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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WORD COUNT: 3781

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.

1 **INTRODUCTION**

2 A major challenge in functional gastrointestinal disorders has been to develop

3 objective biomarkers that can be used to assess treatments more economically than

4 symptoms, which typically show very wide variability(1). Assessment of the small

5 bowel and proximal colon has been particularly difficult owing to its inaccessibility.

6 Capsule technologies, such as endoluminal image analysis(2) and pH monitoring,

7 provide some information on motility and transit. Scintigraphy has been extensively

8 used over the last three decades to show changes in transit through the different

9 regions of the colon(3). It has correctly predicted efficacy in a range of medications

10 with different modes of action on bowel function(4). It has shown acceleration of

11 transit in constipation by stimulant laxatives such as bisacodyl(5) and secretogogues

12 such as lubiprostone(6) however it cannot directly demonstrate whether any effects

13 are due to changes in the balance of absorption/ secretion of fluid from the lumen or

14 changes in motility(7). Transit has been shown to account for 19-27% of the

15 variance in stool form(3) indicating that there are other important parameters in

16 predicting bowel function that may be measurable.

17 Up to 10L/ day of fluids move into, through and out of the gut lumen(8) but

18 objectively measuring this is difficult and requires intestinal intubation which, as we

19 and others have shown, alters fluid volumes significantly(9). Disorders of fecal water

20 content and/ or bowel habit, either diarrhoea or constipation, may result from an

21 imbalance of secretion/ absorption rather than just abnormal motor function.

22 Recently a number of new treatments for constipation stimulating intestinal water

23 secretion have been introduced (10, 11) but assessment of the resulting changes in

24 intestinal fluid content has not previously been possible.

25 We believe this deficit could be corrected using magnetic resonance imaging (MRI)

26 based methods to characterise the contents of patients' small bowel and colon, and

27 to measure whole gut transit time as previously described (12-15). We have also

28 used the relaxation times T1 and T2 to characterise luminal contents (16, 17). T1

29 and T2 are the time constants with which the magnetization of material in the

30 scanner returns to baseline after excitation by the radiofrequency pulse. They are

31 sensitive to the physical and chemical environment of the water protons via

32 interactions with surrounding molecules and in the context of colonic chyme, are

33 expected to fall with a reduction of water associated with more solid chyme.

34 The aim of these studies was to assess the value of these MRI biomarkers by

35 investigating their responsiveness to a well characterised laxative, psyllium husk

36 (ispaghula). Its water-holding properties are known to increase fecal water content

37 and 24-hour fecal weight but reports of its effect on whole gut transit time are

38 inconsistent(18, 19). In some patients it produces unacceptable bloating but whether

39 this reflects distension of intestinal lumen was uncertain.

40 We present two studies, investigating the MRI changes induced by psyllium in

41 healthy volunteers, without constipation, and in patients with constipation. Our

42 hypotheses were that MRI could detect an increase in small bowel water content

43 caused by psyllium preventing water absorption. Furthermore, as this "trapped"

44 water enters the colon, colonic water content would increase.

45

46 **MATERIALS AND METHODS**

47 **Study Design**

48 Two studies were conducted: study 1, performed first, recruited adults without GI

49 disorders (controls); study 2, using similar methodology (see below), recruited adults

50 with chronic constipation (patients). Both were designed as placebo-controlled

51 crossover studies and were conducted according to Good Clinical Practice as

52 determined by the declaration of Helsinki. All authors had access to the study data,

53 and reviewed and approved the final manuscript. The protocols were approved by

54 institutional and national review boards respectively, and prospectively registered on

55 www.clinicaltrials.gov (NCT01805999 and NCT02144376). All subjects gave written

56 informed consent.

57

58 In study 1 three treatments were taken in random order: a placebo (maltodextrin)

59 and two different doses of psyllium. Treatments were separated by washout periods

60 of one week. Investigators were blind to the order of intervention. The patient study,

61 study 2, was designed to be less burdensome and so used only two treatments:

62 placebo and high dose psyllium. In order to ensure return to baseline and avoid any

63 carryover effect from previous treatment in the patient group, both treatment periods

64 were preceded by ≥10 days of usual laxative use, then 8 days without laxatives other

65 than rescue therapy. Rescue therapy (oral bisacodyl 5mg) was permitted in patients

66 who had not opened their bowels for 3 days and were experiencing distressing

67 symptoms, but not in the 48 hours before MRI scans.

68

69 **Study Populations**

70 Controls were recruited through general advertisement between May and August

71 2013. Non-smokers aged 18–65 with BMI 18–30 kg·m^{-2} were eligible. Exclusion

72 criteria included: any given history of GI disease or surgery; antibiotic or probiotic

73 use in the 4 weeks before the study; heavy alcohol intake; pregnancy; lactation;

74 excessive exercise; inability to discontinue medicines likely to alter gastrointestinal

75   transit. Patients were recruited between March 2014 and January 2015 through

76   hospital clinics and advertisement. Eligibility criteria included age ≥18 and a

77   diagnosis of chronic constipation, defined as meeting Rome III criteria for either

78   functional constipation (FC) or constipation-predominant irritable bowel syndrome

79   (IBS-C). Subjects had to pass at least one bowel motion weekly on usual laxatives.

80   Exclusion criteria in addition to those for the control study were: use of morphine or

81   similar opioids; use of open-label psyllium; inability to cease regular laxative use.

82   Patients also underwent a screening period of 2 weeks off laxatives to document

83   normal bowel habit.

84

85   **Treatments and Procedures (Figure 1)**

86   The active treatment used was Metamucil® Original Coarse Fiber (P&G, Cambridge

87   MA USA), a powder containing approximately 3.4g psyllium per 7 g product.

88   Maltodextrin (The Hut Group, Northwich UK) was used as the placebo control.

89   Subjects took 14g of powder three times daily (t.d.s.), either 14 g maltodextrin, 14

90   gm Metamucil (providing 7g psyllium), or a 50:50 mixture 7 g maltodextrin and 7 g

91   Metamucil (providing 3.5g psyllium) , each dose taken with 250mL water. Henceforth

92   in the text we refer to the psyllium doses by their psyllium content i.e. 3.5 or 7 g.

93   Blinding of subjects and investigators was ensured by providing the powders in

94   opaque containers, labelled by independent staff and provided in sealed bags so

95   they could not be recognised. The treatment allocation was according to a computer

96   generated randomisation code. Investigators were blind to the intervention. Subjects

97   were not told which intervention they were taking in any treatment period, although

98   powders did differ subtly in appearance and texture.

