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Original research

Psyllium reduces inulin-induced colonic gas production in IBS: MRI and *in vitro* fermentation studies

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ABSTRACT

Objective Health-promoting dietary fibre including inulin often triggers gastrointestinal symptoms in patients with IBS, limiting their intake. Our aim was to test if coadministering psyllium with inulin would reduce gas production.

Design A randomised, four-period, four-treatment, placebo-controlled, crossover trial in 19 patients with IBS. Subjects ingested a 500 mL test drink containing either inulin 20 g, psyllium 20 g, inulin 20 g+ psyllium 20 g or dextrose 20 g (placebo). Breath hydrogen was measured every 30 min with MRI scans hourly for 6 hours. Faecal samples from a subset of the patients with IBS were tested using an *in vitro* fermentation model. Primary endpoint was colonic gas assessed by MRI.

Results Colonic gas rose steadily from 0 to 6 hours, with inulin causing the greatest rise, median (IQR) AUC_(0–360 min) 3145 (848–6502) mL·min. This was significantly reduced with inulin and psyllium coadministration to 618 (62–2345) mL·min ($p=0.02$), not significantly different from placebo. Colonic volumes AUC_(0–360 min) were significantly larger than placebo for both inulin ($p=0.002$) and inulin and psyllium coadministration ($p=0.005$). Breath hydrogen rose significantly from 120 min after inulin but not psyllium; coadministration of psyllium with inulin delayed and reduced the maximum increase, AUC_(0–360 min) from 7230 (3255–17910) ppm·hour to 1035 (360–4320) ppm·hour, $p=0.007$. Fermentation *in vitro* produced more gas with inulin than psyllium. Combining psyllium with inulin did not reduce gas production.

Conclusions Psyllium reduced inulin-related gas production in patients with IBS but does not directly inhibit fermentation. Whether coadministration with psyllium increases the tolerability of prebiotics in IBS warrants further study.

Trial registration number NCT03265002.

INTRODUCTION

Patients with IBS often report an unpleasant awareness of intestinal gas associated with a sensation of abdominal distension, commonly described as bloating. The connection between intestinal gas and gut symptoms is complex as signalling from gut to brain is influenced by many factors in patients with

Significance of this study

What is already known on this subject?

- Low-fermentable oligo-saccharide, di-saccharide, mono-saccharide and polyols (FODMAP) diets not only reduce IBS symptoms but also alter the gut microbiome reducing *Bifidobacteria* and other beneficial bacteria.
- Ingestion of inulin, a common dietary fibre, increases colonic gas and breath hydrogen.
- Psyllium has been shown in randomised placebo-controlled trials to reduce IBS symptoms.

What are the new findings?

- After studying patients with IBS we have shown that
 - Psyllium produces an increase in colonic volumes without increasing colonic gas or breath hydrogen.
 - Psyllium is only slowly fermented by IBS faecal microbiota.
 - Combining psyllium with inulin reduces both colonic gas and breath hydrogen response in patients with IBS but *in vitro* does not impair fermentation.

How might it impact on clinical practice in the foreseeable future?

- Our findings suggest that by taking viscous, poorly fermented fibres such as psyllium when FODMAP-rich foods are eaten, it may be possible to achieve the reduction of colonic gas symptoms seen on a low-FODMAP diet without disturbing the microbiota or requiring severe dietary restriction.

IBS including mood and somatisation.¹ Although cross-sectional studies of unselected patients with IBS have not shown excessive intestinal gas compared with healthy controls,² increases in gas have been related to symptom induction possibly related to visceral hypersensitivity.³ Inulin-type fructans worsen some symptoms in IBS particularly when given at higher doses.⁴ Despite the apparent clinical benefit of a low-fermentable oligosaccharide,

diaccharide, monosaccharide and polyols (FODMAP) diet,⁵ it inevitably reduces intake of substances which act as prebiotics, leading to a reduction in key bacterial genera in the gut including *Bifidobacteria*,^{6,7} with potential negative consequences.⁸ Alternative approaches to control of colonic fermentation in IBS are therefore being actively pursued.

