TITLE: Demonstration of differences in colonic volumes, transit, chyme consistency and response to psyllium between healthy and constipated subjects using Magnetic Resonance Imaging

SHORT TITLE: Demonstration of effects of psyllium using MRI

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ABBREVIATIONS

AC	Ascending Colon
ACWC	Ascending Colon Water Content
AUC	Area Under the Curve
BMI	Body Mass Index
DC	Descending Colon
FC	Functional Constipation
GI	Gastrointestinal
IBS-C	Constipation-predominant Irritable Bowel Syndrome
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
Q25, Q75	Lower and upper quartile
SBWC	Small bowel water content
SD	Standard Deviation
TC	Transverse Colon
T1	T1 relaxation time
T2	T2 relaxation time
t.d.s.	Three times daily
WAPS24	Weighted Average Position Score at 24 hours

ABSTRACT

Background: In functional gastrointestinal disorders a lack of objective biomarkers limits evaluation of underlying mechanisms. We aimed to demonstrate the utility of Magnetic Resonance Imaging (MRI) for this task using psyllium, an effective constipation treatment, in patients and controls.

Methods: Two crossover studies: 1) adults without constipation (controls, n=9) took three treatments in randomised order for 6 days - maltodextrin (placebo), psyllium 3.5g t.d.s and 7g t.d.s.; 2) adults with chronic constipation (patients, n=20) took placebo and psyllium 7g t.d.s. for 6 days. MRI was performed fasting and postprandially on day 6. Measurements included small bowel and ascending colon water content, colonic volume, transit time and MR relaxometry (T1, T2) to assess colonic chyme. Stool water percentage was measured.

Results: 7g psyllium t.d.s. increased fasting colonic volumes in controls from median 372mL (IQR 284-601) to 578 mL (IQR 510-882), and in patients from median 831mL (IQR 745–934) to 1104mL (847–1316), P<0.05). Mean postprandial small bowel water was higher in controls and patients after 7g psyllium t.d.s. vs. placebo. Whole gut transit was slower in patients than controls (P <0.05). T1 of the descending colon chyme (fasting) was lower in patients [213ms, 176–420] than controls [440ms, 352–884, P <0.05] on placebo, but increased by 7 g psyllium t.d.s. [590ms, 446–1338), P<0.001]. Descending colon T1 correlated with baseline stool water content and stool frequency on treatment.

Conclusions and Inferences: MRI measurements can objectively demonstrate the mode of action of therapy targeting intestinal fluid content in constipation.

Trial Registration Number: www.clinicaltrials.gov NCT01805999, NCT02144376.

Keywords: ispaghula, laxative, relaxometry

KEY POINTS

- MRI can non-invasively simultaneously assess whole gut transit time, colonic volumes and gut content including the composition of chyme, using the relaxation times T1 and T2.
- We show that the volume of the ascending colon and transverse colon is increased and the water content of the descending colon reduced in people with constipation compared to those with normal bowel habit
- MRI demonstrated that psyllium, an effective treatment for constipation, caused an increase in water in the small bowel, colonic volume and a newly identified marker of colonic contents, T1, which correlates with water content of stool.
- Through assessing multiple parameters contributing to constipation in a single test, MRI has the potential to provide clinical characterisation of patients beyond transit alone, leading to more targeted application of current and novel therapies.

INTRODUCTION

A major challenge in functional gastrointestinal disorders has been to develop objective biomarkers that can be used to assess treatments more economically than symptoms, which typically show very wide variability(1). Assessment of the small bowel and proximal colon has been particularly difficult owing to its inaccessibility. Capsule technologies, such as endoluminal image analysis(2) and pH monitoring, provide some information on motility and transit. Scintigraphy has been extensively used over the last three decades to show changes in transit through the different regions of the colon(3). It has correctly predicted efficacy in a range of medications with different modes of action on bowel function(4). It has shown acceleration of transit in constipation by stimulant laxatives such as bisacodyl(5) and secretogogues such as lubiprostone(6) however it cannot directly demonstrate whether any effects are due to changes in the balance of absorption/ secretion of fluid from the lumen or changes in motility(7). Transit has been shown to account for 19-27% of the variance in stool form(3) indicating that there are other important parameters in predicting bowel function that may be measurable.

Up to 10L/ day of fluids move into, through and out of the gut lumen(8) but objectively measuring this is difficult and requires intestinal intubation which, as we and others have shown, alters fluid volumes significantly(9). Disorders of fecal water content and/ or bowel habit, either diarrhoea or constipation, may result from an imbalance of secretion/ absorption rather than just abnormal motor function. Recently a number of new treatments for constipation stimulating intestinal water secretion have been introduced (10, 11) but assessment of the resulting changes in intestinal fluid content has not previously been possible.

We believe this deficit could be corrected using magnetic resonance imaging (MRI) based methods to characterise the contents of patients' small bowel and colon, and to measure whole gut transit time as previously described (12-15). We have also used the relaxation times T1 and T2 to characterise luminal contents (16, 17). T1 and T2 are the time constants with which the magnetization of material in the scanner returns to baseline after excitation by the radiofrequency pulse. They are sensitive to the physical and chemical environment of the water protons via interactions with surrounding molecules and in the context of colonic chyme, are expected to fall with a reduction of water associated with more solid chyme.

The aim of these studies was to assess the value of these MRI biomarkers by investigating their responsiveness to a well characterised laxative, psyllium husk (ispaghula). Its water-holding properties are known to increase fecal water content and 24-hour fecal weight but reports of its effect on whole gut transit time are inconsistent(18, 19). In some patients it produces unacceptable bloating but whether this reflects distension of intestinal lumen was uncertain.

We present two studies, investigating the MRI changes induced by psyllium in healthy volunteers, without constipation, and in patients with constipation. Our hypotheses were that MRI could detect an increase in small bowel water content caused by psyllium preventing water absorption. Furthermore, as this "trapped" water enters the colon, colonic water content would increase.

MATERIALS AND METHODS

Study Design

Two studies were conducted: study 1, performed first, recruited adults without GI disorders (controls); study 2, using similar methodology (see below), recruited adults

with chronic constipation (patients). Both were designed as placebo-controlled crossover studies and were conducted according to Good Clinical Practice as determined by the declaration of Helsinki. All authors had access to the study data, and reviewed and approved the final manuscript. The protocols were approved by institutional and national review boards respectively, and prospectively registered on www.clinicaltrials.gov (NCT01805999 and NCT02144376). All subjects gave written informed consent.

In study 1 three treatments were taken in random order: a placebo (maltodextrin) and two different doses of psyllium. Treatments were separated by washout periods of one week. Investigators were blind to the order of intervention. The patient study, study 2, was designed to be less burdensome and so used only two treatments: placebo and high dose psyllium. In order to ensure return to baseline and avoid any carryover effect from previous treatment in the patient group, both treatment periods were preceded by ≥10 days of usual laxative use, then 8 days without laxatives other than rescue therapy. Rescue therapy (oral bisacodyl 5mg) was permitted in patients who had not opened their bowels for 3 days and were experiencing distressing symptoms, but not in the 48 hours before MRI scans.

Study Populations

Controls were recruited through general advertisement between May and August 2013. Non-smokers aged 18–65 with BMI 18–30 $kg \cdot m^{-2}$ were eligible. Exclusion criteria included: any given history of GI disease or surgery; antibiotic or probiotic use in the 4 weeks before the study; heavy alcohol intake; pregnancy; lactation; excessive exercise; inability to discontinue medicines likely to alter gastrointestinal

transit. Patients were recruited between March 2014 and January 2015 through hospital clinics and advertisement. Eligibility criteria included age ≥18 and a diagnosis of chronic constipation, defined as meeting Rome III criteria for either functional constipation (FC) or constipation-predominant irritable bowel syndrome (IBS-C). Subjects had to pass at least one bowel motion weekly on usual laxatives. Exclusion criteria in addition to those for the control study were: use of morphine or similar opioids; use of open-label psyllium; inability to cease regular laxative use. Patients also underwent a screening period of 2 weeks off laxatives to document normal bowel habit.

Treatments and Procedures (Figure 1)

The active treatment used was Metamucil® Original Coarse Fiber (P&G, Cambridge MA USA), a powder containing approximately 3.4g psyllium per 7 g product. Maltodextrin (The Hut Group, Northwich UK) was used as the placebo control. Subjects took 14g of powder three times daily (t.d.s.), either 14 g maltodextrin, 14 gm Metamucil (providing 7g psyllium), or a 50:50 mixture 7 g maltodextrin and 7 g Metamucil (providing 3.5g psyllium) , each dose taken with 250mL water. Henceforth in the text we refer to the psyllium doses by their psyllium content i.e. 3.5 or 7 g. Blinding of subjects and investigators was ensured by providing the powders in opaque containers, labelled by independent staff and provided in sealed bags so they could not be recognised. The treatment allocation was according to a computer generated randomisation code. Investigators were blind to the intervention. Subjects were not told which intervention they were taking in any treatment period, although powders did differ subtly in appearance and texture.

