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Original research

Combined MRI, high-resolution manometry and a randomised trial of bisacodyl versus hyoscine show the significance of an enlarged colon in constipation: the RECLAIM study

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ABSTRACT

Background Colonic motility in constipation can be assessed non-invasively using MRI.

Objective To compare MRI with high-resolution colonic manometry (HRCM) for predicting treatment response.

Design Part 1: 44 healthy volunteers (HVs), 43 patients with irritable bowel syndrome with constipation (IBS-C) and 37 with functional constipation (FC) completed stool diaries and questionnaires and underwent oral macrogol (500–1000 mL) challenge. Whole gut transit time (WGTT), segmental colonic volumes (CV), MRI-derived Motility Index and chyme movement by 'tagging' were assessed using MRI and time to defecation after macrogol recorded. Left colonic HRCM was recorded before and after a 700 kcal meal. Patients then proceeded to Part 2: a randomised cross-over study of 10-days bisacodyl 10 mg daily versus hyoscine 20 mg three times per day, assessing daily pain and constipation.

Results Part 1: Total CVs median (range) were significantly greater in IBS-C (776 (595–1033)) and FC (802 (633–951)) vs HV (645 (467–780)), p<0.001. Patients also had longer WGTT and delayed evacuation after macrogol. IBS-C patients showed significantly reduced tagging index and less propagated pressure wave (PPW) activity during HRCM versus HV. Compared with FC, IBS-C patients were more anxious and reported more pain. Abnormally large colons predicted significantly delayed evacuation after macrogol challenge (p<0.02), impaired manometric meal response and reduced pain with bisacodyl (p<0.05).

Part 2: Bisacodyl compared with hyoscine increased bowel movements but caused more pain in both groups (p<0.03).

Conclusion An abnormally large colon is an important feature in constipation which predicts impaired manometric response to feeding and treatment responses. HRCM shows that IBS-C patients have reduced PPW activity.

Trial registration number The study was preregistered on ClinicalTrials.gov, Reference: NCT03226145.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ MRI can be used to assess colonic volumes and motor response after a macrogol challenge in patients with constipation.

WHAT THIS STUDY ADDS

⇒ MRI-assessed colonic volumes are greater in both functional constipation (FC) and irritable bowel syndrome (IBS) patients than in healthy volunteers (HVs).

⇒ Large colons (>90th centile for HVs) predict impaired manometric meal response, delayed evacuation after macrogol challenge and reduced pain with bisacodyl.

⇒ Compared with FC, patients with IBS-C show reduced propagated pressure waves in the left colon and report more pain after macrogol and bisacodyl.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ MRI assessment of colonic volumes could contribute to individualised treatment of constipation in secondary/tertiary care.

INTRODUCTION

Constipation is a common symptom affecting approximately 11%–15% of the general population.[1 2] The symptom-based Rome IV classification separates functional constipation (FC) from irritable bowel syndrome with constipation (IBS-C),[3] but this subdivision is controversial[4–7] as symptoms overlap substantially.[8] Treatments targeting these different populations give a number needed to treat varying from 2 to 7,[9] leaving many patients dissatisfied.[10] Many investigators are attempting to improve this by more accurate assessment of the underlying pathophysiology which is recognised to comprise three principal overlapping factors: delayed transit secondary to gut dysmotility, evacuatory dysfunction and abnormal sensory function,

which is often allied to an enlarged or hypercompliant bowel.[7] Current diagnostic tests are based primarily on assessments of transit including scintigraphy and radio-opaque markers and only rarely manometry and barostat owing to the complexity of the latter techniques. By contrast, MRI, which has as yet not been widely used, offers the opportunity to assess many parameters simultaneously. Using MRI, we have developed an objective measure of colonic function, the macrogol challenge[11] which measures colonic volumes (CVs) and maximal motor response (maximal MRI Motility Index, MMI) to macrogol[12] and when combined with non-absorbable markers can reproducibly measure whole gut transit time (WGTT).[13] While there are currently only a few studies using MRI, our pilot study suggested that compared with IBS-C, FC patients had larger CVs, longer transit and reduced motility response,[11] as assessed by either MMI or a colon 'tagging' technique, a recognised measure of movement of colonic content.[14] We hypothesised that MRI assessment of CV and motility would allow better targeting of treatment.

High-resolution colonic manometry (HRCM) provides new insights revealing co-ordinated and often retrograde-moving patterns of colonic contraction—'the cyclic motor pattern' (CMP) particularly in the sigmoid colon, suggesting a 'brake' function.[15] Such recordings also demonstrate that patients with slow-transit constipation show a reduced colonic response to feeding,[16] but it is unclear if this differs from patients with IBS-C.

The primary aims were therefore to (1) compare the non-invasive, patient-acceptable, MRI characterisation of colonic motor function in both FC and IBS-C against the more demanding and invasive HRCM and (2) test in a randomised, double-blinded, cross-over trial of the hypothesis that colonic motility, studied with MRI would predict the difference in response to a colonic motor stimulant (bisacodyl) compared with an antispasmodic (hyoscine butylbromide). The logic behind this comparison was the earlier findings that IBS patients had smaller colons which could have reflected increased colonic tone and motility. This would be expected to respond to an antispasmodic such as hyoscine with reduced symptoms, particularly pain. In contrast, the FC patients in the previous study had both larger colons and reduced motility both of which should have improved with a prokinetic. By using a common endpoint namely pain we aimed to assess a difference between treatments which could be correlated with our MRI measurements.

METHODS

We performed the study in two parts.

Part 1: MRI and manometry

Participants with constipation and healthy volunteer (HV) were recruited at two sites in the UK (Nottingham and London) from both primary and secondary care (online supplemental file A). All participants underwent a 2-week screening period (off laxatives) during which a bowel habit diary including Bristol Stool Form Scale (BSFS) was completed, along with baseline Hospital Anxiety and Depression Scale (HADS) and the Patient Assessment of Constipation-Symptoms (PAC-SYM)[17] score. A modified PAC-SYM (mPAC-SYM) score was calculated using only abdominal pain, discomfort and cramps elements of PAC-SYM. Subjects also underwent a balloon expulsion test to assess the ability to expel a rectal balloon (online supplemental file B1).

All participants attended fasted on two separate occasions for (1) a 2-hour MRI study and (2) a 4-hour HRCM study.

MRI study

Participants consumed 5 transit markers 24 hours before a fasting scan, then ingested oral macrogol provided as MoviPrep (10 mL/kg body weight, minimum 500 mL, maximum 1000 mL), followed by MRI scans at 60 and 120 min. 1 litre MoviPrep contains 100 g of polyethylene glycol (PEG) 3350, 7.5 g sodium sulfate, 2.69 g sodium chloride, 1.01 g of potassium chloride plus aspartame, acesulfame potassium and lemon flavouring, hereafter referred to as macrogol. Images were analysed blind to participant condition, by a single operator (VW-S) to assess total and segmental CVs, motility measures including ascending colon (AC) 'tagging index', AC and descending colon (DC) MMI at 60 and 120 min and WGTT (online supplemental C1,C2).

Primary endpoint

MMI of the AC derived from wall movement at maximum distension to macrogol as previously described.[18]

Secondary endpoints

CVs, peak MMI of the DC, WGTT, assessed by the 'Weighted Average Position Score' (WAPS) of transit markers as previously validated[13] and time to first bowel movement following macrogol.

Exploratory endpoints

Movement of AC colonic chyme as assessed from the 'tagging index'[19] (online supplemental C2), pain scores 0–2 hours after macrogol (0–3 scale).

HRCM study

Participants received a tap water enema to cleanse the left colon prior to flexible sigmoidoscopy and placement of the HRCM catheter.[20] Recordings were performed for 2 hours before and after a 700 kcal meal. Using previously developed software (PlotHRM),[15] manometric traces were examined for the presence of the CMP and high-amplitude propagating contractions (HAPCs) in the hour before and after the meal. Further automated analysis of other motor patterns activity including propagated pressure waves (PPW) was then performed using a Bayesian functional mixed-effects model[21 22] (online supplemental D2.1, figure S5).

Primary endpoint

Percentage time occupied by the CMP (CMP in the sigmoid colon following the meal.

Secondary endpoints

HAPCs per hour and measures of coordinated antegrade and retrograde propagated contractions (analysis detailed in online supplemental D2.1).

Part 2: randomised, placebo-controlled trial comparing bisacodyl and hyoscine

Study design

Constipated subjects from part 1 were invited to take part in a randomised double-blind, double-dummy, cross-over study comparing bowel habit and pain response to a 10-day treatment with either a stimulant laxative, bisacodyl (10 mg daily) or a muscle relaxant, hyoscine butylbromide (20 mg three times per day). Active drug and placebo were provided as identical-appearing overencapsulated capsules, one taken three times daily and one once a day.

Concealed allocation was performed using a numbered container with the sequence bisacodyl versus hyoscine being randomly allocated by Nottingham hospital pharmacy who kept the code, which was not released until data lock. Participants completed a daily diary documenting the number of bowel movements and for each bowel movement, the BSFS and feeling of completeness of evacuation. Each day, they also recorded a pain score (in answer to the question what their 'worst' pain was in that 24-hour period, scored from 1 to 5) and completed a modified mPAC-SYM questionnaire (online supplemental E1.1) before and after the treatment period. Rescue medication (prucalopride, senna or sodium picosulphate based on what they had used before) was allowed if they had no bowel movement for 3 days. Dose reduction was permitted for excessive side effects (see online supplemental E.1). Data were collected on paper CRFs and diaries and collated with both participants and investigators blinded to active ingredient. Unblinding was performed only after completion of data collection and data lock.

Primary endpoint

Difference in average worst daily pain between bisacodyl and hyoscine intervention periods.

Secondary endpoints

The number of complete spontaneous bowel movements (CSBM), mPAC-SYM score and number of days with either hard (BSFS 1 or 2) or no stool.

Exploratory endpoints

We also determined whether any objective MRI or manometry measures could predict clinical response as defined by other authors. A bisacodyl 'responder' was defined as a patient who had an increase in 1 CSBM per week[23] while hyoscine 'responder' had a reduction in (m) PAC-SYM by the previously defined minimal clinically important difference of >0.6 points (ie, reduction in pain).[24]

Statistical analysis

Basic characteristics of the study population, as well as the MRI, HRCM, clinical trial and symptom data, were summarised using frequencies, percentages, means and SD or medians with IQRs as appropriate to the distribution.

Differences between participant groups for continuous variables were assessed using either analysis of variance (ANOVA) or mixed effect models with Tukey's multiple comparisons test for post hoc comparisons between groups and χ^2 tests for categorical data. Comparisons between FC and IBS-C were done using an unpaired t-test or Mann-Whitney U test depending on the distribution of the data. The difference in pain scores between baseline and trial period was analysed separately for each drug (using a Student's t-test if normally distributed or Mann- Whitney U test if not) comparing those with baseline volume >90th centile with those with normal volumes.

Correlations were assessed using the Pearson correlation coefficient for normally distributed data or the Spearman correlation coefficient for non-normally distributed data. Statistical tests were performed using GraphPad Prism V.9 for Windows (GraphPad Software, La Jolla, California, USA).

Sample size considerations

Part 1

Primary objective: We aimed for a level of agreement between MRI and manometry >70% which we could estimate to be within ±10% (95% CI) using 80 patients, assuming a proportion of 0.5 in each group (hypomotile vs normal/hypermotile).

Part 2

There are no previous data on which to base a power calculation so we invited all patients from study 1.

Table 1 Demographics, baseline stool diary and psychological depression and anxiety scores

	HV (n=44)	IBS-C (n=43)	FC (n=38)	P value
Age	33±12	40±13*	46±14*	<0.001
Gender (female %)	39 (89)	41 (95)	36 (95)	0.41
BMI	25±5	26±5	25±5	0.98
Screening stool diary (14-day diary)				
Total BM attempts	17±7	16±13	18±18	0.74
Number of SBM	17±6	14±13	10±11*	0.18
Number of CSBM	15±7	1±2*	2±3*	<0.01
Average BSFS*	4±1	2±1*	2±4*	<0.01
mPAC-SYM	0.2±0.3	2.2±0.8*	1.2±0.9*†	<0.001
Psychological distress (median (IQR))				
HAD Score anxiety	5 (2–8)	7 (4.5–11)*	5 (2.3–7)	0.04
HAD Score depression	1 (0–2.3)	4 (1.5–8)**	2 (1–7)	0.0001

Comparison between groups performed using ANOVA, followed by Tukey's multiple comparisons, apart from sex and % passing balloon expulsion test, which were analysed using the χ^2 test. * p<0.05 vs HVs, ** p<0.001 vs HV, † p<0.05 vs IBS
Data presented as mean±SD except HADs.
*2 week average of daily average stool form, excluding any post rescue therapy.
ANOVA, analysis of variance; BMI, body mass index; BSFS, Bristol Stool Form Scale; CSBM, complete spontaneous bowel movements; HADs, Hospital Anxiety and Depression Scale; HVs, healthy volunteers; IBS, irritable bowel syndrome; mPAC-SYM, modified Patient Assessment of Constipation-Symptoms.

RESULTS PART 1

Clinical characteristics

We enrolled 44 HV, 43 participants with IBS-C and 38 with FC of whom 121 completed part 1 and 72 participated in part 2 (Consort diagram in online supplemental figure S1). Participants were predominantly middle-aged females (116/125) though HVs were younger than the patients (table 1). Participants in all three groups reported similar numbers of attempted bowel movements in the 14-day diary, however, the FC group had fewer spontaneous bowel movements (SBMs) (table 1, online supplemental B3 table S1). Both patient groups with constipation had fewer CSBM and harder stools on the BSFS than HVs. Modified PAC-SYM (mPAC-SYM) scores (see online supplemental E1.1) were significantly higher (indicating worse symptoms of pain, discomfort and cramps) in the IBS-C group compared with FC, both being considerably higher than HVs (table 1). Both patient groups had significantly higher depression scores than HVs with IBS-C patients also having significantly higher anxiety scores. A rectal balloon was expelled in the defined time by 89% of HV, 84% IBS-C and 75% FC (p=0.27) (online supplemental B1).

MRI outcomes

Baseline colon volumes

FC and IBS-C patients had significantly larger mean total baseline CVs than HVs, this difference (approximately 20% increase) being mainly due to increased transverse colon (TC) volumes (TCVs) in both IBS-C and FC with a significant increase in AC volume in IBS-C while DC and rectosigmoid colon (RS) volumes did not differ from HVs (table 2). There were no correlations

Table 2 Total and segmental volumes median (IQR)

Group	n	Ascending colon	Transverse colon	Descending colon	Rectosigmoid	Total CV
HVs	41	205 (143–265)	198 (139–253)	119 (67–163)	106 (64–158)	645 (467–780)
IBS-C	43	261 (203–298)*	292 (187–377)**	119 (73–168)	109 (78–145)	776 (595–1033)**
FC	36	227 (195–292)	313 (198–420)**	107 (76–168)	121 (84–174)	802 (633–951)**

Two-way repeated measures ANOVA showed significant effect of group F=736, p<0.001 and segment F=7.2, p<0.001 and interaction F=6.3, p<0.001.
*p<0.05 vs HV. **p<0.01 vs HV.
ANOVA, analysis of variance; CV, colonic volume; FC, functional constipation; HVs, healthy volunteers; IBS-C, irritable bowel syndrome with constipation.

between baseline CVs and MMI, tagging index or WGTT (online supplemental F2 table S2).