99

100 In each treatment period subjects took the powder for 6 days. Subjects measured

101 out their doses using a plastic spoon and kept a diary of their treatment compliance.

102 Compliance was also assessed by measurement of the total weight of powder

103 consumed, expressed as a % of that expected if compliance was complete.

104 Compliance of 60% was considered acceptable as >12g psyllium daily would be

105 expected to exert some effect. Subjects kept a daily diary of abdominal symptoms

106 and bowel habit.

107

108 On the morning of treatment day 5, subjects swallowed five identical transit markers:

109 cylinder-shaped inert capsules containing 0.4mL 15µM gadoteric acid, a positive MRI

110 contrast agent(15). Ingestion was confirmed in patients by direct observation or via a

111 time-stamped video. On day 6 all subjects attended at 8am, fasted. After an initial

112 MRI scan, during which the intra-luminal position of the transit markers was

113 documented, subjects consumed their morning dose followed by a 330kcal standard

114 rice pudding meal(15, 20). Scans were taken at hourly intervals for 7 hours. Doses of

115 psyllium/ placebo were repeated 165min and 320min after the meal. A second 1000

116 kcal meal was consumed before the final scan. Subjects were scanned supine.

117 Images were acquired using a 1.5T scanner (Achieva, Philips Medical System, Best,

118 The Netherlands). All MRI parameters were measured during a single 15 minute

119 episode in the scanner at each time point. Full details of the MRI methodology are

120 given in the supplementary appendix.

121

122 In the control study, fecal samples for measurement of stool water were taken at

123 enrolment and after the final MRI scan of each treatment period. Patient samples

124 were collected during the run-in period without laxatives before each treatment, and

125 after at least 72h of treatment. Bisacodyl rescue therapy was not permitted in the 48

126 hours prior to MRI scanning.

127

128 **Endpoints**

129 All endpoints were MRI parameters unless reported otherwise. In the control study

130 the primary endpoint was ascending colon free water content (ACWC). Secondary

131 endpoints included: small bowel free water content (SBWC); colonic volume, defined

132 as the sum of the segmental volumes of the ascending, transverse and descending

133 colon (AC, TC, DC); the weighted average position score of the transit markers at

134 24h (WAPS24). This was calculated using the formula (sum of the segment number

135 X the number of markers in each segment divided by the total number of segments)

136 as described previously(15) such that a higher score denotes slower whole gut

137 transit.

138

139 Relaxation times (T1 and T2) of the chyme in the AC and DC were also measured.

140 T1 (longitudinal relaxation time) depends upon the mobility of the water molecules as

141 does T2 (transverse relaxation time) which also depends on exchange between

142 water molecules and surrounding macromolecules. Therefore both of these

143 parameters are expected to decrease as the colonic content becomes more solid.

144 Percentage fecal water content was also determined by freeze drying the stool.

145 Symptoms of flatulence, bloating, abdominal pain and diarrhoea were monitored

146 between MRI scans using 0-100 visual analogue scales(21).

147

148 In the patient study, the primary endpoint was the weighted average position score of

149 the transit markers at 24h (WAPS24). Secondary endpoints included SBWC, ACWC,

150   colonic volume, T1 and T2 of the colonic chyme, and percentage fecal water content.

151   Stool diaries were kept for the period off laxatives immediately before each treatment

152   and during the treatment itself.

153

154   **Sample size and Statistical analysis**

155   Sample size calculation for the control study was based on pilot data in healthy

156   volunteers from a previous study of ACWC(13). Nine subjects would be required in a

157   crossover design to detect an increase in post prandial area under the curve (AUC)

158   of ACWC of 15 L.min (an increase of approximately 10%) with 90% power and

159    $P<0.01$. To allow for withdrawal and noncompliance, 16 subjects were recruited. In

160   the patient study the primary endpoint was WAPS24 since we had pilot data on this

161   endpoint in a relevant patient group by which to power our study. We found, using

162   our MRI marker method, a transit time of mean (SD) 69.2 (32.6) hours in IBS-C(22).

163   We calculated that a study with 20 subjects would have 80% power to detect a

164   change of 21 hours or 30% which is similar to the changes previously reported in

165   constipated subjects treated with psyllium (23, 24) and judged to represent a minimal

166   clinically significant difference. 24 subjects were recruited to allow for attrition.

167

168   Fasting parameters were compared between treatments. Postprandial endpoints

169   were compared using AUC. Data are presented as median (interquartile range) or

170   mean ($\pm$SD). Paired differences were assessed for normality using the Shapiro-Wilk

171   test. 1-way ANOVA with post-hoc Tukey's multiple comparisons test or 1-way

172   Friedman's test followed by Dunn's multiple comparison test were also carried out

173   when appropriate. Data are presented as median (interquartile range) or mean

174   ($\pm$SD). For controls, only subjects with data from all treatment periods are

175   presented. For patients, an intention-to-treat analysis is reported, taking data from

176   subjects who had at least one MRI parameter measured. This analyses the first

177   treatment period of the crossover study only, as if in a parallel group trial, using

178   Mann-Whitney tests. We also report paired analysis of the patient study in those

179   subjects who completed both treatment periods. All comparisons were made by

180   paired t-test unless otherwise stated where paired differences were non-parametric.

181   Statistical analyses were carried out using Prism 6 (GraphPad Software Inc., San

182   Diego, CA, USA) or SPSS version 24 (IBM Corp., Armonk, NY, USA).

183

184   **RESULTS**

185   The demographics of subjects are shown in the Supplementary Appendix. 16 control

186   subjects were randomised and completed the study. Of these, 10 showed adequate

187   compliance. A scanner failure meant that data for one of these were not available so

188   data from only 9 subjects are presented. 37 patients consented, of whom 24 passed

189   screening and were randomised. 4 withdrew before scanning; 4 more withdrew

190   between treatment periods 1 and 2. 20 patients had at least one MRI scan,

191   completing one treatment period (11 psyllium; 9 placebo) and were included in the

192   intention-to-treat analysis (ITT); 16 patients completed both treatment periods with

193   appropriate compliance and were included in the paired analysis (Supplementary

194   Table 1). 15 met Rome III criteria for FC and 1 for IBS-C. Fasting and postprandial

195   results are given in Tables 1 and 2 respectively.

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

197   **Table 2: Area Under the Curve of variables measured on postprandial MRI**

198   **scans during day 6 of treatment**

199

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

225   1). Figure 3 illustrates the changes in the ascending colon that were visible on

226   anatomical scans and water content sequences.