In each treatment period subjects took the powder for 6 days. Subjects measured out their doses using a plastic spoon and kept a diary of their treatment compliance. Compliance was also assessed by measurement of the total weight of powder consumed, expressed as a % of that expected if compliance was complete. Compliance of 60% was considered acceptable as >12g psyllium daily would be expected to exert some effect. Subjects kept a daily diary of abdominal symptoms and bowel habit.

On the morning of treatment day 5, subjects swallowed five identical transit markers: cylinder-shaped inert capsules containing 0.4mL 15μM gadoteric acid, a positive MRI contrast agent(15). Ingestion was confirmed in patients by direct observation or via a time-stamped video. On day 6 all subjects attended at 8am, fasted. After an initial MRI scan, during which the intra-luminal position of the transit markers was documented, subjects consumed their morning dose followed by a 330kcal standard rice pudding meal(15, 20). Scans were taken at hourly intervals for 7 hours. Doses of psyllium/ placebo were repeated 165min and 320min after the meal. A second 1000 kcal meal was consumed before the final scan. Subjects were scanned supine. Images were acquired using a 1.5T scanner (Achieva, Philips Medical System, Best, The Netherlands). All MRI parameters were measured during a single 15 minute episode in the scanner at each time point. Full details of the MRI methodology are given in the supplementary appendix.

In the control study, fecal samples for measurement of stool water were taken at enrolment and after the final MRI scan of each treatment period. Patient samples were collected during the run-in period without laxatives before each treatment, and

after at least 72h of treatment. Bisacodyl rescue therapy was not permitted in the 48 hours prior to MRI scanning.

Endpoints

All endpoints were MRI parameters unless reported otherwise. In the control study the primary endpoint was ascending colon free water content (ACWC). Secondary endpoints included: small bowel free water content (SBWC); colonic volume, defined as the sum of the segmental volumes of the ascending, transverse and descending colon (AC, TC, DC); the weighted average position score of the transit markers at 24h (WAPS24). This was calculated using the formula (sum of the segment number X the number of markers in each segment divided by the total number of segments) as described previously(15) such that a higher score denotes slower whole gut transit.

Relaxation times (T1 and T2) of the chyme in the AC and DC were also measured. T1 (longitudinal relaxation time) depends upon the mobility of the water molecules as does T2 (transverse relaxation time) which also depends on exchange between water molecules and surrounding macromolecules. Therefore both of these parameters are expected to decrease as the colonic content becomes more solid. Percentage fecal water content was also determined by freeze drying the stool. Symptoms of flatulence, bloating, abdominal pain and diarrhoea were monitored between MRI scans using 0-100 visual analogue scales(21).

In the patient study, the primary endpoint was the weighted average position score of the transit markers at 24h (WAPS24). Secondary endpoints included SBWC, ACWC,

colonic volume, T1 and T2 of the colonic chyme, and percentage fecal water content. Stool diaries were kept for the period off laxatives immediately before each treatment and during the treatment itself.

Sample size and Statistical analysis

Sample size calculation for the control study was based on pilot data in healthy volunteers from a previous study of ACWC(13). Nine subjects would be required in a crossover design to detect an increase in post prandial area under the curve (AUC) of ACWC of 15 L.min (an increase of approximately 10%) with 90% power and $P<0.01$. To allow for withdrawal and noncompliance, 16 subjects were recruited. In the patient study the primary endpoint was WAPS24 since we had pilot data on this endpoint in a relevant patient group by which to power our study. We found, using our MRI marker method, a transit time of mean (SD) 69.2 (32.6) hours in IBS-C(22). We calculated that a study with 20 subjects would have 80% power to detect a change of 21 hours or 30% which is similar to the changes previously reported in constipated subjects treated with psyllium (23, 24) and judged to represent a minimal clinically significant difference. 24 subjects were recruited to allow for attrition.

Fasting parameters were compared between treatments. Postprandial endpoints were compared using AUC. Data are presented as median (interquartile range) or mean (±SD). Paired differences were assessed for normality using the Shapiro-Wilk test. 1-way ANOVA with post-hoc Tukey's multiple comparisons test or 1-way Friedman's test followed by Dunn's multiple comparison test were also carried out when appropriate. Data are presented as median (interquartile range) or mean (±SD). For controls, only subjects with data from all treatment periods are

presented. For patients, an intention-to-treat analysis is reported, taking data from subjects who had at least one MRI parameter measured. This analyses the first treatment period of the crossover study only, as if in a parallel group trial, using Mann-Whitney tests. We also report paired analysis of the patient study in those subjects who completed both treatment periods. All comparisons were made by paired t-test unless otherwise stated where paired differences were non-parametric. Statistical analyses were carried out using Prism 6 (GraphPad Software Inc., San Diego, CA, USA) or SPSS version 24 (IBM Corp., Armonk, NY, USA).

RESULTS

The demographics of subjects are shown in the Supplementary Appendix. 16 control subjects were randomised and completed the study. Of these, 10 showed adequate compliance. A scanner failure meant that data for one of these were not available so data from only 9 subjects are presented. 37 patients consented, of whom 24 passed screening and were randomised. 4 withdrew before scanning; 4 more withdrew between treatment periods 1 and 2. 20 patients had at least one MRI scan, completing one treatment period (11 psyllium; 9 placebo) and were included in the intention-to-treat analysis (ITT); 16 patients completed both treatment periods with appropriate compliance and were included in the paired analysis (Supplementary Table 1). 15 met Rome III criteria for FC and 1 for IBS-C. Fasting and postprandial results are given in Tables 1 and 2 respectively.

Table 1: Variables measured on fasting MRI after 5 days treatment

Table 2: Area Under the Curve of variables measured on postprandial MRI scans during day 6 of treatment

Outcomes assessed on fasting scans (Table 1)

On fasting scans, psyllium treatment did not lead to detectable differences from placebo (maltodextrin) in small bowel water content (SBWC) in either study. In the ITT analysis of patients fasting SBWC was 33mL (IQR 9 – 90) on placebo and 54mL (24 – 77) on psyllium Figure 2A & 2B show fasting and post-prandial SBWC for the paired data , Little ascending colon water content (ACWC) was detected with either treatment in either study: one control and one patient had >5 mL detectable on a fasting scan after 5 days of placebo compared to 4/9 (controls) and 4/18 (patients) with >5mL detectable after 5 days of 7 g psyllium t.d.s. In controls, no differences in WAPS24 transit scores between treatments were detected as after 24 hours most markers had passed to the rectosigmoid or been expelled. In the patient study however, where transit was the primary endpoint, scores tended to decrease, indicating faster transit. In the ITT analysis WAPS24 fell from median 4.2 (3.2 – 5.3) on placebo to median 2.0 (1.5 – 4.0) after psyllium (P=0.067). In the paired analysis (n = 16) there was a a mean reduction of 0.8, 95% CI -0.2 to 1.7), a difference that was not statistically significant (figure 2C).

Figure 2D shows fasting colonic volumes for controls and patients. In controls, both psyllium doses increased fasting volume: 7 g t.d.s led to mean 53% increase (220 mL, 95% CI 127 – 312). In patients, ITT analysis showed that the colonic volume increased from 831mL (745 – 934) on placebo to 1104mL (847 – 1316), P<0.05). In the paired analysis the 7 g t.d.s. dose led to mean within-individual increase of 43% (332mL, 95% CI 214 – 451). No difference in segmental colonic volumes in patients was detected in the ITT analysis but in paired analysis both controls and patients showed a significant increase in the fasting AC and transverse colon (TC), with a significant increase in the fasting descending colon (DC) also found in patients(Table

1). Figure 3 illustrates the changes in the ascending colon that were visible on anatomical scans and water content sequences.

Fasting data on T1 relaxation times, where higher values would be expected to reflect increased water content, are shown in Figure 4. In controls, treatment tended to increase fasting T1 in the AC and DC (Figure 4A) but these differences were not significant in the ITT analysis. In patients, $T1_{DC}$ was significantly higher in the DC after psyllium (590ms, 446 – 1338) than placebo (213ms, 176 – 420), P<0.001. Within-individual comparisons of paired data found higher T1 values after psyllium in both AC (P<0.05) and DC (P<0.01). Fasting T2 measurements varied widely in controls without demonstrable difference while differences identified in patients were not consistent across ITT and paired analyses.

Outcomes assessed on serial postprandial scans (Table 2)

Postprandial SBWC showed significant differences between treatments for both groups (Figure 2A & 2B). A dose-response relationship was evident in controls, where postprandial AUC [change from baseline] psyllium 3.5 g t.d.s. led to an increase in postprandial SBWC compared to placebo (P<0.01), albeit less pronounced than that seen with 7 g t.d.s. (P<0.01 versus placebo). An increase with treatment compared to placebo was equally apparent in patients: median AUC for SBWC rose from 13.2 L.min (7.2 – 24.3) with placebo to 42.8L.min (24. – 49.1) with psyllium (P<0.05), with similar values seen in the paired analysis. In our previous work we described a fall in SBWC in the period 0-90 minutes after the test meal(20) but this did not occur with 7 g psyllium t.d.s. After the second meal of the study,

between 360 and 420 minutes, a fall in SBWC was seen with all treatments in both studies.