25 patients with constipation had total CVs exceeding the 90th centile of HVs (923 mL). This increase in CVs was seen equally in all four segments (online supplemental F3 table S3). These patients were equally distributed between IBS-C and FC with no significant difference in age or HADS. However, such patients did tend to have reduced tagging index, harder stools (p=0.06) and slower transit but this was not significant, p=0.2 (table 3).

Effect of macrogol challenge on MRI outcomes, time to first bowel movement and pain

MMI Motility Index

Our primary endpoint, the MMI rose significantly from baseline in all three groups. MMI at T60 median (IQR) for HV was 1732 (1060–3535), 1785 (897–3125) for IBS-C and 2004 (713–3742) for FC. As figure 1A shows there was wide individual variability with no differences between groups. This was also true for the DC (online supplemental figure S6).

Tagging index

Our other measure of motility, the 'tagging index' reflecting movement of colonic chyme was also significantly increased after macrogol in all three groups at 60 and 120 min. However, this index was lower in IBS-C, significantly so at 120 min compared with HVs which did not differ from FC patients (figure 1B).

Colonic volumes

TCVs rose significantly after macrogol (T60 and T120) for all three groups with significant difference between HV and patients (FC and IBS-C) who showed substantial overlap (figure 1C).

Pain after macrogol challenge

Overall, both patient groups reported more pain than the HVs following ingestion of macrogol with greater pain at 60 min for IBS-C compared with FC (figure 2 and online supplemental F4 table S4). The reporting of pain was associated with a significantly higher peak volume 1277 (345) vs 1126 (410) ml but there was a wide scatter, p=0.04, (unpaired t-test) (online supplemental figure S7).

Time to bowel movement after macrogol

This was used to assess overall colonic responsiveness. While most (30/42, 70%) HV had a bowel movement <150 min following macrogol this was only true in 19/40 (47.5%) IBS-C and 14/32 (43.8%) FC (online supplemental figure S8), χ^2 test p=0.028). Patients with abnormal enlarged colons had significantly delayed time to evacuate after macrogol, median (IQR) 180 (118–236), n=22 (3 failed to record) compared with the remaining patients 134 (89–180), n=50 p=0.02 Mann-Whitney U test. When we separately analysed the patients by Rome classification subgroups, two-way ANOVA showed no difference between IBS-C and FC (p=0.88) but a significant effect of enlarged colon, p=0.02.

Whole gut transit

WAPS showed substantial variability but was significantly higher in patients compared with HVs, with a median (IQR) score of 2.2 (0.6–3.4) vs 1 (0–2.3), respectively; p=0.03 (online supplemental figure S9). These scores are equivalent to a WGTT of 69 (21–104) vs 34 (4–69) hours if using the radio-opaque marker technique.[13] However, there was no significant difference between IBS-C and FC patient groups (WAPS median 2.3 (IQR 0.6–4) and 1.7 (0.2–3.1), respectively, p=0.6).

High-resolution colonic manometry

HRCM data were obtained from 97 participants (36 HVs, 36 IBS-C and 25 FC). The CMP was observed in the sigmoid colon in the majority of participants both before and after the meal: 35/36 HVs, 36/36 IBS-C and 24/25 FC. The percentage of time occupied by the CMP in the sigmoid colon following the meal showed wide variability but was significantly lower in the IBS-C but not FC group compared with HVs (figure 3A).

HAPCs were identified in only a minority of participants both before and after the test meal: HV 6 and 8/36; IBS-C 4 and 6/36;

Table 3 Comparison of patients with enlarged colon versus normal-sized colon

	n	IBS-C/FC	TCV mL	Tagging index	Transit hours	CSBM/ wk	BSFS
Enlarged colon	25	15/ 10	1094 (996–1244)	20.9 (6.0)	84 (48)	0.0 (0–1)	1.1 (1.2)
Normal-sized colon	54	28/26	707 (547–793)	25.1 (9.7)	66 (61)	1 (0–3)	2.1 (1.3)
P value		0.62*	<0.0001†	0.06†	0.2†	0.2‡	‡0.06

*Fisher's exact test.
†T-test.
‡Mann-Whitney U test.
BSFS, Bristol Stool Form Score; CSBM, complete spontaneous bowel movements; FC, functional constipation; IBS-C, irritable bowel syndrome with constipation; TCV, transverse colon volume.

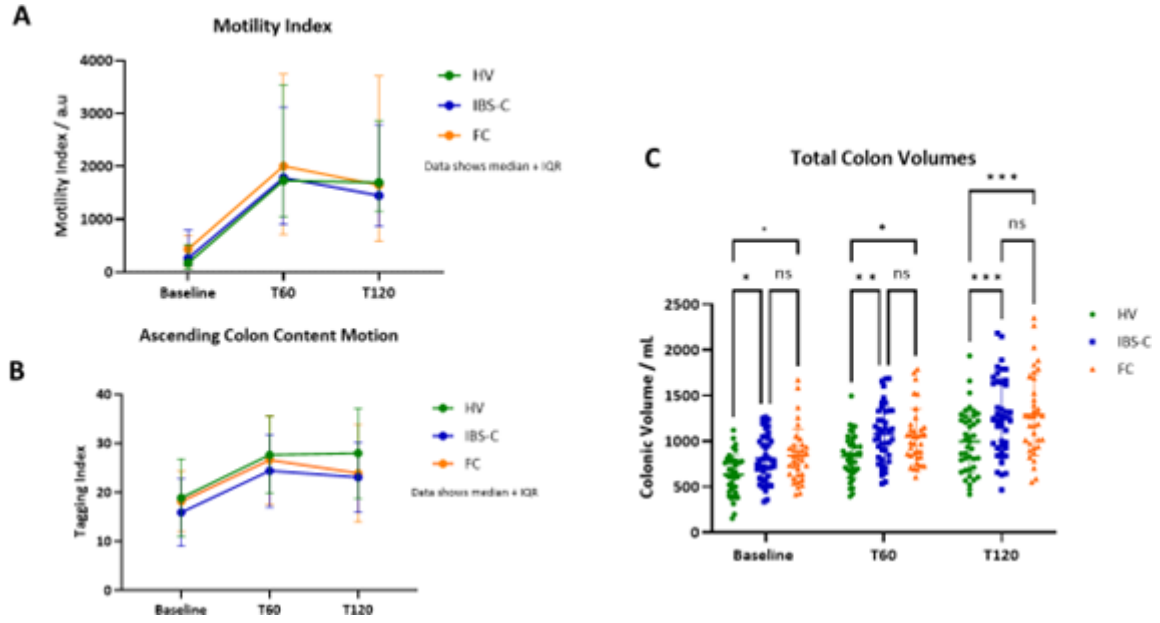

Figure 1 MRI Motility Index (MMI), ascending Colon Content Movement and Total Colonic Volumes. (A) Ascending colon MMI This rose significantly over time (p<0.001) ANOVA showed effect of time p<0.001, effect of group NS, p=0.97. (B) Ascending colon content motion was assessed by Tagging index at baseline and 60 (T60) and 120 min (T120) after macrogol ingestion. Tagging index showed a significant increase over time, which was less than HV in IBS-C (*p=0.02) but not in FC (p=0.08) at 120 min (two-way ANOVA, Tukey's MC.) (C) Total colonic volumes. These rose over time for all groups. Both FC and IBS-C total colonic volumes were greater than HVs but not different from each other two-way ANOVA, Time effect p<0.0001, group effect p=0.0019, post hoc comparisons using Tukey's multiple comparisons *p<0.05, **p<0.01, ***p<0.001 vs HV. ANOVA, analysis of variance; FC, functional constipation; HV, healthy volunteer; IBS-C, irritable bowel syndrome with constipation; NS, not significant.

FC 1 and 5/25, respectively. Due to the low number of subjects with HAPCs, statistical comparisons were not performed.

The meal induced an increase in the power of pressure waves (PW) in all three groups, an effect which did not differ significantly between the groups (online supplemental figure S10). However, when looking at the coordination of PW into propagating PW (PPW)s, significant differences emerged. During both the baseline and postprandial period, the power of PPWs was reduced in the IBS-C group compared with both HV and FC (figure 3B, online supplemental figure S11).

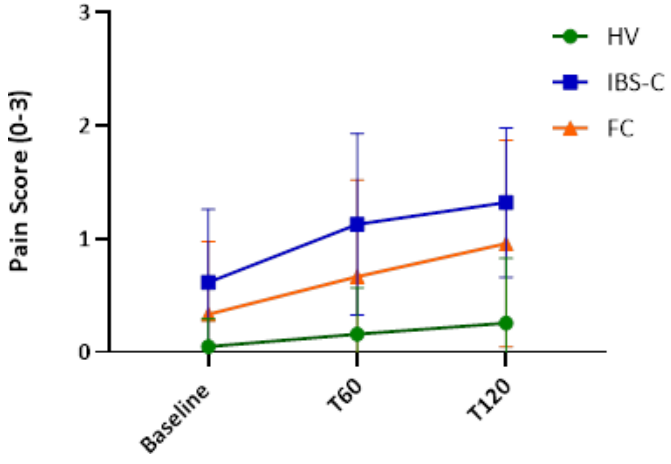

Figure 2 Pain on MRI study day pain score (0–3) is shown at baseline, 60 (T60) and 120 (T120) minutes after macrogol^{R} ingestion. IBS-C and FC had significantly more pain than HVs, p<0.05 at all 3 time points and at T60 IBS-C>FC, p<0.05. Mixed effect model (Restricted maximum likelihood) with Tukey's MC. FC, functional constipation; HVs, healthy volunteers; IBS-C, irritable bowel syndrome with constipation.

Impact of enlarged colon volume on manometric features

HRCM showed striking differences between those with enlarged colons vs those without. As figure 4 shows patients with an enlarged colon at baseline failed to show the normal increase in PW centred around three cps after a meal seen with the remaining subjects.

Examining the PW in more detail using the 2D analysis which analyses the PPWs, both retrograde and antegrade, similarly shows that the enlarged colons fail to show a meal-related increase on both retrograde and antegrade propagated contractions (figure 5).

Manometry versus MRI

There were no significant correlations between the MRI measures of CVs, AC MMI, tagging index or WGTT and the percentage time occupied by CMPs (online supplemental G3 table S5). Classifying participants as hypomotile by MRI (<10th centile of MMI) or manometry (<10th centile of CMPs) showed little agreement and only one subject was hypomotile by both criteria (online supplemental G4 table S6).

RESULTS PART 2: RANDOMISED, PLACEBO-CONTROLLED TRIAL COMPARING BISACODYL AND HYOSCINE

Clinical characteristics

Two patients only completed one arm of the cross-over (one missed bisacodyl and the other hyoscine) leaving 70 sets of paired results. Demographics and bowel habits are shown in online supplemental H1 table S7 and were balanced in both treatment sequences (online supplemental H2 table S8).

RESULTS

Overall, hyoscine was better tolerated than bisacodyl without difference between the two groups. Only 1 FC and 1 IBS-C

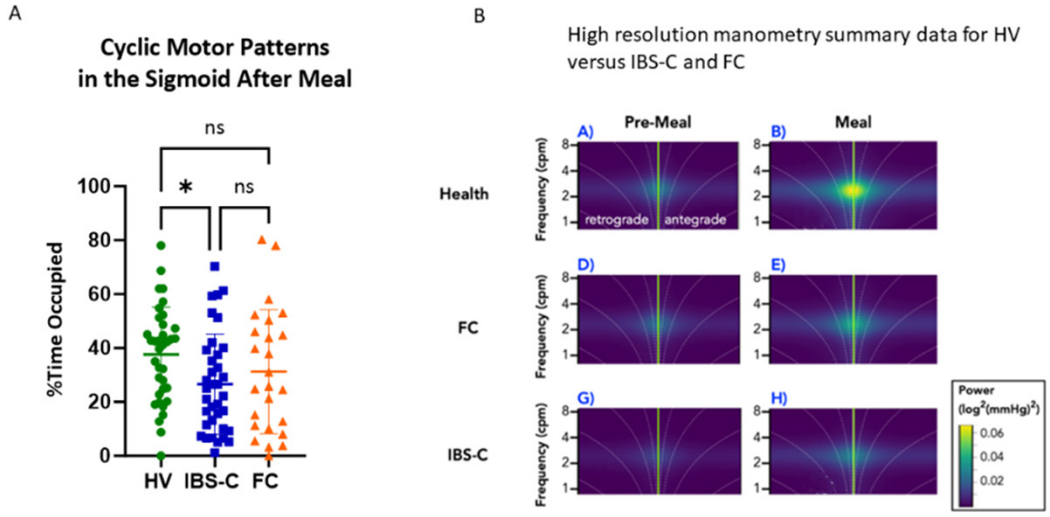

Figure 3 High-resolution colonic manometry before and after meal in HV, IBS-C and FC. (A) Cyclic motor patterns (CMPs) in the sigmoid colon after meal. The percentage of time occupied by the CMPs in the sigmoid colon 1 hour after the meal was significantly lower in IBS-C (27 (SD 19)%) but not FC group (38 (SD 18)%) compared with HVs (38 (18)), * = p=0.049, ANOVA with Tukey's MCs. (B) High-resolution manometry summary data for propagated PWs in HV versus IBS-C and FC. This shows premeal and postmeal frequency distribution on the vertical axis and phase on the horizontal axis. Points to the right of 0 indicate antegrade propagated waves while those to the left indicate retrograde. Higher power is indicated by yellow showing IBS patients had significantly less power than HV or FC, both premeal and postmeal (for full analysis see online supplemental D2 and G2). ANOVA, analysis of variance; FC, functional constipation; HV, healthy volunteer; IBS-C, irritable bowel syndrome with constipation; PW, pressure wave.

taking hyoscine reduced dose due to side effects and no patients stopped early while 26 patients required dose adjustment with bisacodyl (10 FC, 16 IBS-C) with 5 stopping early (2 FC, 3 IBS-C).

The primary endpoint, namely the difference in average worst daily pain scores on bisacodyl versus hyoscine, had a median value (range) of 0.3 (−0.2, 0.8) in IBS-C and 0.7 (−1.2, 1.4) in FC, a difference which was not significant, p=0.2. The correlations between the difference in average worst daily pain scores on bisacodyl and hyoscine and AC MMI and tagging index at 120 min were not statistically significant (Pearson r=−0.16 and 0.13, p=0.22 and p=0.32 for MMI and tagging index, respectively).

Bisacodyl was more effective in both IBS-C and FC in increasing the median number of CSBMs compared with hyoscine. Stools were significantly softer on bisacodyl and the number of days with hard or no stool was significantly less in both groups (table 4). Only 8 participants (5 FC, 3 IBS-C) on bisacodyl required rescue therapy (prucalopride, senna or picosulphate according to patient preference) vs 18 on hyoscine (9 FC, 9 IBS-C). However, both average worse daily pain and mPAC-SYM scores were higher for all patients when taking the bisacodyl (table 4) and for both FC and IBS-C (online supplemental H3table S9).

Considering IBS-C and FC separately, both groups responded similarly to bisacodyl with more CSBM and softer stools compared with both baseline and hyoscine which in contrast produced no significant changes in any of our endpoints (online supplemental table S9). However IBS-C participants reported significantly higher average worse pain compared with FC on bisacodyl, values being 2.7 (2.1–3.3) vs 2 (1.6–2.5) but pain on hyoscine was not significantly different being (1.7 (1.4–2.7) vs 1.5 (1–2), for IBS-C and FC, respectively (mixed-effects ANOVA, effect of treatment F=38.9, p<0.0001, effect of group F=9.4, p=0.003, interaction term not significant).

Impact of MRI and manometry outcomes on response to treatment

Non-responders to bisacodyl (those that failed to increase CSBM by >1) did tend to have larger baseline volumes (893±286, n=33 vs 774±251, n=36, p<0.07). Thus only 33% of patients with enlarged colons were bisacodyl responders v 59% of those with normal colon volume but again this just failed to reach significance, Fisher's exact test p=0.09.