227

228   Fasting data on T1 relaxation times, where higher values would be expected to

229   reflect increased water content, are shown in Figure 4. In controls, treatment tended

230   to increase fasting T1 in the AC and DC (Figure 4A) but these differences were not

231   significant in the ITT analysis. In patients, $T1_{DC}$ was significantly higher in the DC

232   after psyllium (590ms, 446 – 1338) than placebo (213ms, 176 – 420), P<0.001.

233   Within-individual comparisons of paired data found higher T1 values after psyllium in

234   both AC (P<0.05) and DC (P<0.01). Fasting T2 measurements varied widely in

235   controls without demonstrable difference while differences identified in patients were

236   not consistent across ITT and paired analyses.

237

238   **Outcomes assessed on serial postprandial scans (Table 2)**

239   Postprandial SBWC showed significant differences between treatments for both

240   groups (Figure 2A & 2B). A dose-response relationship was evident in controls,

241   where postprandial AUC [change from baseline] psyllium 3.5 g t.d.s. led to an

242   increase in postprandial SBWC compared to placebo (P<0.01), albeit less

243   pronounced than that seen with 7 g t.d.s. (P<0.01 versus placebo). An increase with

244   treatment compared to placebo was equally apparent in patients: median AUC for

245   SBWC rose from 13.2 L.min (7.2 – 24.3) with placebo to 42.8L.min (24. – 49.1) with

246   psyllium (P<0.05), with similar values seen in the paired analysis. In our previous

247   work we described a fall in SBWC in the period 0-90 minutes after the test meal(20)

248   but this did not occur with 7 g psyllium t.d.s. After the second meal of the study,

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

250 studies.

251

252 Ascending colon water content (ACWC) in the control study, where it was the

253 designated primary endpoint, was significant greater in the postprandial phase after

254 7 g psyllium t.d.s. than placebo (P <0.0001), with a clear dose-response relationship

255 (P <0.001, Table 2). In patients, postprandial ACWC was undetectable in most

256 subjects taking placebo, with only 3 volumes >5mL recorded at any point. In this

257 group AUC for postprandial change in ACWC was greater with psyllium (P <0.05) but

258 highly variable, with mean postprandial ACWC ranging from 0 – 57 mL, equivalent to

259 5-10% of colonic volume. Of note, postprandial colonic volumes did not change

260 significantly from fasting baseline with any treatment in either study.

261

262 **Relaxation Times T1 and T2**

263 The AUC [change from baseline] for postprandial $T1_{AC}$ was greater with psyllium in

264 both regions in both groups(Table 2). Figures 4B (controls) and 4C (patients) show

265 the postprandial time course for $T1_{AC}$. The curves for 7 g psyllium t.d.s. show a

266 postprandial increase that returns to fasting levels after 6 hours. A second rise then

267 follows the second challenge meal. These rises did not occur with placebo, nor did

268 $T1_{DC}$ demonstrate such a curve. AUC for patients' $T1_{DC}$ was higher in the ITT

269 analysis but not significant so, although values were significantly greater than

270 placebo in the paired analysis of patients, which was also true for controls taking 7g

271 t.d.s. psyllium.The AUC [change from baseline] for postprandial $T2_{AC}$ was higher

272 after both psyllium doses than placebo in controls, and also higher after psyllium in

273   patients in the paired analysis. Post-prandial $T2_{DC}$ was only found to be higher in

274   patients on paired analysis (Supplementary Table 1).

275

276   **Fecal Water, Bowel Habit and Symptoms**

277   In controls, stool % water content was not higher after placebo treatment than at

278   baseline (baseline median 72%, IQR 69 - 73 vs 73%, 69 – 77 on placebo, P=NSig

279   Wilcoxon). Stool % water was higher than baseline after both psyllium 3.5 g t.d.s.

280   (median 76%, 68 – 80, P<0.05 Wilcoxon) and psyllium 7 g t.d.s. (81%, 75 – 87,

281   P<0.05 Wilcoxon). In patients, stool % water was no different at the start of the

282   placebo and psyllium treatments: 66% (59 – 75) vs. 63% (60 – 70). In this group

283   psyllium 7 g t.d.s increased stool % water by mean 6.2% (SD±7.2, P<0.01, paired t-

284   test) but there was no change after placebo (mean decrease 0.2%, SD±10.0).

285

286   In the patient study stool frequency was similar during pre-treatment periods off

287   laxatives and while taking placebo, but higher while taking psyllium (P <0.05

288   Wilcoxon, Figure 5A). Mean (SD) stool form (Bristol Stool Form Scale) on psyllium

289   was 3.5 (0.83) and 2.6 (1.3) on placebo (P =0.07 Wilcoxon, Figure 5B).

290

291   **Differences between controls and patients**

292   Fasting scans showed a number of differences between controls and patients (Table

293   1). WAPS24 scores were greater for patients than controls, indicating slower transit

294   as expected (Figure 2D). On placebo, fasting colon volumes were larger in patients

295   than controls (median 745 mL, IQR 455 – 844 vs. 372, 284 – 601 P <0.05).

296   Differences were primarily due to larger AC and TC in patients. Fasting T1 of chyme

297   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,

323 preventing absorption from the lumen. Consistent with this, postprandial small bowel

324 water increased with psyllium in both patients and controls. It is worth noting that

325 such a validated, non-invasive test may provide more representative data than older

326 methods requiring aspiration of a non-absorbable marker as the aspiration catheter

327 itself may stimulate intestinal activity, causing changes in absorption or secretion (9,

328 25).

329

330 Volume measurements demonstrated the bulking effect of psyllium. The increases

331 seen exceeded our expectations, in some cases doubling fasting colonic volume,

332 which may explain the bloating that some patients experience. Similar substantial

333 increases in colonic volumes as assessed by MRI have been recently reported in

334 response to high fibre diets by others (26).

335

336 By trapping water, the psyllium appears to abolish the immediate fall in SBWC

337 caused by the rapid absorption of sucrose, glucose and water we have previously

338 observed using the same test meal (20). The fall in SBWC after the second large

339 1000 kcal meal has been observed in most of our previous studies and we believe

340 this reflects the gastro-ileal response to feeding as described previously (27, 28).

341

342 We were unable to confirm an effect of psyllium on transit time although there was a

343 numerical decrease in transit scores in constipated patients. These findings are

344 consistent with other studies of psyllium where its effect on transit is small or non-

345 significant (18, 19, 23, 24). The increase in colonic volume offers an alternative

346 explanation for how psyllium increases stool frequency since there is a greater mass

347   of stool to pass. Total flow (mass/ time) as assessed by daily stool output may,

348   therefore, increase despite little change in speed through the gut (distance/ time).