Ascending colon water content (ACWC) in the control study, where it was the designated primary endpoint, was significant greater in the postprandial phase after 7 g psyllium t.d.s. than placebo ($P < 0.0001$), with a clear dose-response relationship ($P < 0.001$, Table 2). In patients, postprandial ACWC was undetectable in most subjects taking placebo, with only 3 volumes >5mL recorded at any point. In this group AUC for postprandial change in ACWC was greater with psyllium ($P < 0.05$) but highly variable, with mean postprandial ACWC ranging from 0 – 57 mL, equivalent to 5-10% of colonic volume. Of note, postprandial colonic volumes did not change significantly from fasting baseline with any treatment in either study.

Relaxation Times T1 and T2

The AUC [change from baseline] for postprandial $T1_{AC}$ was greater with psyllium in both regions in both groups(Table 2). Figures 4B (controls) and 4C (patients) show the postprandial time course for $T1_{AC}$. The curves for 7 g psyllium t.d.s. show a postprandial increase that returns to fasting levels after 6 hours. A second rise then follows the second challenge meal. These rises did not occur with placebo, nor did $T1_{DC}$ demonstrate such a curve. AUC for patients' $T1_{DC}$ was higher in the ITT analysis but not significant so, although values were significantly greater than placebo in the paired analysis of patients, which was also true for controls taking 7g t.d.s. psyllium.The AUC [change from baseline] for postprandial $T2_{AC}$ was higher after both psyllium doses than placebo in controls, and also higher after psyllium in

patients in the paired analysis. Post-prandial $T2_{DC}$ was only found to be higher in patients on paired analysis (Supplementary Table 1).

Fecal Water, Bowel Habit and Symptoms

In controls, stool % water content was not higher after placebo treatment than at baseline (baseline median 72%, IQR 69 - 73 vs 73%, 69 – 77 on placebo, P=NSig Wilcoxon). Stool % water was higher than baseline after both psyllium 3.5 g t.d.s. (median 76%, 68 – 80, P<0.05 Wilcoxon) and psyllium 7 g t.d.s. (81%, 75 – 87, P<0.05 Wilcoxon). In patients, stool % water was no different at the start of the placebo and psyllium treatments: 66% (59 – 75) vs. 63% (60 – 70). In this group psyllium 7 g t.d.s increased stool % water by mean 6.2% (SD±7.2, P<0.01, paired t-test) but there was no change after placebo (mean decrease 0.2%, SD±10.0).

In the patient study stool frequency was similar during pre-treatment periods off laxatives and while taking placebo, but higher while taking psyllium (P <0.05 Wilcoxon, Figure 5A). Mean (SD) stool form (Bristol Stool Form Scale) on psyllium was 3.5 (0.83) and 2.6 (1.3) on placebo (P =0.07 Wilcoxon, Figure 5B).

Differences between controls and patients

Fasting scans showed a number of differences between controls and patients (Table 1). WAPS24 scores were greater for patients than controls, indicating slower transit as expected (Figure 2D). On placebo, fasting colon volumes were larger in patients than controls (median 745 mL, IQR 455 – 844 vs. 372, 284 – 601 P <0.05). Differences were primarily due to larger AC and TC in patients. Fasting T1 of chyme in both the AC and DC was shorter in patients than controls after placebo (both P

298 <0.05, Figure 4A). After psyllium, values in patients approached those seen in
299 controls on placebo.
300 Comparison of postprandial scans suggested differences between controls and
301 patients in their small bowel responses. Mean SBWC in the postprandial period (0-
302 420 minutes) in controls was 57 ±33 mL on placebo, rising to 106 ±74 mL on 3.5 g
303 psyllium t.d.s. and 147 ±78 mL on 7 g t.d.s. (Friedman's P <0.001). In comparison,
304 patients on placebo had a mean postprandial SBWC of 33 ±17 mL, rising to 81 ±41
305 mL on 7 g psyllium t.d.s. (P <0.001).
306
307 **Correlation of relaxometry with fecal water and symptoms**
308 *Post hoc* analysis of the combined data set for controls and patients showed a
309 correlation between fecal water content and fasting $T1_{DC}$ after placebo treatment
310 (figure 6A; Pearson's r = 0.65, P<0.001 two-tailed). Fasting $T1_{DC}$ also correlated with
311 stool frequency on treatment (Figure 6B; Pearson's r = 0.53, P <0.05 two tailed).
312
313 Controls reported minimal symptoms during the study days. In patients, scores were
314 also low, although fasting scores for bloating were higher after psyllium than placebo
315 (median 5, 0 – 27 vs. 1.5, 0 - 8, P<0.05 Wilcoxon) and remained higher throughout
316 the day.
317
318 **DISCUSSION**
319 By assessing baseline MRI parameters in healthy volunteers and in patients with
320 constipation, and demonstrating their responsiveness to psyllium therapy, our study
321 has not only demonstrated the value of MRI but also revealed some new findings
322 about constipation. The clinical use of psyllium is based on its capacity to bind water,

preventing absorption from the lumen. Consistent with this, postprandial small bowel water increased with psyllium in both patients and controls. It is worth noting that such a validated, non-invasive test may provide more representative data than older methods requiring aspiration of a non-absorbable marker as the aspiration catheter itself may stimulate intestinal activity, causing changes in absorption or secretion (9, 25).

Volume measurements demonstrated the bulking effect of psyllium. The increases seen exceeded our expectations, in some cases doubling fasting colonic volume, which may explain the bloating that some patients experience. Similar substantial increases in colonic volumes as assessed by MRI have been recently reported in response to high fibre diets by others (26).

By trapping water, the psyllium appears to abolish the immediate fall in SBWC caused by the rapid absorption of sucrose, glucose and water we have previously observed using the same test meal (20). The fall in SBWC after the second large 1000 kcal meal has been observed in most of our previous studies and we believe this reflects the gastro-ileal response to feeding as described previously (27, 28).

We were unable to confirm an effect of psyllium on transit time although there was a numerical decrease in transit scores in constipated patients. These findings are consistent with other studies of psyllium where its effect on transit is small or non-significant (18, 19, 23, 24). The increase in colonic volume offers an alternative explanation for how psyllium increases stool frequency since there is a greater mass

of stool to pass. Total flow (mass/ time) as assessed by daily stool output may, therefore, increase despite little change in speed through the gut (distance/ time).

Psyllium's main benefit may be through increased water content of colonic chyme and stool, making feces softer and hence easier to pass. Free water was more readily detected in the ascending colon of controls, and in both groups after psyllium, but in individual patients it was often undetectable. This may have resulted from avid water absorption in the constipated colon, or mixing of free water into the colonic contents where the MRI signal of the water gets quickly reduced by interactions with bacteria and tiny pockets of gas from fermentation.

A major finding in this study was the demonstration of the value of T1 in assessing colon contents. This has not been previously reported. While free water was only detectable in a few cases, the parameter T1, reflecting the physical and chemical environment of the water molecules, was readily measurable in all subjects and normally distributed. T1 largely reflects the freedom of water molecules to move so higher values should reflect increased water content of the chyme, as we have shown previously with Moviprep(16). This is borne out by our observations: values in the colon were greater proximally than distally, consistent with progressive water absorption during transit; values were lower in constipation than health but increased with psyllium, supporting the mechanism of action of psyllium as currently understood, and again suggesting that free water was mixed with the colonic contents. Further evidence for this effect is the postprandial rise in T1 seen in the ascending colon with psyllium during the study day. The increase in T1 seen is consistent with delivery into the colon of small bowel water that was prevented from

absorption by the presence of the fibre. Such an effect was not seen in the descending colon, being further removed from episodic influxes associated with feeding.

The correlation of T1 in the descending colon with both fecal water content and stool frequency supports the clinical relevance of the parameter. Many patients define their constipation by straining to pass hard, dry stools. Fecal water content can be measured directly but provides no information on changes more proximally in the colon. T1 provides a potential method to assess fecal consistency *in vivo*, and to act as a non-invasive parameter for evaluation of constipation therapies. The fact that ascending colon T1 is decreased shows that the dehydration in constipation is found not only in the stool but throughout the colon. This is concordant with earlier studies reporting slow orocaecal transit in constipation pointing to an important role for the small bowel in constipation(29).

A major limitation of MRI has been expense compared with scintigraphy which has an established track record of evaluation of a range of drugs designed to treat functional gastrointestinal disorders. However, as we show here, MRI does provide extra information on mode of action, particularly the impact on regional volumes and fluid distribution which can only be inferred from changes in transit. This will be of particular value in evaluating agents with novel modes of action like Tenapanor(30, 31), an inhibitor of the sodium-proton exchanger NHE3, or plant derived inhibitors of aquaporins like rhein(32).

