*=0.011).

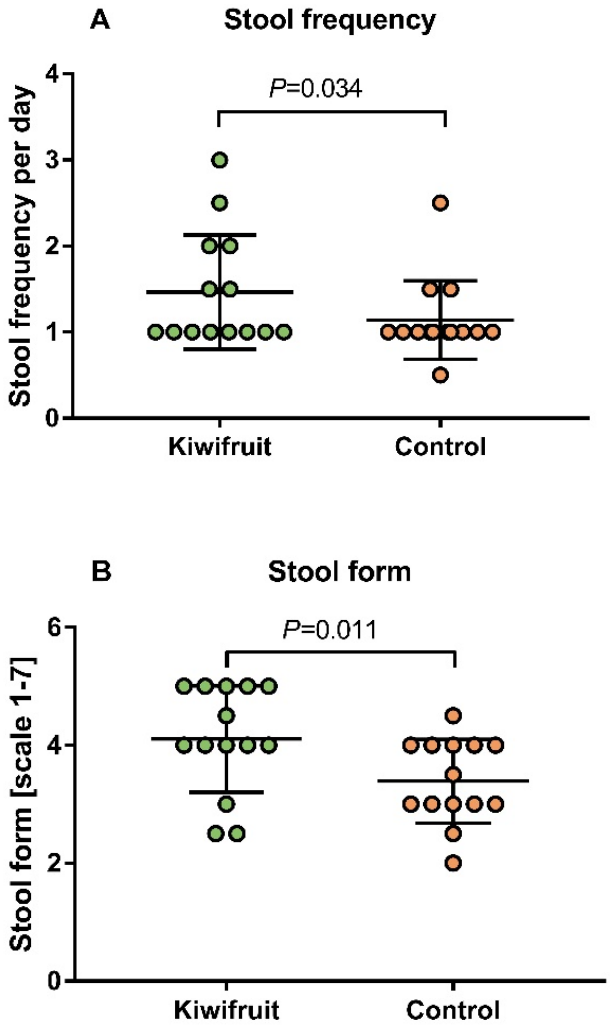


Figure 20: Stool frequency per day during each of the three study periods (A) and stool form, self-assessed following the Bristol chart, ranging from 1 for severe constipation to 7 for extreme diarrhoea, during each of the three study periods (B)

There was no significant difference in the scores for any adverse symptoms between the kiwifruit consumption, control consumption and baseline (Appendix A). As both kiwifruit and

the control maltodextrin drinks were well tolerated, participants reported few instances of these symptoms, with no symptom reported to be higher in severity than level 2 (moderate).

Discussion

This study aimed to use MRI techniques to identify the mechanism of action behind the therapeutic benefit seen from kiwifruit in previous studies. We hypothesised that some of the physicochemical properties of kiwifruit would increase intestinal water, and we have found this to be the case.

Colonic water content, reflected by T1 relaxation time, showed some increase in the ascending colon. This is likely due to water trapping by the fruit fibre in the small intestine. The timing of the increase in colonic water content in the second half of the study day is in accordance with when the meal contents would be predicted to reach the caecum.

In the descending colon measuring T1 relaxation time was challenging, as the descending colon was sometimes empty. There was no difference in T1 found here and the values were generally lower than those in the ascending colon. This is as previously reported[138] and in keeping with the function of the colon in progressively dehydrating colonic contents as they move distally. It also suggests that the kiwifruit fibre is

degraded sufficiently by this point to no longer exert any effect here.

Regarding the small bowel water content, the pattern seen on the control arm was similar to that observed previously[137,188] with an initial drop in content likely to reflect the rapid absorption of water with the glucose, fructose and sucrose molecules in the orange juice in the test meal, followed by a later rise due to intestinal and pancreatic secretions accompanying the gastric emptying of the solid phase of the test meal into the small bowel. The maltodextrin did not appear to increase small bowel water content over baseline despite being given with 250ml of water behaving as we have observed with pure glucose[137] suggesting it was rapidly digested. In contrast the water content on the kiwifruit arm rose initially, with two peaks seen to coincide with the ingestion of the kiwifruit doses. This suggests kiwifruit impairs water absorption as it does for glucose absorption as previously demonstrated by reduced postprandial hyperglycaemia[204]. The second peak was greater than the first – which may reflect retention of the initial fibre within the small bowel with additional water trapping by the second dose. The larger increase after the second dose may be also due to the fact that the sugars in Meal A may have enhanced water absorption immediately after ingestion of the first dose.

There was no significant difference in small bowel water content at the fasting time point despite pre-dosing for 2 days, which suggests that the effect of the kiwifruit disappears overnight when the small bowel contents are emptied into the colon by the migrating motor complexes which occur during overnight fasting[205].

Colonic volumes were increased by kiwifruit ingestion which may reflect the increased volume of water arriving in the colon. Although flow through the colon is not uniform, colonic volumes can be approximated from the formula: Volume = flow (24 h stool excretion) x transit time. The rise in colonic volumes despite unchanged transit time implies therefore an increase in 24-hour stool excretion in keeping with the more frequent passage of looser stools we observed. It is worth noting that while the colon volume average as assessed by the AUC 0-420 min throughout the study day rose by 16% this is much less that the 50% increase in total colonic volume we previously reported after 10.5g of psyllium daily[138]. This may suggest that kiwifruit has prokinetic effects in addition to simple water trapping and hence less colonic distension when compared to ispaghula. Whether this translates into less bloating would require a head to head direct comparison study.

Acceleration of transit, when compared to psyllium (7.5g/day), by 2 kiwifruit daily was observed in the Japanese arm[29] of the multicentre study already described above[16] . The fact that we did not observe a significantly accelerated transit time may in part be due to a "floor effect" in this healthy volunteer population in whom transit, as we have previously observed, is relatively fast at 28 h (IQR 4-50) with some subjects with a low or zero weighted average position score and hence difficult to demonstrate further accleration[156]. We did observe that there was a higher proportion of subjects who had expelled all the marker pills at the 24-hour mark (giving a score of 0) in the kiwifruit arm (six participants) compared to control (two participants). Similar findings were seen in another of our recent studies assessing the effect of psyllium on transit in which transit times were not seen to be significantly increased in healthy volunteers as most markers had passed to the rectosigmoid colon or had been expelled at 24 hours, whereas a significant increase in speed was seen in participants with constipation whose baseline transit was slower[138].

Despite using healthy volunteers rather than constipated patients the change in bowel habit (increased frequency with a normal stool form) and low side-effect profile are similar to what has been seen in previous patient studies[190–192].

Limitations

This mechanistic study was limited by small sample size which was exacerbated by some participants' withdrawal from the study. Our subjects were young adults, so caution is needed in extrapolating our results to the elderly. However, these data will serve as a basis for power calculations for future MRI studies of digestive function.

We also did not control any other aspects of diet – for example limiting any other fruit consumption (beyond asking not to eat kiwifruit in the control week) or other sources of fibres. We gave two meals on the study day to simulate how kiwifruit are normally consumed as part of a mixed diet. Both have been used in previous studies, so their effect is known, however the nutrients they contain may have interacted with the digestion of the kiwifruit and vice versa.

The kiwifruit we used were top quality and somewhat larger than the average on sale at present. Furthermore, the dose of two kiwifruit twice daily was large compared to normal consumption but ensured a clear mechanistic result in the limited intervention time with the small numbers it was feasible to scan. The clinical trials show that more reasonable doses i.e. 2 daily are also clinically effective[190–192,199]. The maltodextrin

acted as a control for calorie content but did not control for other aspects like chewing and salivation.

Future work and Conclusions

Future work may want to look at whether other fruits have similar mechanisms of action or whether these are unique to the kiwifruit. Kiwifruit contain substantial amounts of the protease actinidin which has the potential to activate protease activated receptors found on enterocytes, lymphocytes and enteric nerves which have been shown to play an important role in visceral sensitivity. Kiwifruit also contain raphides (needle-shaped calcium oxalate crystals found in the tissues of kiwifruit). It is unclear whether these are all destroyed in the acid gastric environment or whether some enter the small bowel where they might stimulate water secretion. Whether raphides or actinidin alter colonic absorption, secretion or motility remains to be determined. The effect of the kiwifruit on the microbiota may also be another area worth studying in more detail since preliminary studies show its consumption increases lactobacilli and bifidobacteria[207].

In conclusion, these MRI data suggests that consumption of kiwifruit in healthy volunteers increases water content in the small bowel and ascending colon, as well as an increase in colonic bulk. These data are consistent with the observations of

an increase in stool frequency and looser stool consistencies, suggesting that kiwifruit could be used as a dietary alternative to laxatives in mild constipation.

SECTION 3: RECLASSIFYING CONSTIPATION USING IMAGING AND MANOMETRY

The final section of this thesis will focus on studies using MRI and manometry techniques to assess the motility of the colon in both healthy volunteers and patients with constipation. The first of which assessed the reproducibility of the MRI measures and the second which looks at comparing the results of both.

Chapter 6: The MRI Colonic Motility Test: Reproducibility of the Macrogol Stimulus Challenge

This project was accepted for publication to Neurogastroenterology and Motility and is in press (DOI 10.1111/nmo.13942):

Abstract

Background

Previous work developed a novel diagnostic test using magnetic resonance imaging (MRI) to assess colonic response to a macrogol challenge drink to define underlying mechanisms of severe constipation. We aimed to measure the intra-subject reproducibility of objective MRI measures of colonic function, which have not previously been explored.

Methods

Healthy participants aged ≥18 attended for MRI on two occasions (identical protocols, minimum 1 week apart). They underwent a fasted scan then consumed the macrogol drink. Subjects were scanned at 60 and 120 minutes, with maximum value reached used for comparison. The colonic volume, water

content, mixing of colonic content and the movement of the colon walls were measured. Coefficients of variation and intraclass correlation coefficients (ICC) were calculated.

Results

12 participants completed the study: 9 females, mean age 26 years (SD 5) and body mass index 24.8kg/m2 (SD 3.2). All measures consistently increased above baseline following provocation with macrogol. The volume, water content and content mixing had good intra-subject reproducibility (ICC volume=0.84, water content=0.93, mixing=0.79, $p<0.001$). With the wall movement, the response to the challenge was generally large, but more variable between visits resulting in a lower ICC overall (Ascending Colon=0.65, Descending Colon=0.76, $p<0.001$).

Conclusions

The colonic response to the macrogol stimulus as assessed by MRI is heterogeneous but large compared to baseline, with moderate to good reproducibility, making the test suitable to study potential pathologies underlying GI disorders such as constipation. More data are needed to better define the normal range for comparison with patient groups who may have both hypo- and hyper-motile responses.

Introduction

Constipation is a common condition, affecting on average 14% of the world population[208]. For those affected it can have a serious impact on quality of life[10] and associated economic cost[3].

The latest Rome Criteria (Rome IV) split primary constipation into Functional Constipation (FC) and Irritable bowel syndrome with constipation (IBS-C), distinguished by the presence or absence of pain in association with bowel habit. These conditions may have different underlying mechanisms, requiring quite different treatments. Distinguishing these conditions in the clinic based mostly on patient report leads to trial and error treatments which may explain why nearly 50% of patients are dissatisfied with their treatment[5].

A novel diagnostic test using Magnetic Resonance Imaging (MRI) based on the consumption of a macrogol challenge drink has been developed to define the underlying mechanisms of severe constipation[32,180]. This looked at assessment of the colonic volumes, water content and mixing of colonic chyme, as well as movements of the colon walls, critical components in defining organ function. This showed that there may be distinct differences between the two groups of patients, and if this is the case then they would likely need different treatments.

It is currently unknown; however, how reproducible the response of the colon is to this stimulus test. Previous studies have looked at the variability and reproducibility of other GI measurements such as gastric emptying and transit, as well as manometry techniques and have found considerable inter- and intra-subject variability using a variety of analytical methods including coefficients of variation, intra-class correlation coefficients and Bland Altman analysis[14,209–212].

The main aim of this study was to measure the intra-subject reproducibility of these MRI colon function measures using an open-label study design in a group of healthy volunteers. A secondary aim was to assess the feasibility of measuring the motor function of the descending colon (not studied in previous work) since abnormalities of distal colon function are also likely to be an important determinants of bowel function.

Methods

Study Design

This was an open-label study examining the reproducibility of the response of the large intestine to acute ingestion of 500mL to 1000 mL of poly-ethylene glycol and electrolyte solution (Moviprep, Norgine Pharmaceuticals Ltd, Harefield, UK). The study was approved by the Ethics Committee of the University

of Nottingham (Nottingham, UK) [UoN FMHS D10052016 SPMIC MR]. The study was carried out according to Good Clinical Practice principles and all participants gave written informed consent.

Subjects

Twelve healthy volunteers were recruited from the general campus population at the University of Nottingham by advertisement. The inclusion criteria were male or female, 18–55 years of age, body mass index between 18 and 30 kg m^{-2}. The exclusion criteria were pregnancy, any history of serious acute or chronic illness especially gastrointestinal, use of medication known to affect gastrointestinal transit, such as opiates and constipating drugs, substance abuse, and unsuitability for MRI scanning (such as pacemaker). Prior to each study visit participants were asked to complete the Talley bowel habit questionnaire[213] and screening diary to ensure they did not suffer from recurrent abdominal pain and bowel frequency was normal (defined as bowel movements > 2 and < 10 over the 3 days prior to each MRI study day).

Study Day Protocol

Each participant attended the study centre on two occasions, at least 1 week apart. They were asked to avoid strenuous exercise, dietary supplements and alcohol for 24 hours prior to

the study visit and caffeine for 18hrs. On the morning of the scan, participants arrived fasted (from 10.30pm the night before) at the study centre.

After completing the daily eligibility questionnaire participants underwent a 20-30-minute MRI baseline scanning session. Participants then drank 10 mL per kg of body weight (rounded to 100 mL) of Moviprep, at a rate of 2.5 mL kg-1 per 15 minutes. This was based on previous experience that using fixed doses of 1000 or 2000 mL made smaller subjects unduly nauseated so that they could not reliably consume the prescribed amounts introducing an uncontrolled source of variation between subjects. Participants underwent two further 20-30-minute scan sessions at 60 (T60) and 120 (T120) minutes after starting the macrogol drink (see schematic Figure 21).

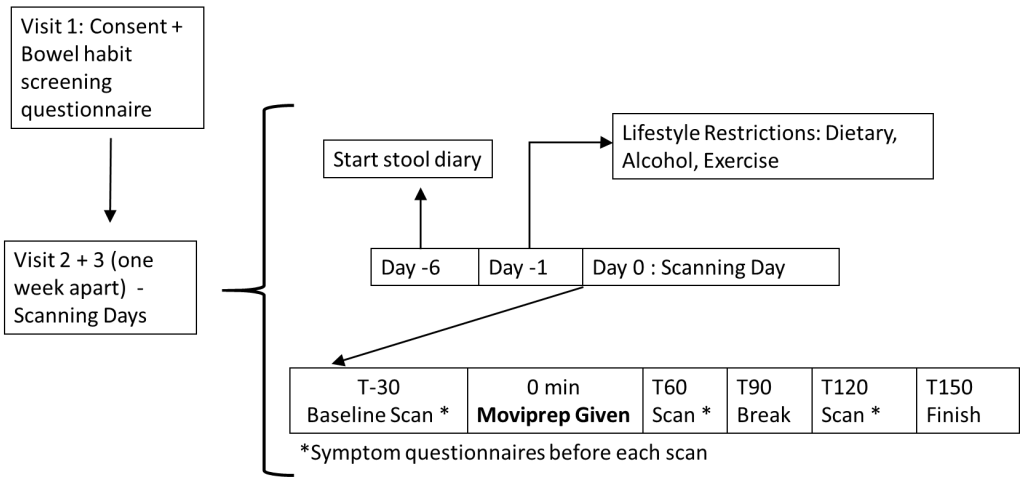


Figure 21: Schematic of the study day

Participants were asked to rate their symptoms of pain, bloating and flatulence as absent=0, mild =1, moderate =2 and severe=3 using the questionnaire of Tornblom[150] at baseline, immediately after finishing the drink (T60) and 60 minutes later (T120) (Appendix C). Participants were also asked to record the time of their first bowel movement following the drink (TBM).

MRI Protocol

Imaging was carried out on a 3.0T Ingenia wide-bore scanner (Philips, Best, The Netherlands) with a parallel imaging SENSE abdominal body receiver coil. A range of MRI sequences were used to image the abdomen including:

1) Colonic regional and total volumes: a 3D coronal dual echo fast field echo sequence with mDIXON reconstruction[203].
2) Bowel Water Content: A single shot, coronal fast spin echo sequence (rapid acquisition with relaxation enhancement, RARE).
3) MRI content mixing measurement: A single 10 mm slice cine balanced turbo field echo (bTFE) with tag lines 12 mm apart.
4) MRI wall movement measurement: Cine bTFE.

Full sequence information is detailed in Appendix B. Data were acquired on an expiration breath-hold with duration between 18

and 24 seconds depending on the sequence (for the first 3 sequences), monitored using a respiratory belt. The colon wall measurement sequence was duration 10 minutes with a temporal resolution of 1 sec acquired during gentle free breathing.

Image Analysis

Analyses were performed with automated and semi-automated image analysis software (Analyze9® (Mayo Clinic, Rochester, USA), and software written using Matlab® (The MathWorks Inc, Natick, USA) and IDL® (Research Systems Inc., Boulder, USA). Colonic volumes and water content (mL) were measured as previously described[31,136,162]. For the colonic volume data, the slice thickness was increased to 5.4 mm by averaging 3 consecutive slices to reduce the number of images to be used in the analysis from 111 to 37. Colonic content mixing within the colon was assessed using the average coefficient of variance (%) for the colonic region of interest in the tagged cine MRI data[162]. This was calculated from mean and standard deviation maps of the voxel intensities measured across the time series cine data, with a user defined region of interest drawn round the colon contents on the mean intensity map.