349

350   Psyllium's main benefit may be through increased water content of colonic chyme

351   and stool, making feces softer and hence easier to pass. Free water was more

352   readily detected in the ascending colon of controls, and in both groups after psyllium,

353   but in individual patients it was often undetectable. This may have resulted from avid

354   water absorption in the constipated colon, or mixing of free water into the colonic

355   contents where the MRI signal of the water gets quickly reduced by interactions with

356   bacteria and tiny pockets of gas from fermentation.

357

358   A major finding in this study was the demonstration of the value of T1 in assessing

359   colon contents. This has not been previously reported. While free water was only

360   detectable in a few cases, the parameter T1, reflecting the physical and chemical

361   environment of the water molecules, was readily measurable in all subjects and

362   normally distributed. T1 largely reflects the freedom of water molecules to move so

363   higher values should reflect increased water content of the chyme, as we have

364   shown previously with Moviprep(16). This is borne out by our observations: values in

365   the colon were greater proximally than distally, consistent with progressive water

366   absorption during transit; values were lower in constipation than health but increased

367   with psyllium, supporting the mechanism of action of psyllium as currently

368   understood, and again suggesting that free water was mixed with the colonic

369   contents. Further evidence for this effect is the postprandial rise in T1 seen in the

370   ascending colon with psyllium during the study day. The increase in T1 seen is

371   consistent with delivery into the colon of small bowel water that was prevented from

372 absorption by the presence of the fibre. Such an effect was not seen in the

373 descending colon, being further removed from episodic influxes associated with

374 feeding.

375

376 The correlation of T1 in the descending colon with both fecal water content and stool

377 frequency supports the clinical relevance of the parameter. Many patients define

378 their constipation by straining to pass hard, dry stools. Fecal water content can be

379 measured directly but provides no information on changes more proximally in the

380 colon. T1 provides a potential method to assess fecal consistency *in vivo*, and to act

381 as a non-invasive parameter for evaluation of constipation therapies. The fact that

382 ascending colon T1 is decreased shows that the dehydration in constipation is found

383 not only in the stool but throughout the colon. This is concordant with earlier studies

384 reporting slow orocaecal transit in constipation pointing to an important role for the

385 small bowel in constipation(29).

386 A major limitation of MRI has been expense compared with scintigraphy which has

387 an established track record of evaluation of a range of drugs designed to treat

388 functional gastrointestinal disorders. However, as we show here, MRI does provide

389 extra information on mode of action, particularly the impact on regional volumes and

390 fluid distribution which can only be inferred from changes in transit. This will be of

391 particular value in evaluating agents with novel modes of action like Tenapanor(30,

392 31), an inhibitor of the sodium-proton exchanger NHE3, or plant derived inhibitors of

393 aquaporins like rhein(32).

394 Our study had limitations. Scanner failure and subject withdrawal reduced our

395 numbers. Drop outs always raise the concern of selection so we report analysis of

396 those 20 who completed the first arm of the cross-over as an ITT analysis. We also

397   performed a paired analysis limited to those who completed the protocol and on

398   whom we had adequate scans; supplementary data tables provide these results.

399   Reassuringly we find very similar results in the paired analysis to the ITT analysis

400   suggesting that drop out was random and not a source of bias. Sample sizes were

401   hence small, limiting statistical confidence, but our data will enable more accurate

402   power calculations for future studies. Heterogeneity in response and variation in

403   dietary fibre intake may have obscured a treatment effect. Comparisons between the

404   studies may be affected by differences in age and gender since controls were

405   younger and predominantly male, while patients were mainly female. In a previous

406   study increased height was associated with larger colons, suggesting larger colons

407   in men, although height-standardised colons in women were larger(12). More data

408   will be needed to understand the impact of age and gender compared to other

409   physiological factors.

410

411   One advantage of the crossover designs used, and therefore reason to report both

412   ITT and paired analyses, is the reduction of such sources of variation in assessment

413   of an intervention's effect. Such a design is less practical for trials where symptoms

414   are the primary endpoint as establishment of a symptom pattern generally takes

415   time. An objective point metric, such as volume or chyme T1, avoids this delay in

416   assessment and so allows shorter periods of intervention and washout.

417

418   There may have been other opportunities to introduce bias: blinding was imperfect

419   due to the nature of the intervention and compliance cannot not be guaranteed

420   without direct observation. These issues are readily addressable with pharmaceutical

421   therapies where plasma drug concentrations can be measured. The objective nature

422   of MRI outcomes somewhat mitigated these issues compared to other methods of

423   assessing treatment efficacy particularly since MRI analysis was undertaken by a

424   single operator (KAM) to reduce variation. Our previous work on intra-observer

425   variation in colonic volume measurement found a coefficient of variation <5%.

426   Fasting SBWC values were lower than we observed previously (13, 20) which may

427   be a result of our small sample size here.

428

429   In a short scanning session we assessed fasting volume, relaxation times and

430   transit. Transit itself reflects the composite effects of propulsive forces, volume of

431   material and resistance to flow. The two patients with the longest $T1_{AC}$ both had a

432   transit score in the normal range, and may have a different aetiology for their

433   symptoms that would respond to different treatment. High scores for bloating and

434   flatulence during the psyllium study day, but not during the placebo day, were also

435   seen. Objective assessment of physiological changes offers the chance to further

436   separate out disorders of the defecatory process from visceral hypersensitivity, as

437   set out in the Rome IV criteria(1). The prospect of assessing, and predicting,

438   response to therapy with a single MRI may be enhanced further by developments

439   such as assessment of colonic wall motion through cine MRI(33, 34).

440

441   These techniques require further validation in larger cohorts and randomised

442   controlled trials. Nevertheless, this work clearly demonstrates the potential of a

443   comprehensive MRI panel to measure objective differences in luminal content

444   between controls and patients with chronic constipation, both in their natural state

445   and in response to therapeutic modulation. This might be of particular value in

446   demonstrating the site of action of secretogogues now being introduced into therapy.

447   The application of MRI has the potential to generate new insights into intestinal

448   function, provide a platform for early phase evaluation of new treatments and provide

449   an objective approach to evaluation of patients with functional disorders not

450   responding to simple empirical therapy.