Our study had limitations. Scanner failure and subject withdrawal reduced our numbers. Drop outs always raise the concern of selection so we report analysis of those 20 who completed the first arm of the cross-over as an ITT analysis. We also

performed a paired analysis limited to those who completed the protocol and on whom we had adequate scans; supplementary data tables provide these results. Reassuringly we find very similar results in the paired analysis to the ITT analysis suggesting that drop out was random and not a source of bias. Sample sizes were hence small, limiting statistical confidence, but our data will enable more accurate power calculations for future studies. Heterogeneity in response and variation in dietary fibre intake may have obscured a treatment effect. Comparisons between the studies may be affected by differences in age and gender since controls were younger and predominantly male, while patients were mainly female. In a previous study increased height was associated with larger colons, suggesting larger colons in men, although height-standardised colons in women were larger(12). More data will be needed to understand the impact of age and gender compared to other physiological factors.

One advantage of the crossover designs used, and therefore reason to report both ITT and paired analyses, is the reduction of such sources of variation in assessment of an intervention's effect. Such a design is less practical for trials where symptoms are the primary endpoint as establishment of a symptom pattern generally takes time. An objective point metric, such as volume or chyme T1, avoids this delay in assessment and so allows shorter periods of intervention and washout.

There may have been other opportunities to introduce bias: blinding was imperfect due to the nature of the intervention and compliance cannot not be guaranteed without direct observation. These issues are readily addressable with pharmaceutical therapies where plasma drug concentrations can be measured. The objective nature

of MRI outcomes somewhat mitigated these issues compared to other methods of assessing treatment efficacy particularly since MRI analysis was undertaken by a single operator (KAM) to reduce variation. Our previous work on intra-observer variation in colonic volume measurement found a coefficient of variation <5%. Fasting SBWC values were lower than we observed previously (13, 20) which may be a result of our small sample size here.

In a short scanning session we assessed fasting volume, relaxation times and transit. Transit itself reflects the composite effects of propulsive forces, volume of material and resistance to flow. The two patients with the longest $T1_{AC}$ both had a transit score in the normal range, and may have a different aetiology for their symptoms that would respond to different treatment. High scores for bloating and flatulence during the psyllium study day, but not during the placebo day, were also seen. Objective assessment of physiological changes offers the chance to further separate out disorders of the defecatory process from visceral hypersensitivity, as set out in the Rome IV criteria(1). The prospect of assessing, and predicting, response to therapy with a single MRI may be enhanced further by developments such as assessment of colonic wall motion through cine MRI(33, 34).

These techniques require further validation in larger cohorts and randomised controlled trials. Nevertheless, this work clearly demonstrates the potential of a comprehensive MRI panel to measure objective differences in luminal content between controls and patients with chronic constipation, both in their natural state and in response to therapeutic modulation. This might be of particular value in demonstrating the site of action of secretogogues now being introduced into therapy.

The application of MRI has the potential to generate new insights into intestinal function, provide a platform for early phase evaluation of new treatments and provide an objective approach to evaluation of patients with functional disorders not responding to simple empirical therapy.

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AUTHOR CONTRIBUTIONS: RCS obtained funding; RCS, JMJ, LM, PAG, GM, AS-S, CK, GS, CLH and KAM were involved in study concept and design. KAM collected the data and supervised the study days. GM, KAM, CLH and GS were responsible for analysis, GM, KAM, LM, PAG and RCS were responsible for interpretation of data. GM and KAM had primary responsibility for writing the manuscript. RCS was responsible for the final content. All authors contributed to the completion of the manuscript and read and approved the final version.

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599 # SUPPORTING INFORMATION

600 ## Study 1 CONSORT diagram

601

602 **Enrolment**

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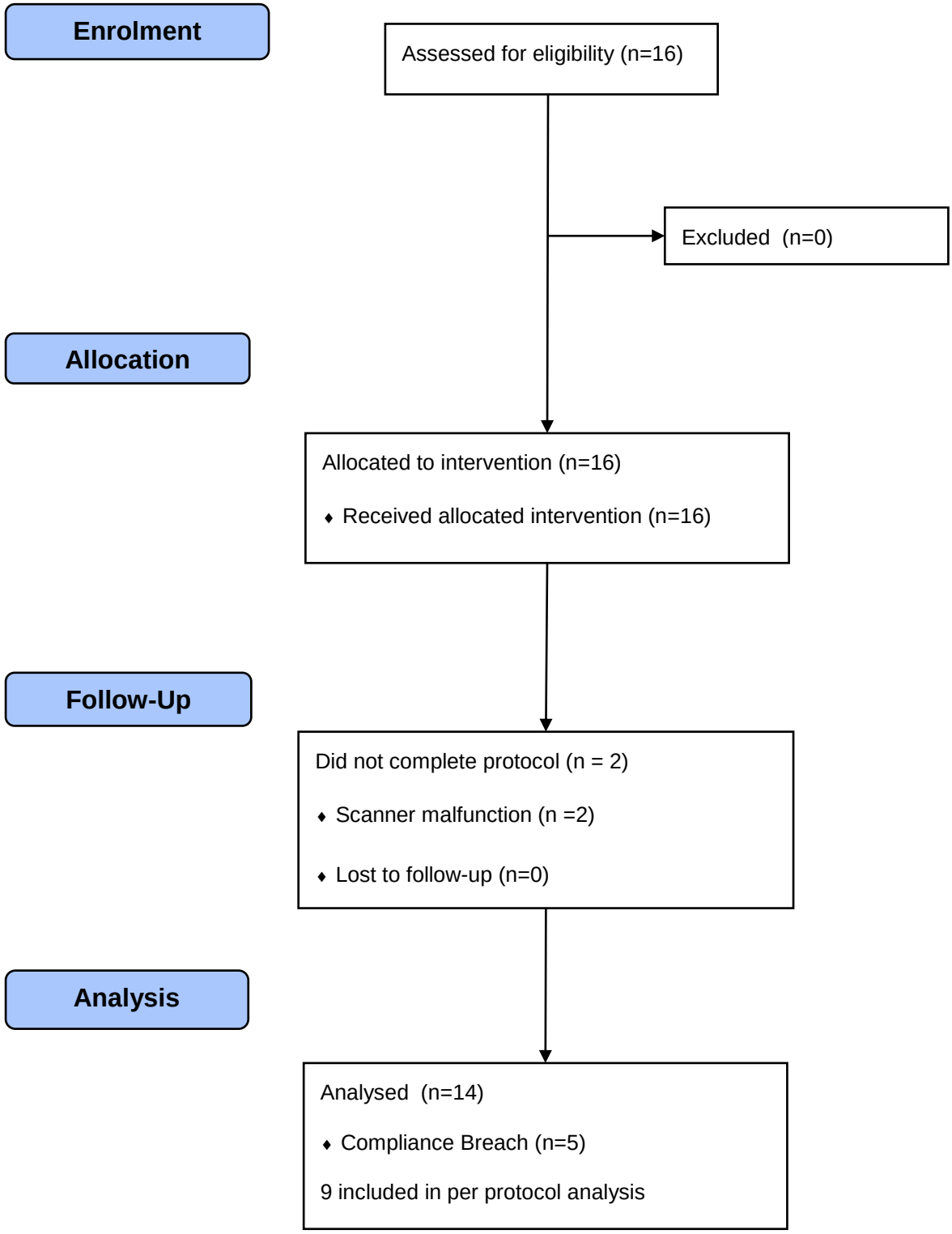
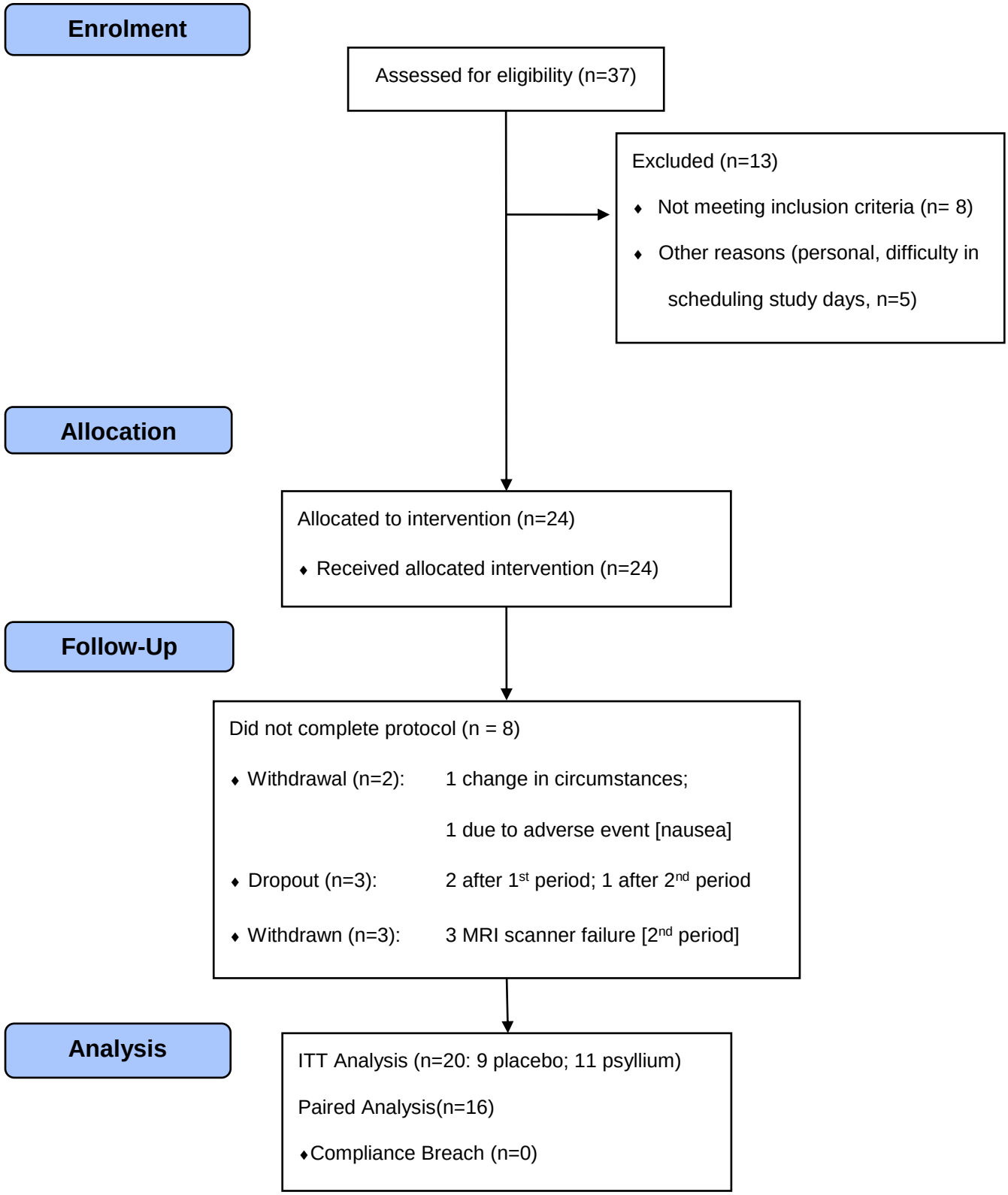
604