Impact of enlarged colon on response to treatment

Those with an enlarged colon had significantly less increase in pain as assessed by mPAC-SYM on bisacodyl. They tended to have fewer CSBMs but this was not significant (table 5). There was no difference in response to hyoscine (online supplemental H4 table S10).

DISCUSSION

MRI provides a novel approach to assessing colonic function, the utility of which this study attempted to determine. Despite disproving some of our original hypotheses we were able to show that constipation is associated with an enlarged colon and that those with colon size exceeding the 90th centile of HVs (33% of our constipated cohort) did show a delay in defaecation after macrogol administration and significantly impaired motor response to feeding. They also had significantly less pain and a tendency to less CSBMs with bisacodyl. The significance of an enlarged colon complements studies in constipated paediatric patients showing sigmoid dilatation in a proportion of sufferers in whom underlying organic pathology has been ruled out,[25] and

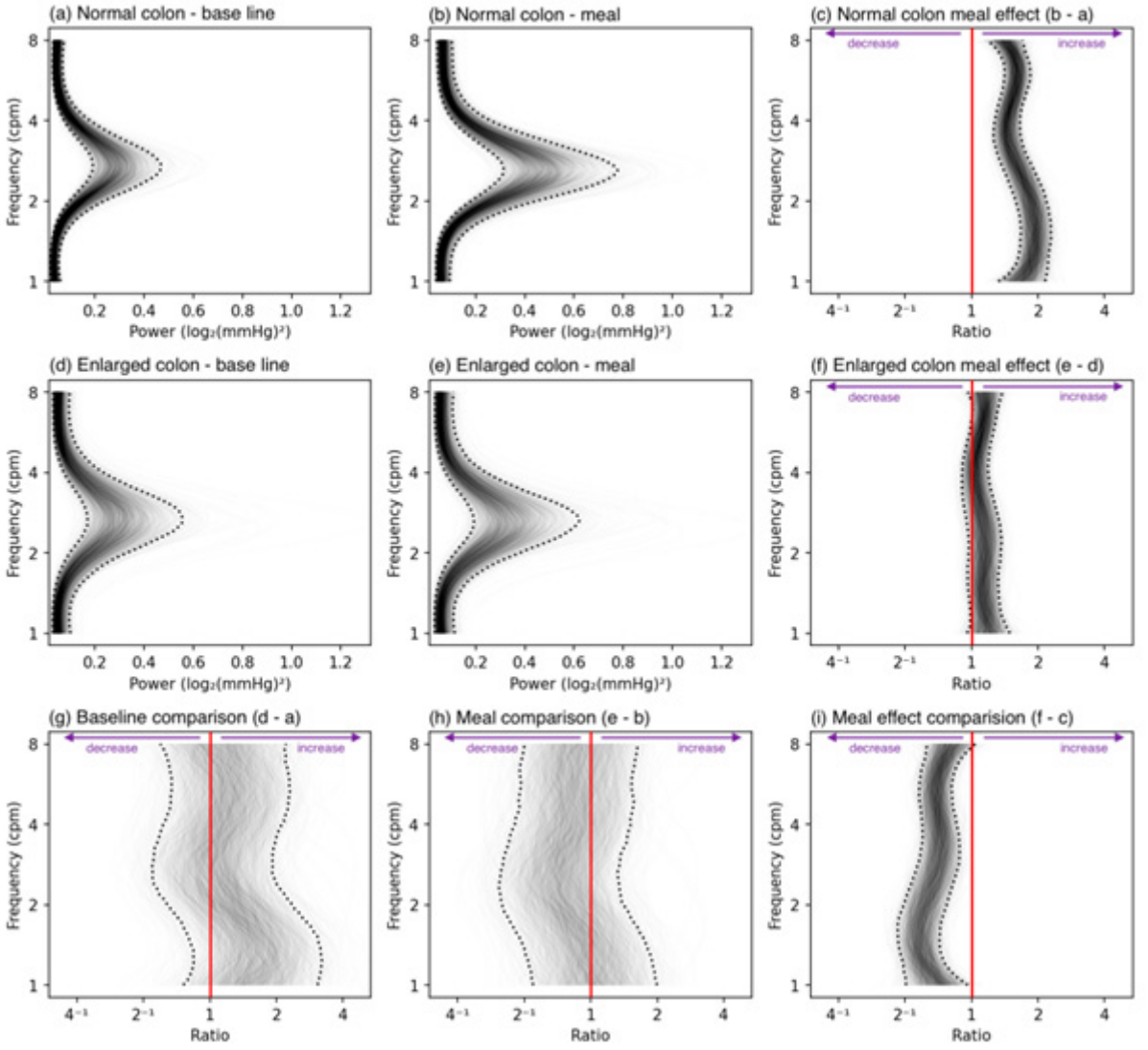

Figure 4 One-dimensional (1D) analysis of pressure waves (PW) at frequencies between 1 and 8 cycles per minute (cpm) in the sigmoid colon. Patients with a normal volume colon (top row; a–c) and patients with an enlarged volume colon (middle row; d–f) left column (a, d, g) shows baseline and middle column (b, e, h) the meal periods. In each image, the frequency of PW is shown on the y-axis. In (a, b, d, e) power is shown on the x-axis. The power refers to the prevalence of the PW at any of the calculated frequencies. 2000 overlapping grey lines in each panel represent posterior samples, and the dotted black lines form envelopes of 95% credible intervals. (g, h) The power ratio across the frequency range, between the enlarged and normal colons. When the entire envelope lies to one side of the vertical red line (which represents a ratio of 1), this shows a significant deviation (to the left a decrease in PWs in the enlarge compared with normal colons; to the right of the red line indicates an increase in PWs). During baseline and a meal period the red lines in (g, h) lie entirely within the grey envelope indicating no significant difference. (c, f) The ratio of the power of baseline activity to meal activity. In the normal colons (c), the grey envelope lies to the right of the red line indicating that the meal induced a significant increase in power in frequencies between 1 and 8 cpm. In patients with an enlarged colon (f), no meal response is seen (red line lies within the grey envelope). (i) The meal effect between the two groups, as the grey envelope lies to the left of the red line indicates that patients with an enlarged colon have a significantly reduced meal response compared with those with a normal diameter colon.

also extends a growing body of literature demonstrating that rectal hyposensitivity (present in 25% of constipated adults)[26] is secondary to an enlarged or hypercompliant rectum in the majority.[27]

The macrogol challenge, which approximately doubles CVs, is designed to be a substantial reproducible stimulus to proximal colonic motility,[12] something our current study confirms. Although it enables us to non-invasively assess the motility of both the ascending and DC, our study shows that this did not correlate with the response to a meal using high-resolution manometry of the distal colon. However, HRCM is a difficult technique and there are considerable obstacles to using it widely in clinical practice including the availability and expense of the equipment and the patients' dislike of invasive procedures. Although MRI after macrogol cannot produce the same details as HRCM its convenience and high patient acceptability may lead to it being more widely used in the future.

This large study recruiting from both primary and secondary care in multiple sites found CVs and WGTT were highly variable and did not differ between FC and IBS-C, though both were

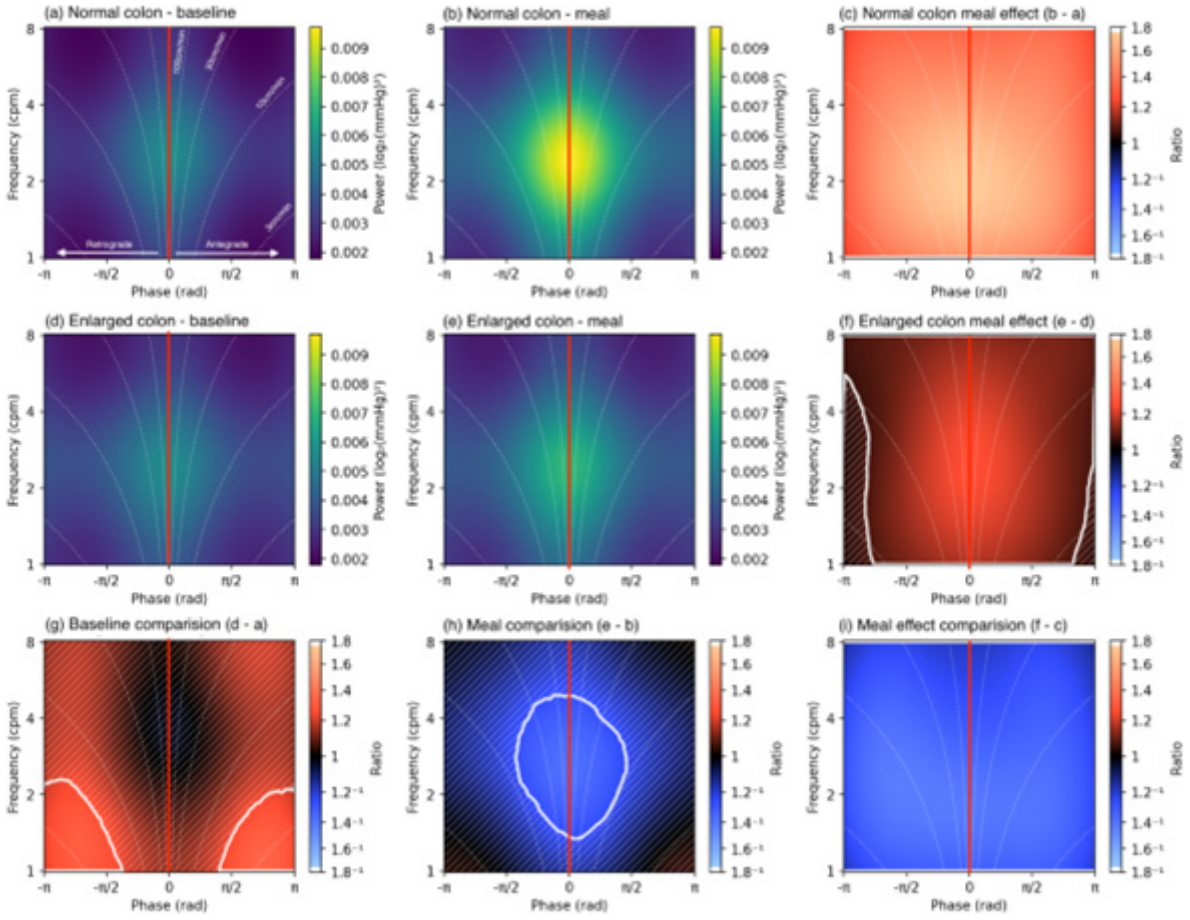

Figure 5 Two-dimensional (2D) analysis of propagating pressure waves (PPW) in the sigmoid colon at frequencies between 1 and 8 cpm. In each panel, the vertical line at 0 on the x-axis indicates synchronous (non-propagating) activity. Retrograde propagation is to the left of the midline and antegrade to the right. The curved dotted lines indicate the speed of propagation, from 3 cm/min to 100 cm/min. (a, b, d, e) The green pixels represent the increasing power of propagated activity. The first column represents baseline data, the second column meal data. Patients with a normal diameter colon are shown in the top row, patients with an enlarged colon in the second row. The bottom row compares PPW power across the frequency range between the normal and enlarged colon during baseline (g) and meal (h) periods. (g) The orange area demarcated by the solid white line indicates a significant increase in antegrade and retrograde PPW at <3 cpm in the enlarged compared with normal volume colons. (h) The blue area demarcated by the solid white line indicates a significant decrease in antegrade and retrograde PPW between 2 and 4 cpm in patients with enlarged compared with normal volume.

significantly greater than HVs. Thus, we did not confirm our earlier smaller study suggesting that IBS-C had smaller colons possibly because this previous study recruited extremes from tertiary care less representative of general clinical practice.[11] IBS-C did, however, have a lower tagging index after macrogol suggesting their motor response was less efficient at moving colonic contents. However, the main difference was pain (IBS-C>FC) both at baseline and 60 min after macrogol as well as during bisacodyl and hyoscine treatment.

HRCM is more demanding for both patient and investigator and less widely available but can provide very detailed information on colonic contractile activity. This is one of the largest

Table 4 Clinical endpoints of RCT of bisacodyl versus hyoscine (all patients: n=70 paired) median (IQR)

	Bisacodyl	Hyoscine	P value
Pain			
Average worst daily pain (range 1–5)	2.3 (1.8–3.1)	1.6 (1.3 to 2.3)	<0.001
mPAC-SYM (abdominal pain, discomfort and cramps) after intervention (range 0–4)	2 (1.3–3)	1 (0.3 to 2)	<0.001
Stool frequency and consistency (over 10-day period)			
CSBM	4.0 (0.0–9.0)	0.0 (0.0 to 2.0)	<0.001
Average BSFS over the 10 days (excluding BMs following rescue, BSFS 1–7)	5.3 (4.7–6.0)	2.4 (1.3 to 3.8)	<0.001
Days with hard (BSFS one or 2) or no stool, or needing rescue	2 (1–5)	5.6 (3–7)	<0.001

BSFS, Bristol Stool Form Scale; CSBM, complete spontaneous bowel movements; mPAC-SYM, modified Patient Assessment of Constipation-Symptoms; RCT, randomised controlled trial.

Table 5 Effect of enlarged colon on response to bisacodyl

	n	Basal mPAC-SYM	Change in mPAC-SYM	Weekly CSBM	Change in BM
Enlarged colon	22	1.2 (0.8)	0.6 (1.0)	1.4 (0.0–3.7)	0.5 (0.0–2.8)
Normal-sized colon	49	1.1 (0.8)	1.0 (0.9)	2.3 (0.0–5.3)	2.3 (0.0–5.3)
P for difference		0.6*	0.05*	0.06‡	0.12‡

CSBM, complete spontaneous bowel movement; mPAC-SYM, modified Patient Assessment of Constipation-Symptoms.

such studies and our data showed that while a meal resulted in a significant increase in PW in all three groups, the coordination of these PW into PPW was significantly reduced in IBS-C patients, compared with both HV and FC. Uncoordinated contractions particularly during the postprandial period when there is an increase in contractile activity could cause pain in IBS patients, but this requires further study.

We had hypothesised that the difference in pain score on a stimulant (bisacodyl) versus a smooth muscle relaxant (hyoscine) would be greater in those with hypermotility, but in the event, it did not correlate with either MMI, tagging index, WGTT nor HRCM. We did, however, show that although bisacodyl is an effective laxative, it does increase pain in IBS patients more than hyoscine, which in contrast did not alter any of the recorded symptoms and required rescue laxatives for most of our patients.

Limitations of this study include the fact that for both expense and patient comfort reasons using MRI one can only record motility from the colon for short periods using the macrogol challenge. However, from HRCM studies, we know that colonic motility is erratic and needs prolonged recording to get reliable results. Furthermore, the mechanism of response to the distension induced by macrogol is quite different from the more physiological response following the meal we used in the HRCM study, which probably accounts for the lack of correlation between the two measures. Another concern relates to the image registration of successive cine images required to overcome artefact generated by the movement of the diaphragm and abdominal contents in a free-breathing subject. While this works well with relatively shallow breathing, large deviations of the diaphragm can cause changes to the colon wall that are not associated with wall contractions, leading to an artificial increase in the MMI. The tagging technique does overcome this limitation as it is a breath-hold scan and may be a more reliable measure though it does assess the movement of chyme rather than wall movement per se and also over a much shorter duration. This insight should be further investigated in large clinical cohorts to test its utility and ability to predict response to treatments.