451

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598

SUPPORTING INFORMATION

Study 1 CONSORT diagram

601

602   **Enrolment**

603

604

Assessed for eligibility (n=16)

Excluded (n=0)

Allocation

Allocated to intervention (n=16)

♦ Received allocated intervention (n=16)

Follow-Up

Did not complete protocol (n = 2)

♦ Scanner malfunction (n =2)

♦ Lost to follow-up (n=0)

Analysis

Analysed (n=14)

♦ Compliance Breach (n=5)
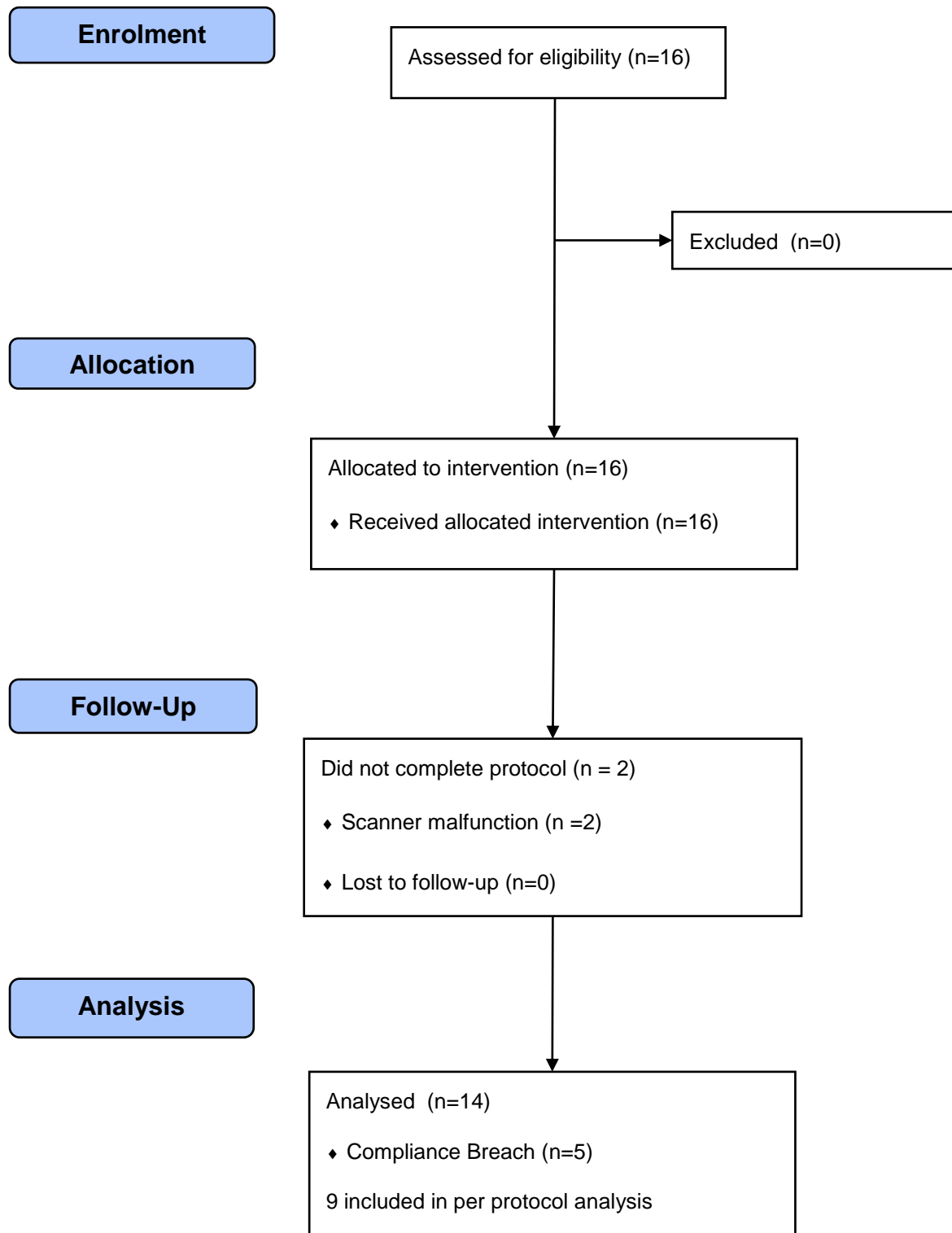
9 included in per protocol analysis

605 **Study 2 CONSORT diagram**

606

607 **Enrolment**

608 Assessed for eligibility (n=37)

609

Excluded (n=13)

610 ♦ Not meeting inclusion criteria (n= 8)

611 ♦ Other reasons (personal, difficulty in

612 scheduling study days, n=5)

Allocation

Allocated to intervention (n=24)

♦ Received allocated intervention (n=24)

Follow-Up

Did not complete protocol (n = 8)

♦ Withdrawal (n=2):   1 change in circumstances;

1 due to adverse event [nausea]

♦ Dropout (n=3):   2 after 1st period; 1 after 2nd period

♦ Withdrawn (n=3):   3 MRI scanner failure [2nd period]

Analysis

ITT Analysis (n=20: 9 placebo; 11 psyllium)

Paired Analysis(n=16)

♦Compliance Breach (n=0)

613 **Demographics of Participants**

614

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)

615

616

617 **MRI protocol and analysis**

618

619 All images were acquired using a whole-body 1.5T scanner (Achieva, Philips Medical

620 System, Best, The Netherlands). Each imaging period lasted for 15 – 20 minutes and

621 volunteers were positioned supine in the magnet with a 16-element receiver coil

622 wrapped around the abdominal region. Between scans, patients and volunteers were

623 asked to sit upright away from the scanner.

624

625 The volume of freely mobile water in the small bowel (Small Bowel Water Content,

626 SBWC) was measured and analysed as described previously (1) using a coronal

627 single-shot turbo spin-echo sequence, acquiring 24 slices in a single 24 second

628 breath hold (TR/TE$_{eff}$ = 8000/320 ms, 512x512 reconstructed matrix, reconstructed

629 voxel size 0.78x0.78x7 mm^3). Ascending colon water content (ACWC) was

630 measured and analysed similarly. Colonic volume measurements, as described in

631 detail previously (1, 2), were obtained using a coronal dual-echo gradient echo

632 sequence (TR/TE1/TE2 = 157/2.3/4.6 ms, 256x256 reconstructed matrix,

633 reconstructed voxel size1.76x1.76x7 mm^3).