Viscous fibres such as psyllium, an arabinoxylan polymer that resists digestion in the human upper gastrointestinal tract,⁹ improve symptoms in unselected IBS¹⁰ though until now the mechanism was unclear. We have recently used MRI to show that psyllium's open network of polymers traps water in the small bowel and increases ascending and descending colon water content. We believe that this is the basis of its laxative effect, softening stool so that it is easier to pass.¹¹ However, the small bowel effects may also be important since psyllium forms a highly viscous solution in the upper small intestine which interferes with absorption.¹² Psyllium is only slowly fermented so its viscosity effects are likely to persist for some hours after it reaches the colon. We have previously used radio-isotopic imaging to demonstrate that psyllium 3.5 g three times a day substantially reduced lactulose-associated acceleration of proximal colonic transit in healthy volunteers, but limitations of the method used means the mechanisms of action was unclear.¹³

The current study uses MRI to test the hypothesis that psyllium can reduce the increase in colonic gas induced in IBS by inulin. Inulin is a macropolymer which reaches the colon largely intact. There it is rapidly fermented producing large amounts of gas¹⁴ which correlates with symptoms of flatulence, bloating, discomfort and pain in patients with IBS.³ We further investigated the potential mechanisms of action by assessing the viscosity of test substances, known to affect fibre functionality,^{15,16} and their *in vitro* gas production by microbiota derived from stool donated from the participants with IBS.

MATERIALS AND METHODS

Two studies were performed, both investigating the effect of psyllium and inulin, both alone and when coadministered, on gas production in IBS. An *in vivo* study used MRI and breath hydrogen in patients with IBS and an *in vitro* study measured the viscosity of test solutions and their fermentation in a laboratory model of colonic fermentation using stool samples from participants in the *in vivo* study.

The carbohydrates used were inulin (Orafti HP, Beneo, Mannheim, Germany) which is 99.9% pure with average degree of polymerisation >23 and psyllium husk (Supernutrients, Bath, UK) which is 88.9% dietary fibre with 23.5% being soluble,¹⁷ degree of polymerisation being >2800.¹⁸ The placebo used was food grade dextrose (Thornton & Ross, Huddersfield, UK).

Human MRI study

This was a single-centre, four-period, four-treatment, placebo-controlled, crossover trial. Each treatment was administered once to each participant, with randomisation of treatment order. The treatments were test drinks containing inulin, psyllium, inulin and psyllium and dextrose as a control.

Participants

Participants were recruited through the digestive diseases services at Nottingham University Hospitals NHS Trust and the Circle Treatment Centre, through primary care via the NIHR Clinical Research Network in England and through general advertisement. Eligible participants were aged over 16 years, fulfilling Rome IV criteria¹⁹ for either diarrhoea-predominant

or constipation-predominant IBS. Exclusion criteria included: pregnancy, contraindications to MRI scanning, inability to cease use of supplementary fibres or laxatives, inability to stop drugs affecting GI motility, history of other pre-existing gastrointestinal disorders or resection, intention to change smoking habit during the study, excess alcohol intake or anyone who in the opinion of the investigator would be unable to comply with the study protocol.

Test drinks and controlled diet

Participants were provided with a low-fibre, low-FODMAP meal to eat at home the evening prior to and during the study day in order to standardise intake of foods likely to increase colonic gas (see online supplemental tables S1A,B for nutritional information).

The test drinks contained (a) 20 g inulin powder, (b) 20 g psyllium powder, (c) 20 g inulin powder and 20 g psyllium powder and (d) 20 g dextrose powder as a placebo control. Inulin and dextrose were dissolved in boiling water which was subsequently cooled in a refrigerator overnight. Test drinks containing psyllium were divided into four 125 mL drinks with a quarter of the psyllium added to each just prior to ingestion to avoid gelling which gave the solutions an unpleasant texture. The other drinks were imbibed at the same rate so that the 500 mL total for all test substances was consumed over 5 min. All test substances were stored in a sealed container in a cool, dry and dark environment prior to consumption.