Previous assessments of CV in vivo have either used the volume required to fill a colon during barium enema or used ionising radiation (X-ray/CT scanning). MRI provides a much more acceptable way of assessing volume in the undisturbed colon. The ability to assess specific regional volumes may prove an advantage when dealing with the rare but difficult to manage patients with severe constipation and underlying megarectum and/or megacolon since it may guide the choice of surgical or medical therapies.[28] The underlying pathophysiology of an enlarged colon remains to be determined but this can be assessed using normal MRI scanners available in many hospitals. Further investigation of the causes of constipation including the association between an enlarged colon and manometry will require larger numbers but could easily include CV assessed using MRI. The development of MRI-compatible fibreoptic manometry tubes[29] will allow the simultaneous imaging and pressure measurement of the range of manometry patterns including HAPCs. While waiting for spontaneous or meal-induced HAPCS is not feasible with MRI owing to their low frequency (approximately 4–5 per day[30]), expense and patient discomfort related to prolonged scanning, an agent like bisacodyl, which produces a rapid response,[31] will make such studies possible. These will allow a non-invasive assessment of the impact of HAPCs on colonic tone, motility and contents and also identify MRI patterns characteristic of patients who fail to respond to bisacodyl. Future studies could also include novel prokinetic agents to allow better evaluation of their mode of action.

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Contributors Conception or design: RCS, LM, PG, CLH, AM, SMS, PD, SAT, DA and CC. Data collection: VW-S, SMS, CLH, LM and MC. Data analysis and interpretation: VW-S, RCS, CLH, CC, SMS and PD. Drafting article: VW-S, RCS, SMS, PD and CC. Critical revision: All authors. Final approval: all authors. RS is the guarantor of the integrity of this article.

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Competing interests RCS: Grants/research supports: Sanofi-Aventis Deutschland, Nestle Consultant to Enterobiotix. MC: Consultant: Sanofi, Takeda, Biocodex, Mayoly. SMS: honoraria for teaching for the Laborie Group. SAT: grant support from Takeda. Consultancy fees AstraZeneca, Sanofi-Aventis. Share holder Motilent.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Ethics approval The protocol was approved by a 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 in accordance with the Declaration of Helsinki.

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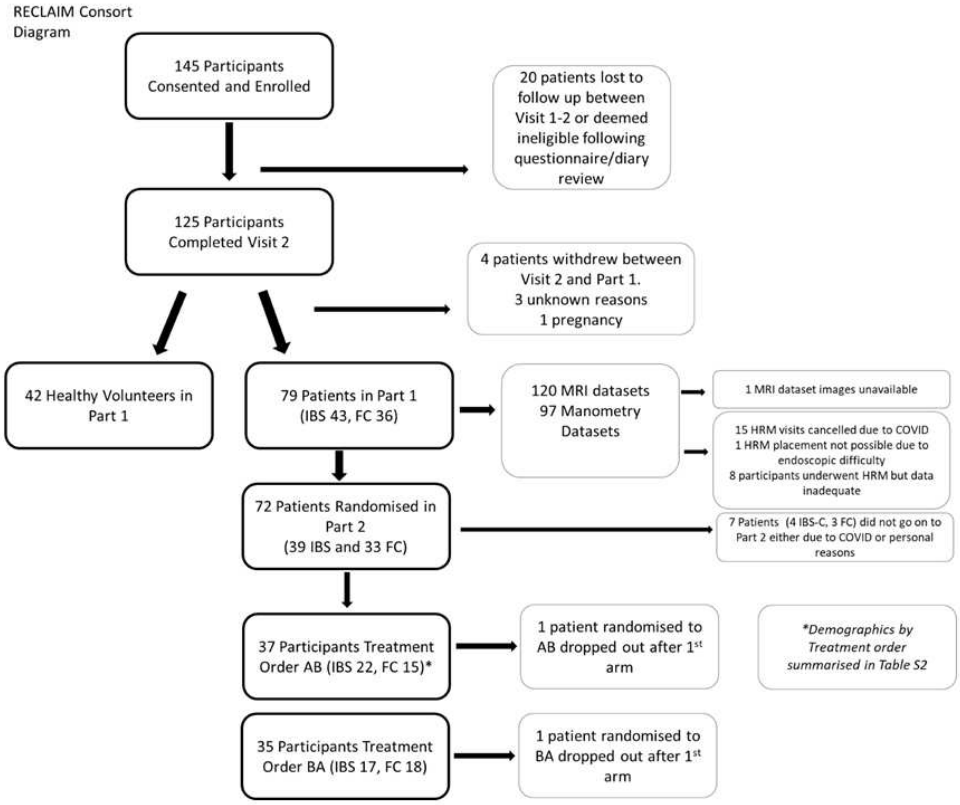
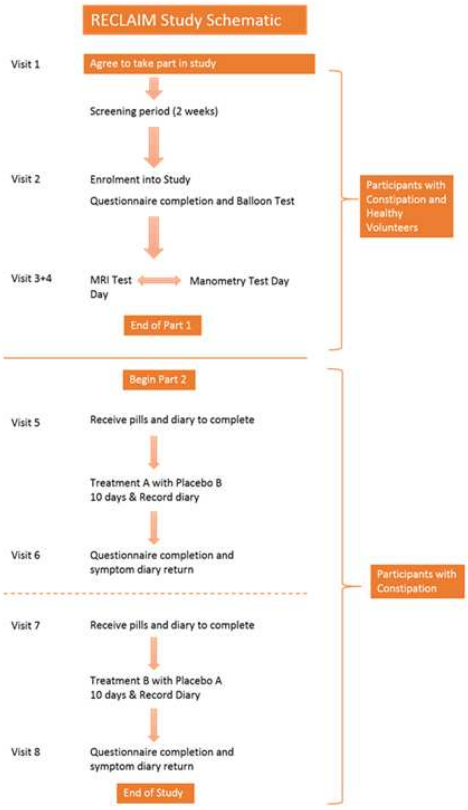
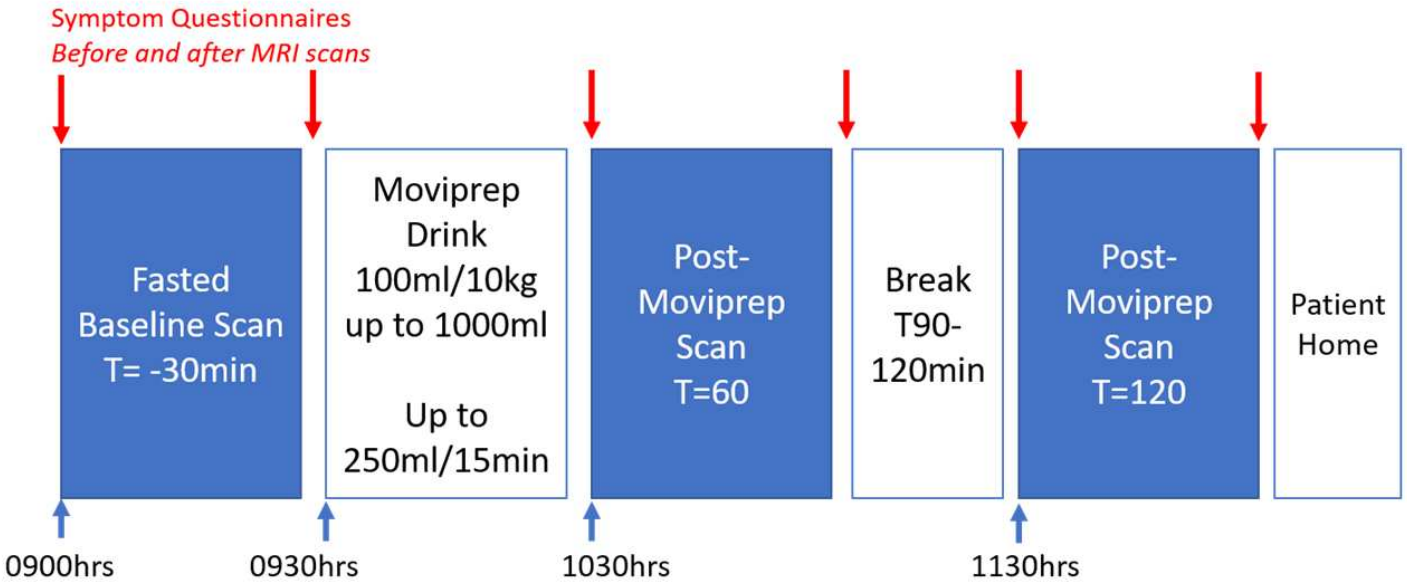
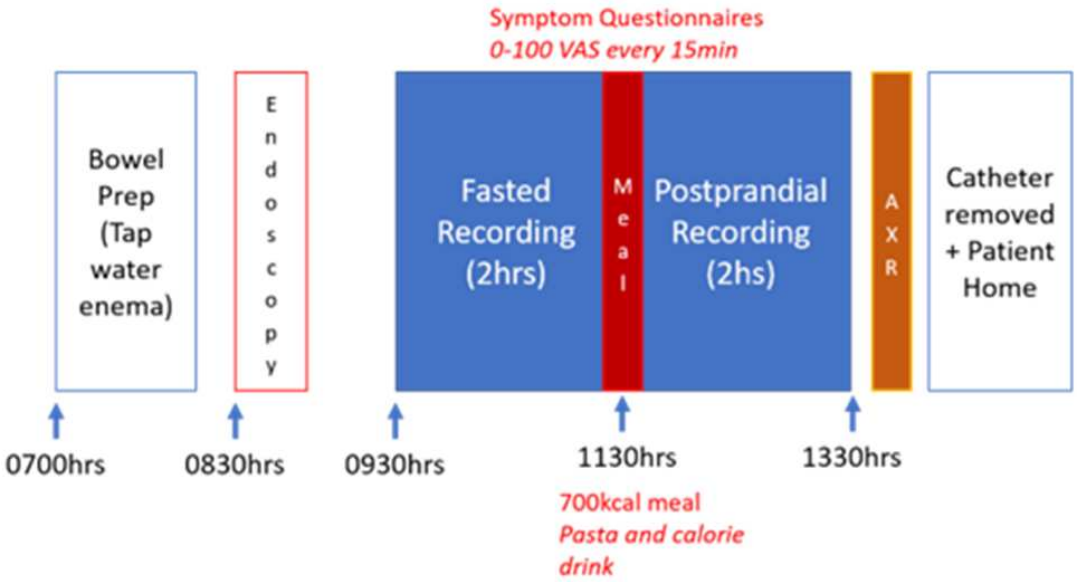
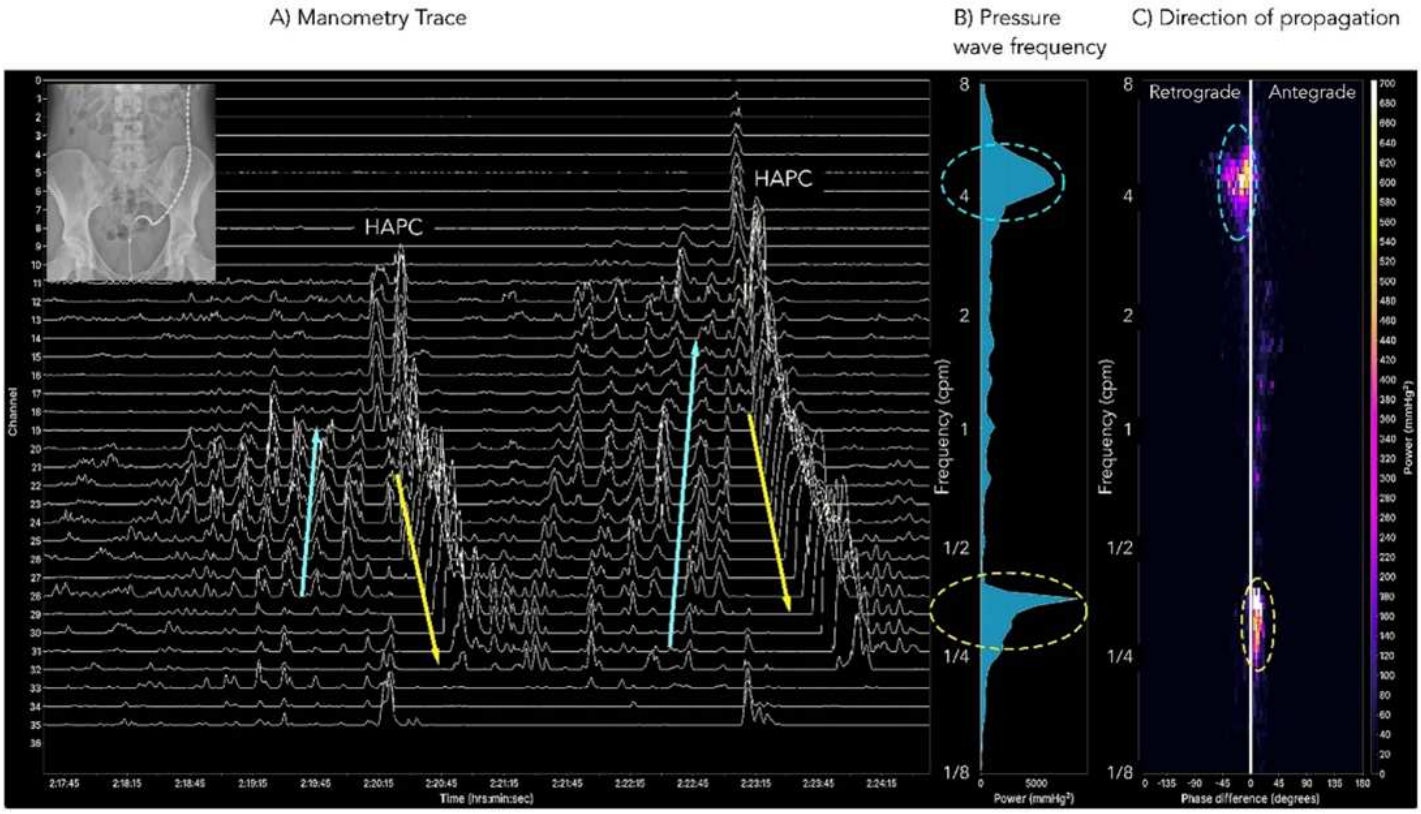
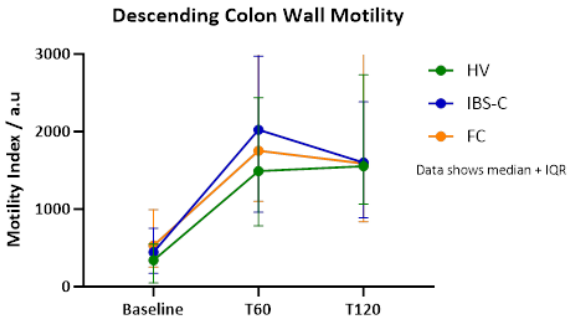

RECLAIM Consort Diagram
145 Participants Consented and Enrolled
20 patients lost to follow up between Visit 1-2 or deemed ineligible following questionnaire/diary review
125 Participants Completed Visit 2
4 patients withdrew between Visit 2 and Part 1.
3 unknown reasons
1 pregnancy
42 Healthy Volunteers in Part 1
79 Patients in Part 1 (IBS 43, FC 36)
120 MRI datasets
97 Manometry Datasets
1 MRI dataset images unavailable
15 HRM visits cancelled due to COVID
1 HRM placement not possible due to endoscopic difficulty
8 participants underwent HRM but data inadequate
72 Patients Randomised in Part 2 (39 IBS and 33 FC)
7 Patients (4 IBS-C, 3 FC) did not go on to Part 2 either due to COVID or personal reasons
37 Participants Treatment Order AB (IBS 22, FC 15)*
1 patient randomised to AB dropped out after 1st arm
Demographics by Treatment order summarised in Table S2
35 Participants Treatment Order BA (IBS 17, FC 18)
1 patient randomised to BA dropped out after 1st arm