For the colonic wall movements, the untagged cine data were non linearly registered across the time course using GIQuant®

(Motilent Ltd, London, UK)[161]. The spatio-temporal motility technique (STMM) was then used to assess changes in luminal diameter with time (due to contractions) along the AC and DC using software written in Matlab[160,214] and this data were used to calculate the combined velocity distance motility index (a.u)[214]. Briefly, the speed of the wall motion was multiplied with the normalised luminal diameter changes to generate a plot highlighting both deformation (expansion and contraction) and motion of the bowel wall. The area under this plot above a threshold value (which excluded breathing noise artefacts in the data) was then calculated. This method was previously validated by comparison against a visual scoring system[160,214].

Statistical Analysis

Basic characteristics of the study population were summarised using frequencies, percentages, means and standard deviations as appropriate. The maximum value after the drink for each MRI parameter was determined from T60 and T120 data for each subject to allow for the fact that oro-caecal transit times of the drink, and hence time of peak colonic distension, could differ between the two visits. Normality of the data was assessed using the D'Agostino & Pearson normality test and all statistical tests were performed using GraphPad Prism version 7.03 for Windows, (GraphPad Software, La Jolla California USA).

The reproducibility of these MRI parameters (volumes, water content, content mixing and wall movement) as well as time to first bowel movement (TBM) was determined by carrying out Bland-Altman analysis[215]. Coefficients of variation ((Standard Deviation / Mean) x 100) for intra-subject data and intra-class correlation coefficients (ICC) (using two way mixed models with absolute agreement) were calculated for the baseline and maximum values acquired by T=120 minutes from starting the drink, analysed from the 2 visits. Inter-subject coefficient of variance was also assessed to determine whether the intra-subject variability was similar to the inter-subject variability.

Bland-Altman Plots were generated with dotted lines representing bias and 95% limits of agreement, and a paired t-test or Wilcoxon test (if non-normal data) was used to assess the statistical significance of this bias. For ICC, the 95% confidence interval of the ICC estimate is used as the basis to evaluate the level of reliability using the following general guideline:

"Values less than 0.5 are indicative of poor reliability, values between 0.5 and 0.75 indicate moderate reliability, values between 0.75 and 0.9 indicate good reliability, and values greater than 0.90 indicate excellent reliability"[216].

We also looked at the use of a 'responder rate' to assess the wall movements response to the stimulus, using the 90% centile of baseline values as a cut off for a definition of 'responder'; we determined whether participants responded to the macrogol stimulus the same way on both occasions. This follows the assumption that the baseline data represent the no or very low motility state with its inherent measurement error due to breathing and positioning of the lines to generate the motility metric. A 90% limit to this baseline data would therefore give a realistic upper limit to the no motility state. Response to stimulus was also tested in all MRI parameters at both visits using either the paired T-test or Wilcoxon test of baseline and maximum values, to determine whether a significant increase in each parameter was measured after the macrogol drink.

This was a pilot study to enable us to make power calculations for the MRI parameters for future studies. Given the expense of these studies we chose a sample size of 12 which we felt was a reasonable compromise and has some justification based on rationale around feasibility and precision of estimates[217]. A previous manometry reproducibility study showed good reproducibility in ambulatory manometry readings with just 7 participants[210].

Results

12 participants were recruited: 9 female, 3 males, mean age 26 (SD 5) and body mass index 24.8 (SD 3.2) kgm^{-2}. Examples of images obtained for analysis are shown in Figure 22, highlighting the features extracted from the MRI data.

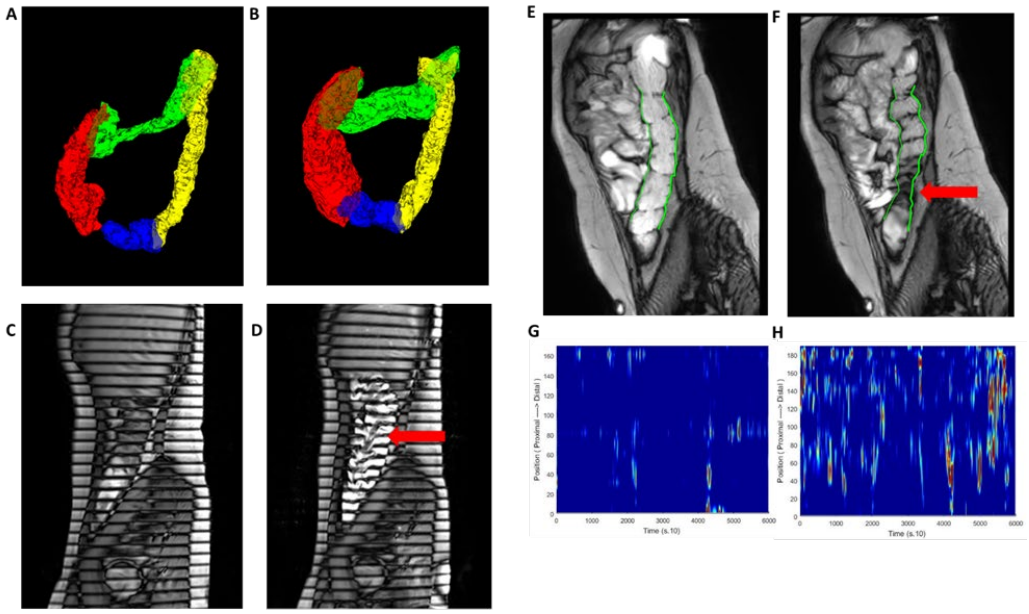


Figure 22: A+B: Pre and post stimulus anatomical 3D rendered images for analysis of colonic volume. C+D: Pre and Post stimulus tagging images (content mixing) of the AC, in Figure 2a the tagging lines remain horizontal whereas in 2b they are distorted by fluid flow in the AC (arrow). E+F: Images from wall movement analysis, vertical lines are drawn at the edge of the descending colon which move with contractions (arrow). G+H: Pre and Post stimulus STMM Maps produced from wall movement analysis, showing meal response.

Reproducibility Measures

The details of the summary data for our MRI parameters and TBM as well as coefficients of variation and ICC are shown in Table 5. All MRI endpoints changed significantly from baseline to maximum values following the macrogol challenge.

In all parameters the intra-subject CoV was smaller than the inter-subject CoV. The ICCs were more varied with all the ICC's above 0.6 (moderate) however some of the lower limits of the 95% CI dropping to 0.3. The ICC for volumes and water content performed best with colonic volume ICC at 0.84, maximum total gut water content and maximum colonic water content were 0.92 and 0.85, respectively. Reproducibility for mixing and movement measurements of both the contents and walls in both the AC and DC performed less well (ranged from 0.62 to 0.76). The time to bowel movement had an ICC of 0.98.

Bland-Altman plots are shown in Figure 23 for the maximum post stimulus data only, which show no statistically significant bias in the data, however the 95% limits of agreement varied greatly. Correlation graphs of baseline and maximum values, with line of identity shown, Visit 1 vs Visit 2, are shown in Figure 24 and show the large effect of the macrogol stimulus on the MRI parameters measured.

Table 5: Reproducibility Results

Measurement	Time point	Visit 1	Visit 2	CoV (Intra-subject) %	CoV (Inter-subject) %	Intraclass Correlation Coefficient (ICC) (Baseline and Maximum , Visit 1 vs Visit 2)	Lower	Upper	
Colon Volume (mL)†	Baseline	728 ± 267	768 ± 246	14	34	0.84	0.67	0.93	<0.001
	Maximum	1226 ± 336	1199 ± 242	11	24				
Paired T-test (Base-max)		p<0.0001	p<0.0001						
Total Water Content (mL) †	Baseline	113 ± 63.3	108 ± 49	34	51	0.93	0.84	0.97	<0.001
	Maximum	1071 ± 266	1060 ± 313	18	27				
Paired T-test (Base-Max)		p<0.0001	p<0.0001						
Colonic Water content (mL)	Baseline	0 (0)	0 (0)	*	*	0.85	0.7	0.93	<0.001
	Maximum	538±278	540±242	29	48				
Paired Wilcoxon (Base-Max)		p=0.0005	p=0.0005						
Content Mixing AC (%)	Baseline	18±8	16±6	14	41	0.76	0.49	0.89	<0.001
	Maximum	35±10	30±7	13	27				
Paired T-test (Base-Max)		p<0.0001	p<0.0001						
Content Mixing DC (%)	Baseline	23±8	22±7	15	33	0.62	0.3	0.81	<0.001
	Maximum	31±11	28±7	16	29				
Paired T-test (Base-Max)		p=0.0106	0.0177						
Wall Movement AC (a.u)	Baseline	272 (37-710)	135 (37-264)	85	94	0.65	0.35	0.83	<0.001
	Maximum	1886 (955-3669)	1877 (1613-2163)	32	70				
Paired Wilcoxon (Base-Max)		p=0.0015	p=0.0005						
Wall Movement DC (a.u)	Baseline	258 (104-460)	441 (167-911)	64	76	0.76	0.53	0.89	<0.001
	Maximum	2249 (1786-2834)	1941 (1454-3864)	32	53				
Paired Wilcoxon (Base-Max)		p=0.0005	p=0.0005						
Time to Bowel Movement (min)		95 (95-113)	90 (51-133)	110	19	0.98	0.94	0.99	<0.001

** CoV unable to be calculated due to the large number of zero data*

† Further segmented data in supplementary information

All individual time point post-stimulus data in supplementary information

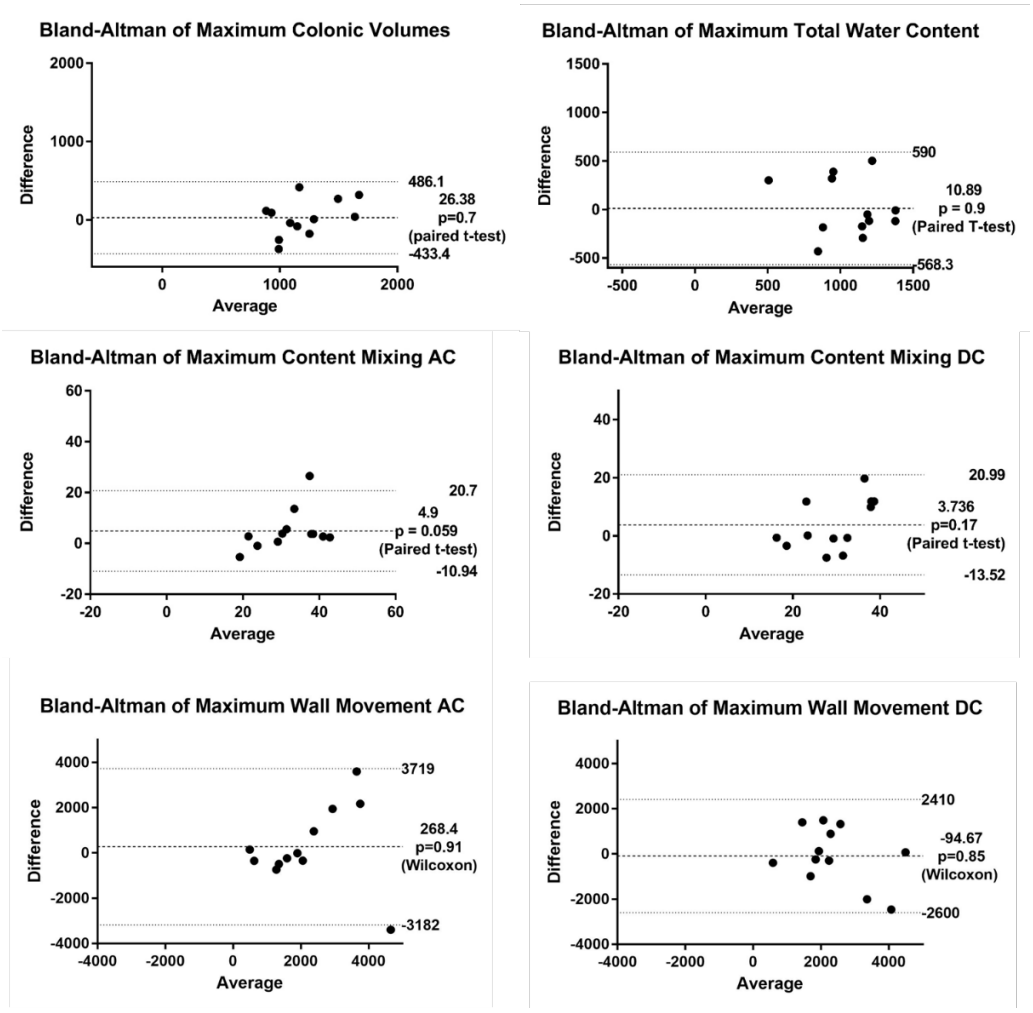


Figure 23: Bland-Altman Plots showing difference vs average with dotted lines representing bias and 95% limits of agreement, and a paired t-test or Wilcoxon test (if non-normal data) used to assess for significance of this bias.

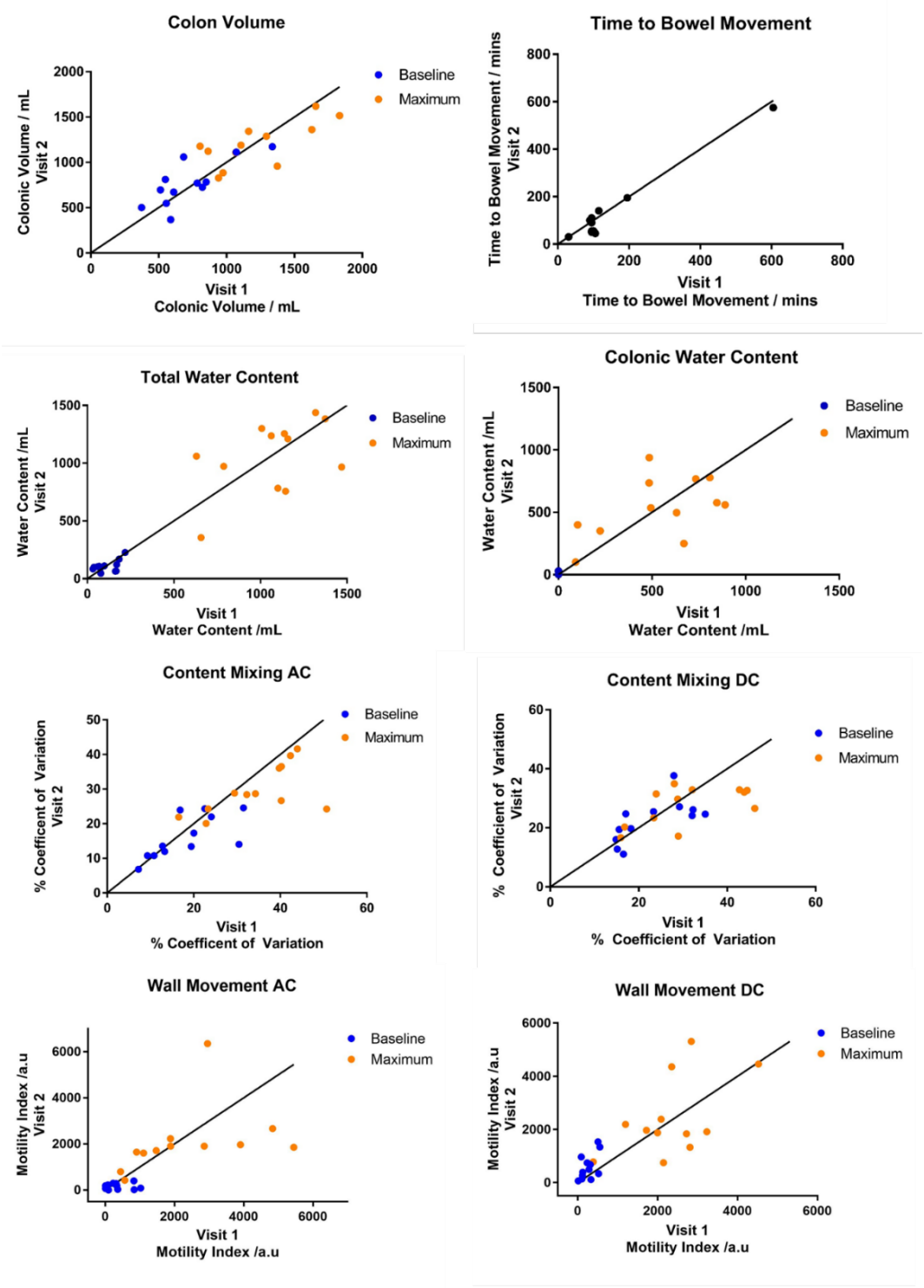


Figure 24: Correlation all endpoints at baseline and maximum, with line of identity shown, Visit 1 vs Visit 2

Responder Rate

Using the 90% centile of baseline values as a cut off for a definition of 'responder' to the stimulus, the results of the individual peak values with a responder line for both AC and DC wall movement are shown in Figure 25. This shows that for the AC, all participants kept their responder status and in the DC one participant changed responder status across visits.

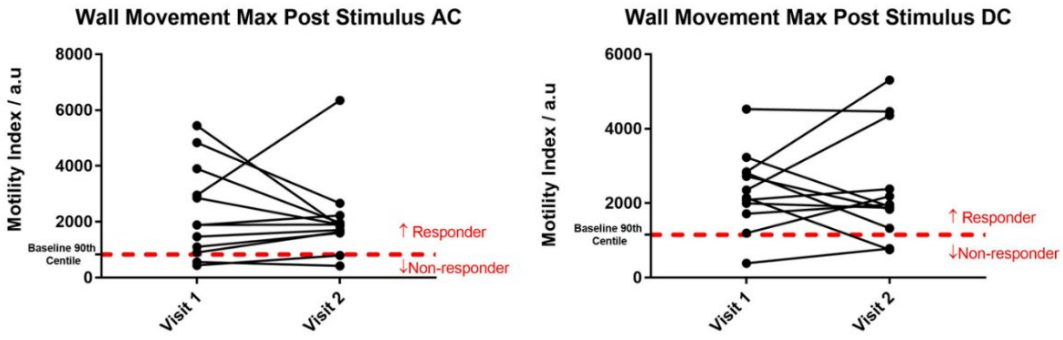


Figure 25: Graph of responders/non-responders for colonic wall movement

Symptom Scores

Symptom scores for abdominal pain, flatulence and bloating were all low. All participants scored zero at baseline for all symptoms. For both visits at T60 and T120, median scores were all <0.5 for all symptoms. There was no significant difference found between visit 1 and 2 scores for any symptoms at both time points (p values between p=0.44 and p>0.9, Wilcoxon test).