Protocol

Participants were provided with written information about the study at least 24 hours prior to informed consent. Eligibility was confirmed and information including medical history, current medications, height, weight and smoking history was recorded. Their IBS subtype was confirmed according to Rome IV criteria using a 1-week stool diary documenting stool frequency and consistency on the Bristol Stool Form Scale (BSFS).¹⁹ Participants completed the Hospital Anxiety and Depression Scale²⁰ and the Patient Health Questionnaire 12 Somatic Symptom Scale²¹ questionnaires. Eligible participants were randomised to the order of test drink intake using www.randomization.com and given a dietary advice sheet for the day preceding each study day. Participants were provided with a low-fibre, low-FODMAP evening meal to eat at home before 20:00 on the evening prior to MRI scans (online supplemental table S1A).

Participants fasted from 20:00 the day prior to the study day and were allowed only sips of water for essential tablets as necessary. On the morning of the study they attended the University of Nottingham Sir Peter Mansfield Imaging Centre and underwent a fasted MRI scan on a 1.5 T GE MRI scanner using a parallel imaging 12-element torso coil (see online supplemental material MRI endpoints and methods for details). They also had breath hydrogen measured by exhaling into a gas analyser (GastroCH4ECK, Bedfont, UK) and recorded symptoms of flatulence, bloating and abdominal pain using a modified Gastrointestinal Symptom Rating Scale.²² This asks subjects to rate their symptoms on a 7-point scale 0–3 with intervals of 0.5 units, with anchors being 0=none, 1=mild, 2=moderate and 3=severe. Participants then consumed the allocated test drink. MRI scans were performed immediately after the drink and then hourly for 6 hours, while hydrogen breath tests and symptom scoring occurred every half hour (see online supplemental figure S1 for study day details). After 210 min, participants received a 338 kcal meal of rice pudding, jam and orange juice (see

online supplemental table S1B for nutritional information) and completed a hydrogen breath test and symptom score. Participants spent approximately 15 min inside the MRI at any one time. A washout period of at least 6 days was used between study days. Subjects collected their own stool samples at home using a dedicated stool collection kit (see online supplemental material for details). Samples were double bagged and frozen at -20°C in a domestic freezer before being brought to the unit in an insulated bag on an ice pack to avoid thawing. Samples were consecutive samples requested from all subjects and were thus a random sample with no selection criteria.

The primary endpoint was the change from fasting values in colonic gas assessed from the area under the curve (AUC) from 0 to 360 min in arbitrary units with secondary endpoints being colonic volume AUC (L·min) and small bowel water content (SBWC) AUC (L·min), all measured by MRI. Other secondary endpoints included breath hydrogen in parts per million and severity of pain, bloating and flatulence, each scored 0–3.

In vitro fermentation study

Gas production from the fermentation of the test substrates was measured using the ANKOM RF gas production system (ANKOM Technology, Macedon, New York, USA).

Fermentation bottles were seeded with faecal samples from eight of the individuals with IBS from the human MRI study (four constipation-predominant IBS (IBS-C), four diarrhoea-predominant IBS (IBS-D)) as previously described.¹⁷ Faecal samples were frozen at -80°C and defrosted at room temperature prior to testing. Test substance samples were diluted 1 in 10 in prerduced phosphate-buffered saline (10% wt/vol), homogenised in a stomacher and strained to remove particulates. Fermentation bottles containing dextrose (0.5 g acting as a control of known fermentability), psyllium (0.5 g), inulin (0.5 g) or both inulin and psyllium (0.5 g+0.5 g) were inoculated with 3 mL of slurry, sealed so anaerobic and incubated at 37°C in a shaking water bath (80 rpm) for 5 days. Gas pressure was automatically measured every 15 min for 48 hours using the ANKOM RF system. Gas production from fibre was calculated using previous methods.²³ Data are reported as cumulative gas volume produced during fermentation. Further technical details are given in online supplemental material.