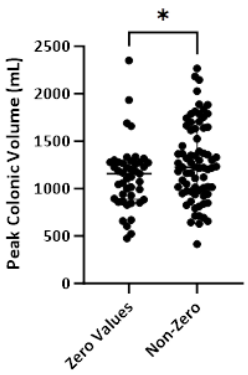

RECLAIM Study Schematic
Visit 1
Agree to take part in study
Screening period (2 weeks)
Visit 2
Enrolment into Study
Questionnaire completion and Balloon Test
Visit 3+4
MRI Test
Manometry Test Day
Day
End of Part 1
Participants with Constipation and Healthy Volunteers
Begin Part 2
Visit 5
Receive pills and diary to complete
Treatment A with Placebo B
10 days & Record diary
Visit 6
Questionnaire completion and symptom diary return
Visit 7
Receive pills and diary to complete
Treatment B with Placebo A
10 days & Record Diary
Visit 8
Questionnaire completion and symptom diary return
End of Study
Participants with Constipation

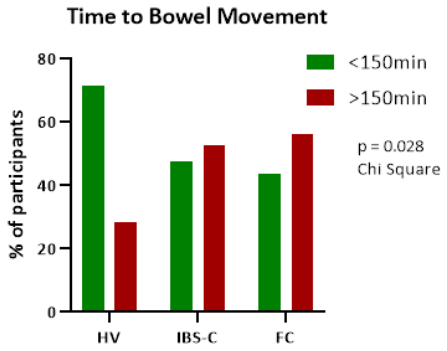

Symptom Questionnaires
Before and after MRI scans
Fasted Baseline Scan T= -30min
Moviprep Drink 100ml/10kg up to 1000ml
Up to 250ml/15min
Post-Moviprep Scan T=60
Break T90-120min
Post-Moviprep Scan T=120
Patient Home
0900hrs
0930hrs
1030hrs
1130hrs


Symptom Questionnaires
0-100 VAS every 15min
Bowel Prep (Tap water enema)
Endoscopy
Fasted Recording (2hrs)
Meal
Postprandial Recording (2hs)
AXR
Catheter removed + Patient Home
0700hrs
0830hrs
0930hrs
1130hrs
1330hrs
700kcal meal
Pasta and calorie drink


A) Manometry Trace
B) Pressure wave frequency
C) Direction of propagation
HAPC
HAPC
Channel
Time (hrs:min:sec)
Frequency (cpm)
Power (mmHg²)
Retrograde
Antegrade
Phase difference (degrees)


Descending Colon Wall Motility
Motility Index / a.u
3000
2000
1000
0
Baseline
T60
T120
HV
IBS-C
FC
Data shows median + IQR


*
2500
2000
1500
1000
500
0
Peak Colonic Volume (mL)
Zero Values
Non-Zero


Time to Bowel Movement
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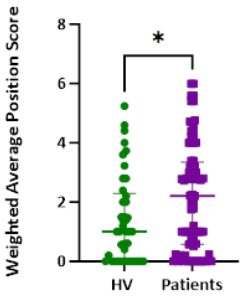
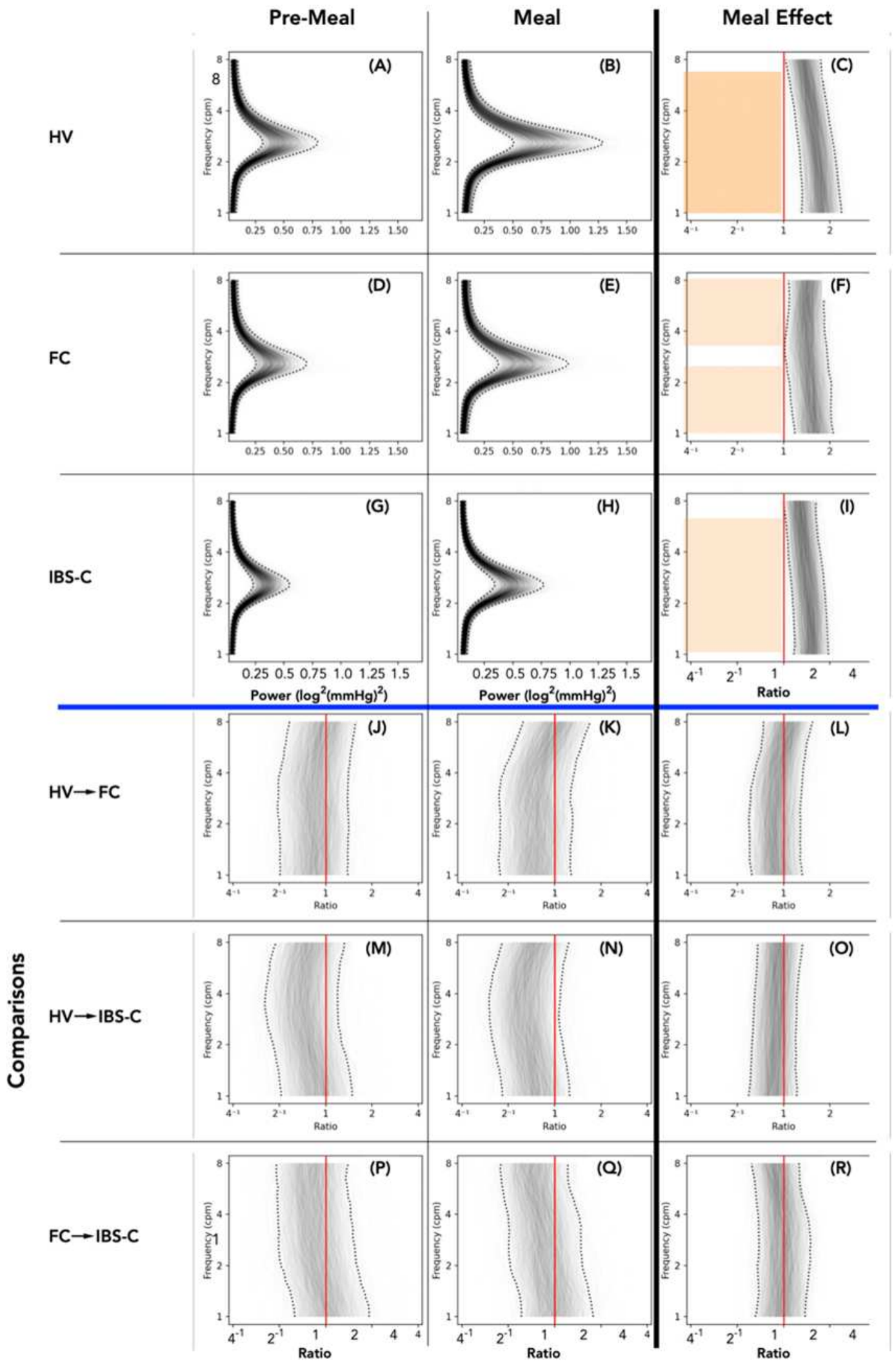
>150min
p = 0.028
Chi Square
% of participants
80
60
40
20
0
HV
IBS-C
FC


Weighted Average Position Score
8
6
4
2
0
*
HV
Patients


Pre-Meal
Meal
Meal Effect
HV
FC
IBS-C
Comparisons
HV→FC
HV→IBS-C
FC→IBS-C
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
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(K)
(L)
(M)
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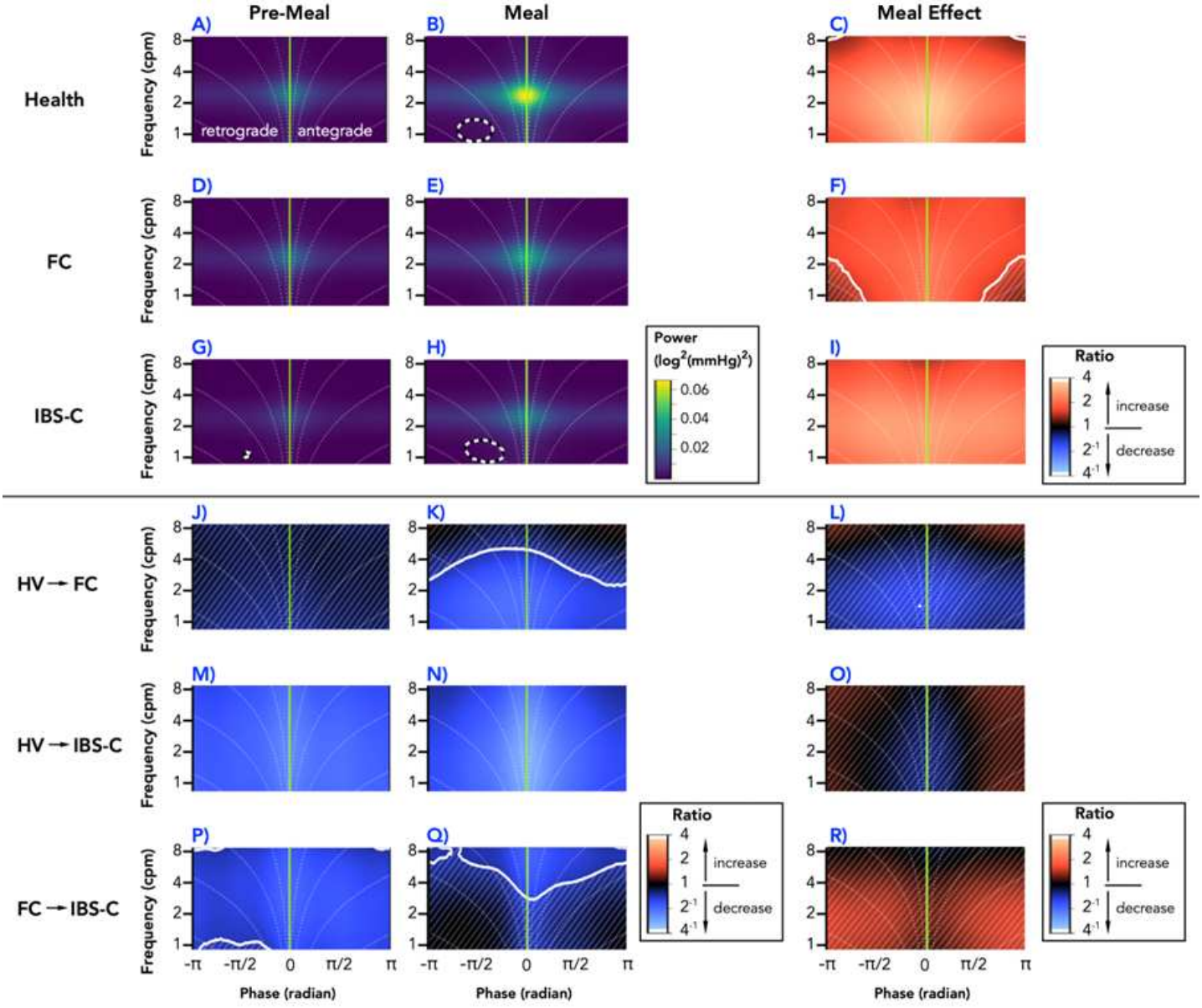
(O)
(P)
(Q)
(R)
Frequency (cpm)
Power (log²(mmHg)²)
Ratio


Pre-Meal
Meal
Meal Effect
Health
FC
IBS-C
HV → FC
HV → IBS-C
FC → IBS-C
A)
B)
C)
D)
E)
F)
G)
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M)
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O)
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Frequency (cpm)
retrograde
antegrade
Power (log²(mmHg)²)
0.06
0.04
0.02
Ratio
increase
decrease
-π
-π/2
0
π/2
π
Phase (radian)

Supplementary data

A Methods

A. Recruitment

Participants with constipation and healthy volunteers were recruited at 2 sites in the UK (Nottingham and London) from both primary and secondary care, and also through social media. After consent we confirmed potential eligibility as either a HV or a patient with constipation using the Cleveland Clinic constipation Score (HV cut off was ≤5). Those whose diary confirmed they had < 3 complete spontaneous bowel movements (CSBM) per week on average were enrolled as patients. HVs were required to have >3 CSBMs per week on average. Constipated patients were classified into either FC or IBS-C based on their responses to the ROME IV criteria questionnaire.

A.1 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

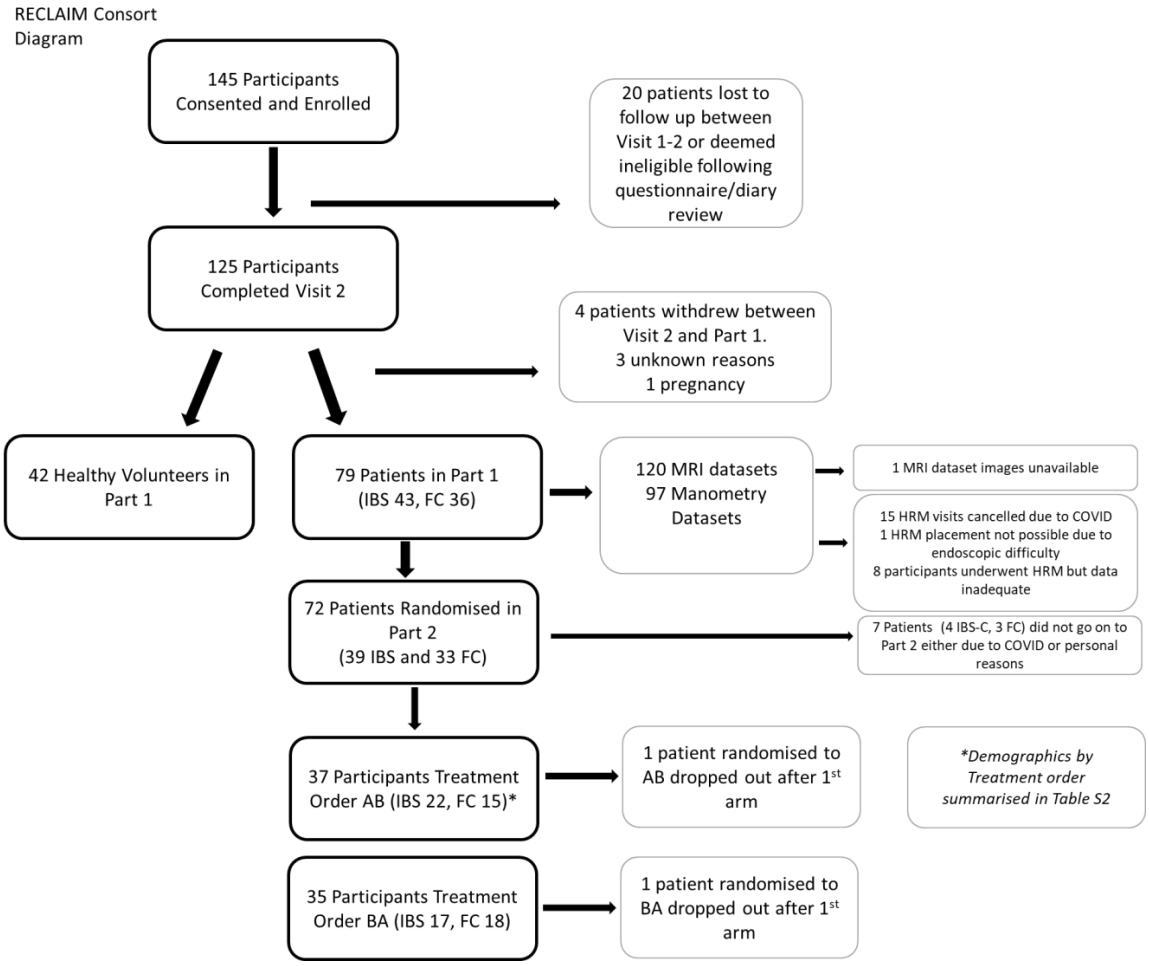
6. Any reported history of gastrointestinal resection (excluding appendicectomy or cholecystectomy)
7. Presence of an intestinal stoma
8. Causes of secondary constipation disorders (e.g., systemic sclerosis / Parkinson's disease)
9. Inability to cease use of medicines that cause constipation or alter 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 Moveprep)
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

A 2 Participant flow

As reported in the Consort Diagram in Figure S1 below, a total of 145 participants were consented and enrolled across both sites, of whom 125 eligible participants completed Visit 2 (HV 44, IBS 43, FC 38). Recruitment started August 1st 2017 and the last patient completed December 10 2020

121 participants then proceeded to complete at least one visit of Part 1 of the study (HV 42, IBS 43, FC 36). 72 patients with constipation (39 IBS and 33 FC) then went on to participate in Part 2.