Study 2 CONSORT diagram



613 **Demographics of Participants**

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Median and interquartile range (Q25 – Q75)	**Study 1: CONTROLS**	**Study 2: PATIENTS**
Age	23.5 (21.25 – 25)	39 (26 – 47.5)
Gender (M:F)	12 : 4	2 : 22
Body Mass Index ($kg.m^{-2}$)	22.5 (21.2 – 25.3)	25.9 (21.4 – 28.8)
Current smoker (Y:N)	0 : 16	6 : 18
HADS Anxiety Score (normal <8)	4 (2.25 – 5.75)	4.5 (3 – 6.75)
HADS Depression Score (normal <8)	1 (0 – 1.75)	2 (0 – 4.75)
Patient Health Questionnaire (PHQ-12)	2 (1 – 3)	3.5 (1 – 6.5)

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616

MRI protocol and analysis

All images were acquired using a whole-body 1.5T scanner (Achieva, Philips Medical System, Best, The Netherlands). Each imaging period lasted for 15 – 20 minutes and volunteers were positioned supine in the magnet with a 16-element receiver coil wrapped around the abdominal region. Between scans, patients and volunteers were asked to sit upright away from the scanner.

The volume of freely mobile water in the small bowel (Small Bowel Water Content, SBWC) was measured and analysed as described previously (1) using a coronal single-shot turbo spin-echo sequence, acquiring 24 slices in a single 24 second breath hold (TR/TE_{eff} = 8000/320 ms, 512x512 reconstructed matrix, reconstructed voxel size 0.78x0.78x7 mm^3). Ascending colon water content (ACWC) was measured and analysed similarly. Colonic volume measurements, as described in detail previously (1, 2), were obtained using a coronal dual-echo gradient echo sequence (TR/TE1/TE2 = 157/2.3/4.6 ms, 256x256 reconstructed matrix, reconstructed voxel size1.76x1.76x7 mm^3).

Transit times were measured and scored as described previously (3) using a T_1 weighted 3D FFE sequence (TE/TR = 1.5/3.8 ms, FA = 10^o, FOV= 250 x 371x 200 mm^3) and the ascending and descending colon relaxation times were acquired using a single slice bTFE sequence. T_1 data were acquired with a preparatory 180^o inversion pulse at 8 different inversion times (TI) ranging from 100 – 5000 ms (4), while T_2 data were obtained with a preparatory spin echo pulse before acquiring from 10 different echo times (TE) ranging from 20 – 637 ms (5). For both sequences,

there was a 10 second gap between each acquisition to allow the system to return to equilibrium. Small regions of interest were drawn on the resulting images to calculate the mean signal intensity for the region at each different TI or TE at the top, middle and bottom of the colonic segments. The relaxation times were determined by fitting the signal intensity data to a model of the signal evolution of the tissue after application of all the preparation and imaging radio-frequency pulses.

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TABLES

Table 1: Variables measured on fasting MRI after 5 days treatment

Median (Q25-Q75)	CONTROLS			PATIENTS †	
	Maltodextrin	**Psyllium 10.5g/d**	**Psyllium 21g/d**	**Maltodextrin n = 9**	**Psyllium n = 11**
WAPS24 (transit)	1.0 (0.1 – 2.2)	1.4 (0.2 – 2.1)	0.6 (0 – 1.9)	4.2 (3.2 – 5.3)$^{+}$	2.0 (1.5 – 4.0)
Small Bowel Water Content (mL)	51 (15 – 75)	58 (15 – 138)	47 (42 – 157)	33 (9 – 90)	54 (24 – 77)
Relaxation times (ms)					
$T1_{AC}$	720 (572 – 904)	690 (594 – 911)	966 (667 – 1093)	509 (472 – 670)$^{+}$	890 (478 – 1030)
$T1_{DC}$	440 (352 – 884)	570 (473 – 700)	763 (575 – 985)	213 (176 – 420)$^{+}$	590 (446 – 1338)**
$T2_{AC}$	70 (56 – 79)	73 (62 – 86)	83 (67 – 88)	58 (42 – 73)	72 (51 – 105)
$T2_{DC}$	53 (40 – 67)	54 (45 – 70)	74 (56 – 80)	42 (34 – 52)	66 (54 – 86)**
Colon Volume (mL)					
AC	138 (114 – 208)	213 (152 – 285)*	251 (191 – 301)**	270 (174 – 361)$^{+}$	390 (320 – 412)
TC	132 (99 – 188)	215 (119 – 332)**	228 (163 – 362)**	362 (221 – 438)$^{++}$	366 (267 – 547)
DC	111 (60 – 185)	142 (117 – 213)	132 (87 – 225)	221 (130 – 278)	246 (221 – 336)
Total	372 (284 – 601)	559 (411 – 807)**	578 (510 – 882)**	831 (745 – 934)$^{++}$	1104 (847 – 1316)*

WAPS24 = weighted averaged position score at 24 hours(15). AC = ascending colon; TC = transverse colon; DC = descending colon.

† ITT analysis

Within-group comparison to maltodextrin *P<0.05; **P<0.01; ***P<0.001.

Between-groups comparison (controls vs. patients) of maltodextrin results $^{+}$P<0.05, $^{++}$P<0.01

Table 2: Area Under the Curve of variables measured on postprandial MRI scans during day 6 of treatment

Median (Q25-Q75) or Mean (±SEM)	CONTROLS			PATIENTS †	
	Maltodextrin	**Psyllium 10.5g/d**	**Psyllium 21g/d**	**Maltodextrin n = 9**	**Psyllium n = 11**
Free Water AUC (L.min)					
ACWC	0.2 (0.1 – 0.6)	4.0 (2.4 – 7.0)**	7.4 (2.8 – 16.5)**	0.13 (0.01 – 0.66)	3.41 (0.10 – 7.69)
SBWC	21.3 (12.9 – 33.7)	28.7 (25.2 – 64.5)**	46.5 (37.0 – 82.8)**	13.2 (7.2 – 24.3)	42.8 (24.0 – 49.1)*
Relaxation times AUC (s.min)					
$T1_{AC}$	215 ± 18	303 ± 18 *	374 ± 23***	247 (205 – 306)	411 (265 – 513)*
$T1_{DC}$	160 ± 15	188 ± 17	277 ± 35*	94 (76 – 211)	275 (210 – 377)
$T2_{AC}$	21 ± 2	30 ± 1**	38 ± 2****	25 (20 – 30)	34 (32 – 50)
$T2_{DC}$	17 (13 – 22)	18 (16 – 22)	24 (20 – 25)	18 (16 – 22)	25 (24 – 29)

AC = ascending colon; TC = transverse colon; DC = descending colon. WC = water content

Units of area under the curve expressed as function of time, either litre.minutes or, for relaxation times, second.minutes

† ITT analysis

All comparisons to Maltodextrin *p < 0.05; ** p< 0.01; ***p<0.0005 ; ****p<0.0001

Comparisons by Wilcoxon signed rank test or paired t-test

673 **Supplementary Table 1: Paired analysis of patient data**

674 Paired analysis of fasting and post-prandial MRI variables from patients who undertook treatment periods of both psyllium 7g t.d.s.

675 and placebo (maltodextrin) 7g t.d.s. for 6 days (n = 16).