Viscosity assessment

Substrate viscosity was assessed using an AR-G2 rheometer (TA Instruments, Delaware, USA) using a cup and vane geometry. Analysis was performed at the same concentration as predicted to occur *in vivo* (4% solution assuming 20 g is distributed in a colonic volume of around 500 mL) and *in vitro* (0.5% solution). Inulin was solubilised in boiling H_2O and stored at 4°C overnight. Psyllium was mixed with water or the inulin solution immediately prior to analysis at a frequency of 6.28 rad/s (1 Hz). The frequency was determined using a strain-sweep experiment to be within the linear viscoelastic range.

Statistical analysis

The sample size was calculated using data from a previous study of the effect of inulin on colonic gas in IBS.³ To detect an increase of 40 mL of gas at 80% power and alpha of 1.66%, we calculated 20 participants would be required to complete the study.²⁴ We planned to enrol up to 25 participants to allow for 20% dropout. We considered 40 mL to be the minimally clinically important difference, representing approximately a 20% increase in ascending colon volume. All statistical analysis was

performed using GraphPad Prism V.8.2.1 or later for Windows (GraphPad Software, La Jolla, California, USA). Normally distributed data are presented as mean \pm SD and non-normally distributed data as median (IQR) and analysed using parametric and non-parametric statistical tests, respectively.

Profiles of values over time are summarised as AUC from 0 to 360 min for the MRI study and from 0 to 48 hours for the *in vitro* fermentation studies. Differences between the AUC of the change from fasting values for MRI colonic gas, AUC for breath hydrogen and for *in vitro* gas production were compared for the four test carbohydrates using the non-parametric Friedman test with Dunn's multiple comparisons as a post hoc test. The differences in colonic volume AUC, SBWC AUC and patient symptoms at 6 hours were normally distributed and analysed using repeated measures one-way analysis of variance (RM ANOVA) with Tukey's multiple comparisons as the post hoc test. Comparisons of data between the two IBS subtypes were performed using Mann-Whitney test.

Gas production from inulin and psyllium coadministration was measured in the *in vitro* fermentation studies and theoretical values were calculated by summation of measured values for inulin alone and psyllium alone. Calculated inulin and psyllium was treated as an additional variable for all analyses. Comparisons of *in vitro* data between IBS subtype per substrate were performed using Mann-Whitney test.

MRI images were anonymised to ensure the analysis was done blind as to the treatment. The methods used were as previously published for SBWC,²⁵ colonic gas²⁶ and colonic volumes²⁷ (see online supplemental material).

Patient and public involvement (PPI)

Patients were not involved in the details of the study design and endpoints; however, these were very similar to previous studies³ which had benefited from PPI.

RESULTS

Human MRI study participants

Overall, 26 patients attended the initial screening visit, of which 19 completed the study (for Consolidated Standards of Reporting Trials diagram see online supplemental figure S2). Overall, 15 patients were female and 4 were male, with a median age of 39 (min–max 19–65) years. Overall, 9 participants suffered from constipation-predominant IBS (IBS-C) and 10 from diarrhoea-predominant IBS (IBS-D). There were no significant differences in age, gender, anxiety or depression scores between IBS-C and IBS-D (table 1). Patients with IBS-D had a higher body mass index (BMI) ($35.0\pm 7.1\text{ kg/m}^2$ vs $26.2\pm 5.7\text{ kg/m}^2$, $p=0.009$) and as expected had a greater frequency of bowel movements ($p=0.02$) and higher BSFS Score ($p=0.02$) than patients with IBS-C. All tolerated the study procedures well.