A.3 Figure S1: Consort Diagram



B. Study Visit Protocols

The study took place over 8 visits. The first visit was to check eligibility and consent before the screening period commenced.

All participants completed a daily study diary documenting whether they had attempted to open their bowels, whether they had passed stool, the number of bowel movements (BMs) in the day, the stool form score for each BM and whether the bowel movement was a complete spontaneous bowel movement (CSBM), defined as "a bowel motion with the feeling of complete evacuation, without using your laxative/rescue therapy". They also recorded if they had taken any rescue medication.

At the second visit the results of the diary were reviewed and, if eligible, the participant completed the HADS and PAC-SYM questionnaire and underwent the balloon expulsion test.

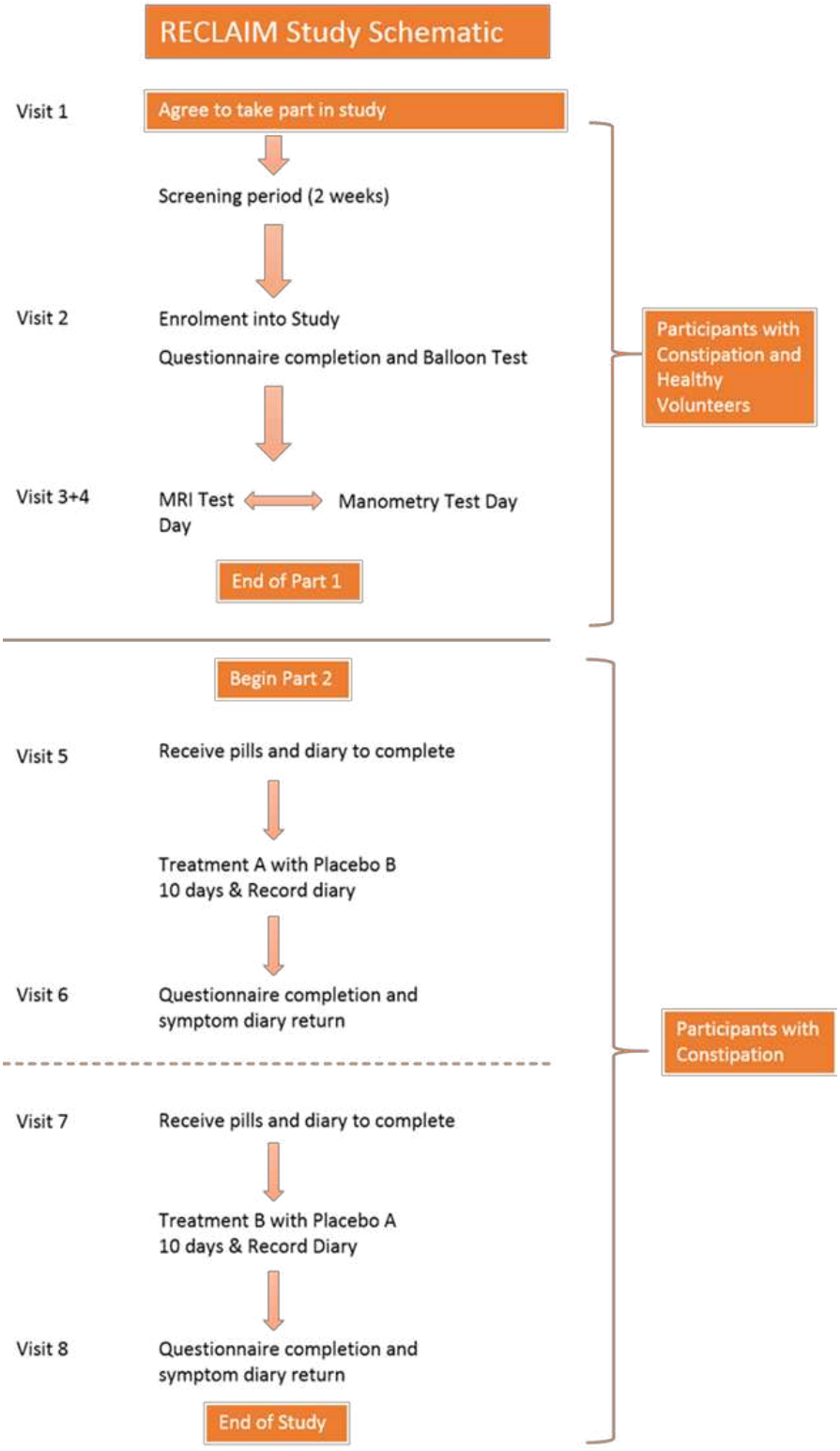
B1 Balloon expulsion test

This was completed using a 50mL water filled balloon, and the patient allowed up to 2 mins to attempt in the seated position with adequate privacy.

Visits 3 and 4 were the MRI and manometry visits, details outlined below. These were performed in a random order.

Visits 5-8 involved the crossover trial, with an initial visit to dispense the blinded tablets and questionnaire, a 10-day treatment period, followed by a minimum 7-day wash-out period before commencing the second set of tablets. The study visits are summarised in Figure S2.

B.2 Figure S2: Study Schematic



B.3 Table S1: Baseline Data on Bowel Habit and Balloon Expulsion Test

	HV (n=44)	IBS-C (n=43)	FC (n=38)	
Screening Stool Diary (14-day diary)				p
Total BM Attempts	17±7	16±13	18±18	0.743
Total BM Inc those after rescue medication	17±7	13±11	11±9*	0.17
Number of SBM	17±7	12±12	10±11*	0.05
Number of CSBM	15±7	1±2*	2±3*	<0.01
Days with hard (BSFS 1 or2) or no stool	2±3	12±3*	12±3*	<0.01
Average stool consistency ‡	4±1	2±1*	2±4*	<0.01
Balloon expulsion test result				
BET (% Pass)	89%	84%	75%	0.27

C MRI Protocol

The participants were provided with 5 plastic MRI visible marker pills to swallow 24 hours before attending the local test site at 9am on the morning of the test. They did not eat or drink anything from the night before, apart from sips of water for essential medicines. The patient cohort were also asked to refrain from laxatives for 48hrs prior to the visit. Participants completed an eligibility questionnaire to confirm the above and that there were no new contraindications to MRI before undergoing the first series of MRI scans (outlined below) while fasted, lasting approximately 30 minutes.

30 minutes after the start of the scan participants started drinking a poly-ethylene glycol and electrolyte solution (Moveprep^{R}, Norgine Pharmaceuticals Ltd, Harefield, UK). They drank 10ml/kg body weight, rounded to the nearest 100ml within the 500ml-1000ml range, ¼ of the total to be drunk every 15 minutes. Moveprep^{R} 1 litre contains 100 g macrogol with 182 mmol Na+, 52.8 mmol SO4, 59.8 mmol Cl-, 14.2 mmol K+ and 56.5 mmol ascorbate.

Immediately after finishing the Moveprep^{R}, 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 Moveprep^{R}, was also recorded wherever possible. If participants did not

open their bowels during the study session, they were asked to note this and inform us, if they did not this was recorded as >150mins.

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.

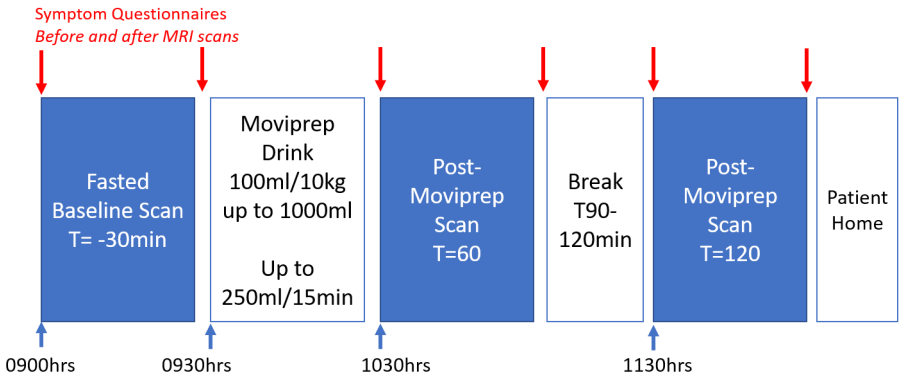
C1 MRI analysis

The principal MRI techniques and analyses were as previously described:

1) MRI colonic volumes. A 3D coronal dual echo fast field echo sequence with mDIXON reconstruction. (1)
2) MRI wall motility measurement. Cine bTFE data were acquired over 10 minutes (2 ,3).
3) MRI content mixing measurement – "tagging index". 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. Variation in the pixel intensity was assessed from the average coefficient of variation (%COV) for ascending colon which is the "tagging index". (4)
4) completed tagged scans of the ascending colon.
5) MRI transit measurement. Whole Gut Transit Time (WGTT) was assessed from the Weighted Average Position Score (WAPS) of MRI transit markers 24 hours after ingestion, as we have previously validated, where a higher score equates to a longer transit time.(5) This was assessed at the baseline scan before ingestion of the Moviprep^{R}.

Additional sequences were taken in order to position the scan.

C 2 Figure S3: Schedule for MRI study day



C 3 Manometry Protocol

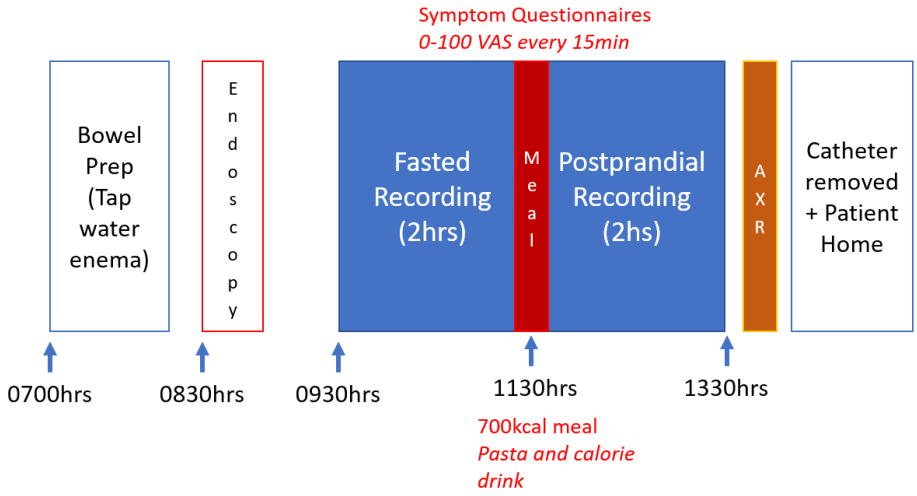
On the morning of the test the participant attended the local test site, fasted from the night before, apart from sips of water for essential medicines. Prior to the endoscopy procedure a tap water enema was administered to cleanse the left side of the colon. Once the study team were satisfied that bowel preparation had been satisfactorily achieved, the participant was transferred to an endoscopy procedure room to undergo colonoscopy. The colonoscope was then passed and the flexible manometry catheter (~4 mm in diameter with 36 recording sites spaced 1 cm apart) 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. 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 (Abbotts Nutrition) nutrient drink (400Kcal) and a pasta meal (300Kcal).

The recording was then continued for a further 2 hours. 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. They then returned to the investigation room and the catheter was removed by gentle traction.

For the high-resolution manometry, the catheter and software system used was purchased from Medical Measurement Systems. This consists of a catheter which was inserted endoscopically and a software system for collection of data. The catheter used was the "Unisensor UniTip High Resolution Catheter" which is CE-marked for non-surgical invasive use in the area of Gastroenterology. As this manometry system is CE marked and being used within its intended purposes we did not require a letter of no objection from the MHRA for usage.

C4 Figure S4: Schedule for manometry study day



D. Clinical Trial Protocol

The interventions used were Buscopan 20mg taken orally three times daily for 10 days and 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 a placebo. Whichever capsule was the placebo in the first 10 days trial was replaced by the active drug in the second part. The order in which they were taken was randomised according to a code generated by the pharmacy team at Nottingham University Hospitals NHS Trust (lead pharmacy site) so neither participant nor the research workers knew which active drug they are taking for each trial period. The code linking patient to drug sequence was kept in pharmacy and only released after data lock.

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 from prucalopride 2mg tablet, taken orally, senna 7.5mg tablet, 1-4 taken orally and sodium picosulfate 5mg -10mg liquid, taken orally.

If during treatment in either arm, a participant felt they were getting intolerable side effects, first a dose adjustment was permitted (in line with clinical practice) – initially a halving of the dose (this would be done for all tablets at all timepoints) and potentially reduction in frequency (every other day dosing) to a level acceptable. If still unable to tolerate, a subject was advised to discontinue but still advised to complete the diary and return for subsequent visits (this did not exclude the patient from continuation in the trial). Treatment order was balanced and there were no differences between those that received A or B first (See Table 3.2).

Modified PAC-SYM (mPAC-SYM) score

This was calculated using only abdominal pain, discomfort and cramps elements of PAC-SYM since the bowel function element was more precisely measured using the stool diary and the tenesmus and rectal bleeding are more local phenomena less clearly related to colonic function.

D.1 Supplementary Table S2: Schedule of treatments in randomised cross-over trial of bisacodyl versus buscopan

Intervention A = Bisacodyl 10mg once daily; Intervention B = Buscopan 20mg 3 times daily						
Visit 5		**Visit 6**		**Visit 7**		**Visit 8**
Start of intervention period 1	**10 days therapy**	End of intervention period 1	Washout	Start of intervention period 2	**10 days therapy**	End of intervention period 2
Assignment to intervention *order* ***AB or BA*** *Baseline measures*	***Active A & Placebo B*** *or* ***Active B & Placebo A*** *Daily symptom record*	*Final measures*	*At least 7 days*	*Baseline measures*	***Active A & Placebo B*** *or* ***Active B & Placebo A*** *Daily symptom record*	*Final measures*

D. 2 Supplementary Table S3: Demographics of participants by treatment sequence

Data shows mean ± SD	A followed by B (bisacodyl first) n=37	B followed by A (hyoscine first) n=35	Significance of difference p
Age	44 ± 14	43 ± 14	0.81 (unpaired t-test)
Gender	35 F 2M	33 F 2M	0.95 (Chi-Square)
HADS anxiety score	6.9 ± 4.1	7.7 ± 4.7	0.47 (unpaired t test)
HADS depression score	4.3 ± 3.7	4.6 ± 4.0	0.75 (unpaired t test)
(m) PAC-SYM	1.9 ± 0.9	1.9 ± 0.8	0.74 (unpaired t test)
Screening Stool Diary (14-day diary)			
Total BM Attempts	20 ± 17	17 ± 15	0.52 (unpaired t test)

Number of CSBM	1.7 ± 2.9	1.7 ± 2.4	0.91 (unpaired t test)
Average stool consistency (BSFS) ‡	2.1 ± 1.3	1.8 ± 1.3	0.31 (unpaired t test)

D3 Statistical analysis plan created before code break

Part 1

We will first confirm that the AC MRI motility index (MMI) differs between the FC and IBS-C participants using an unpaired t-test or Mann-Whitney U test depending on the distribution of the data. We will use the same test to compare the FC and IBS-C groups with the control group.