Discussion

Our results indicate that colonic volumes, content mixing and wall movement all consistently increased above baseline post stimulus. This is consistent with previously published data on healthy volunteers using different quantities of the macrogol challenge drink[32,162,180]. MRI measures showed large inter-subject variability with coefficients of variation across the cohort ranging from 33% to 94%. Importantly, the intra-subject variability was lower than the inter-subject variability for all parameters measured (range 11-85%). This means that all individuals' data had more in common with itself than with the group as a whole.

The colonic volume and AC content mixing data had good intra-subject reproducibility. Fasting colonic volumes have already been shown to be consistent on different occasions[218,219], however the fact that these volume and content mixing measurements were also reproducible post stimulus suggests that these responses are relatively consistent and change quite slowly over the measurement time. This may suggest an element of autoregulation of the colon itself, whereby it uses feedback mechanisms to generate propulsion and ensure conditions remain constant despite variable input. This is particularly the case for the AC content mixing measurement, a

dynamic flow measurement, also known as tagging, which is effectively acquired for just 40 seconds (20 secs acquisition with a gap of 1 hour). If this content mixing were more erratic a bigger variation in the data would be observed. In addition to this, by allowing a maximum value of the response to be used in this protocol has removed some of the variation that changes in oro-caecal transit time between visits could introduce, which would in turn impact on the response of the colon to the stimulus.

AC and DC wall movement were reasonably repeatable at baseline, with a low metric measured compared to the post stimulus data, but the response to the challenge was variable between study days resulting in a lower ICC overall and indicating variability in the physiological response to the stimulus, likely due to the irregular nature of colonic contractions following the macrogol drink. It is known from manometry data that in resting condition colonic contractions are erratic; a study in healthy volunteers performed over 4 hours found that high amplitude contractions make up only 1.4% of the contractile activity of the colon[164]. Simultaneous detection of these high amplitude contractions from manometry with MRI has been previously shown by Kirchhoff et al[220] who also showed low motility in their basal data prior to bisacodyl instillation in the DC. They also showed that following the

bisacodyl not all subjects' colons produced high amplitude contractions during their 24-minute measurement period, with some subjects producing multiple contractions and others none at all. Given the fact that the wall movement scanning acquisition time was just 10 minutes each scan session, it perhaps is not that surprising that larger variability was found for these data using this method. The contractions seen in the wall of the AC and DC following the macrogol drink are not continuous, but sporadic and will depend on several factors. These would include the rate of delivery of the macrogol to the AC, mixing of the macrogol with the colonic contents already present, and the absorption rate of any fluid from the macrogol. The macrogol itself also contains a number of poorly absorbed and possibly irritant anions (such as sulphate and ascorbate) which may have other effects of motility, although this has never been studied.

All these factors will influence the distension of the colon wall which is probably the main trigger for the wall motion observed. The total water content (which included SB) measurements showed there was larger variability of this measure, across the two visits, compared to the colonic volumes (although individual colonic segments showed larger variation (supplementary information)) and colonic content mixing, again supporting the

assumption of variable oro-caecal transit of the macrogol to the colon and its subsequent transport and absorption.

However, with our definition of responder (that is, those whose post stimulus response was greater than the 90% centile of baseline values), all subjects kept the same status for AC wall movements and only 1 changed for DC across visits. The DC definition of responder was set much higher than the AC due to larger amounts of movement present during the baseline scan for this region of the colon. There is also more susceptibility of the DC to artefacts from the motion in the neighbouring small bowel which may erroneously increase the index. On the other hand, this may well be representative as manometry studies do show that DC activity is often greater than AC, with a proximal to distal gradient observed previously[164]. However, with our definition of responder (that is, those whose post stimulus response was greater than the 90% centile of baseline values defining the 'no motility' state), all subjects kept the same status for AC wall movements and only 1 changed for DC across visits. The DC definition of responder was set higher than the AC due to larger amounts of movement present during the baseline scan for this region of the colon. There is also more susceptibility of the DC to artefacts from the motion in the neighbouring small bowel which may erroneously increase the index. Since intra- and inter-individual variability in baseline

motility were very similar, we felt it reasonable to use a single threshold of 90% of group values rather than expressing response as a fixed % change from individual baseline. This approach is likely to be used in clinical practice when looking for abnormalities in motility in patient populations.

The time to first bowel movement also showed low variability across the visits, however it is worth noting that due to the nature of the study days patients were more likely to go in the half hour periods they had between scans and no participants needed to go whilst in the scanner itself (or at least did not ask to be taken out the scanner mid scan) which constrains the data to limited values and may artificially improve its reproducibility. This parameter has obvious face validity and is highly relevant to patient management, however although a useful objective clinical measure it does not define the abnormality of colonic function. Being a large stimulus, it overcomes the uncontrolled variability in baseline values to yield a value which is highly reproducible compared to baseline parameters. It could be useful as a screening test to determine who would benefit from the more expensive and detailed MRI test.

This study was limited by the relatively small sample size, especially as the data are so heterogeneous, and other factors such as diet in the preceding days were not controlled, and this

could have had an impact on the baseline state of the bowel resulting in larger variability. Equally the data were only collected over a relatively short period (within either breath holds or over 10 mins for the wall movements) and it is possible that these time periods are not representative of the overall effect. In comparison, data collected from the traditional manometry techniques can record from 2-4hrs up to a 24hr period, however this is impractical for MRI studies, both due to cost and the practicality of lying still within the scanner for such a prolonged period.

No standardisation of the baseline condition was carried out due to impracticalities of undertaking this approach in clinical practice. Variable transit times of constipation patients would mean that dietary interventions prior to the scan day may influence some patients more than others. In addition, defecation prior to starting the scanning would be of limited use as almost all of the constipation patients would not be able to comply. Some of the variability seen in the measurements may have come from intra-observer analyses of the data. This source of variation in the measurements were beyond the scope of the study. Previous limits of agreement data for water content measurements have been measured at -11% to 13% (inter-observer) and -4% to 3% (intra-observer)[136], and for total colonic volumes inter-observer variation is around 5%

(unpublished data). However intra- and inter-observer variations will be an important determinant for the use of the individual MRI parameters in future studies and as a clinical test and will be investigated in future studies.

In conclusion, the colonic response to the macrogol challenge as assessed by MRI is heterogeneous but large compared to baseline data, and has moderate to good reproducibility, making it a suitable test to study potential pathologies underlying GI disorders such as constipation. More data are needed to better define the normal range for comparison with patient groups who may have both hypo- and hyper-motile responses to the challenge drink and the reproducibility of the test in patients will also need investigating.

Chapter 7: Reclassifying Constipation Using Imaging and Manometry (RECLAIM Study)

This work was carried out between August 2016-June 2019. The study is still ongoing and so results of an interim analysis of the validation study are presented.

Introduction

Approximately 50% of patients with constipation are not satisfied by current medical management. Whether this is because medications are directed to treatment of symptoms and not underlying pathophysiological mechanisms is unclear. The RECLAIM study uses MRI and high-resolution manometry to define colonic responses in individuals with different subtypes of constipation and examines their different response to prokinetic and antispasmodic treatment in a randomised clinical trial.

Aim & Hypothesis

The overall aim of the study was to determine if the more complex method of manometry could be replaced by MRI, a more widely accessible test and be useful in clinical practice.

We planned to achieve this in two stages. The first stage was to determine whether the two techniques correlated or if we could use them to classify patients based on their underlying physiology and produce similar groupings (Validation Study). The second stage was a clinical trial of two medications to see whether MRI or HRM was a better predictor of clinical response (Crossover Study).

Our primary hypothesis was that MRI parameters would correlate with HRM findings and there would be no difference in performance of MRI and HRM as a predictor of clinical response.

For this thesis, I will present the results of an interim analysis of the validation study which will address the first part of this hypothesis. The results of the crossover trial assessing clinical response are not yet available.

Methods

Study Design

This was a two-part study, with the first part a validation study of the two techniques and second part a crossover study of two medications. The study was sponsored by the University of Nottingham and funded by a grant from the MRC (ref: MR/N026810/1). The protocol was approved by the Research Ethics Committee (East Midlands – Nottingham 1, 17/EM/0032)

and all participants gave written informed consent. The study was carried out according to Good Clinical Practice as defined by the Declaration of Helsinki. The study was registered on clinicaltrials.gov, reference: NCT03226145.

Study Participants

A total of 120 participants were recruited for the study (40 HV, 40 IBS-C and 40 FC) across two main recruiting sites (Nottingham and London). Healthy volunteers only participated in the first part of the study (validation).

Initial inclusion and exclusion criteria are detailed in table 6 below. Participants were asked to complete a two-week bowel habit diary to confirm their symptoms prior to being deemed eligible.

Table 6: Inclusion and exclusion criteria for the RECLAIM Study

Eligibility criteria
Inclusion criteria
1. Aged ≥ 16 years
2. Capacity to give informed consent for participation
3. Ability to understand written and spoken English
4. For Constipation Group: Symptoms of constipation meeting Rome IV criteria for functional constipation or constipation-predominant irritable bowel syndrome

5. For Control Group: No symptoms of constipation. This will be defined as a score of 5 or less on the Cleveland Clinic Score

Exclusion criteria

1. Participation in any clinical trials in the past 3 months
2. Inability to understand written and spoken English
3. Pregnancy, assessed by a urinary pregnancy test, or current breastfeeding
4. History of significant adverse reaction or hypersensitivity, or known contra-indication to any of the medicinal products or equipment used in the study
5. History declared by the candidate of certain pre-existing gastrointestinal disorders, including:
   i. inflammatory bowel disease
   ii. coeliac disease
   iii. cancer of the gastrointestinal tract
6. Any reported history of gastrointestinal resection (excluding appendicectomy or cholecystectomy)
7. Presence of an intestinal stoma
8. Causes of secondary constipation disorders (e.g. systemic sclerosis / Parkinson's disease)
9. Inability to cease use of medicines that cause constipation or alter colonic contractility (e.g. opioids, smooth muscle relaxants)
10. Antibiotic use in the last 3 months
11. Comorbidity that would prevent safe adherence to the protocol (e.g. inability to lie flat, kidney disease contraindicating use of Moviprep)
12. Judgement by the PI that the candidate who will be unable to comply with the full study protocol (e.g. diabetes, severe COPD)
13. Contraindication to MRI or colonic manometry
    - *Examples for MRI* include claustrophobia, metallic implants, pacemakers, history of metallic foreign body in eye(s) and penetrating eye injury

- *Examples for manometry* include diagnosis of previous complications of diverticular disease or previous endoscopic complications

14. Contraindication to Medicines to be used in study
    - Examples include prostatism or glaucoma
15. Clinical evidence of significant pelvic organ prolapse syndromes
16. Inadequate screening diary following review
    i. Control Group: A screening diary that records <6 complete spontaneous bowel motions in the fortnight.
    ii. Constipation Group: A screening diary that records >6 complete spontaneous bowel motions in the fortnight

Study Day Protocols

The study was made up of 8 visits, 4 for each part of the study. These visits were as follows and are summarised in Figure 26:

Visit 1 – Screening and Initial Eligibility

Once a potential participant had given consent, they were assessed against eligibility criteria. Institutional records were accessed as necessary for confirmation. Where records, including GP records, were not immediately available, participants were included for screening and further information sought during the screening period. If participants were on or had recently been on any courses of antibiotics the commencement of the screening period was delayed until a period of 3 months had passed. A blood test for renal function was taken if none was available within the preceding 3 months.

The participant then completed the Cleveland Clinic Constipation Score and Rome IV questionnaire.

Participants were then given a 14- day dairy on which to record their bowel habit. They made a record of each toilet visit to open their bowels, including the time of day and predominant stool form, based on the Bristol Stool Form Scale (1 score only). Participants were asked to cease regular laxative use during this time. Rescue medication was available should the participant not open their bowels for 3 consecutive days and consider themselves to have intolerable discomfort.

They provided stool samples during this time. They were provided with the necessary kit to do this at home when able and provided with storage instructions (samples were frozen) and brought the samples back at Visit 2.

Visit 2 – Confirmation of Eligibility and Balloon Expulsion Test

This was scheduled for at least 2 weeks after Visit 1. The research team reviewed the diary for eligibility and adequate data recording. If the participant was eligible then arrangements were made for visits 3 and 4. The participant completed the PAC-SYM (symptoms) questionnaire and the HADS (Hospital Anxiety-Depression Score) questionnaire. They recommenced normal laxative use. Participants also underwent

the balloon expulsion test on this visit (details in the interventions & procedures section).

Visits 3 and 4: Imaging and manometry

Visits 3 and 4 involved Magnetic Resonance Imaging (MRI) or High-Resolution Manometry (HRM). The order of assessment was determined at random to reduce any order effect. The visits were at least 7 days apart. Participants were asked not to use laxatives or other agents used to modulate gut neuromuscular function for 2 days before each assessment. Standardised dietary advice was provided to the participant for what they were allowed to eat / drink on the preceding evening (full details of the study days outlined in the interventions and procedures section to follow).

Visit 5 (Start of Crossover Study)

Patients were assigned to treatment order (See blinding section to follow for details). They then started their first treatment period. They were provided with blinded treatment packs and instructions to take them as well as a stool and symptom diary.

Visit 6

This was arranged at the end of the first treatment period. They returned their diaries and symptom questionnaires.

There was then a washout period of at least 14 days to minimise any carryover effect between the two treatment periods.

Visit 7

This was the start of the second treatment period. Provided with further blinded treatment pack and instructions to take them, as well as the stool and symptom diary.

Visit 8

This was the end of the second treatment period. They returned their diaries and symptom questionnaires.

Participants completed the following assessments at each visit: Bowel habit and pain diary throughout the treatment periods, PAC-SYM questionnaire at beginning and end of each treatment period.

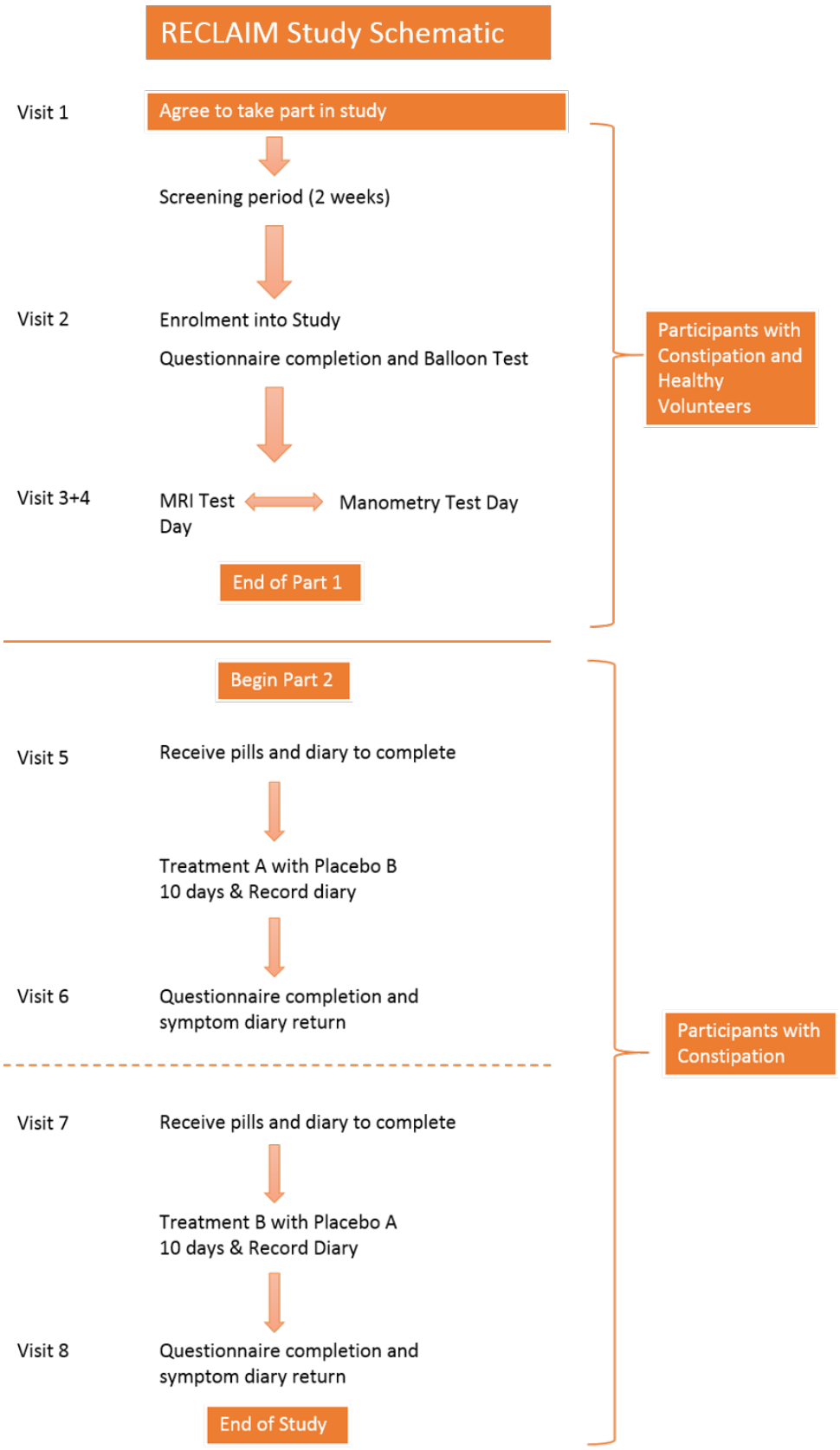


Figure 26: RECLAIM Study Schematic

Interventions and Procedures

Balloon expulsion test (Visit 2)

A non-latex balloon made of inert, elastic material, attached to a thin catheter, was inserted in the participant's rectum with appropriate lubrication. 50mL of warm water was instilled into the balloon. The participant was then asked to expel the balloon per rectum, sitting on the toilet in privacy. The time taken to expel the balloon (up to 120sec) was recorded on the CRF as a baseline measurement.