Colonic gas and colonic volume

Colonic gas, as measured by MRI, rose steadily during the course of the study period, reaching a maximum at 360 min (figure 1A). The AUC of the change in colonic gas from fasting to 360 min (AUC gas, the primary outcome) was significantly affected by the fibre given (figure 1B) (Friedman test, $p=0.005$). Inulin caused the greatest rise in colonic gas (AUC gas 3145 (848–6502) mL·min) which was significantly reduced by the coadministration of inulin and psyllium (618 (62–2345) mL·min, $p=0.02$), the latter not being significantly different from dextrose ($p>0.99$).

Table 1 Characteristics of study participants by IBS subtype

Characteristic	Constipation-predominant IBS (n=9)	Diarrhoea-predominant IBS (n=10)	P value
Age, median (min–max)	39 (19–65)	43 (19–65)	0.92
Gender, male/female	2/7	2/8	>0.99
BMI (kg/m ²), mean±SD	26.2±5.7	35.0±7.1	0.009
HADS-A, mean±SD	6.8±6.3	5.6±3.7	0.62
HADS-D, mean±SD	2.8±3.1	3.6±2.4	0.34
PHQ-12SS, mean±SD	4.6±4.4	7.7±3.4	0.10
Weekly bowel frequency, mean±SD	7.7±3.0	12.4±5.0	0.02
Weekly Bristol Stool Form Scale Score, mean±SD	2.1±0.8	5.3±0.8	0.02

BMI, body mass index; HADS-A, Hospital Anxiety and Depression Scale-Anxiety subscale; HADS-D, Hospital Anxiety and Depression Scale-Depression subscale; PHQ-12SS, Patient Health Questionnaire-12 Somatic Symptom Scale.

IBS subtype influenced the AUC gas which was larger in IBS-D compared with those with IBS-C for both inulin (Mann-Whitney test, $p=0.01$) and inulin with psyllium coadministration ($p=0.03$), but not for dextrose or psyllium alone (figure 2 and online supplemental table S2).

Total colonic volume remained unaltered by dextrose, rose steadily for both psyllium alone and inulin alone but the greatest rise occurred with inulin and psyllium coadministration (figure 3A). RM ANOVA showed a significant difference between test drinks ($p=0.01$); post hoc analyses showed greater AUCs for inulin (353.8 ± 85.0 L·min) and inulin and psyllium coadministration (362.5 ± 109.0 L·min) compared with dextrose (300.2 ± 73.0 L·min, $p=0.002$ and $p=0.005$), respectively. Since the effect of increased small bowel water inflow would be maximal in the ascending colon, we also measured just the ascending colon at 360 min when the differences between the test drinks were highly significant ($p=0.0016$). Coadministration of inulin and psyllium increased ascending colon volume at 360 min compared with inulin alone or psyllium alone ($p=0.0026$ and $p=0.018$, respectively, figure 3B, see online supplemental table S3 for regional AUCs). The differences in regional AUCs between the test drinks are most obvious proximally as the test material would not have progressed beyond the transverse colon during the 6-hour study period. Unlike colonic gas however, there were no differences in colonic volumes in response to the test drinks between IBS subtypes (online supplemental table S2).

Breath hydrogen

Psyllium produced no discernible increase in breath hydrogen, whereas breath hydrogen began to rise within 60 min of ingestion of inulin (figure 4). Coadministration of inulin and psyllium both slowed and reduced the maximum rise in breath hydrogen during the study period.

Significant differences were found in AUC breath hydrogen between the test drinks ($p<0.0001$). Post hoc analysis showed AUC for breath hydrogen after inulin, 7230 (3255–17910) ppm·hour, was significantly greater than after both psyllium (555 (180–915) ppm·hour, $p<0.0001$) and dextrose (750 (180–1140) ppm·hour, $p<0.0001$). Coadministration of inulin and psyllium reduced the AUC to 1035 (360–4320) ppm·hour, which was significantly lower than inulin alone, $p=0.0065$. No differences were found in AUC for breath hydrogen between IBS subtypes for any of the test drinks (online supplemental table S2).