We will then calculate correlations between the manometry parameters and AC and DC MMI separately using the Pearson or Spearman correlation coefficient depending on the distribution of the data; this will be evaluated in all 80 subjects with FC or IBS-C.

We will calculate a lower limit of 'normal' motility, defined as <10th centile, for both methods in our healthy volunteer group. We will then calculate a measure of agreement (Cohen's kappa statistic) between a classification into hypomotile (reduced motility) versus normal / hypermotile (normal or exaggerated motility) [categorised using the 10th centile values from the control group] comparing the gold-standard manometry classification versus one based on either AC or DC MMI in the 80 participants in the FC and IBS-C groups.

We will also calculate overall % agreement and sensitivity / specificity values using the manometry classification as a gold standard.

Part 2

We will calculate a correlation coefficient (Pearson or Spearman correlation coefficient as appropriate) between the difference in pain scores between the buscopan and bisacodyl trial periods and the MMI measured in Part 1, to test the hypothesis that there is a positive association between the MMI and pain score changes on buscopan compared with bisacodyl. This will assess whether those with normal / hypermotility experience greater pain relief on Buscopan, while those with hypomotility will do better on bisacodyl.

We will also use linear mixed models to estimate the difference in pain scores comparing buscopan with bisacodyl, to account for the cross-over design, and will include an interaction term between the treatment group and the patients' MMI to test whether the difference in pain scores between the drugs varies according to the motility index.

We will analyse the difference in pain scores between baseline and trial period for each drug separately (using a Student's t test if normally distributed or Mann Whitney U test if not) comparing those with motility <10th centile (Group A) with those with normal / hypermotility (Group B) to determine if patients in group A respond

D4 Sample size considerations

Part 1

Primary objective:

A level of agreement >70% would be sufficiently accurate to allow a choice of the best MRI method and would be in line with the accuracy of most clinical assessments. With a total of 80 participants (with functional constipation (FC) or irritable bowel syndrome with

constipation (IBS-C) we could estimate a level of agreement of 70% to within ±10% (95% confidence interval), assuming a proportion of 0.5 in each group (hypomotile versus normal/hypermotile).

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

Secondary objectives:

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 calculate we need 27 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 difference of 0.045.

We plan to recruit 40 per group to allow for technical errors in recordings or subject drop-out. We will also recruit 40 volunteers without constipation matched for age and gender with the patient cohort, in order to meet secondary objective 2.

Part 2

There are no previous data on which to base a power calculation. We will ask all patients with FC or IBS-C from study 1 to take part, giving us potentially 80 subjects. Allowing for refusals and dropout we expect a sample size of 60 patients for this study.

This will 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 is a reasonable minimal clinically significant difference .

E: Supplementary Manometry and MRI data Analysis

While every attempt was made to place the manometry catheters so that sensors spanned the rectum, sigmoid and descending colon, invariably in some patients there were too few or no sensors in the rectum or descending colon. However, in all subjects, sensors were present in the sigmoid colon, and therefore this was used the primary site for the manometry analysis. In addition, while all had studies recorded manometry data 2 hours prior to and after the meal, we have found in our previous published work, that the colonic response to a meal is rapid, occurring within a minute of starting to eat. Therefore, in our previous studies (6 ,7) and in this study we have limited the analysis to the 1 hr period prior to and after the meal.

Manual analysis: Using our previously developed software (PlotHRM) (8) the manometric traces were examined for the presence of:

1. The Cyclic Motor Pattern (CMP); repetitive propagating pressure events with a cyclic frequency of 1-8/min in either a retrograde or antegrade direction or aligned synchronously across ≥3 sensors. In each trace the start and end time of each episode of the CMP was recorded and then the time occupied by the CMP prior to and after the meal was obtained.

2. High-amplitude propagating contractions (HAPCs); an array of propagating pressure events with at least 2 component pressure waves having a trough- to-peak amplitude of >100 mm Hg. Once identified the following characteristics were obtained, average amplitude of pressure waves, extent, and speed of propagation.

Automated analysis: In previous analysis we have shown that pressure waves at frequencies between 1-8cpm dominate in the sigmoid colon of healthy controls, patients with constipation and patients with diarrhoea pre-dominant irritable bowel syndrome(6 ,9). Therefore, here we focused upon pressure waves in this frequency range. The automated analysis was performed using a Bayesian functional mixed-effects model (described in detail elsewhere(7). This approach has been previously used in the analysis of data from patients with slow transit constipation(7) and patients with diarrhoea predominant irritable bowel syndrome (15). The automated approach was broken into 2 distinct sections.

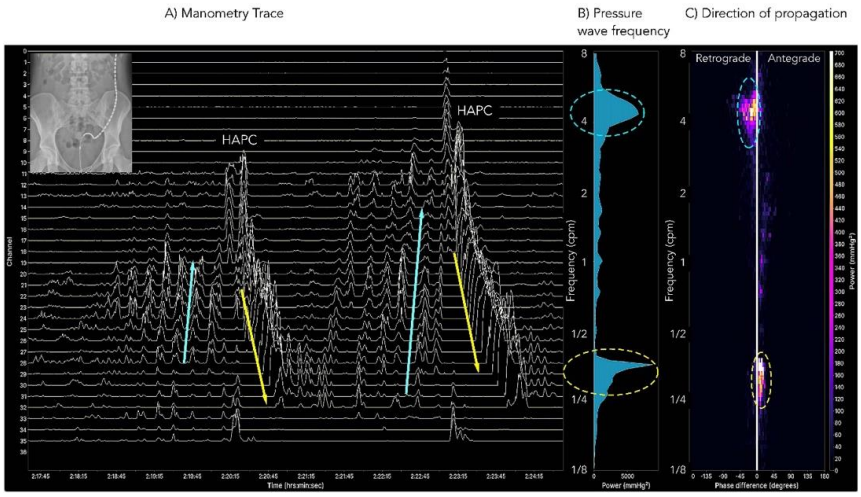
1Dimensional (1D) analysis: Provides an indication of the power of pressure waves (PW) between 1-8 cpm in one hour prior to and after the meal. This analysis did not consider whether the pressure waves are temporally associated with pressure waves in an adjacent channel (i.e. whether the pressure wave is part of a propagating contraction). An increase in the power of PW in response to a meal, indicates that there was an increase in number of PW after a meal. While an increase in the amplitude of the PW may contribute to the

increased power, the current analysis approach does not distinguish between these two (count and amplitude) characteristics.

2 Dimensional (2D) analysis: The data used in the 1D analysis were then re-analysed using a 2D group analysis. This analysis provides an indication of the power of propagating pressure waves (PPWs), both antegrade and retrograde, between 1-8 cpm in one hour prior to and after the meal. The 2D analysis assessed the potential temporal relationship between every pressure wave in a channel with pressures waves in the adjacent channel (both the channel above and below). A temporal association between pressure waves in adjacent channels was determined if the duration of the pressure waves in adjacent channels overlapped. If this condition was met the pressure wave formed part of a PPW. PPWs were not defined by distance propagated or the amplitude of the pressure wave. As with the 1D analysis, an increase in the power of PPW in response to a meal, indicates that there was an increase in number of PPW after a meal.

E.1 Figure S5: Manometry Trace Example

Manometry trace example and analysis**:** Automated analysis on a segment of a single manometry recording.



A) shows a section of the manometry trace with 2 HAPCs (yellow arrows) and two clusters of the retrogradely propagating cyclic motor pattern. The X-ray insert shows the location of the catheter for this recording, with sensors in the descending and sigmoid colon).

B) shows the dominant frequencies of pressure waves within the manometry window. A peak can be seen at ~4cpm (the frequency of the cyclic motor pattern) and another at 1/3cpm* (the frequency of the HAPCs).

C) shows the direction of propagation of the dominate frequencies. The 4cpm activity propagates in a retrograde direction (see blue arrows in A) and the 1/3cpm propagates in an antegrade direction (HAPCs, Yellow arrows in A)

* Note that the group analysis in this study focused upon frequencies between 1 – 8cpm. Lower frequencies were excluded.

E.2 Supplementary MRI Data

E.2.1 Figure S6 Ascending and Descending Colon Wall Motility

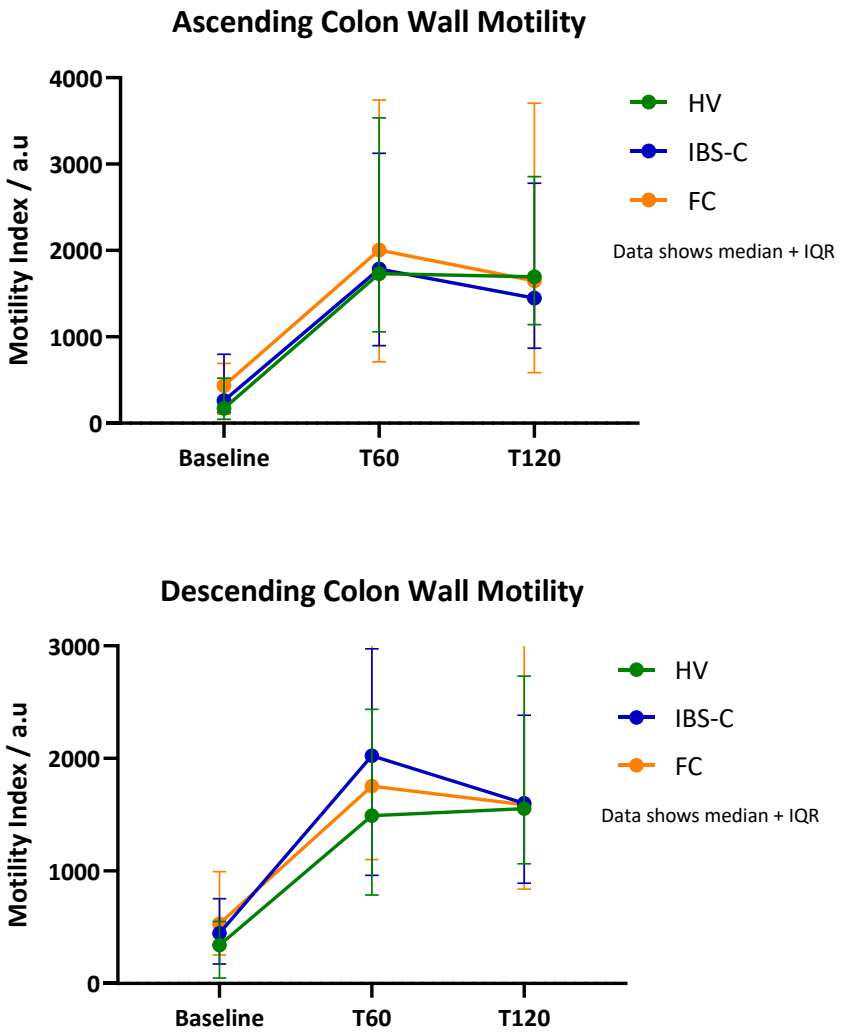
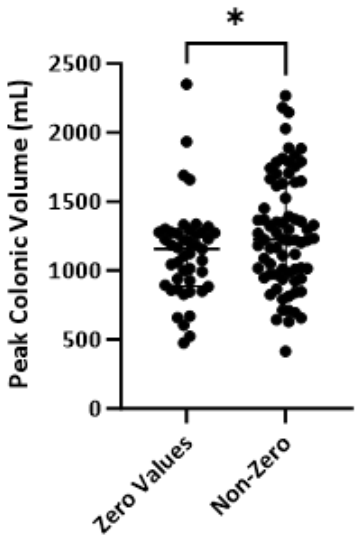


Figure S6 shows the AC and DC motility index which rose following the meal but as can be seen there was no difference between the groups with wide individual variability (2-way ANOVA, Time effect p<0.0001 for both AC and DC, group effect N Sig AC p=0.8, DC p=0.25) .

E.2.2 Figure S7: Peak volume vs pain on MRI day

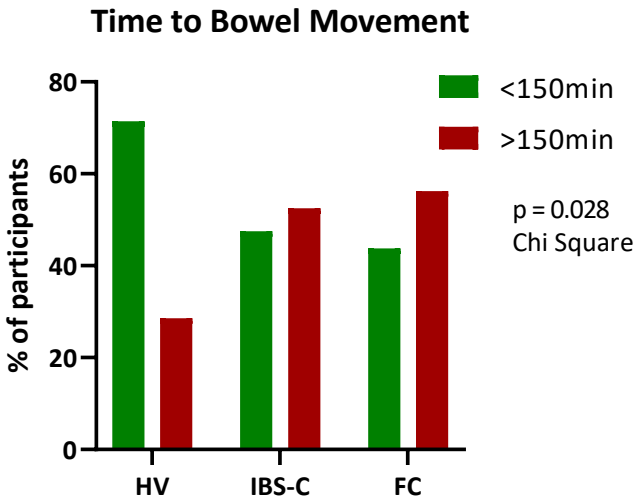
This shows the peak volume in all participants in those with pain (Non-Zero) versus those without pain (Zero values) on the study day. We had planned to correlate the volume of the colon and participants' reporting of pain, however due to the high number of zero values a direct correlation was not possible. 74 participants reported pain at their peak volume vs 45 who did not.



The presence of pain was associated with a significantly higher peak volume 1277(345) versus 1126(410) ml but there was a wide scatter, p=0.04, (unpaired t-test).

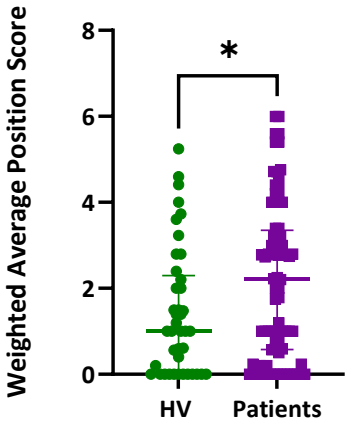
E2.3 Figure S8 Time to Bowel Movement

Compared to healthy volunteers significantly fewer patients opened their bowels within 150 minutes of Moviprep^{R} ingestion after which time they were free to go home.



E.2.4 Figure S9: Whole gut transit time

Whole gut transit time, as measured by a weighted average position score (WAPS), was significantly longer when all patients were compared to healthy volunteers, with a median (IQR) score of 2.2 (0.6-3.4) and 1 (0-2.3), respectively; p=0.03 (Mann-Whitney)



E.2.5 Supplementary Table S3: Correlation of baseline Colonic Volumes with other MRI parameters

	Baseline Colonic Volume vs.T120 AC Motility	**Baseline Colonic Volume vs. Tagging AC T120**	**Baseline Colonic Volume vs. Transit Score**	**Baseline Colonic Volume vs. Time to Bowel Movement**
Spearman R	0.18	-0.26	-0.048	0.37
95 % CI	-0.053 to 0.39	-0.47 to -0.033	-0.28 to 0.19	0.15 to 0.56
P value	0.1181	0.0217	0.6830	0.0012

E.2.6 Supplementary Table S4: Pain Scores during Moviprep Challenge

At both Time 60- and 120-minutes post ingestion both patient groups experienced significantly more pain than HVs. FC reported less pain than IBS at 60 minutes, but this difference was lost by 120 minutes.