676

Median (Q25-Q75) or Mean (±SEM)	FASTING			POST-PRANDIAL		
	Maltodextrin	**Psyllium 21g/d**			**Maltodextrin**	**Psyllium**
WAPS24	3.4 (1.6 – 4.8)$^{+}$	2.2 (1.5 – 3.0)				
SBWC	32 (11 – 71)	32 (15 – 60)		**SBWC (L.min)**	13.8 ± 1.8	34.2 ± 4.3 ***
Colonic Volume (mL)	745 (455 – 844)$^{++}$	951 (849 – 1233)***		**ACWC (L.min)**	0.02 (0.001 – 0.1)	1.13 (0.3 – 7.4)**
AC	241 (173 – 296)$^{+}$	370 (260 – 415)**				
TC	242 (152 – 372)$^{++}$	404 (287 – 537)**				
DC	173 (116 – 218)	232 (217 – 320)**				
Relaxation times (ms)				**Relaxation times AUC (s.min)**		
$T1_{AC}$	550 (492 – 609)	820 (440 – 1136)*		**$T1_{AC}$**	232.8 ± 14.8	386.3 ± 35.8 **
$T1_{DC}$	230 (187 – 549)$^{+}$	566 (319 – 778)**		**$T1_{DC}$**	143.3 ± 23.1	247.6 ± 30.3 ***
$T2_{AC}$	62 (45 – 70)	72 (54 – 94)*		**$T2_{AC}$**	25.5 ± 1.0	36.8 ± 2.8 **
$T2_{DC}$	44 (38 – 58)	58 (50 – 67)		**$T2_{DC}$**	19.6 ± 1.0	23.7 ± 1.1 ***

WAPS24 = weighted averaged position score at 24 hours(15). AC = ascending colon; TC = transverse colon; DC = descending colon.

Within-group comparison to maltodextrin *P<0.05; **P<0.01; ***P<0.001 (two-tailed).
Between-groups comparison (controls vs. patients) of maltodextrin results $^{+}$P<0.05, $^{++}$P<0.01

FIGURE LEGENDS

Figure 1: Schematic of events during each treatment period.

Each such period was separated by a washout period of at least one week in the control study. In the patient study, subjects additionally recommenced usual laxative use for at least 10 days, followed by 8 days off laxatives prior to each study treatment.

Figure 2: Changes in MRI parameters of volume and transit.

A & B) Small bowel water content during a study day in A) controls, B) patients treated with placebo (maltodextrin 7 g), psyllium 3.5 g or psyllium 7 g three time daily. Ingestion of doses marked by an arrow ↓.

C) Fasting colonic volume after 5 days treatment.

D) WAPS24 transit score after 5 days treatment. Higher scores reflect slower transit.

Figure 3: Changes in ascending colon content in response to 5 days psyllium

A) Single shot balanced gradient echo sequence (bTFE/ TrueFISP) showing increase in fasting volume in one patient after 5 days treatment with psyllium 7 g t.d.s compared to maltodextrin placebo.

B) Heavily T2-weighted single shot fast spin sequence (RARE/ SSFSE) showing excess colonic water content in one patient after 5 days treatment with psyllium 7 g t.d.s compared to maltodextrin placebo.

Figure 4: changes in MRI relaxometry parameters

A) T1 of the chyme in the ascending colon and descending colon after 5 days treatment.

B & C) T1 of the ascending colon during a study day in B) controls, C) patients treated with placebo (maltodextrin 7 g), psyllium 3.5 g or psyllium 7 g t.d.s. Ingestion of doses marked by an arrow ↓.

Figure 5: changes in stool frequency and form

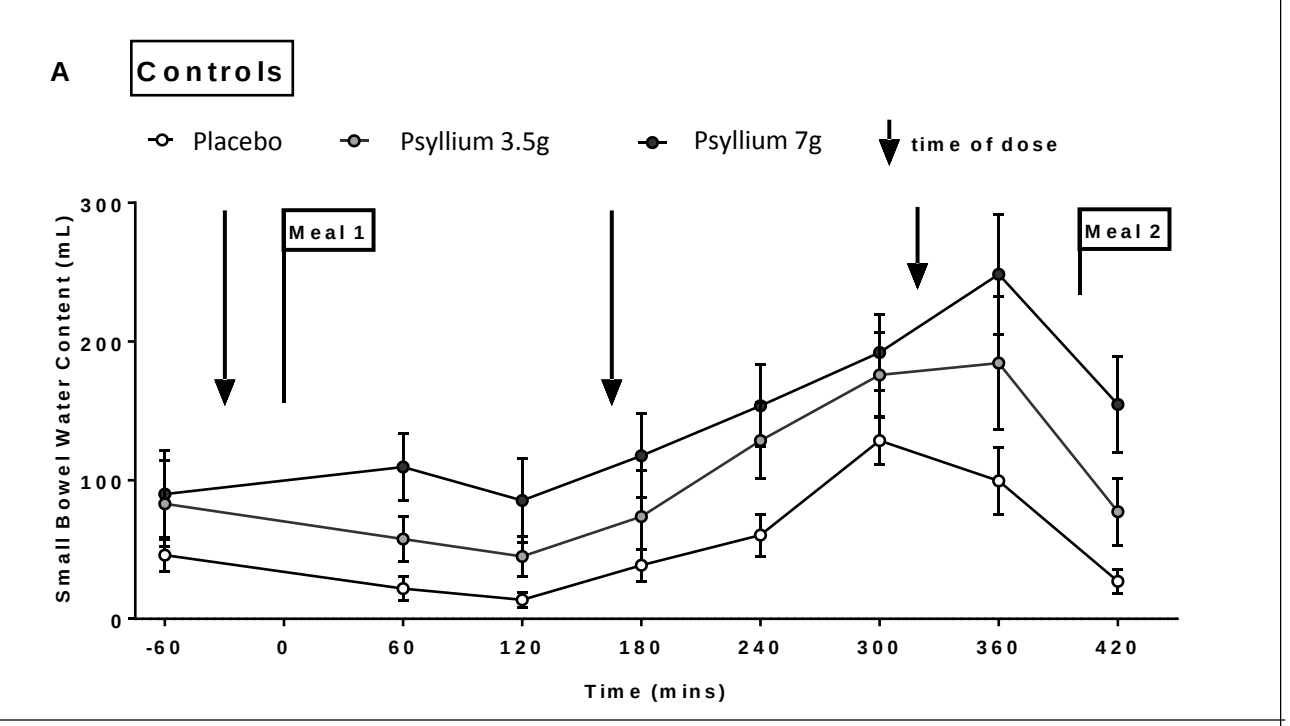
A) Mean stools frequency for patients during 6-day run-in periods (baseline) and 6 days on treatment with maltodextrin placebo or 21g/day psyllium

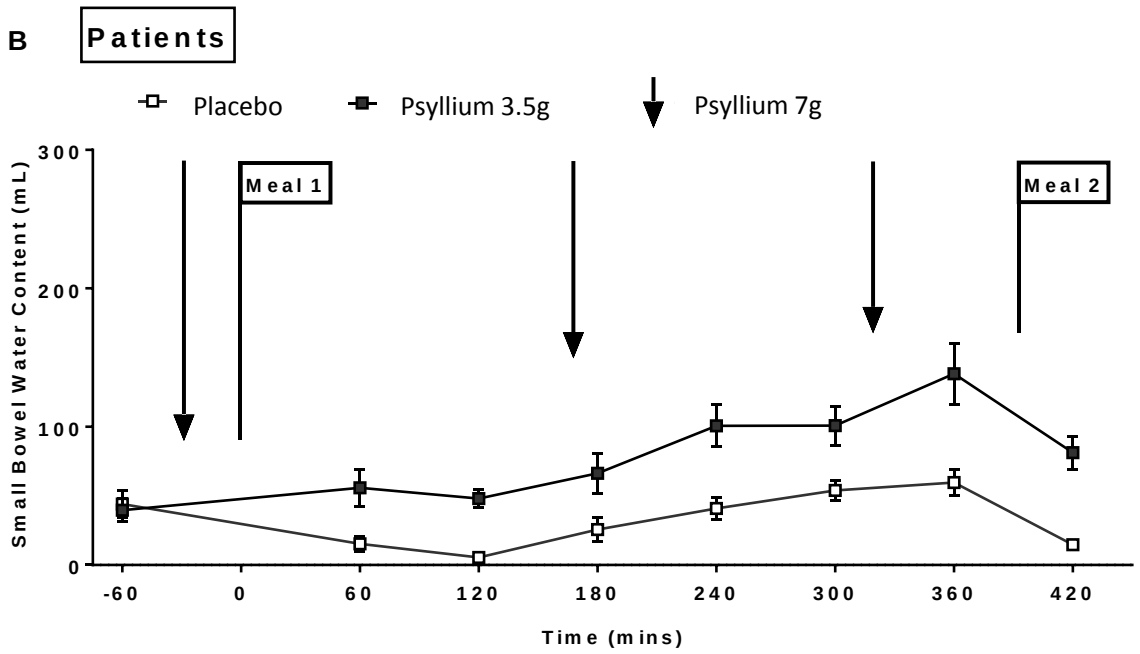
B) Mean stool consistency according to the Bristol Stool Form Scale