634

635 Transit times were measured and scored as described previously (3) using a T$_1$

636 weighted 3D FFE sequence (TE/TR = 1.5/3.8 ms, FA = 10°, FOV= 250 x 371x 200

637 mm^3) and the ascending and descending colon relaxation times were acquired using

638 a single slice bTFE sequence. T$_1$ data were acquired with a preparatory 180°

639 inversion pulse at 8 different inversion times (TI) ranging from 100 – 5000 ms (4),

640 while T$_2$ data were obtained with a preparatory spin echo pulse before acquiring from

641 10 different echo times (TE) ranging from 20 – 637 ms (5). For both sequences,

642   there was a 10 second gap between each acquisition to allow the system to return to

643   equilibrium. Small regions of interest were drawn on the resulting images to calculate

644   the mean signal intensity for the region at each different TI or TE at the top, middle

645   and bottom of the colonic segments. The relaxation times were determined by fitting

646   the signal intensity data to a model of the signal evolution of the tissue after

647   application of all the preparation and imaging radio-frequency pulses.

648

649   **Supplementary References**

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666

667

668 **TABLES**

669 **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

670

671   **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					

672

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 ↓.

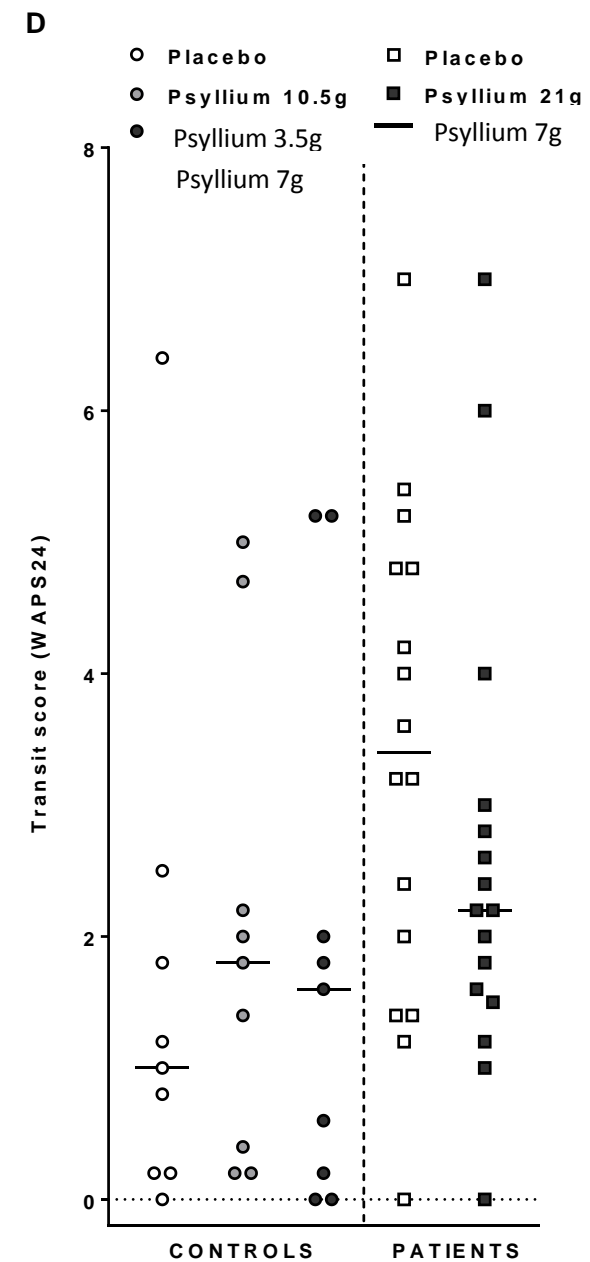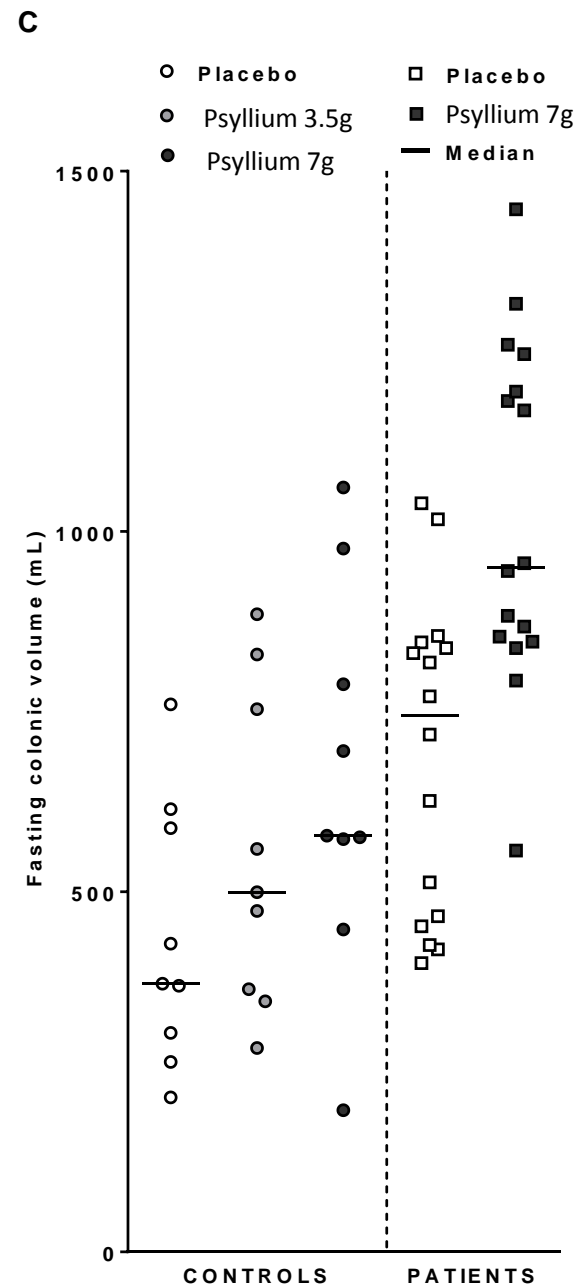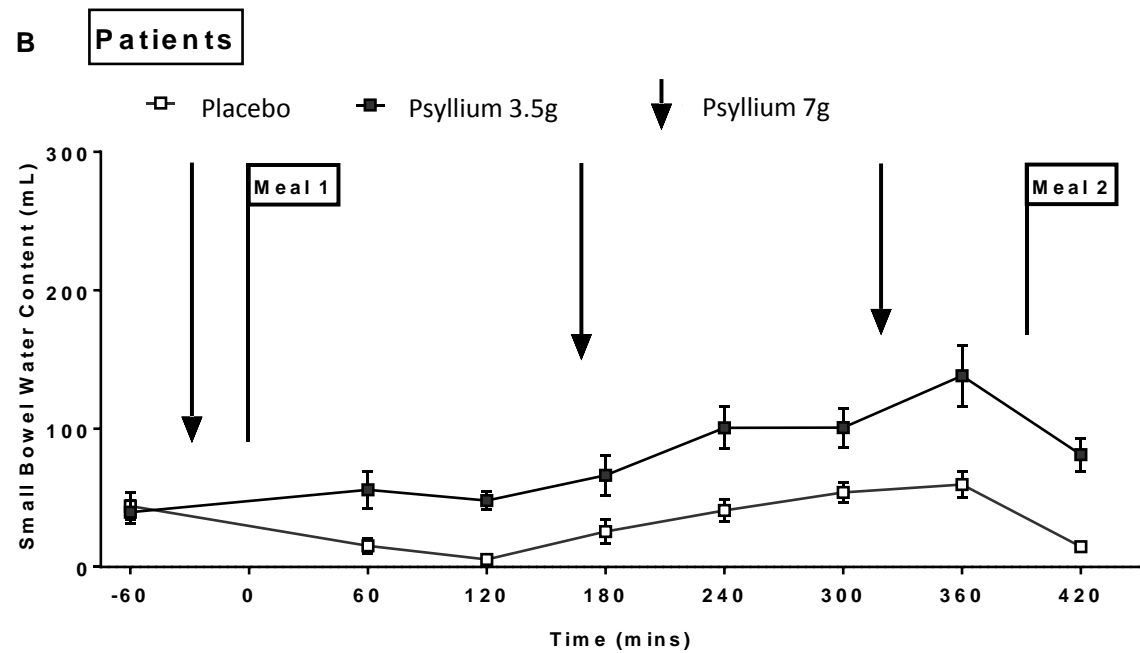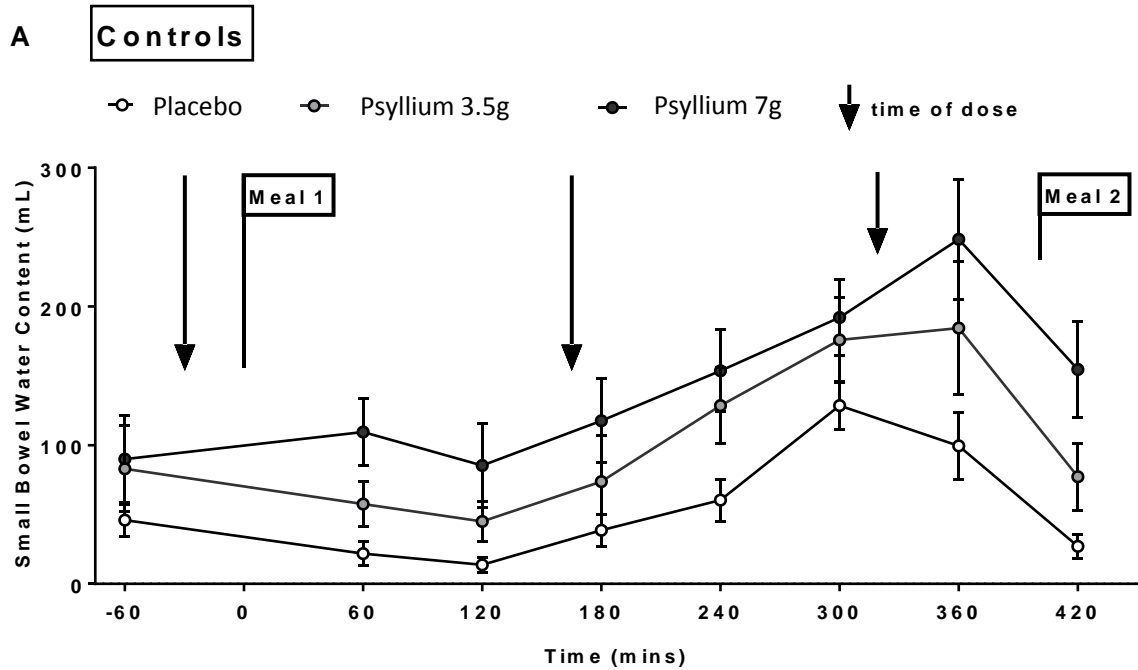**Figure 5: changes in stool frequency and form**