Using the time for breath hydrogen to rise more than 10 ppm from baseline, we can estimate that inulin, when given alone, passed rapidly through the small bowel reaching the caecum within 143 ± 86 min ($n=19$). Adding psyllium to the inulin slowed this somewhat to 176 ± 86 min ($n=8$) but the change was not significant, $p=0.26$. However, it should be noted that in 11 subjects, breath hydrogen after psyllium plus inulin did not rise by 10 ppm and so transit time could not be calculated nor could a transit time for psyllium or dextrose alone. Using MRI detected arrival of fluid in the ascending

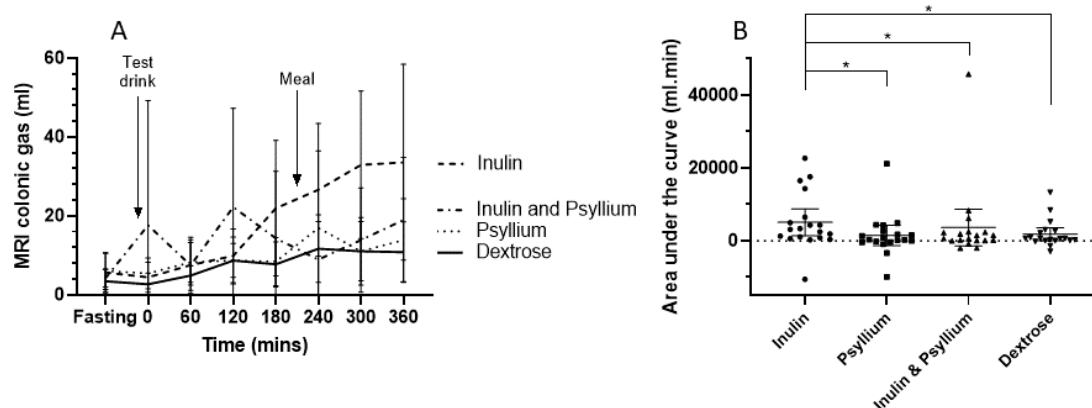


Figure 1 Change in MRI colonic gas from fasting values for each test drink ($n=19$). Data shown are mean±95% CI. (A) Time course over the duration of the study, showing significantly greater gas production for inulin compared with psyllium, dextrose and inulin and psyllium coadministration at 360 min ($p=0.0097$). (B) Area under the curve (AUC) for individual participants after each test drink (on the x axis). Inulin produced a significantly larger AUC than the other three test drinks. * $p<0.05$.

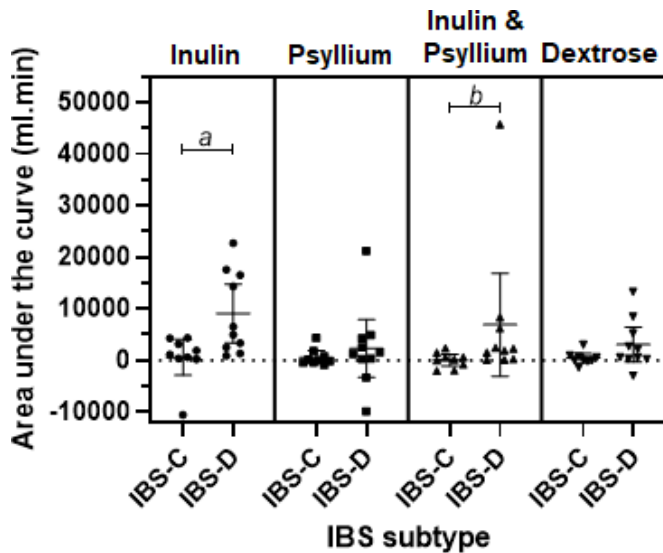


Figure 2 Area under the curve (AUC) change in MRI colonic gas from fasting values for IBS-C versus IBS-D (n=19), demonstrating significantly greater AUCs for IBS-D after both inulin and inulin and psyllium coadministration test drinks. Data shown are mean \pm 95% CI. a, $p=0.01$. b, $p=0.03$. IBS-C, constipation-predominant IBS; IBS-D, diarrhoea-predominant IBS.

colon as a marker of oro-caecal transit was not possible with inulin as it does not trap fluid and so does not produce a clear increase in signal intensity to mark caecal arrival.