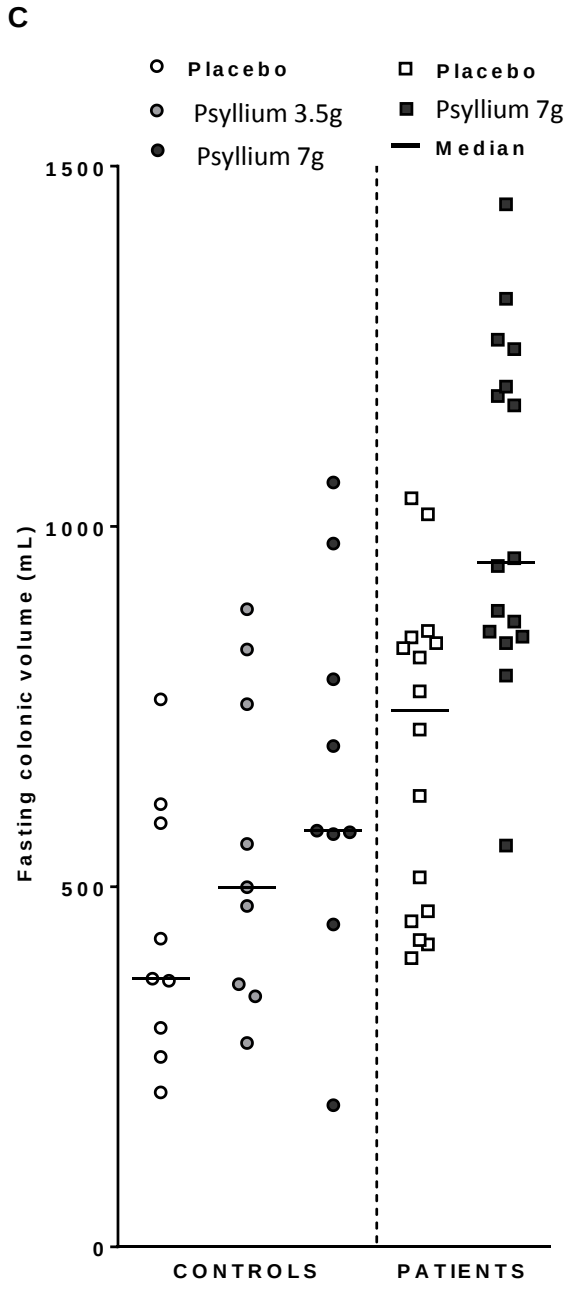
Figure 6: Correlations of relaxometry with fecal water content and stool frequency

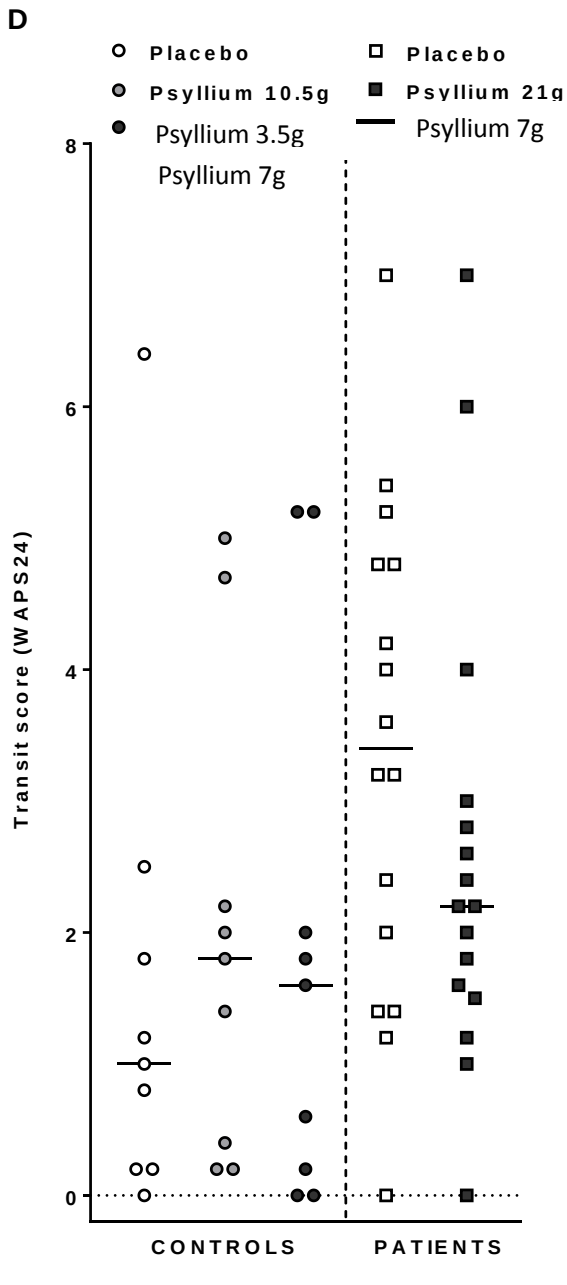
A) Fasting T1 relaxometry of descending colon content ($T1_{DC}$) plotted against fecal water content measured by freeze drying, in controls and patients after 5 days treatment with maltodextrin placebo.

B) Mean stool frequency plotted against fasting T1 relaxometry of descending colon content ($T1_{DC}$)

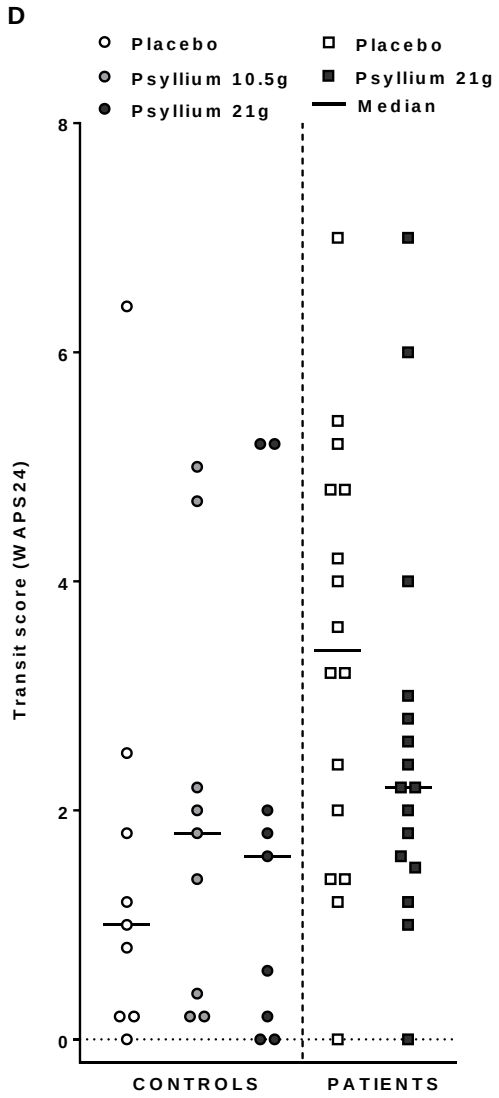

A
Controls
Placebo
Psyllium 3.5g
Psyllium 7g
time of dose
Meal 1
Meal 2
Small Bowel Water Content (mL)
300
200
100
0
-60
0
60
120
180
240
300
360
420
Time (mins)


B
Patients
Placebo
Psyllium 3.5g
Psyllium 7g
Meal 1
Meal 2
Small Bowel Water Content (mL)
300
200
100
0
-60
0
60
120
180
240
300
360
420
Time (mins)


C
Placebo
Psyllium 3.5g
Psyllium 7g
Placebo
Psyllium 7g
Median
Fasting colonic volume (mL)
1500
1000
500
0
CONTROLS
PATIENTS


D
Placebo
Psyllium 10.5g
Psyllium 3.5g
Psyllium 7g
Placebo
Psyllium 21g
Psyllium 7g
Transit score (WAPS24)
8
6
4
2
0
CONTROLS
PATIENTS

D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Psyllium 10.5g
Psyllium 21g
Placebo
Psyllium 21g
Median
Transit score (WAPS24)
8
6
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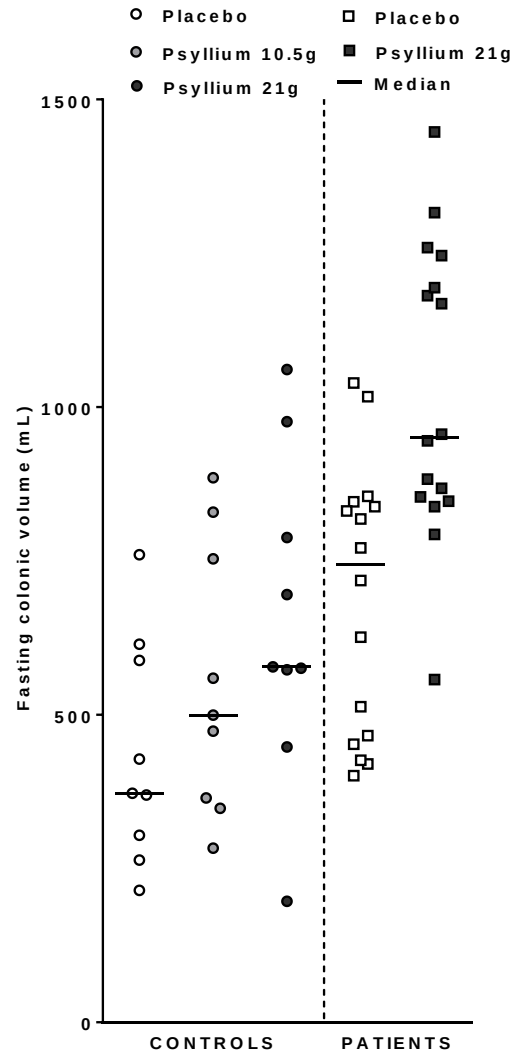
CONTROLS
PATIENTS