A) Mean stools frequency for patients during 6-day run-in periods (baseline) and 6

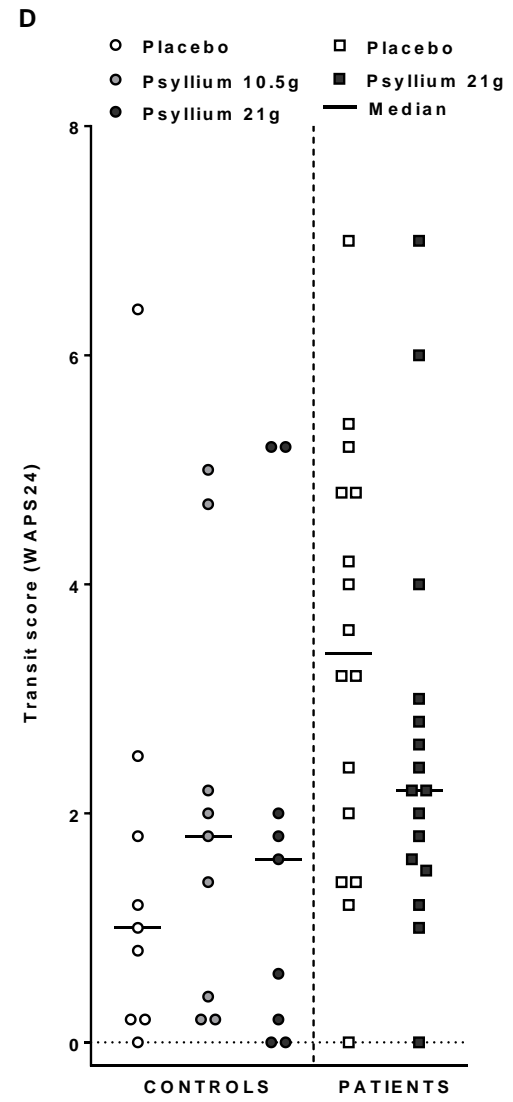days on treatment with maltodextrin placebo or 21g/day psyllium