Small bowel water content

Test drinks containing psyllium resulted in greater SBWC than the dextrose control. Significant differences were found in SBWC AUC between test drinks (RM ANOVA, $p<0.0001$); the values with inulin alone (47.2 ± 22.9 L \cdot min) did not significantly differ from those with dextrose (42.4 ± 23.4 L \cdot min, $p=0.63$) but adding psyllium to inulin caused a significant increase to 86.9 ± 42.8 L \cdot min, $p=0.0007$. Psyllium alone induced the greatest SBWC 102.7 ± 49.1 L \cdot min, significantly greater than seen with inulin and psyllium coadministration ($p=0.03$) (online supplemental figure 3). There were however no differences in

SBWC between IBS subtype for any of the test drinks (online supplemental table 2).

IBS symptoms

Symptoms were typically mild to moderate, the main symptom being flatulence which rose steadily during the study period for all test drinks. Six hours post ingestion there were significant differences between test drinks for flatulence ($p=0.01$), inulin leading to more severe symptoms than psyllium (1.2 ± 0.8 vs 0.5 ± 0.5 , $p=0.04$). Coadministration of inulin and psyllium resulted in intermediate values which were not significantly different from inulin alone. Considering all patients with IBS, no differences were found between test drinks for abdominal pain or bloating. However, when subgroups IBS-C and IBS-D were compared, IBS-D showed greater pain and bloating after inulin alone ($p=0.04$ and $p=0.01$, respectively) and greater pain after inulin and psyllium coadministration ($p=0.03$ (online supplemental table S2)).

In vitro fermentation study

Bacterial fermentation of all test fibres and the placebo dextrose resulted in the production of gas which was greatest for dextrose (AUC 4500 ± 1144 mL \cdot hour), significantly higher than for inulin alone and psyllium alone ($p<0.001$ for both (figure 5)).

Of the substrates likely to reach the colon intact, coadministration of inulin and psyllium to the fermentation model generated the most gas (AUC of 2991 ± 1169 mL \cdot hour), whereas inulin alone produced less gas (1122 ± 758 mL \cdot hour), although the difference was not significant, $p=0.056$. Psyllium alone resulted in the lowest amount of gas production (324 ± 146 mL \cdot hour), which was significantly lower than coadministration of inulin and psyllium ($p=0.02$). The calculated values for inulin and psyllium gas production (the sum of inulin alone plus psyllium alone) gave a lower mean AUC (1446 ± 704 mL \cdot hour) than that actually measured during coadministration, although this difference was not significant ($p=0.1$).

There were no differences in gas production for any fibre between IBS subtypes (online supplemental figure 4). Gas production *in vitro* and *in vivo* were both highly variable but correlated strongly for inulin ($r^2=0.58$, $p=0.03$) but not for