C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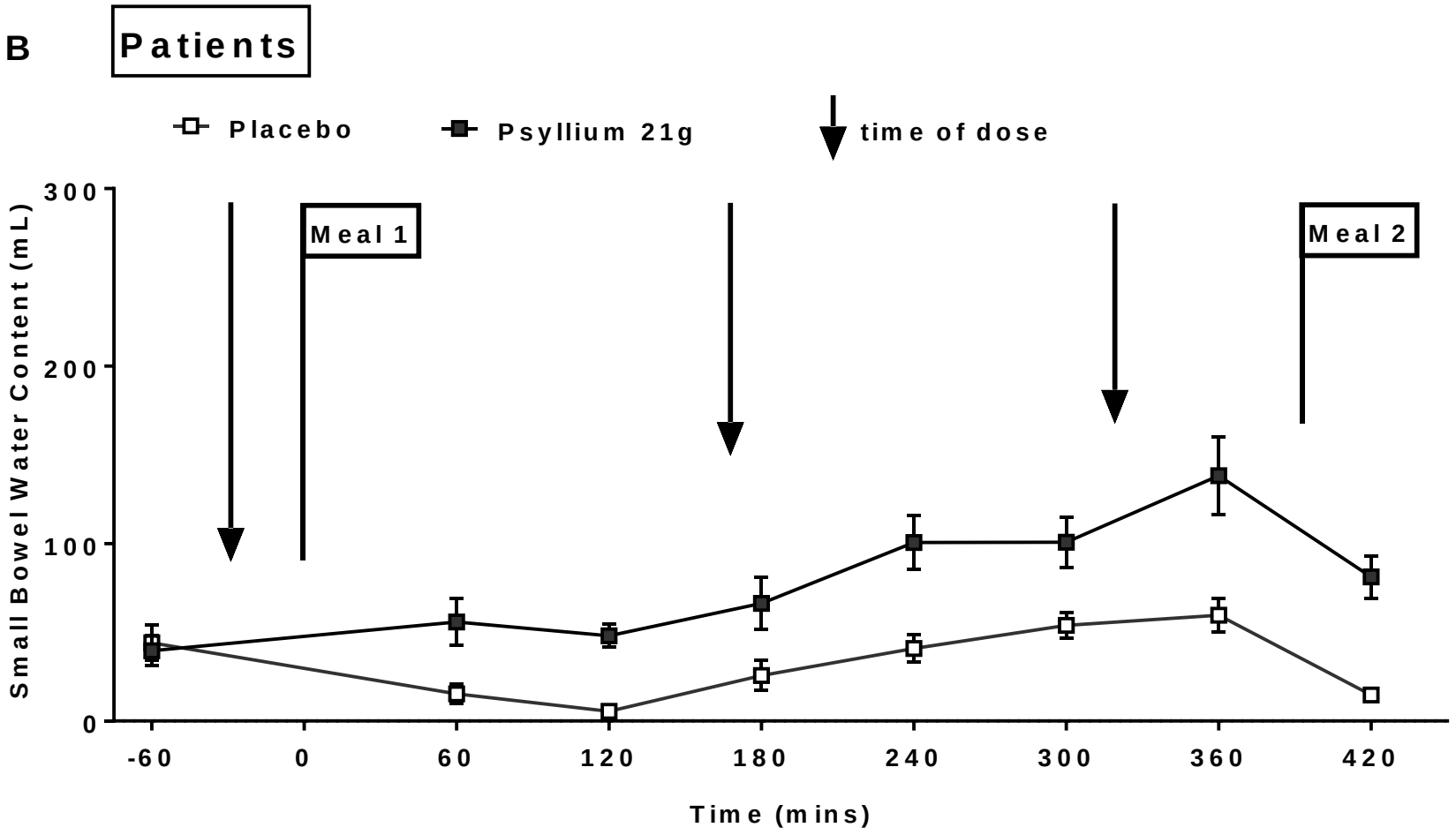
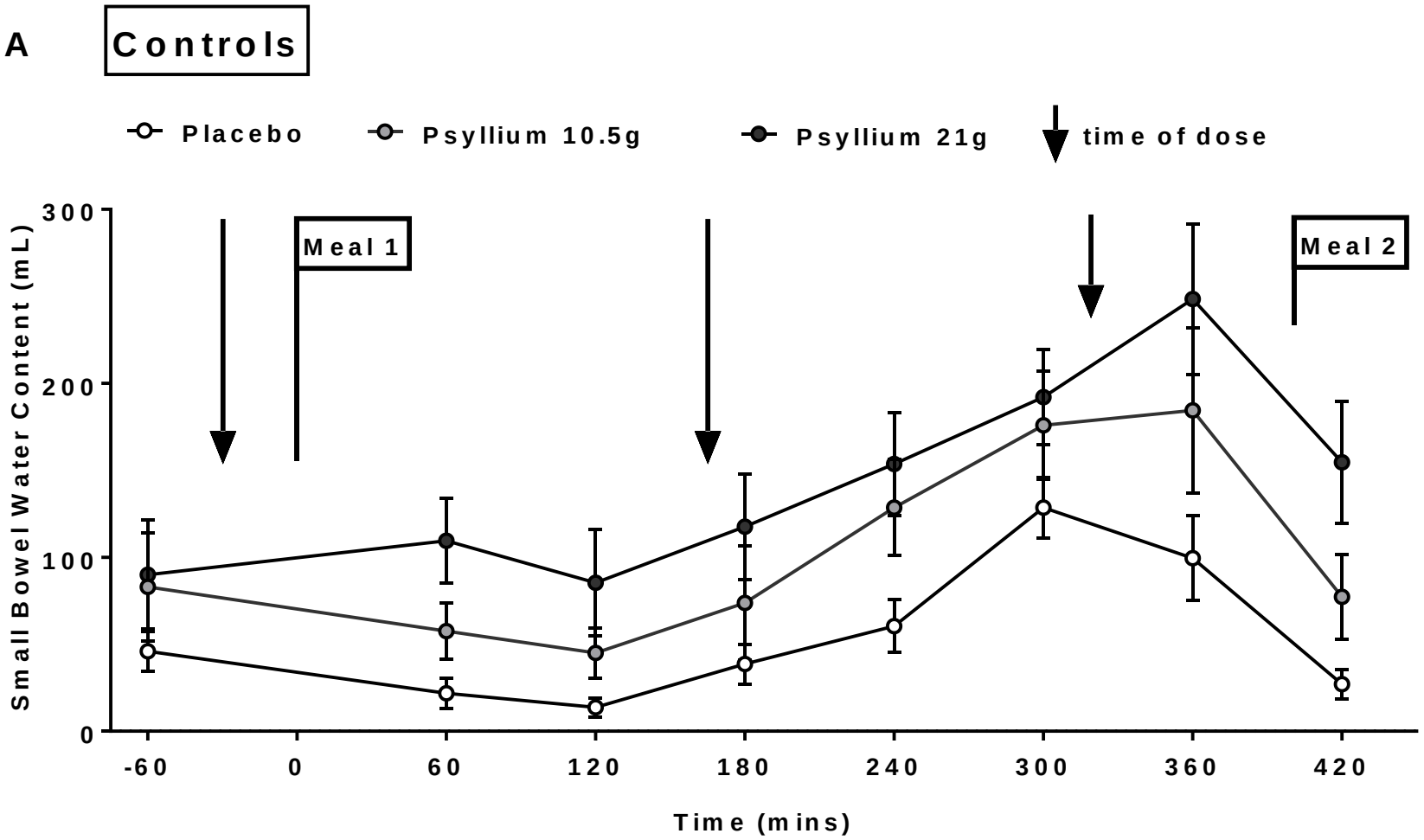
Psyllium 21g
Placebo
Psyllium 21g
Median
1500
1000
500
0
Fasting colonic volume (mL)
CONTROLS
PATIENTS


B
Patients
Placebo
Psyllium 21g
time of dose
Meal 1
Meal 2
Small Bowel Water Content (mL)
300
200
100
0
-60
0
60
120
180
240
300
360
420
Time (mins)


A
Controls
Placebo
Psyllium 10.5g
Psyllium 21g
time of dose
Meal 1
Meal 2
Small Bowel Water Content (mL)
300
200
100
0
-60
0
60
120
180
240
300
360
420
Time (mins)