Manometry visit (Visit 3 or 4)

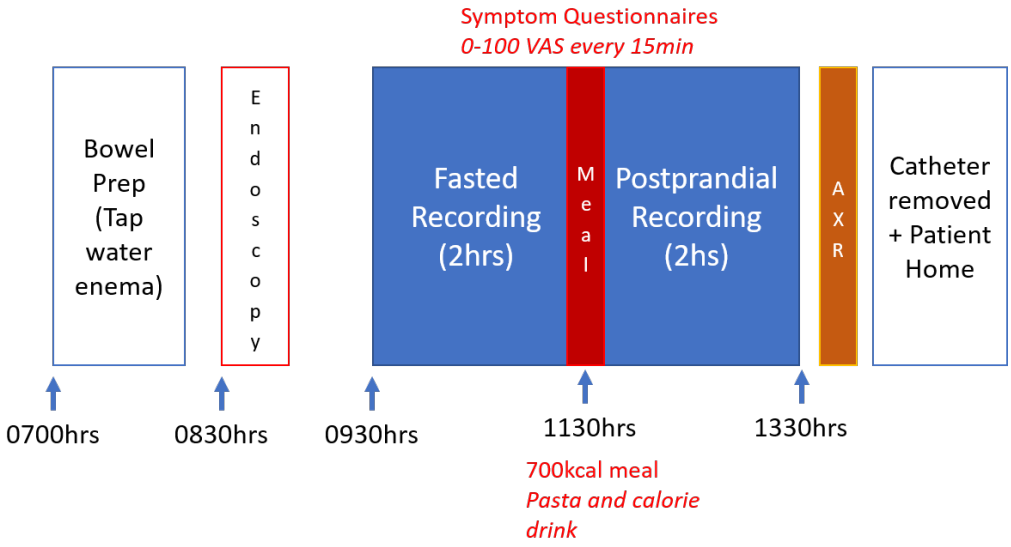


Figure 27: Manometry Study Day Schematic

On the morning of the test the participant attended the local test site. They were fasted from the night before, apart from sips of water for essential medicines. Heart rate and blood pressure was measured on arrival. After removing their own

clothes, and dressing in a hospital gown, a tap water enema (water at room temperature; maximum 1 L) was administered to cleanse the left side of the colon. If an adequate bowel movement had not been induced within 15 minutes, further tap water enemas were given, with the amount dictated by patient comfort.

Once the study team were satisfied that bowel preparation had been satisfactorily achieved, the participant was transferred to an endoscopy procedure room to undergo colonoscopy. Prior to insertion of the colonoscope a clinical digital rectal examination was performed, this is standard procedure for all colonoscopy and findings were documented in a standardised format. The colonoscope was then passed and the flexible manometry catheter (~4 mm in diameter) was inserted alongside it. Once in place, about 35 cm into the bowel, a thread attached to the tip of the catheter was secured to the lining of the bowel with a fine metal clip, deployed through the colonoscope. Following this, up to four biopsies were taken from the colonic wall mucosa during removal of the scope. Once the colonoscope was removed, the catheter was taped in place at the anus using an adhesive dressing.

After approximately 30 mins (to allow for a recovery period following colonoscopy), and with the participant in a semi-

reclining position on a hospital bed, the catheter was connected to the recording equipment and monitoring commenced. Following a 2 hour 'rest' (baseline) period, the participant was supplied with a standard 700 Kcal meal and asked to consume this within 10 minutes.

The meal consisted of a 200 mL of Ensure TwoCal nutrient drink (400Kcal) and a pasta meal (300Kcal).

The recording was then continued for a further 2 hours. The participant completed a VAS score of symptoms every 15 mins during the recording (Appendix C).

At the end of the recording period the participant was transferred by wheelchair to have an abdominal X-ray. This confirmed the position of the catheter (Figure 28). They then returned to the investigation room and the catheter was removed by gentle traction.

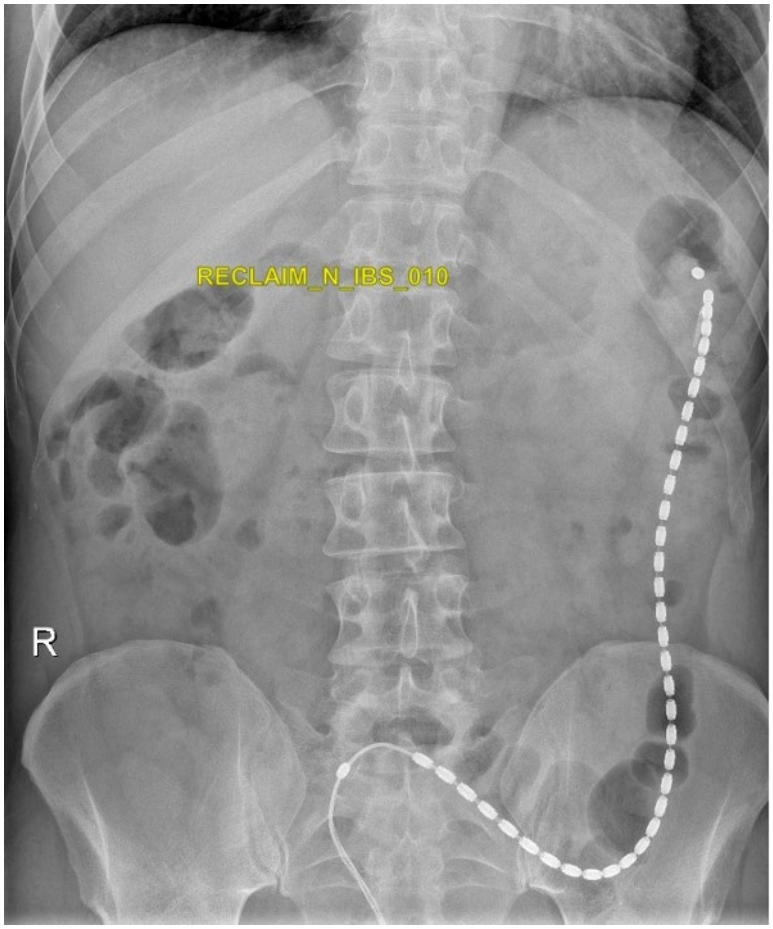


Figure 28: Abdominal radiograph showing placement of the manometry catheter in the descending and sigmoid colon

MRI visit (Visit 3 or 4)

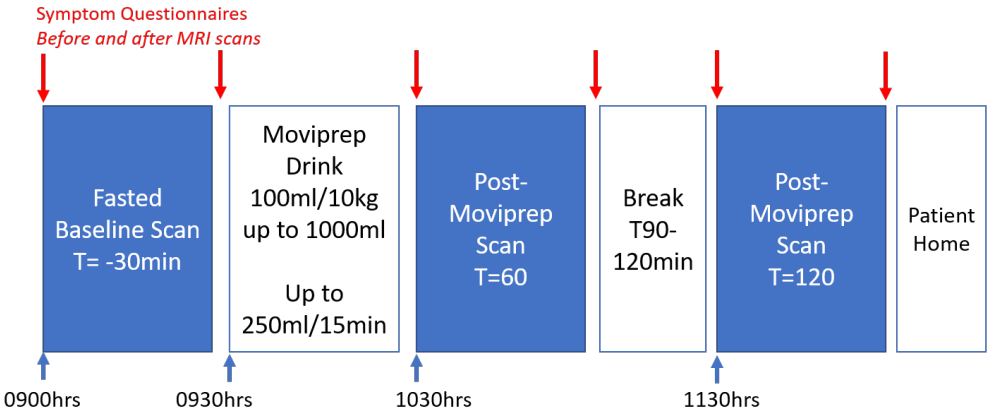


Figure 29: MRI Study Day Schematic

The participants were provided with 5 plastic marker pills to swallow the day before attending at 9am. They were asked to either attend the unit for this or, if more convenient, provided us with time-stamped photographic evidence of swallowing the pills at home.

On the morning of the test the participant attended the local test site. They did not eat or drink anything from the night before, apart from sips of water for essential medicines. Participants confirmed that no contraindications to MRI scanning had arisen via a safety checklist, then changed out of their own clothes into hospital scrubs.

Participants then underwent a series of MRI scans while fasted, lasting approximately 30 minutes.

30 minutes after the start of the scan participants started drinking a poly-ethylene glycol and electrolyte solution (Moviprep, Norgine Pharmaceuticals Ltd, Harefield, UK). They drank 10ml/kg body weight, rounded to the nearest 100ml within the 500ml-1000ml range, graduated as ¼ of the total to drink every 15 minutes.

Immediately after finishing the moviprep, participants had a further set of MRI scans (T=60). A further scan then taken an hour later (T=120). The time to first bowel movement after drinking the macrogol was also recorded wherever possible.

Crossover Study Interventions (Visits 5-8)

The interventions used were:

- Buscopan 20mg taken orally three times daily for 10 days
- Bisacodyl 10mg taken orally once daily for 10 days

We provided 2 sets of capsules: A and B.

One of the capsules contained either Buscopan or Bisacodyl and the other an inert drug, the placebo. Whichever capsule was the placebo in the first 10 days trial was replaced by the active drug in the second part. A placebo was required as the number of capsules taken per day (two tablets once a day versus three times a day) was different for each medication and this allowed

for proper blinding to treatment allocation for the crossover design.

The order in which they are taken was randomised so neither participant nor the research workers knew which active drug they are taking for each trial period.

Rescue Therapy

Rescue therapy was available if participants did not open their bowels for 3 days during any screening or treatment periods and could not tolerate resulting symptoms. A choice of commonly used medications for constipation was given and participants were given one which they had previously used.

Rescues: Prucalopride 2mg tablet, taken orally

Senna 7.5mg tablet, 1-4 taken orally

Sodium Picosulfate 5mg -10mg liquid , taken orally

MRI Protocol + Image Analysis

Imaging was carried out on a 3.0T Ingenia wide-bore scanner (Philips, Best, The Netherlands) with a parallel imaging SENSE abdominal body receiver coil.

Alignment of methods between London and Nottingham

The MRI scan card was set up in Nottingham on the Ingenia RX scanner and then transferred to the Centre for Medical Imaging, University College London (UCL), to be uploaded on their scanner.

Scan operators from UCL attended a scanning session in Nottingham to ensure alignment of technique.

Any files transferred between Nottingham and UCL were via secure channels – this was either via secure external hard-drive/memory stick or a secure web-based service.

The principal MRI analyses undertaken were:

MRI colonic volumes

A 3D coronal dual echo fast field echo sequence with mDIXON reconstruction. This is the same technique outlined in detail in Chapter 6.

MRI wall motility measurement

Cine bTFE data were acquired over 10 minutes. An observer drew a line along the main axis of the AC and DC which was used to create 15 lines at 90º to this axis. These 15 lines were 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 were smoothed, and a Motility Index was defined as the percentage of the time points at which there is a change in length (i.e. wall velocity) of greater than 0.5 mms-1 between consecutive time points. This is the same technique outlined in detail in Chapter 6.

MRI content mixing measurement

A single slice cine bTFE with tag lines 12 mm apart was acquired over a 20 s breath-hold positioned oblique-sagittally through the AC. This acquisition was also repeated oblique-coronally. The amount of mixing and flow of the colonic contents was determined using the mean co-efficient of variance over the visible AC in the image, calculated on a voxel-by-voxel basis across the time-series of data. This is the same technique outlined in Chapter 6.

MRI transit measurement

Whole Gut Transit Time (WGTT) was assessed from the Weighted Average Position Score (WAPS) of the MRI transit markers 24 hours after ingestion, as we have previously validated, where a higher score equates to a longer transit time. This was assessed at the baseline scan before ingestion of the Moviprep Drink.

Additional sequences were taken in order to position the scan.

Manometry Analysis

Anonymised recordings were uploaded to a secure file sharing site. Analysis was performed by Dr Phil Dinning at the Flinders Medical Centre in Australia. Analysis parameters included:

- percentage of recording time occupied by cyclical retrograde propagated contractions
- number of high amplitude propagating contractions (HAPCs)
- frequency, duration, amplitude and polarity of other occurring contractile activities

Stool and Mucosal Samples

During the study, stool and mucosal samples were collected from participants as described above. These will be analysed for microbiota and metabolites at a later date and will not be described in detail in this thesis.

Randomisation and Blinding

The physiological assessments in the validation study, MRI and HRM, were conducted with randomisation of the order of assessment. This randomisation was performed using the randomization.com sequence generator ⟨http://www.randomization.com⟩ and stratified by site

(Nottingham and London). Separate sequences were used for patients and controls. Participants could not be blind to the assessment they are having, nor the research nurses and staff responsible for implementing assessment. Data analysis was blind for this study as it was undertaken at distance, both in time and geography, from the initial assessment.

The crossover trial was a two-period, two-intervention design. Participants and investigators were blind to whether the participant is taking the active formulation of intervention A or B. The placebo of the second intervention was taken during each period of the crossover. The order of active intervention was randomised. Participants therefore followed a regimen that was either: A+B- // A-B+ or A-B+// A+B-. Randomisation was performed using the randomization.com sequence generator ⟨http://www.randomization.com⟩ and stratified by site (Nottingham and London) and used randomly permuted blocks of size 4 or 6. Intervention sequence generation and storage of the intervention code was handled by NUH Clinical Trials Pharmacy. Access to the intervention sequence was restricted to members on the delegation log within NUH Clinical Trials Pharmacy, who then allocated interventions to participants (independently from those enrolling the participants who were researchers in the team).

Delegated pharmacy staff at other recruiting sites were provided with an intervention sequence generated by NUH pharmacy and were responsible for allocating interventions to recruited participants and secure storage of the intervention sequence.

Data Analysis

For this thesis, only an interim analysis of results from part 1 of the study was carried out. No results from the crossover study are available yet.

Primary Outcome

The primary outcome was correlation of MRI motility and volume scores after stimulation with the motility scores as assessed by HRM. However, as these tests do look at distinctly different parts of the bowel, we will also assess whether we are able to use these tools to group patients into normal or hyper/hypo-motile categories.

Secondary Outcomes

We also will look at the two techniques independently to determine differences between patient groups and HVs both before and after the stimuli. This will include:

MRI Measures:

- MRI Colonic Volume

- MRI Wall Motility (including responder rate as defined in Chapter 6 and relationship between Volume and Wall Motion)
- MRI Content Motility
- MRI Transit Time
- Time to Bowel Movement after Moviprep

Manometry Measures:

- HAPCs
- Cyclic Contractions
- Pan-colonic Pressurisations

Power & Sample Size

nQuery v7.0 was used for the sample size calculations.

Primary Outcome: A level of agreement >70% would be sufficiently accurate to allow the more accessible MRI technique to replace manometry to classify constipation and would be in line with the accuracy of most clinical assessments. With a total of 80 participants (with FC or IBS-C) we could estimate a level of agreement of 70% to within ±10% (95% confidence interval).

A kappa statistic of 0.7 (indicating good agreement) would be estimated to within ±0.16 with 80 participants, assuming a

proportion of 0.5 in each group (hypomotile versus normal/hypermotile).

Secondary outcomes: To compare physiological activity of the colon, assessed by MRI and HRM, in people with constipation, and assess for any relationship between symptom profiles (IBS-C versus FC) and physiology

Our pilot study data showed an ascending colon MRI motility index mean (SD) of 0.055 (0.044) for functional constipation versus 0.107 (0.07) for constipation-predominant irritable bowel syndrome.

Using these values, we calculated we needed 28 participants per group to detect such a difference with 90% power with $\alpha < 0.05$ (two-sided). 35 participants per group would be needed to detect a mean difference of 0.045.

We planned to recruit 40 per group to allow for technical errors in recordings or subject drop-out. We also recruited 40 volunteers without constipation matched for age and gender with the patient cohort.

There were no previous data on which to base a power calculation for part 2 of the study. We asked all patients with FC or IBS-C from the validation study to take part, giving us

potentially 80 subjects. Allowing for refusals and dropout we expected a sample size of 60 patients for this part of the study.

This would give us >95% power to detect a correlation coefficient of 0.50 between motility and pain score differences with a significance level of 0.05, indicating that motility accounts for 25% of variance in pain scores which would be a reasonable minimal clinically significant difference.

Statistical Analysis

Basic characteristics of the study population were summarised using frequencies, percentages, means and standard deviations where appropriate. Correlations were performed using Pearson correlation if normally distributed and Spearman if not. Normality of the data was assessed using the D'Agostino & Pearson normality test and all statistical tests were performed using GraphPad Prism version 7.03 for Windows, (GraphPad Software, La Jolla California USA).

For the MRI measures, the maximum colonic volume value after the drink were determined from T60 and T120 data for each subject to allow for different oro-caecal transit times of the drink over the two visits. This time point was then used as a reference point for analysis of the motility data. Time from

MoviPrep™ to first bowel movement (TBM) was recorded using a cut off at 150min (average time spent at study centre).

Results

Baseline Demographics and Questionnaires

Baseline data from 87 enrolled participants ([age 36±13; BMI 25.5±5]: 41 HVs [36F], 28 IBS-C [27F] and 18 FC [17F]) were analysed. Age and BMI were similar between groups.

Both patient groups have significantly higher constipation and depression scores than HV; IBS-C also had higher anxiety scores (Table 7). IBS-C present a similar number of SBM to health but a lower number of bowel movements they considered complete, whereas FC have lower numbers of both SBM and CSBM. The ability to expel the balloon varied between groups with 87% success in HV group, 78% in IBS-C and 61% in FC.