Maximum pain scores during Moviprep challenge Median (range)						
Group	Pain score baseline	Number (%) with >0 score	Pain score T=60	Number (%) with >0 score	Pain score T=120	Number (%) with >0 score
HV (n=41)	0 (0-0)	2 (5)	0 (0-0)	7 (17)	0 (0-0)	9 (22)
IBS-C (n= 43)	0.5 (0-1.0)*	25 (58)	1 (0.5-2.0)* †	35 (81)	1 (1.0-2.0)*	39 (91)
FC (n=36)	0 (0-0.4)*	9 (25)	0.5 (0-1.0)*	19 (53)	1 (0-1.5)*	24 (67)
* p<0.05 vs HV, † p<0.05 vs FC						

E.2.7 Figure S9: Peak volume vs pain on MRI day

This shows the peak volume in all participants in those with pain versus those without pain on the study day. We had planned to correlate the volume of the colon and participants' reporting of pain, however due to the high number of zero values a direct correlation was not possible. 74 participants reported pain at their peak volume vs 45 who did not.

The presence of pain was associated with a significantly higher peak volume 1277(345) versus 1126(410) ml but there was a wide scatter, p=0.04, (unpaired t-test).

E.2.8 Supplementary Table S5 Agreement between MMI and manometry defined hypomotility.

Table S 6 Number of participants with MMI <10th centile of HVs and / or % time cyclical motility <10th centile of HVs

	N total	Peak MMI <952	% time cyclical motility <15.1	Meeting both criteria of hypomotility
HV	35	4	4	0
FC	25	6	6	1
IBS	36	5	5	0

Only 1 FC patient was hypomotile on both measures. None of the remaining was defined as hypomotile by both measures.

E2.9 Segmental colonic volumes in patients with enlarged colon versus normal sized colon

Table S7 Segmental colonic volumes in patients with enlarged colon versus normal sized colon

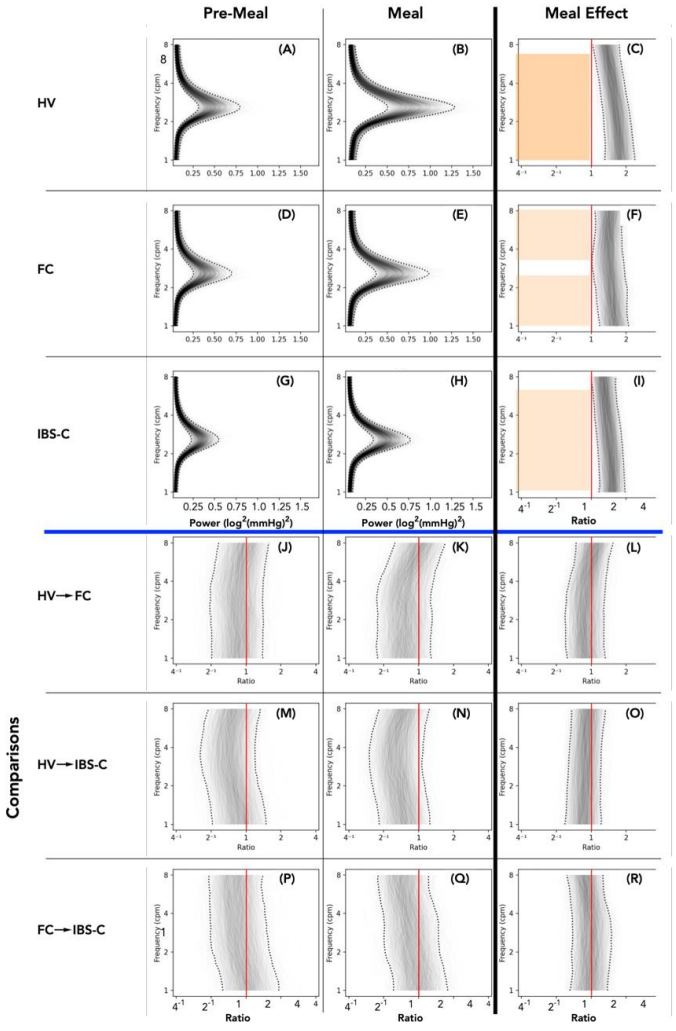
	AC	TC	DC	RS	Total
Enlarged colon	323 (348-289)	399 (495-328)	195 (252-164)	145 (225-113)	1076 (1241-987)
Normal sized colon	251 (260-194)	228 (315-155)	86 (120-66)	106 (140-68)	703 (793-544)
P difference Mann Whitney test	<0.0001	<0.0001	<0.0001	0.0004	<0.0001

E. 3 Manometry Data

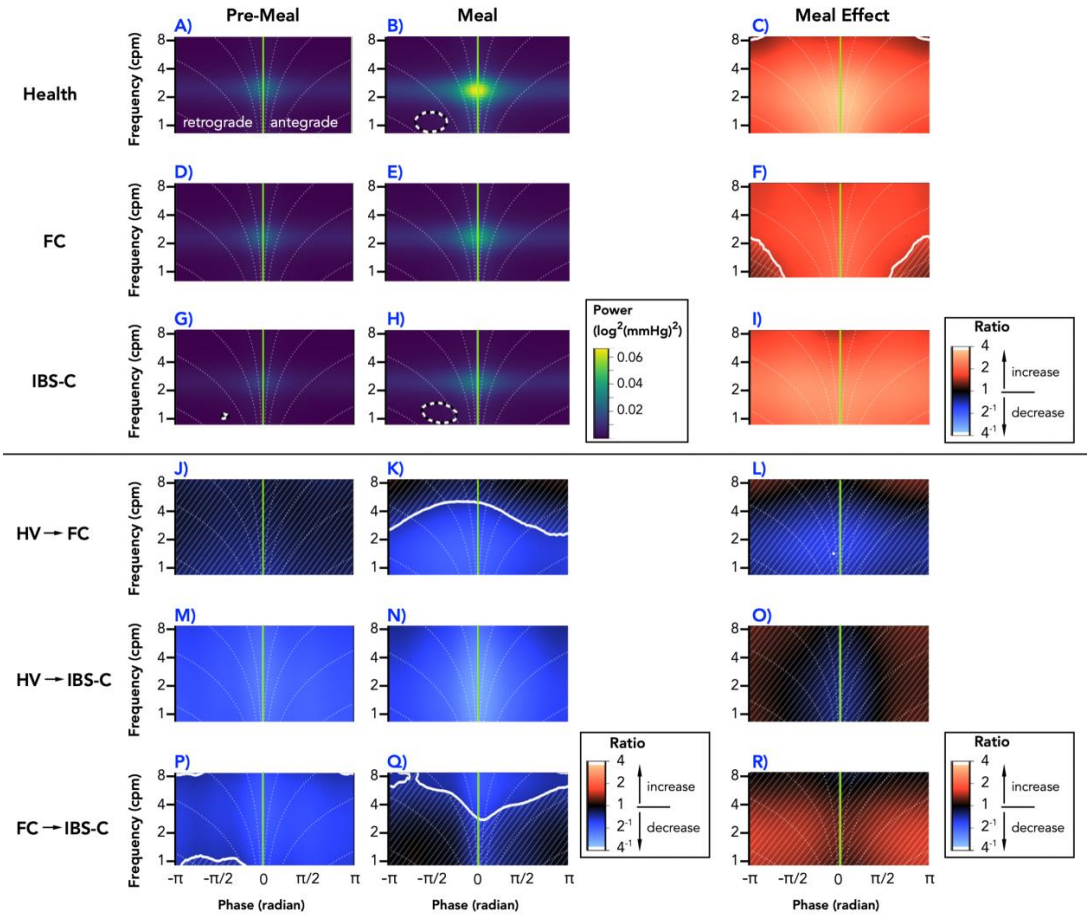
E.3.1 Supplementary Table S8Correlation of Manometry and MRI Measures

Correlations of % Time occupied CMPs vs MRI Measures	CMP vs. Total Baseline Volume	CMP vs. Total T120 Volume	CMP vs. Peak Motility AC	CMP vs. Tagging Max	CMP vs. Transit Score (WAPS)
Spearman r	-0.039	-0.032	0.099	-0.0040	0.0088
95% confidence interval	-0.24 to 0.17	-0.24 to 0.18	-0.11 to 0.30	-0.21 to 0.21	-0.20 to 0.22
P value	0.7065	0.7598	0.3394	0.9702	0.9331
Number of XY Pairs	96	95	95	92	94

E.3.2 Figure S10: One-dimensional (1D) analysis of pressure waves at frequencies between 1 – 8 cycles per minute (cpm) in the sigmoid colon of patients with a normal diameter colon (top row; a-c), FC patients (middle row; D-F) and IBC-patients (Bottom row; G-I). Baseline data is shown the left column and meal data in the middle colon. In each image, frequency of pressure waves is shown on the Y-axis. In (a, b, d, e, g, h)) power is shown on the X-axis. 2000 overlapping grey lines in each panel represent posterior samples, and the dotted black lines form envelopes of 95% credible intervals. The meal effect for each patient group is shown in c, f & i. When the entire grey envelope lies to one side of the vertical red line (which represents a ratio of 1), this shows a significant deviation (to the left a decrease meal response and to the right of the red line an increase meal response.



Comparisons between the three groups are shown below the solid blue line. The first row compares HV to FC (J & K); the second row HV to IBS-C (M & N) and the final row FC to IBS-C (P & Q). A comparison of the meal effects between groups is shown in the final column (L, O, R). In all images below the blue line the red line at the ratio of 1 lies entirely within the grey envelope, indicating there were no significant differences between groups or between meal effects. Main message of the figure; the meal induced an increase in the power of PW in all three groups, however this increase did not differ between the groups.



E.3.3 Figure S11: 2-Dimensional analysis of propagating pressure waves (PPW) in the sigmoid colon occurring at frequencies between 1-8cpm. In each panel the vertical line at 0 on the x axis indicates synchronous (non-propagating) activity. Retrograde propagation is to the left of the midline and antegrade propagation to the right. In panels (a, b, d, e, g.h) the green pixels represent increasing power. The first column is baseline data, the second column is data after the meal. Healthy adults are shown in the top row, patients with FC in the second row and those with IBS-C in the 3rd row. The bottom 3 rows (below the solid blackline) compare power across the frequency range between the 3 groups, during premeal (J, M, P) and postmeal (K, N, Q) periods. For each of rows 4 to 6 blue indicates a reduction in PPW power in the second named group compared to the first. For example, in the 4th row HV is named first and FC second. Therefore, blue regions indicate a reduction in power in FC patients compared to HV. In panels J, K, M, N, P, Q, the blue regions demarcated by the solid white line indicate significant reductions. The faint diagonal hatching in these panels indicates regions of non-significance. The meal effect is shown in panels (C-I). The extensive orange/red regions indicates that propagating activity increased in power after the meal. The area demarcated by the solid white lines indicates a significant increase. Comparison of the meal effects between the 3 groups are shown in final panels of the 4th to 6th rows (panels; L, O, R). For each of these panels, blue indicates a reduced meal response in the second group compared to the first. For example, in row 4, the blue in panel (L) indicates the meal effect is FC was reduced compared to HV. However, as the faint diagonal hatching in present throughout the panel this reduction is not significant. The diagonal hatched lines are also present throughout panels O and R, and therefore the meal effect did not differ between any of the groups.

Main message of the Figure: The meal caused a significant increase in the PPWs in all three groups and this meal effect did not differ between the groups (L. O, R). However, during both the baseline and meal periods the PPWs were significantly reduced in IBS-C compared to both HV and patients with FC.

F Clinical Trial Data

F. 1 Supplementary Table S 9 Clinical Trial Data by Traditional Subgroup

Clinical Endpoints	Buscopan	Bisacodyl	Statistical Analysis (Mann-Whitney)
(IBS n=38 (unpaired – 2 subjects only completed 1 arm)			
Pain			
Average worst daily pain	1.7 (1.4-2.72)	2.7 (2.1-3.3)	p <0.001
Days with Severe or Very severe Pain	0 (0-2)	2 (0-4)	p <0.001
Modified (Pain Qs only) PAC-SYM before intervention (range 0-4)	1.67 (0.92-2.33)	1.67 (1.0-2.0)	p = 0.874
Modified (Pain Qs only) PAC-SYM after intervention (range 0-4)	1.33 (0.67-2)	2.67 (1.67-3)	p <0.001
Change in PAC-SYM (Post intervention vs Pre)	0 (-1 – 0.33)	1 (0.3-1.67)	p <0.001
Frequency & Consistency			
Weekly average number of bowel movements considered complete (excluding those following rescue)	0.35 (0-2.1)	1.75 (0-5.95)	p = 0.031
Change in weekly CSBMs vs Screening Period	0 (0-1)	1.15 (0-5.15)	p = 0.027
Weekly average days with constipation (either no bowel movements or at least one type 1-2, or needing rescue)	4.55 (2.8-7)	1.75 (0-3.5)	p <0.001
Change in weekly average of days with constipation vs screening period	-0.7 (-2.23-0)	-3.7 (-5.03--2.68)	p <0.001

Average stool form over the 10 days (exc BMs following rescue, Bristol Stool Chart Scale 1-7)	2.05 (1.07-3.51)	5.5 (4.9-6)	p <0.001

Clinical Endpoints	**Hyoscine**	**Bisacodyl**	**Statistical Analysis (Wilcoxon)**
FC (Paired data n=33)			
Pain			
Average worst daily pain	1.5 (1-2)	2 (1.6-2.5)	p <0.001
Days with Severe or Very severe Pain	0 (0-1)	0.5 (0-1.75)	p = 0.026
Modified (Pain Qs only) PAC-SYM before intervention (range 0-4)	0.33 (0.17-1.33)	0.67 (0.33-1.17)	p = 0.891
Modified (Pain Qs only) PAC-SYM after intervention (range 0-4)	0.67 (0-1.67)	1.67 (0.58-2.33)	p = 0.005
Change in PAC-SYM (Post intervention vs Pre)	0 (-0.33 – 0.67)	0.67 (0-1.67)	p = 0.019
Frequency & Consistency			
Weekly average number of bowel movements considered complete (excluding those following rescue)	0 (0-1.93)	2.8 (0-6.3)	p <0.001
Change in weekly CSBMs vs Screening Period	0 (-0.55-0.7)	1.5 (0-4.5)	p <0.001
Weekly average days with constipation (either no bowel movements or at least one type 1-2, or needing rescue)	5.6 (3.68-6.83)	1.4 (0.7-2.8)	p <0.001
Change in weekly average of days with constipation vs screening period	-0.6 (-2.2-0)	-4.1 (-5.7--2.35)	p <0.001
Average stool form over the 10 days (exc BMs following rescue, Bristol Stool Chart Scale 1-7)	2.43 (1.46-3.97)	5.19 (4-6.1)	p <0.001

consort F2 Supplementary Table S10 Effect of enlarged colon on response to hyoscine

Table S 9					
	n	Basal mPAC-SYM	Change in mPAC-SYM	Weekly CSBM	Change in CSBM
Enlarged colon	21	1.0 (1.0-2.0)	0.0 (-0.3-0.7) (-1.0-0.2)	0.0 (0.0-2.1)	0.0 (-0.2-1.1)
Normal sized colon	50	1.0 (0.3-1.8)	0.0 (-0.3-0.7)	0.7 (0.0-2.1)	0.0 (0.0-0.9)
P		0.3‡	0.6‡	0.5‡	0.9‡

↑ t test ‡Mann Whitney test

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