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

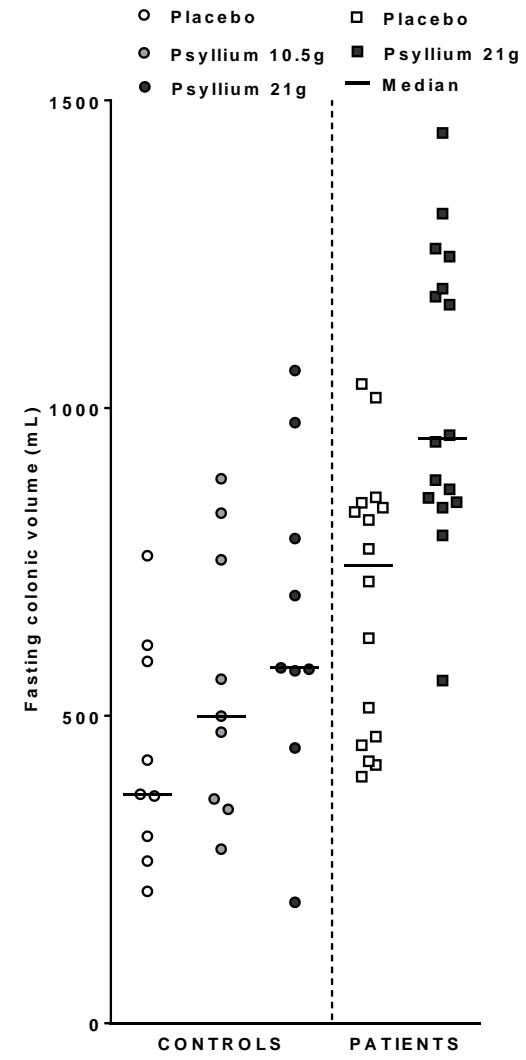**Figure 6: Correlations of relaxometry with fecal water content and stool**

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
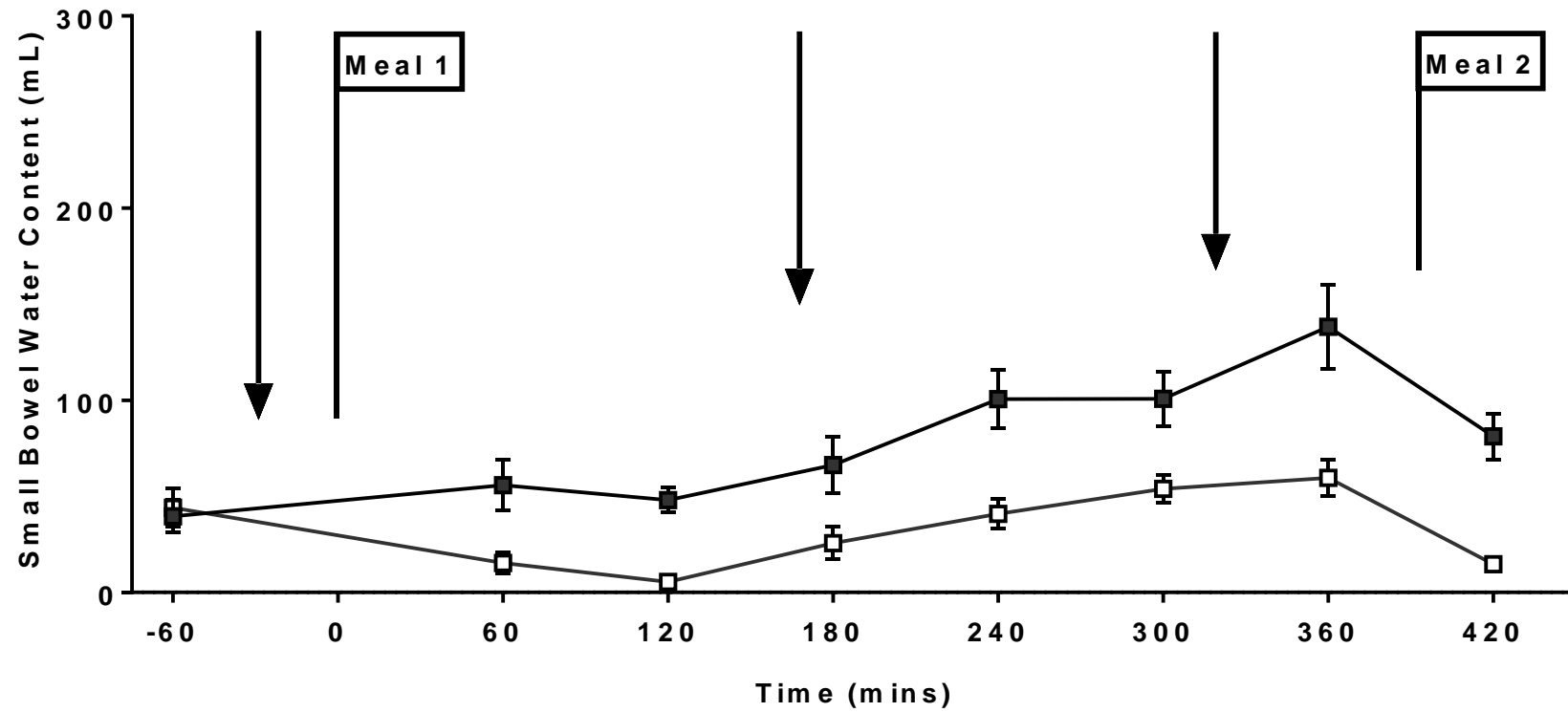
content ($T1_{DC}$)

A Controls

- ○ Placebo
- ◐ Psyllium 3.5g
- ● Psyllium 7g
- ↓ time of dose

Small Bowel Water Content (mL) vs Time (mins), with Meal 1 and Meal 2 markers.

B Patients

- □ Placebo
- ■ Psyllium 3.5g
- ↓ Psyllium 7g

Small Bowel Water Content (mL) vs Time (mins), with Meal 1 and Meal 2 markers.

C

- ○ Placebo
- ◐ Psyllium 3.5g
- ● Psyllium 7g
- □ Placebo
- ■ Psyllium 7g
- — Median

Fasting colonic volume (mL) for CONTROLS and PATIENTS.

D

- ○ Placebo
- ◐ Psyllium 10.5g
- ● Psyllium 3.5g
- Psyllium 7g
- □ Placebo
- ■ Psyllium 21g
- — Psyllium 7g

Transit score (WAPS24) for CONTROLS and PATIENTS.

C

A Controls

Placebo Psyllium 10.5g Psyllium 21g time of dose

Meal 1 Meal 2

Small Bowel Water Content (mL)

300

200

100

0

Time (mins)

-60   0   60   120   180   240   300   360   420