Table 7: Demographic and Questionnaire Data

Demographics	HV (n=41)	IBS (n=28)	FC (n=18)	P-Value
Age	33 (12)	37 (13)	42 (13)	0.06 ANOVA
BMI	25.3 (5)	25.4 (5)	26.3 (4)	0.8 ANOVA
M:F	5:36	1:27	1:17	
Questionnaires				
CCCS	2 (2)	17.6 (4.4)*	15.5 (4.3)*	<0.0001 ANOVA
PAC-SYM	0.2 (0.2)	2.3 (0.7)*	2.0 (0.6)*	<0.0001 ANOVA
HADS-A	5.3 (3.5)	7.8 (4)*	7.2 (5)	0.03 ANOVA
HADS-D	2.1 (2.3)	5.1 (3.7)*	4.6 (4.3)*	0.0005 ANOVA
Bowel movements (over 2-week period)				
SBMs	17 (7)	14 (12)	7 (5)*†	0.0003 ANOVA
CSBMs	16 (6)	1 (1)*	2 (2)*	<0.0001 ANOVA
Balloon Expulsion Test				
Able to expel in <2 min	36 (87%)	22 (78%)	11 (61%)	Chi-square for trend p=0.02

Tukey's Multiple Comparisons: * p<0.05 vs HV, † p<0.05 vs IBS
Data presented as mean (SD)

MRI vs HRM Comparison

62 out of 87 enrolled participants had completed both MRI and manometry studies at the point of analysis (29 HV, 22 IBS-C, 11 FC). Using the data for the MRI Wall Motility Score (AC+DC), Content Motility and Peak Volume we compared against the Total Manometry Score for time occupied across the DC/SC, we calculated quartiles of the data and plotted them below (Figures 30-32). Direct correlation analyses of these did not reveal significant relationships between the data (Table 8).

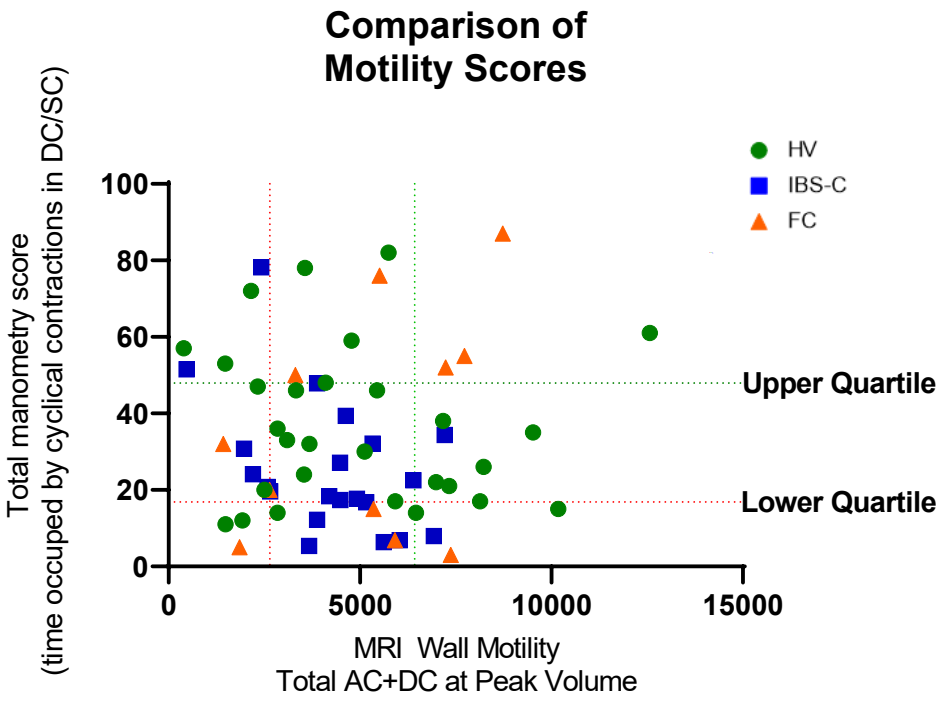


Figure 30: MRI Wall Motility vs Manometry Scores

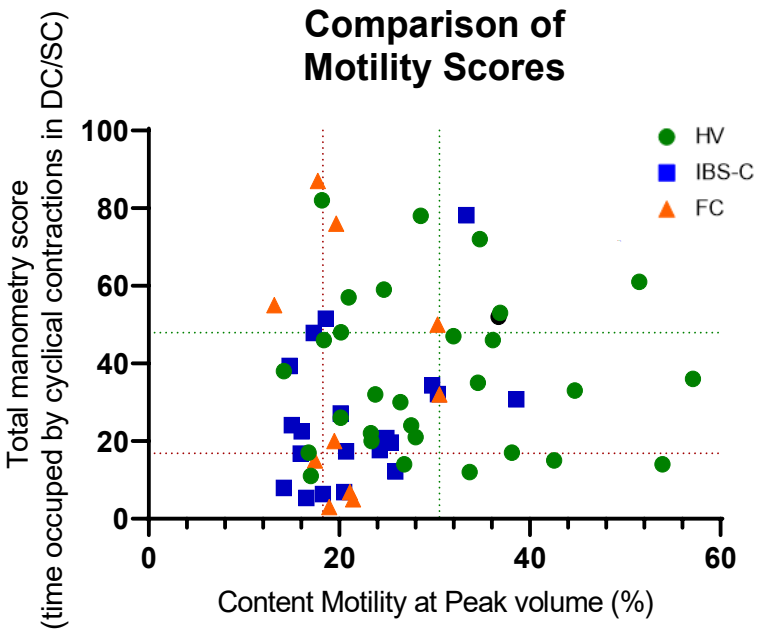


Figure 31: MRI Content Motility vs Manometry Scores

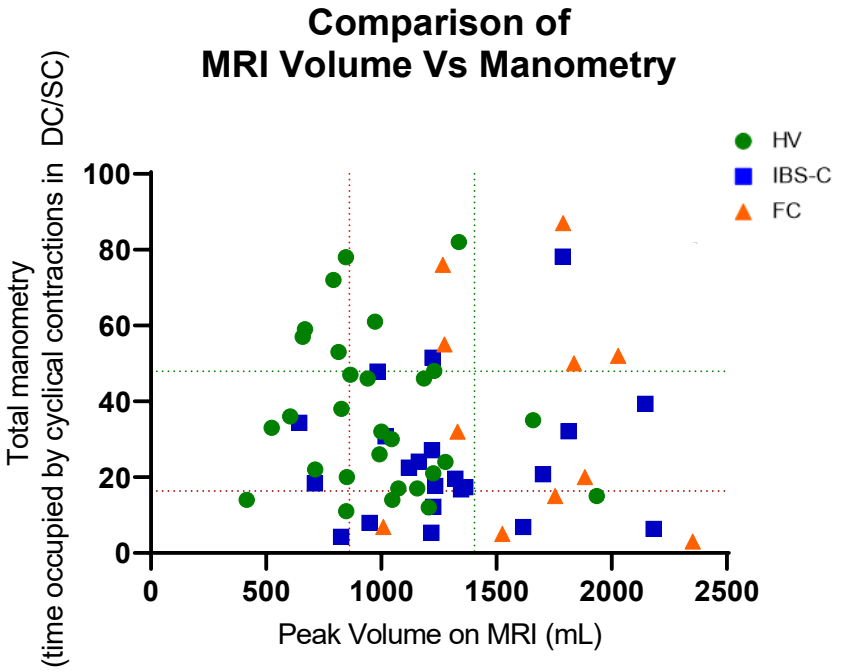


Figure 32: MRI Volume vs Manometry Scores

Table 8: Correlation Analyses of MRI and Manometry Measures

Group	Pearson r	95% CI	R squared	P Value
Manometry vs Wall Motility				
HV	-0.11	-0.46 to 0.27	0.012	0.57
IBS-C	-0.43	-0.73 to -0.0004	0.187	0.05
FC	0.42	-0.24 to 0.81	0.180	0.20
Manometry vs Content Motility at Peak Volume				
HV	-0.05	-0.41 to 0.33	0.002	0.81
IBS-C	0.36	-0.08 to 0.69	0.130	0.11
FC	-0.086	-0.71 to 0.61	0.007	0.83
Manometry vs MRI Volume				
HV	-0.15	-0.49 to 0.23	0.022	0.44
IBS-C	0.15	-0.29 to 0.54	0.022	0.51
FC	-0.1	-0.66 to 0.53	0.010	0.76

MRI Measurements

Of the 87 participants enrolled to date 66 have complete MRI datasets, 31 HVs, 23 IBS-C and 12 FC.

MRI Colonic Volumes

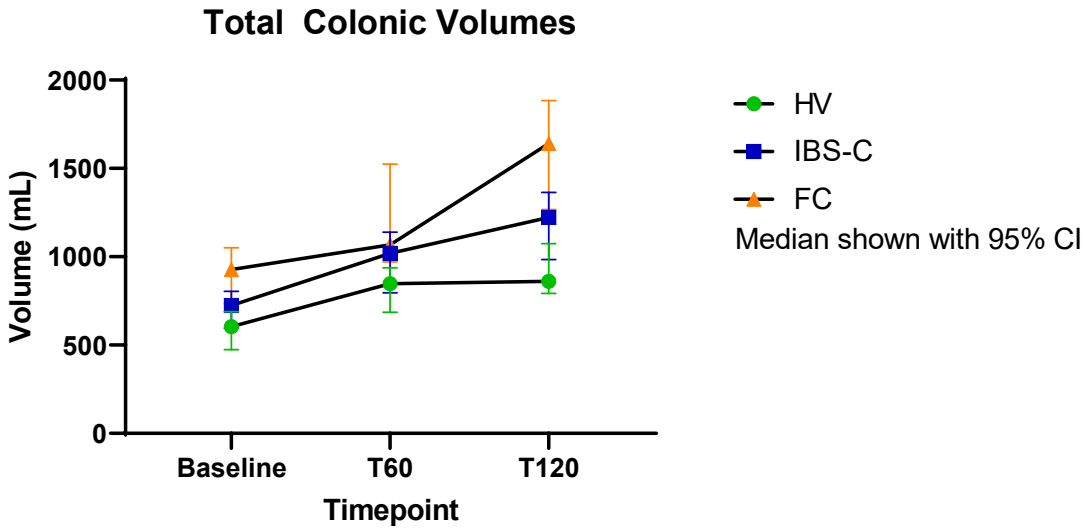


Figure 33: Total Colonic Volumes shown at timepoints throughout study day

Total colonic volume increased from baseline after consumption of the macrogol drink in all groups (Figure 33), overall interaction in 2way ANOVA p=0.0006, with both main effects of time and group being significant at p<0.0001. Multiple comparisons analysis showed significant differences between HV and each patient group at all three timepoints, however no difference between the patient groups themselves (Tukey's multiple comparisons, Table 9). However, when the peak volume is considered rather than separate time points there is a difference between the IBS-C and FC groups. When the

separate colonic segments are considered the significant differences are seen in the AC and TC at all timepoints, with DC only significantly larger in the FC group compared to HV at T120 (Table 10).

Table 9: Total Colonic Volumes

Timepoint	HV (n=31)	IBS-C (n=23)	FC (n=12)
Baseline	599±210mL	760±233 mL*	953±275 mL*
T60	827±244mL	1033±287 mL*	1143±355 mL*
T120	942±335mL	1277±442 mL*	1586±424 mL*
Peak	999±315	1310±407*	1585±424*†

**** p<0.05 vs HV data at same timepoint***
†p<0.05 vs IBS-C (Tukey's MC)
Data presented as mean ±SD

Table 10: Colonic Volumes shown by segment

Timepoint	Colon Segment	HV (n=31)	IBS-C (n=23)	FC (n=12)
Baseline	AC	198±95	259±78*	303±103*
	TC	196±106	283±139*	369±95*
	DC	108±50	117±52	166±98
	SC	98±51	100±48	115±48
T60	AC	268±114	366±122*	403±158*
	TC	266±104	372±142*	416±150*
	DC	150±64	171±65	201±108
	SC	143±87	123±66	123±77
T120	AC	299±148	429±152*	540±145*
	TC	297±141	447±194*	541±192*
	DC	188±69	228±97	325±138*
	SC	158±87	173±94	180±86

p<0.05 vs HV data at same segment/timepoint (Tukey MC).

Volume data in mL and presented as mean ± SD

MRI Wall Motility

For the MRI wall motility measures gathered so far, there were no significant differences between the groups after the moviprep. There was significant variability in the post- drink values obtained across all three groups.

"Responder rate"

If we use the responder definition from the reproducibility study (that is those whose post stimulus motility is greater than the 90% centile of HV baseline data, which is now calculated from a larger pool of HVs) then we see the below (Figure 34/35):

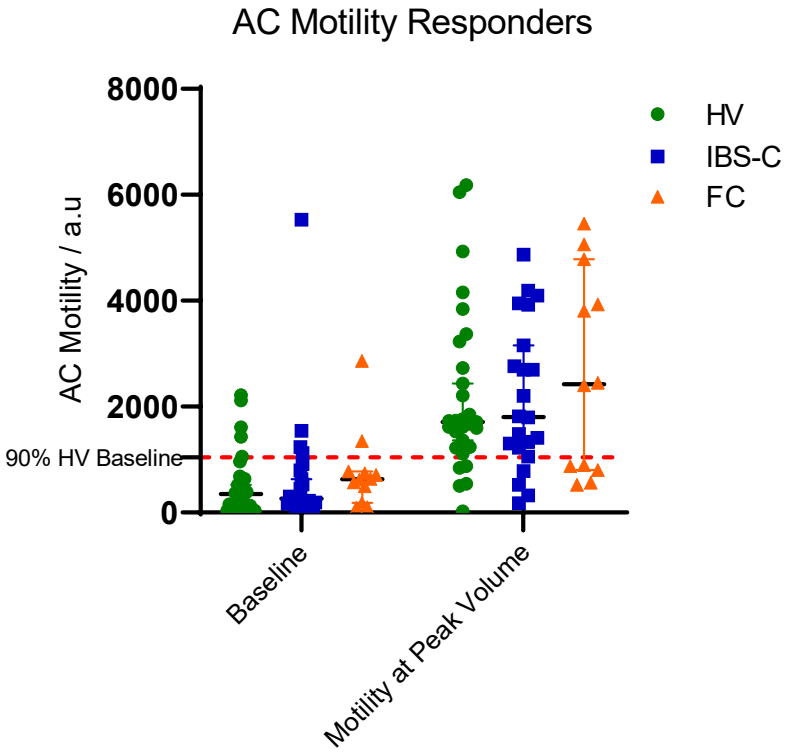


Figure 34: AC Motility Scores at Baseline and Peak across the groups, with a responder line drawn at the 90% centile of HV baseline data

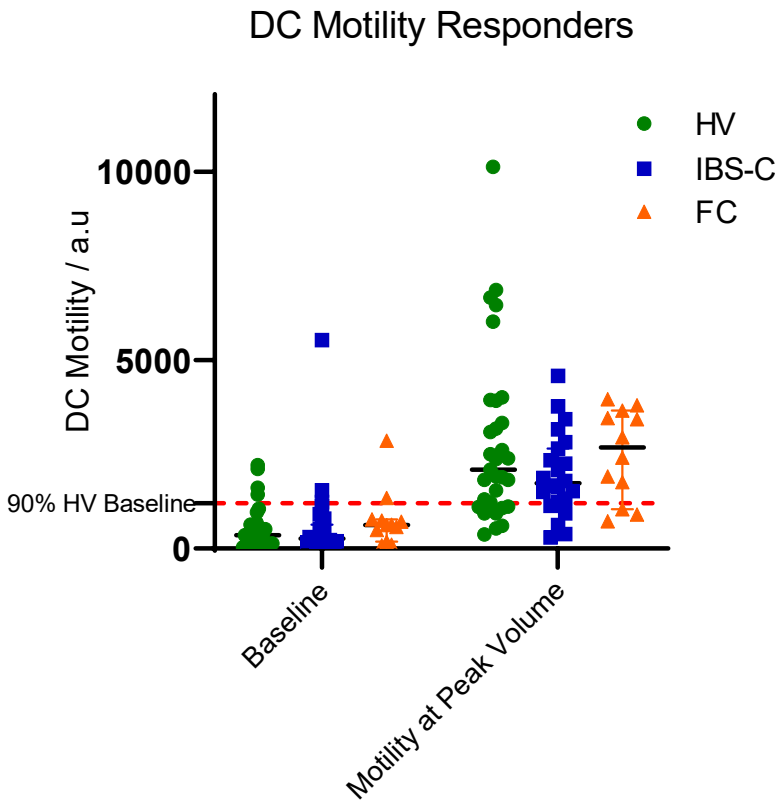


Figure 35: DC Motility Scores at Baseline and Peak across the groups, with a responder line drawn at the 90% centile of HV baseline data

We have used the motility obtained at peak volume as this is likely where the maximal stimulus is felt and allows consistency with use of time points across the different motility measurements.

For the AC, 17% were "non-responders" in the HV group compared to 18% in IBS-C and 42% in FC. In the DC it was 26% in HV compared to 23% in IBS-C and 25% in FC.

Volume vs Motility

Correlation of motility scores with volume calculated at the same timepoint across all subjects showed a weak to moderately positive correlation with r values between 0.17 and 0.36 over the different time points (Table 11).

Table 11: Correlations of MRI Volume and Wall Motility Across Groups and Timepoints

Timepoint	Segment	Spearman r	95% CI	P Value
Healthy Volunteers				
Baseline	AC	0.20	-0.19 to 0.54	0.3
	DC	0.45	0.08 to 0.7	0.02*
	AC+DC	0.32	0.06 to 0.54	0.01*
T60	AC	0.35	-0.03 to 0.64	0.06*
	DC	0.28	-0.09 to 0.59	0.12
	AC+DC	0.31	0.06 to 0.53	0.01*
T120	AC	0.29	-0.1 to 0.6	0.13
	DC	0.45	0.1 to 0.7	0.01*
	AC+DC	0.39	0.14 to 0.6	0.002*
IBS-C				
Baseline	AC	0.27	-0.17 to 0.62	0.21
	DC	0.31	-0.15 to 0.66	0.17
	AC+DC	0.28	-0.03 to 0.54	0.07*
T60	AC	0.36	-0.07 to 0.68	0.09*
	DC	-0.06	-0.48 to 0.38	0.78
	AC+DC	0.19	-0.12 to 0.46	0.22
T120	AC	0.31	-0.14 to 0.65	0.16
	DC	-0.29	-0.64 to 0.17	0.20
	AC+DC	0.06	-0.25 to 0.36	0.71
Functional Constipation				
Baseline	AC	-0.34	-0.77 to 0.31	0.29
	DC	0.13	-0.5 to 0.66	0.70
	AC+DC	-0.13	-0.52 to 0.3	0.55
T60	AC	0.13	-0.49 to 0.67	0.68
	DC	-0.36	-0.78 to 0.28	0.25
	AC+DC	-0.06	-0.46 to 0.37	0.80
T120	AC	0.31	-0.33 to 0.76	0.32
	DC	-0.22	-0.71 to 0.42	0.50
	AC+DC	0.09	-0.34 to 0.48	0.69

MRI Content Motility (Ascending Colon)

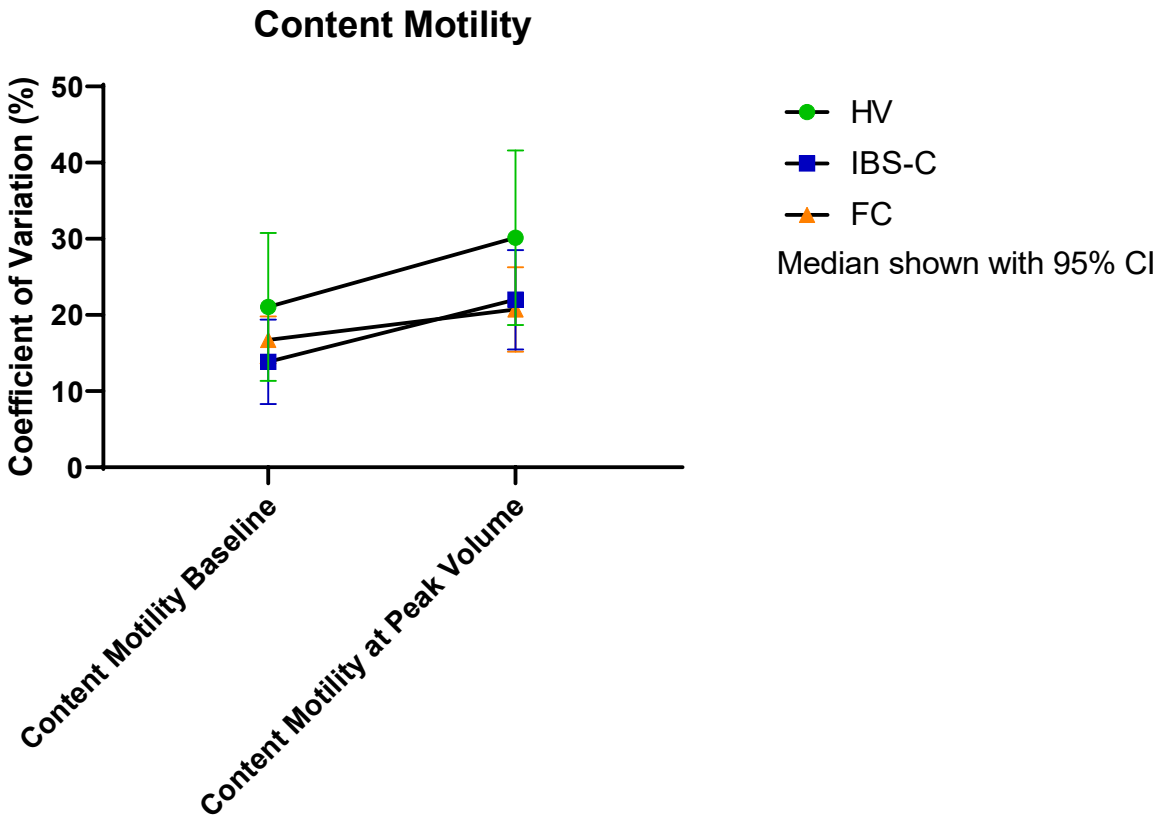


Figure 36: AC Content Motility Scores

For the AC content motility (Figure 36) a two-way ANOVA of the effect of both time (baseline vs peak) and group (HV, IBS-C and FC) on the content motility score showed the main effects of time yielded an F ratio of $F(1,58)=45.69$ $P<0.0001$ and participant group was $F(2,58)=7.378$ $P=0.0014$. However, the overall interaction effect was statistically insignificant ($F(2, 58)=1.773$, $P=0.1790$).

Transit Time

Transit for healthy volunteers was 1.33±1.5 (mean+/-SD) and 2.64±1.8 in IBS-C and 2.57±1.25 in FC. 1-way ANOVA showed p =0.0061 with Tukey's multiple comparisons showing significant difference between HV and IBS-C (adjusted p value 0.0097) (Figure 37). This can be put in hrs (as previously validated[156]) to be: 44±43hrs in HV, 82±54hrs in IBS-C and 80±37hrs in FC.

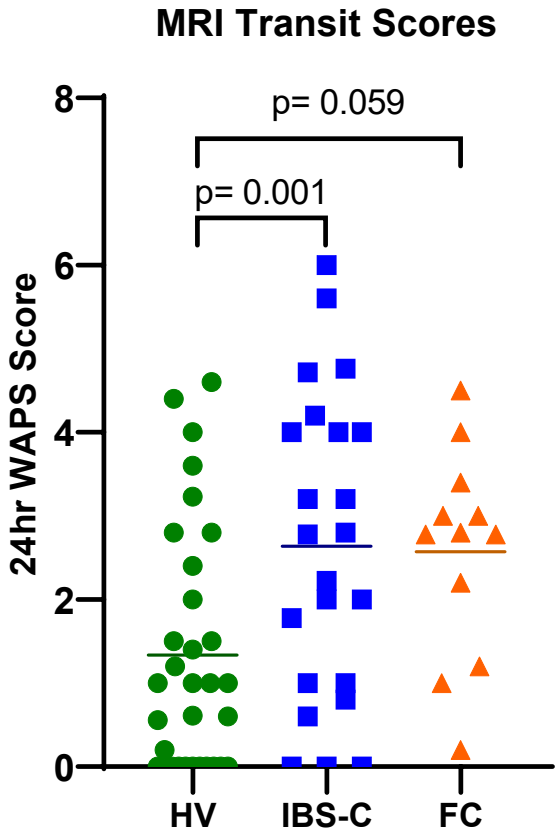


Figure 37: MRI Transit Scores

Time to bowel movement

Time to First Bowel Movement was measured by questionnaire to the participants, however if this did not occur within the study day this was not always completed/returned after the visit. We have therefore used 150min as a cut off for this measure, as this represented the average visit time as well as the upper end of the IQR from our HV pilot data.

In this cohort, HVs had a TBM of <150 min in 22/31 (71%) cases vs 8/23 (35%) in IBS-C and 3/12 (25%) in FC (p=0.002 Chi Square for trend) (Figure 38).

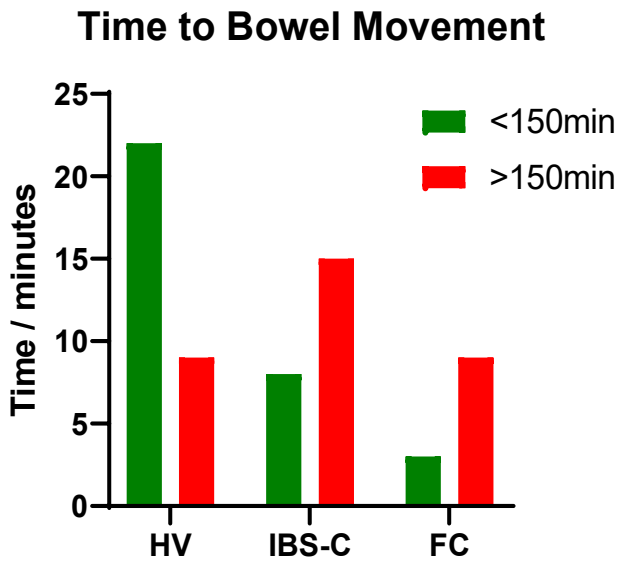


Figure 38: Time to Bowel Movement following consumption of macrogol drink, with cut off at 150mins used.

Manometry Measures

Manometry data were available for 80 participants (37 HV, 16 FC patients, and 27 IBS-C patients). Manual data analysis of both types of motor patterns observed and time occupied by the cyclic patterns are shown in table 12 below:

Table 12: Number of participants with each motor pattern observed before and after a meal

	HV (n=37)		**IBS-C (n=27)**		**FC (n=16)**	
	Before	*After*	*Before*	*After*	*Before*	*After*
HAPCs	4 (11%)	8 (22%)	3 (11%)	3 (11%)	0	0
Cyclic	13 (35%)	28 (76%)	5 (19%)	11 (41%)	4 (25%)	8 (50%)
PCP	19 (51%)	24 (65%)	18 (67%)	18 (67%)	14 (88%)	15 (94%)

All types of contraction pattern appeared to either remain the same or increase in frequency after the meal, across all three groups. The most common pattern observed were the pan-colonic pressurisations, which were present in the majority of participants in all groups both before and after the meal. No HAPCs were observed at any point in the FC group either before or after the meal.

For the cyclic activity, an increase was seen in all three groups in the descending colon (Table 13, Figure 39). Within the sigmoid colon there was an increase in activity in the HV and IBS-C groups however not in the FC group. A decrease in activity was observed in the rectum in both the HV and FC group but not the IBS-C group. The only significant difference in effect between the groups was observed in the sigmoid colon, in which the FC group had significantly less of an activity increase than the HV group.

Table 13: Mean time occupied by the cyclic contractions in the hour pre and post meal and the difference (mins)

Group	**Pre-Meal**			**Post-Meal**			**Difference**		
	Desc	*Sig*	*Rectum*	*Desc*	*Sig*	*Rectum*	*Desc*	*Sig*	*Rectum*
HV	12 ±9	17 ±9	21 ±11	20 ±12	23 ±10	15±9	8 ±8	7±7	-6±7
IBS-C	5±6	11±9	18 ±10	11 ±10	15 ±10	16 ±10	7 ±6	4±6	-2.0 ±10
FC	9 ±11	20 ±12	20 ±10	16 ±14	22 ±15	14±7	7 ±7	1±9*	-5.7 ±10
***p<0.05 vs HV (unpaired t-test)**									

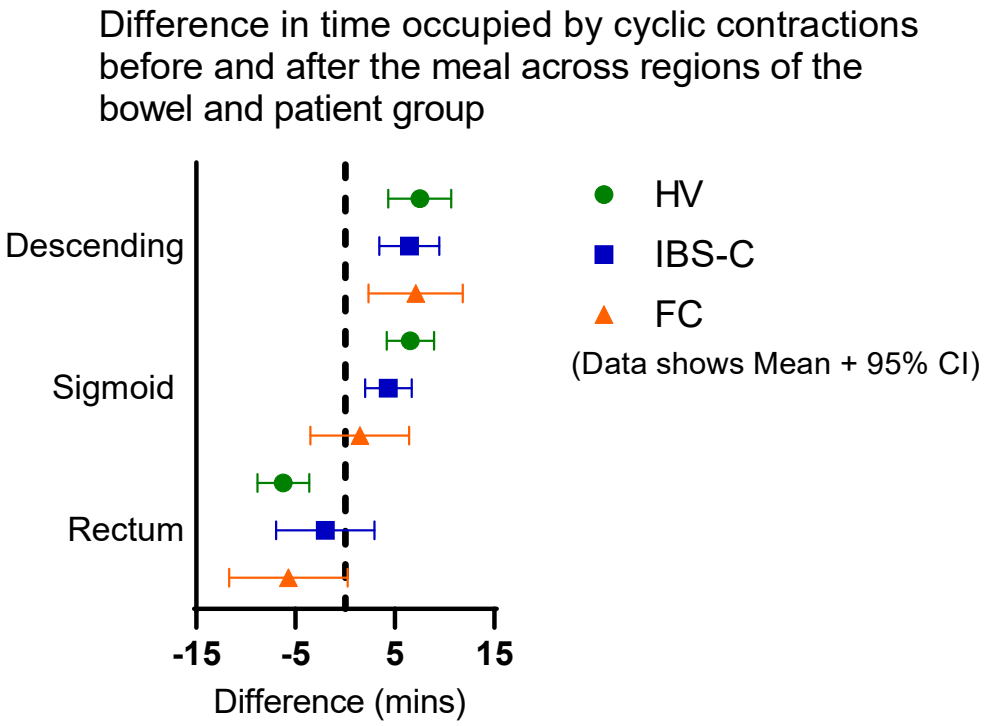


Figure 39: Differences in Time Occupied by Cyclic Contractions

Discussion

As this is only an interim analysis, one needs to be aware that these results may not be representative of the entire dataset and is currently underpowered. However, bearing that in mind, it still represents one of the largest sets of this type of data currently available, and the first that has looked at both these measures in the same cohort.

Demographic Data

The demographics of the recruited participants was similar to that seen in previous literature (discussed in my first chapter) with a majority of female participants of pre-menopausal age.

As expected, both constipated groups scored higher in the CCCS and PAC-SYM questionnaire and had higher anxiety and depression scores. Sensation of completeness of evacuation appeared selectively impaired in IBS-C, with no difference in SBMs noted between health and IBS, only those that were complete, in comparison to FC group in which bowel movements were globally reduced. This may reflect differences in rectal sensitivity which has been shown to be increased in IBS compared to FC[108,221].

Differences in BET success were noted across the groups, with the highest success rate in HVs followed by IBS-C, then FC

however how this may relate to overall motility elsewhere in the colon is not yet clear, however is something we plan to explore. As outlined in earlier chapters, the relationship between constipation and obstructed defecation has been studied previously however has proven complicated to disentangle. We did however standardise the size and consistency of the balloon, so this potential effect on the participants ability to expel a simulated stool that has been previously described[53] should have been reduced/eliminated.

MRI vs HRM Comparison

Correlation of manometry and MRI data is so far in the early stages, as only half the total data are collected so far. It does suggest differences in the way patient groups behave, with some suggestion that there are people at both the hyper and hypo-motile ends of the spectrum in both forms of measurement, however equally some participants with opposite scores (low MRI scores and high manometry and vice-versa) with little in the way of pattern emerging at this point. This may well become clearer with more data or indeed more heterogenous. It is also important to remember that the two techniques are not directly comparable in that they measure different sections of bowel (MRI AC/DC and manometry DC/SC/rectum) in different environments, albeit both in a

baseline state and after some form on stimulation (macrogol vs meal). There is also previous evidence[153,164] that the motility varies in different areas of the colon anyway – and this may relate to the differing functions of the regions of the colon, one may expect for example more mixing type motion in the AC (as more liquid content is acted upon by bacteria and fermentation processes occur) whereas later in the colon the focus may switch to a more propulsive action to get stool to the rectum in preparation for defecation. Our MRI techniques at this stage are unlikely to be sensitive enough to differentiate between these, whereas the manometry techniques as shown may be starting to.

However, without the results of the second part of the study (clinical trial of treatments), whether these data will be clinically useful is yet to be determined.

MRI Data

The MRI volume data are consistent with what has previously been observed[32] with largest volumes and increase from baseline seen in FC group.

The positive relationship between volume and motility appears as what one might expect in HVs, as contractions may be stimulated by colonic distention. Such a correlation is not seen

with FC which may imply an impaired response to distension while IBS shows an intermediate and weaker correlation.

It is still not clear how this relates to the patient's symptoms and experience, as motility observed in those with distended colons may not be actually propulsive in nature and therefore may not lead to effective defecation and relief of symptoms.

Our motility scores (both wall and content) in general had a lot of variability post-stimulus and were not significantly different between groups. The "non-responder" numbers are interesting in that HV and IBS-C proportions appear similar in both AC and DC, whereas the FC "non-responder" group is greater in AC only. However, again with such small numbers it is difficult to say whether this will show to be a significant finding when the full dataset is available.

Transit was slower in the patient groups as well as time to bowel movement, which is again, what we would expect and in-keeping with previous studies. However, in contrast to these we did not find a difference between the two patient groups. It is worth bearing in mind that, as previously noted, our groups were underpowered and the number in the FC considerably smaller than both the IBS-C/HV group and that observed with previous study. It may well be that with greater numbers (that

are currently in the process of recruitment and assessment) this changes.

Manometry Data

The manometry data do suggest that there are underlying differences in the motor patterns in both the IBS-C and FC group in both the sigmoid and possibly the rectum. Again, with the addition of more data we would hope that these may become clearer. Early automated analysis software currently being developed also suggests there are differences in the coordination of motor patterns (how each sensor relates to its neighbouring sensors) and that this may be decreased in the IBS group. This fits with our hypothesis that while IBS patients may have similar amounts of activity to HV, the co-ordination of this is important and if impaired could contribute to their symptoms. The directionality of the contractions may also be important here, as found in previous work – with retrograde and anterograde cyclic contractions differing in frequency, however this information is not available from the manual analysis at present but will be examined in more detail using automated software as more data are collected.

The data from this study, in particular the manometry data, appear to be extremely heterogenous, more so than previous data seen by members of the study team. And one of the

interesting observations in this dataset is the high proportion of pan-colonic pressure waves observed, which have not been seen in such number in previous studies in vivo. There are a number of possible explanations for this – it may be related to the gas content, possibly from the endoscopy itself as post-procedure XR did show large variation in gas in the bowel, or residual bowel content. It is worth noting that this is one of the first manometry studies of the colon in which full bowel preparation was not used and therefore the quality of the bowel preparation as well as remaining contents in the ascending/transverse colon varied considerably and may well have impacted the contraction patterns along the length of the bowel. This is something we are planning to look into further when the complete dataset is available.

Future Work

As mentioned previously, recruitment and analysis are still ongoing and we are hoping to have recruited our target numbers by the end of 2020, as well as completing the medication trial arm of the study. This should help us understand how underlying motor patterns may influence response to medication and how this may be a better way to target therapies for individualised patient care.

We will also be looking into analysis of the microbiota (as mentioned in the methods) which will no doubt be of interest as this is a growing field.

Manometry and MRI techniques are an evolving process, as more data are gathered and experience gained, there are modifications and improvements that can be made to both the acquisition and analysis of the data. We had thought that perhaps MRI motility might replace manometry, however our early data suggest that whilst the MRI certainly provides some useful information and may help distinguish FC from IBS-C, the manometry provides different data which may be complementary such as demonstration of incoordination.

The experiences from this study have helped shape protocols for further studies and work is currently ongoing on creating more automated analysis software for both the manometry and MRI that would be more easily translated into a clinical setting down the line.

Chapter 8: Future Uses of MRI: Strengths and Limitations

This thesis set out to explore and evaluate the role of novel imaging and recording techniques in the evaluation and possibly treatment of constipation. In this final chapter I hope to summarise the key qualities of MRI in particular which may lend it to applications in clinical practice.

Strengths of MRI

One of the key strengths of MRI is the lack of ionising radiation, which allows us to perform repeated measures over time without the associated radiation risks to the participant. This has allowed us to gather "real-time" data for example as contents make their way through the GI tract.

Whilst cost and availability are a significant limitation, these are being minimised as MRI gets cheaper and more and more hospitals acquire them for a wide range of applications. Almost all DGHs in the UK will now have invested in some form of MRI scanner (make/strengths do vary) and whilst their workload is increasing in other areas as well, if there were validated and clinically useful tests available there is the potential for widespread uptake and integration into existing pathways.

The data gathered in these and previous experiments have shown than MRI can generate accurate measurements of a variety of variables. These are objective and not necessarily operator dependent (as similar non-ionising techniques such as ultrasound imaging can be).

The ability of a clinician to provide a patient with such objective information should also not be under-estimated, particularly those in these functional cohorts. Many of these patients have a large amount of psychological distress associated with their symptoms and the ability to undergo a test that may well demonstrate that their colon works 'as normal' may provide much needed reassurance, especially if this can also be provided visually with an image or even a video.

Limitations of MRI

As stated, MRI does remain an expensive and sometimes challenging test. The cost of the scanner itself is often in the region of £1-2 million not including the cost of upkeep and employment of staff to both perform the scanning and report it, and so whilst many hospitals will have already invested in a scanner, it represents a significant cost burden and so clinical cost-effectiveness must be shown.

The logistical aspect of the MRI scanner can also present its own challenge – any metal implants must be MR safe or compatible or else they pose significant risk, and whilst progress has been made in the creation of more MR safe devices (such as pacemakers) in recent years, this can make certain participants unable to undergo this. Even MR safe implants will cause a degradation artefact in some sequences.

Some patients may suffer with claustrophobia or become distressed by the loud noise created by the scanner and so may not tolerate the scan, and certainly longer sequences and time spent in the scanner can exacerbate these feelings. Lying still for prolonged periods as well as the breath holding can also create artefact or cause discomfort.

The analysis of the MRI images does at present represent a significant obstacle to its widespread use, as for the majority of these techniques, an element of human driven analysis is performed to generate the values. These can vary in difficulty, require a certain amount of anatomical as well technical knowledge, and can take significant time to complete. The transit measurements are the simplest but still require a number of steps from obtaining the correct slices on the scanner, to reconstructing a 3D visualisation from which to identify the capsules before running these through an equation

to generate the value (takes around ~10min for each participant). The colonic volumes at the other end of the scale currently require manual drawing around the segments of the colon through a large number of thin slices, which can take over an hour in some cases, especially if the anatomy is challenging. Some of these steps could be automated in future, but full automation may require more advanced technology such as Artificial Intelligence (AI).

Development into a Novel Imaging Biomarker

A Consensus review by O'Connor et al in 2017[222] outlined a roadmap (Summarised in Figure 43) for how imaging techniques could be developed as biomarkers in cancers. These principles however are applicable to a wider range of disorders and it is worth considering how this work could fit into this framework. They describe how there are 3 overall domains of discovery, validation and qualification covering 3 parallel tracks (technical validation, clinical validation and cost effectiveness) that enable imaging biomarkers (IB) to overcome the translational gaps which currently prevent a number of these promising potential IBs crossing over from the academic sphere into widespread clinical use.

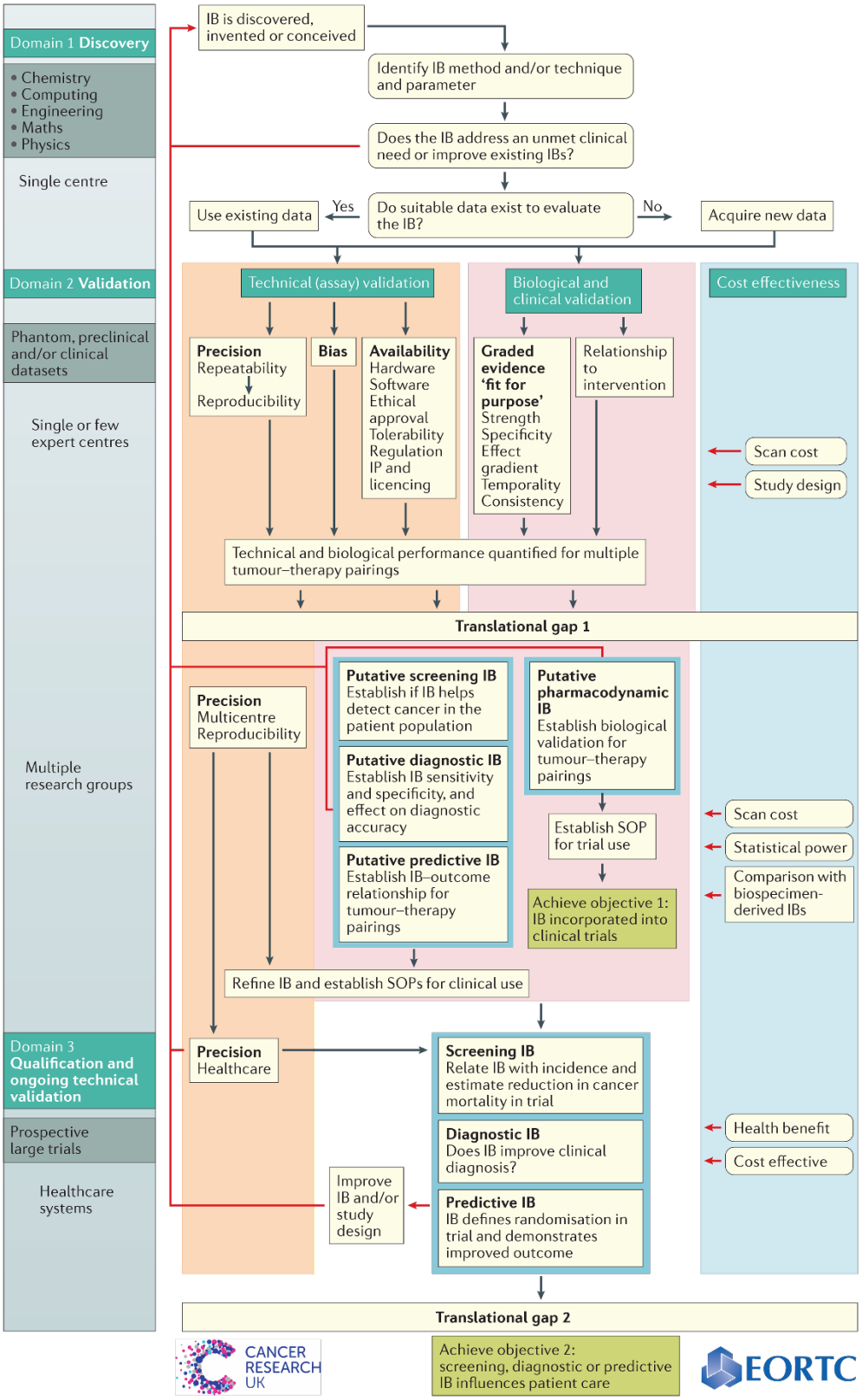


Figure 40: The Imaging Biomarker Roadmap. Image reproduced from http://www.cancerresearchuk.org/sites/default/files/imaging_biomarker_roadmap_for_cancer_studies.pdf

The work described in chapters 6 and 7 (COLONMOT study and RECLAIM study) describe how we have taken MRI sequences that have already gone through discovery phase of this model and moved them into the second of the domains, validation, as we start to address all three tracks.

The COLONMOT study represents the first step in the technical validation of these techniques as a potential IB for constipation. This focusses on the precision (repeatability and reproducibility) of the test. We also briefly touched on the availability (incorporating feasibility, safety, and tolerability) for its use in healthy volunteer population.

The RECLAIM study then expanded upon this work – moving into the clinical validation as well as building on the technical validation work already done. This encompassed aspects of reproducibility across multiple sites (albeit theoretically on identical scanner models) – and we will have the opportunity to establish how this may impact the ability of this MRI tool to be used on a wider scale. We also expanded from the healthy volunteer population into our anticipated patient population. This allows us to potentially examine both the availability to them and move into the clinical utility and validation. If we can show, as we hope to with the full dataset, that MRI results have a relationship with clinical symptoms and potential treatment

strategies the this will pave the way to move into the next stages of the roadmap.

There are also other areas within this overall validation stage which we have not explored, such as whether these sequences could be adapted for other brands or strengths of MRI scanner, and availability of analysis software with assessment of inter-rater variability and other measures of technical performance.

The final steps would be to conduct a definitive and well powered prospective study that could link the MRI outcomes to clinical outcomes as well as robust cost-effectiveness analysis and necessary regulatory approvals to enable widespread adoption of the technique and application of the IB.

A role for colonic manometry?

Whilst the data we have gained and are continuing to analyse about the colonic movements from the manometry recording are fascinating and giving us new insights into physiology and pathology, whether this will be a valuable clinical tool still remains to be seen. Even compared to MRI, it is logistically challenging, expensive, and uncomfortable.

If it were to be used in a clinical practical setting, it is likely to be extremely limited to a tertiary or an even more specialist level centre. It requires such a range of different skillsets to perform a single test from endoscopists to experienced nursing staff to someone who can interpret the data. Much more work is likely needed here to establish its clinical utility.

The Future

One step that would dramatically improve the chances of these techniques becoming clinically useful is AI. As discussed, one of the significant limitations of these techniques at present is the manpower needed to analyse the data generated by the scanner. Recently it has been proposed that some of these sequences could be automated to improve efficiency and a certain amount would be feasible through programming and code, however the ability, for example, to identify, draw around and segment colonic segments for volume analysis, requires knowledge as well as pattern recognition. This is an area that machine learning and artificial intelligence may prove of benefit and is currently being researched and investigated in other areas of medical imaging and radiology. There is the potential downside in the loss of accuracy – however in these situations you are likely looking for a large difference rather than marginal ones. For example, if you were using this technique to evaluate

a patient in consideration for colectomy you would need a very large difference in values from normal, and whilst AI vs manual drawing may well provide a slightly different value this is not likely to be of clinical significance. This is certainly something that would need to be assessed in more detail however if these techniques were to be developed.

Whilst this thesis has primarily focussed on constipation, it is obvious that these techniques could be applied to a wide range of other conditions, both organic and functional, of the GI tract. These sequences and similar have been used in the investigation of IBD, coeliac disease, functional diarrhoea and more both in our centre and elsewhere.

Equally they may be a useful tool in future studies looking at drugs and their actions in the gut, for example newer secretagogues could be given to participants and effects demonstrated in vivo using the small bowel water techniques.

Considering that over the last 20 years the scientific community has made enormous strides in the field of MR imaging, the possibilities ahead are vast and so I hope that this work, in part, may contribute to the use of MRI as a valuable clinical tool in the future, for the evaluation of GI disorders including, but perhaps not limited to, constipation.

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APPENDICES

Appendix A: Supplementary Data used in KIWI Study

Table 1: Time course data for MRI endpoints

Table 2: Gastrointestinal symptoms monitored during the study

Figure 1: Total (A), ascending (B), transverse (C) and descending (D) colon volumes shown for each scan time point.

Appendix B: Supplementary Data used in COLONMOT Study

Table 1: Summary data of Colonic Volume by Segment

Table 2: Summary data of Water Contents of the Bowel by Segment

Table 3: Summary data of key parameters for post stimulus time points only.

Table 4: MRI sequence parameters

Appendix C: Questionnaires used in these Studies

Constipation Scoring System (Cleveland Clinic)

ROME IV Diagnostic Criteria

Hospital Anxiety and Depression Scale (HADS)

PAC-SYM

Symptom Questionnaire MRI Day

Symptom Questionnaire Manometry Day (VAS 0-100)

Appendix A: Supplementary data for KIWI study

Table 1: Time course data for MRI endpoints

MRI Variable	Test Product	Time point								
		Fasting	**T0**	**T60**	**T120**	**T180**	**T240**	**T300**	**T360**	**T420**
T1 AC (sec)	Kiwifruit	0.75	0.72	0.73	0.75	0.73	0.71	0.69	0.8	0.87
		±0.4	±0.3	±0.3	±0.3	±0.2	±0.2	±0.2	±0.3	±0.3
	Control	0.58	0.58	0.64	0.64	0.67	0.56	0.57	0.65	0.67
		±0.3	±0.3	±0.3	±0.3	±0.3	±0.2	±0.2	±0.3	±0.3
T1 DC (sec)	Kiwifruit	0.58	0.55	0.49	0.52	0.56	0.56	0.49	0.52	0.57
		±0.3	±0.3	±0.3	±0.3	±0.3	±0.3	±0.3	±0.3	±0.3
	Control	0.49	0.55	0.53	0.53	0.53	0.48	0.52	0.6	0.5
		±0.2	±0.3	±0.3	±0.3	±0.3	±0.3	±0.3	±0.4	±0.3
SBWC (ml)	Kiwifruit	110	181	85	111	212	290	240	196	53
		±54	±75*	±76*	±64*	±125*	±107*	±93*	±84*	±46*
	Control	83	49	38	60	70	85	107	120	28
		±61	±34	±25	±46	±53	±53	±51	±57	±18
Colon Volume (ml)	Kiwifruit	559	600	583	570	573	586	598	618	638
		±166	±137*	±129*	±111*	±132*	±137*	±131*	±140*	±136*
	Control	525	509	489	501	503	491	505	531	525
		±113	±101	±103	±101	±102	±104	±103	±130	±130

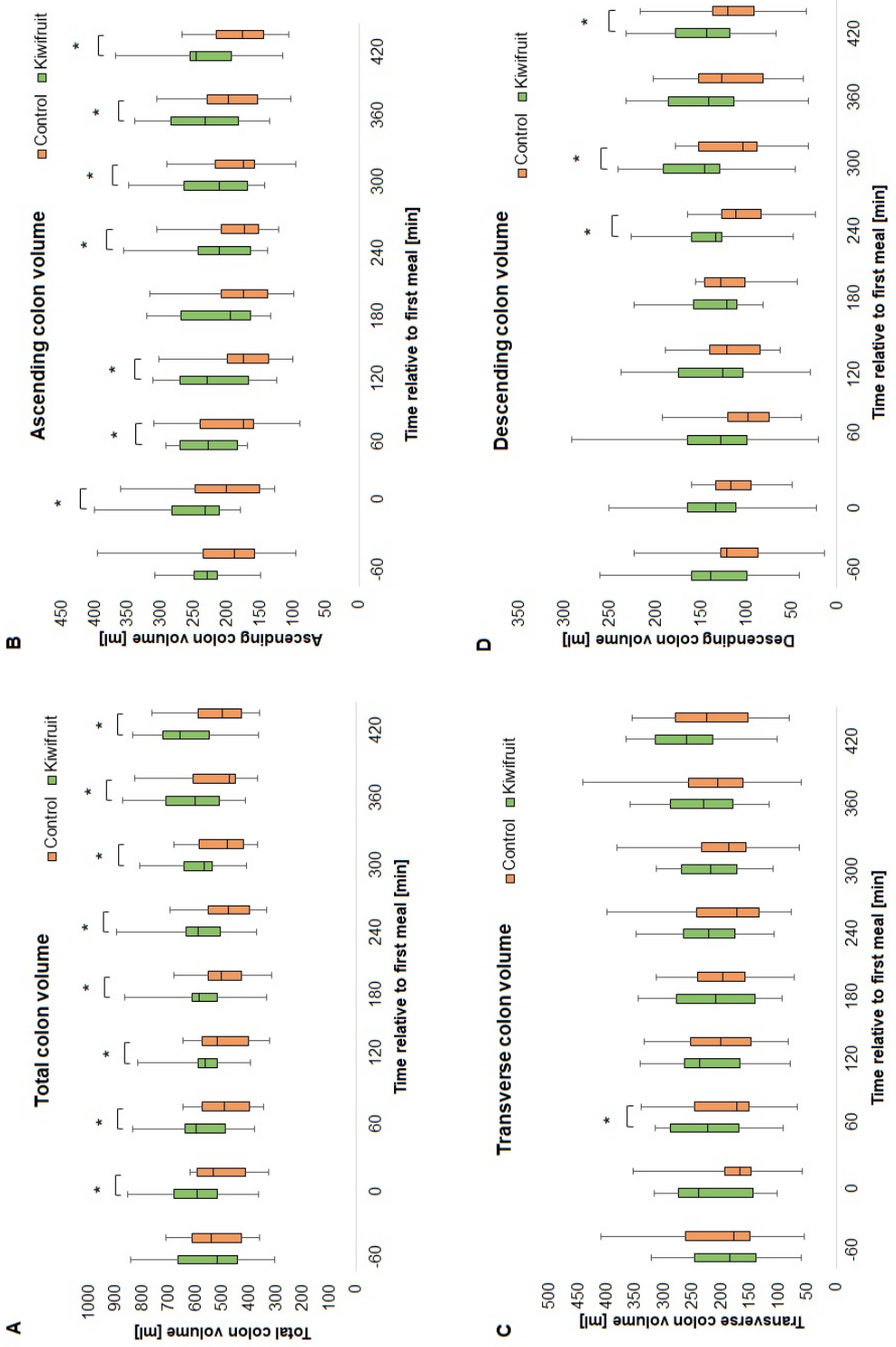
* Indicates p<0.05 vs control at matched time point (paired t-test)

T1 – T1 relaxation time; AC – ascending colon; DC – descending colon; SBWC – small bowel water content

Table 2: Gastrointestinal symptoms monitored during the study. Data are mean severity levels (range from 0, symptom not present, to 3, severe and disabling) for each symptom and study period. Kiwifruit and control drink were consumed for four days each by each participant, whilst days 1, 2, and 7 of both arms of the study were combined to make up the baseline data, i.e. six days per participant, n=14

	Kiwifruit	Control	Baseline
Gas	0.54	0.61	0.46
Bloating	0.11	0.14	0.14
Abdominal pain	0.00	0.04	0.07
Nausea	0.07	0.04	0.00

Figure 1: Total (A), ascending (B), transverse (C) and descending (D) colon volumes shown for each scan time point. Asterisks indicate $P<0.05$



Appendix B: Supplementary data for COLONMOT study

Table 1: Summary data of Colonic Volume by Segment

	Visit 1 Volume (mL)	Visit 2 Volume (mL)	ICC (baseline and maximum V1 vs V2)	95% CI	p value
Ascending Colon					
Baseline	263±92	287±86	0.816	0.618-0.916	<0.0001
Peak	483±122	461±103			
Transverse Colon					
Baseline	197±120	209±86	0.767	0.529-0.892	<0.0001
Peak	313±167	304±118			
Descending Colon					
Baseline	175±86	189±82	0.713	0.439 – 0.865	<0.0001
Peak	299±68	296±68			
Sigmoid Colon					
Baseline	93±33	90±46	0.633	0.302-0.827	<0.0001
Peak	170±39	178±51			
Values are mean ± SD if normally distributed, median (IQR) otherwise					

Table 2: Summary data of Water Contents of the Bowel by Segment

	Visit 1 Water Content (mL)	Visit 2 Water Content (mL)	ICC (baseline and maximum V1 vs V2)	95% CI	p value
Small Bowel					
Baseline	113±63	105±51	0.928	0.840-0.913	<0.0001
Peak	564±174	563±202			
Ascending Colon					
Baseline	0	0	0.822	0.629-0.919	<0.0001
Peak	327±159	332±126			
Transverse Colon					
Baseline	0	0	0.909	0.801-0.960	<0.0001
Peak	111±82	113±85			
Descending/Sigmoid Colon					
Baseline	0	0	0.655	0.344-0.835	<0.0001
Peak	148±122	149±107			
Values are mean ± SD if normally distributed, median (IQR) otherwise					

Table 3: Summary data of key parameters for post stimulus time points only.

	Visit 1	Visit 2	ICC (baseline and post stimulus time point V1 vs V2)	95% CI	p value
Total Colon Volume (mL)					
T60	1133±262	1116±245	0.825	0.636-0.920	<0.001
T120	1125±394	1093±317	0.895	0.773-0.953	<0.001
Total Water Content (mL)					
T60	1016±274	1015±344	0.915	0.814-0.962	<0.001
T120	848±292	863±290	0.866	0.714-0.940	<0.001
Colon Water Content (mL)					
T60	463±258	475±214	0.883	0.748-0.948	<0.001
T120	455±260	494±274	0.788	0.569-0.902	<0.001
Content Mixing AC (%)					
T60	31±9	27±7	0.628	0.298-0.825	<0.001
T120	29±11	24±7	0.299	-0.103-0.627	0.074
Content Mixing DC (%)					
T60	28±10	23±8	0.305	-0.111-0.634	0.076
T120	30±9	26±6	0.490	0.108-0.49	0.008
Wall Movement AC (a.u)					
T60	1188 (524-2426)	1531 (701-2163)	0.704	0.424-0.861	<0.001
T120	888 (492-2421)	1346 (834-1886)	0.416	0.016-0.699	0.022
Wall Movement DC (a.u)					
T60	1795 (1179-2630)	1618 (884-1955)	0.663	0.368-0.838	<0.001
T120	1273 (934-2511)	1274 (702-2053)	0.338	-0.080-0.650	0.054
Values are mean ± SD if normally distributed, median (IQR) otherwise					

Table 4: MRI sequence parameters

	Wall Motility	Content Motility	Colonic Volume	Water Content
Sequence Type	Cine bTFE	Cine bTFE	Dual echo FFE with mDIXON reconstruction[203]	RARE
2D/3D	2D (2 stacks)	2D	3D (2 stacks overlapping 30 mm in HF direction)	2D multi-slice
Orientation	Sagittal Oblique	Sagittal Oblique	Coronal	Coronal
Slice Thickness (mm)	10	15	3.6 acquired 1.8 interpolated	7 (0 gap)
No. of slices	2	1	111	20
FOV (mm^2)	330x227	330x259	250 x 375	400 x 400
Resolution (mm^2)	1.5x1.6	0.98 x 0.98	0.98 x 0.98	0.78 x 0.78
FA	50	45	10	90 and Refocussing of 108
Breath hold/Free Breathing (duration)	Free breathing (10 mins)	BH (20 s)	2 x BH (18 s) 1 BH per stack	BH (24 s)
Temporal Resolution (s)	1	0.6	N/A	N/A
Number of cine scans per slice	600	33	N/A	N/A

bTFE = balanced Turbo Field Echo
FFE = Fast Field Echo
RARE = Rapid acquisition with relaxation enhancement

Appendix C: Questionnaires Used in These Studies

Constipation Scoring System

Frequency of bowel movements
0 □ 1-2 times per 1-2 days
1 □ 2 times per week
2 □ Once per week
3 □ Less than once per week
4 □ Less than once per month

Difficulty: painful evacuation effort
0 □ Never
1 □ Rarely
2 □ Sometimes
3 □ Usually
4 □ Always

Completeness: feeling incomplete evacuation
0 □ Never
1 □ Rarely
2 □ Sometimes
3 □ Usually
4 □ Always

Pain: abdominal pain
0 □ Never
1 □ Rarely
2 □ Sometimes
3 □ Usually
4 □ Always

Time: minutes in lavatory per attempt
0 □ Less than 5
1 □ 5-10
2 □ 10-20
3 □ 20-30
4 □ More than 30

Assistance: type of assistance
0 □ Without assistance
1 □ Stimulative laxatives
2 □ Digital assistance or enema

Failure: unsuccessful attempts for evacuation per 24 hours
0 □ Never
1 □ 1-3
2 □ 3-6
3 □ 6-9
4 □ More than 9

History: duration of constipation (yr.)
0 □ 0
1 □ 1-5
2 □ 5-10
3 □ 10-20
4 □ More than 20

Rome IV

1. In the last 3 months, how often did you have pain anywhere in your abdomen?

- ☐ Never
- ☐ Less than one day a month
- ☐ One day a month
- ☐ Two to three days a month
- ☐ Once a week
- ☐ Two to three days a week
- ☐ Most days
- ☐ Every day
- ☐ Multiple times per day or all the time

2. How often did this pain in your abdomen happen close in time to a bowel movement -- just before, during, or soon after? (Percent of times with pain)

- ☐ 0% Never
- ☐ 10%
- ☐ 20%
- ☐ 30%
- ☐ 40%
- ☐ 50%
- ☐ 60%
- ☐ 70%
- ☐ 80%
- ☐ 90%
- ☐ 100% Always

3. How often did your stools become either softer than usual or harder than usual when you had this pain? (Percent of times with pain)

- ☐ 0% Never
- ☐ 10%
- ☐ 20%
- ☐ 30%
- ☐ 40%
- ☐ 50%
- ☐ 60%
- ☐ 70%
- ☐ 80%
- ☐ 90%
- ☐ 100% Always

4. How often did your stools become either more frequent than usual or less frequent than usual when you had this pain? (Percent of times with pain)

- ☐ 0% Never
- ☐ 10%
- ☐ 20%
- ☐ 30%
- ☐ 40%
- ☐ 50%
- ☐ 60%
- ☐ 70%
- ☐ 80%
- ☐ 90%
- ☐ 100% Always

5. Has it been 6 months or longer since you started having this pain?

- ☐ Yes
- ☐ No

6. In the last 3 months, how often did you have hard or lumpy stools that looked like Type 1 or 2 in the picture below? (Percent of all bowel movements)

- ☐ 0% Never
- ☐ 10%
- ☐ 20%
- ☐ 30%
- ☐ 40%
- ☐ 50%
- ☐ 60%
- ☐ 70%
- ☐ 80%
- ☐ 90%
- ☐ 100% Always

7. Did you have hard or lumpy stools (like Type 1 or 2) when you were not taking drugs for diarrhoea?

- ☐ No, or rarely
- ☐ Yes

8. In the last 3 months, how often did you have fewer than three bowel movements a week without taking a laxative medication or enema? (Percent of weeks)

- ☐ 0% Never
- ☐ 10%
- ☐ 20%
- ☐ 30%
- ☐ 40%
- ☐ 50%
- ☐ 60%
- ☐ 70%
- ☐ 80%
- ☐ 90%
- ☐ 100% Always

9. In the last 3 months, how often did you strain during bowel movements? (Percent of bowel movements)

- ☐ 0% Never
- ☐ 10%
- ☐ 20%
- ☐ 30%
- ☐ 40%
- ☐ 50%
- ☐ 60%
- ☐ 70%
- ☐ 80%
- ☐ 90%
- ☐ 100% Always

10 In the last 3 months, how often did you have a feeling of incomplete emptying after bowel movements? (Percent of bowel movements)

- ☐ 0% Never
- ☐ 10%
- ☐ 20%
- ☐ 30%
- ☐ 40%
- ☐ 50%
- ☐ 60%
- ☐ 70%
- ☐ 80%
- ☐ 90%
- ☐ 100% Always

11 In the last 3 months, how often did you have a sensation that the stool could not be passed (was blocked), when having a bowel movement? (Percent of bowel movements)

- ☐ 0% Never
- ☐ 10%
- ☐ 20%
- ☐ 30%
- ☐ 40%
- ☐ 50%
- ☐ 60%
- ☐ 70%
- ☐ 80%
- ☐ 90%
- ☐ 100% Always

12 In the last 3 months, how often did you press on or around your bottom, or remove stool with your fingers, in order to have a bowel movement? (Percent of bowel movements)

- ☐ 0% Never
- ☐ 10%
- ☐ 20%
- ☐ 30%
- ☐ 40%
- ☐ 50%
- ☐ 60%
- ☐ 70%
- ☐ 80%
- ☐ 90%
- ☐ 100% Always

13 Did any of the symptoms of constipation listed in questions 6-12 above begin more than 6 months ago?

- ☐ No
- ☐ Yes

14 Are you currently taking a prescription medication for pain?

- ☐ No
- ☐ Yes

15 In the last 3 months, how often did you have mushy or watery stools that looked like Type 6 or 7 in the picture below when you were not using drugs or other treatment for constipation? (Percent of all bowel movements)

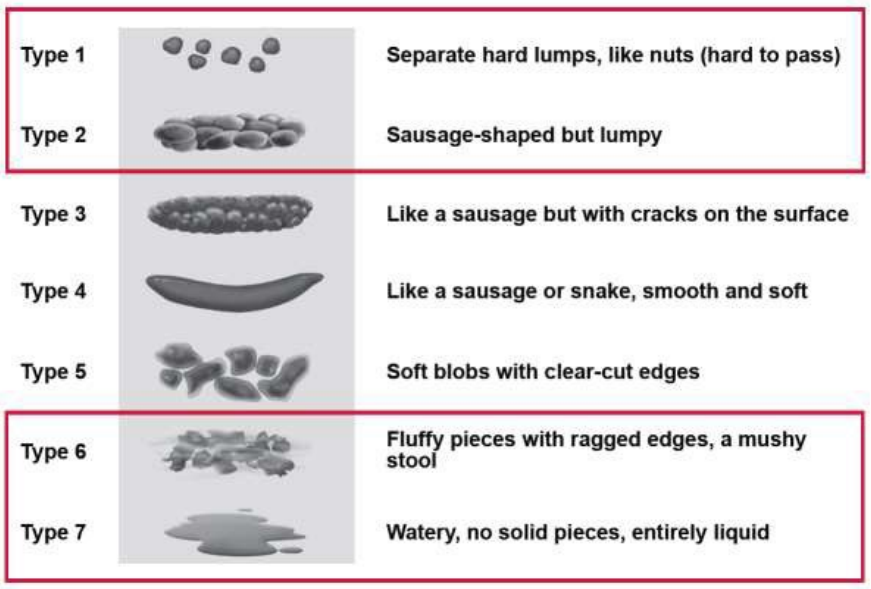
- ☐ 0% Never
- ☐ 10%
- ☐ 20%
- ☐ 30%
- ☐ 40%
- ☐ 50%
- ☐ 60%
- ☐ 70%
- ☐ 80%
- ☐ 90%
- ☐ 100% Always

16 Did you have mushy and watery stools (like Type 6 or 7) when you were not using drugs or other treatment for constipation?

- ☐ No, or rarely
- ☐ Yes

17 In the last 3 months, when you had abnormal stools, what were they usually like?

- ☐ Usually constipation (like Type 1 or 2 in the picture)
- ☐ Usually diarrhoea (like Type 6 or 7)
- ☐ Both diarrhoea and constipation - that is, more than 1/4 of all the abnormal bowel movements were constipation and more than ¼ were diarrhoea
- ☐ Not applicable, because I never or rarely had abnormal bowel movements



Hospital Anxiety and Depression Scale (HADS)

Please complete each of the following questions, checking the one response that comes closest to how you have been feeling **in the past week.**

(A) I feel tense or 'wound up':
3 □ Most of the time
2 □ A lot of the time
1 □ Sometimes
0 □ Never

(D)I still enjoy the things I used to enjoy:
0 □ Definitely as much
1 □ Not quite as much
2 □ Only a little
3 □ Hardly at all

(A) I get a sort of frightened feeling as if something awful is about to happen:
3 □ Definitely and quite badly
2 □ Yes, but not too badly
1 □ A little, but it doesn't worry me
0 □ Never

(D)I can laugh and see the funny side of things:
0 □ As much as I always could
1 □ Not quite as much now
2 □ Definitely not as much now
3 □ Never

(A)Worrying thoughts go through my mind:
3 □ All of the time
2 □ A lot of the time
1 □ Sometimes, but not too often
0 □ Rarely

(D)I feel cheerful:
3 □ Never
2 □ Not often
1 □ Sometimes
0 □ Most of the time

(A)I can sit at ease and feel relaxed:
0 □ Definitely
1 □ Usually
2 □ Not often
3 □ Never

(D)I feel as if I am slowed down:
3 □ Nearly all the time
2 □ Very often
1 □ Sometimes
0 □ Never

(A)I get a sort of frightened feeling like 'butterflies' in the stomach:
0 □ Never
1 □ Occasionally
2 □ Quite often
3 □ Very often

(D)I have lost interest in my appearance:
3 □ Definitely
2 □ I don't take as much care as I should
1 □ I may not take quite as much care
0 □ I take just as much care as ever

(A)I feel restless, as if I have to be on the move:
3 □ Very much
2 □ Quite a lot
1 □ Not Very much
0 □ Never

(D)I look forward with enjoyment to things:
0 □ As much as I ever did
1 □ Somewhat less than I used to
2 □ Definitely less than I used to
3 □ Hardly at all

(A)I get sudden feelings of panic:
3 □ Very often
2 □ Quite often
1 □ Not very often
0 □ Never

(D)I can enjoy a good book or TV program:
0 □ Often
1 □ Sometimes
2 □ Not often
3 □ Rarely

PAC-SYM

This questionnaire asks you about your constipation in the past 2 weeks. Answer each question according to your symptoms, as accurately as possible. There are no right or wrong answers.

For each symptom below, please indicate how severe your symptoms have been during the past 2 weeks. If you have not had the symptom during the past 2 weeks, check 0.

How severe have each of these symptoms been in the last 2 weeks	**Absent**	**Mild**	**Moderate**	**Severe**	**Very Severe**
	0	**1**	**2**	**3**	**4**
Discomfort in your abdomen					
Pain in your abdomen					
Bloating in your abdomen					
Stomach cramps					
Painful bowel movements					
How severe have each of these symptoms been in the last 2 weeks	**Absent**	**Mild**	**Moderate**	**Severe**	**Very Severe**
	0	**1**	**2**	**3**	**4**
Rectal burning during or after a bowel movement					
Incomplete bowel movement, like you didn't "finish"					
Bowel movements that were too hard					
Bowel movements that were too small					
Straining or squeezing to try to pass bowel					
Feeling like you have to pass a bowel movement but you couldn't (false alarm)					

Symptoms on MRI Day

Throughout the study days we want you to record any abdominal symptoms you experience including pain, bloating, flatulence. Please record each of these on scale 0 – 3 where:

0 – absent,

1 – mild and easily ignored,

2 – moderate and with a negative impact on daily activity,

3 – severe and disabling, profoundly interfering with daily activity

Timepoint	**Abdominal Pain**	**Bloating**	**Flatulence**
Before Scan 1 (T=-30min)			
After Scan 1 (T=0min) *MOVIPREP GIVEN*			
Before Scan 2 (T=60min)			
After Scan 2 (T=90min)			
Before Scan 3 (T=120min)			
After Scan 3 (T=150min)			

Time to first Bowel Movement: _____

Symptoms on Manometry Day

VAS Score : To be completed at baseline prior to any intervention, then every fifteen minutes during manometry recording period, both before and after the meal, which is given at T=120.

Sensation of abdominal gas

none extreme

Abdominal discomfort

none extreme

Desire to evacuate gas (flatus)

none extreme

Desire to defecate

none extreme

Urgency to defecate

none extreme

Abdominal pain

none extreme

Other..... (please describe)

none extreme