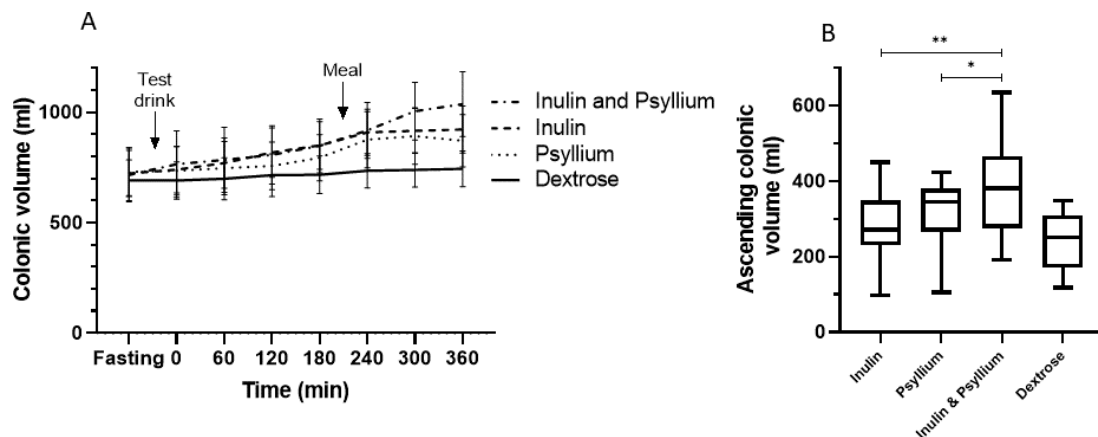


Figure 3 Colonic volumes after the test drink (n=19). (A) Total colonic volumes after dextrose remained stable but rose significantly after the other three test drinks. Area under the curve for both inulin and inulin and psyllium coadministration were significantly greater than dextrose, $p=0.002$ and $p=0.005$, respectively. Data shown are mean \pm 95% CI. (B) Ascending colonic volume 360 min after each test drink (on the x axis), represented by Tukey box and whiskers plot. Inulin and psyllium coadministration significantly increased the volume compared with either inulin or psyllium alone ($p=0.003$ and $p=0.02$, respectively).

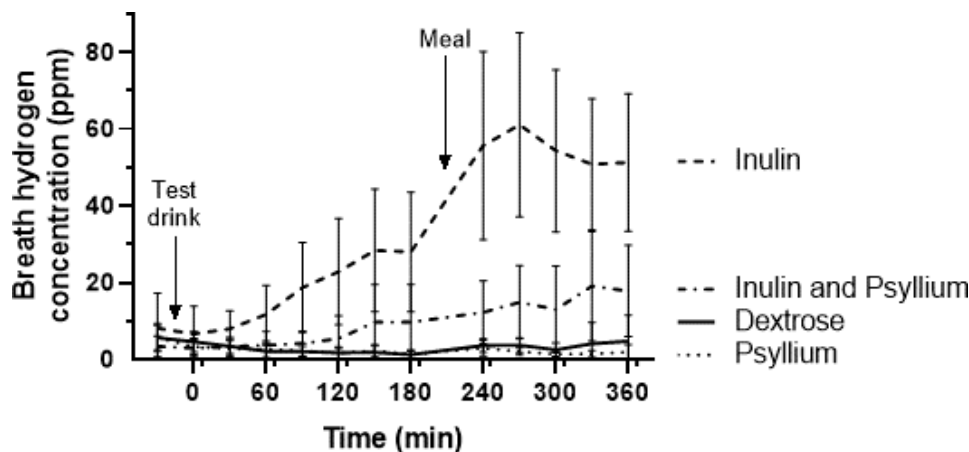


Figure 4 Breath hydrogen concentration (ppm) at fasting and every 30 min after the test drink (n=19). Breath hydrogen rose steadily after 30 min with the inulin test drink but area under the curve analysis demonstrated a significantly reduced rise when inulin was coadministered with psyllium, $p=0.0065$. Both psyllium and dextrose produced significantly less breath hydrogen than inulin alone, both $p<0.0001$. Data shown are mean \pm 95% CI.

psyllium ($r^2=0.14$, $p=0.35$) or coadministration of inulin and psyllium ($r^2=0.003$, $p=0.89$ (figure 6)).

Viscosity of substrates

Coadministration of inulin to psyllium did not alter the viscosity when compared with psyllium alone. Concentrations used *in vitro* and *in vivo* both resulted in formation of a weak gel as indicated by the storage modulus (solid-like behaviour) (G') values being higher compared with the loss modulus (liquid-like behaviour) (G'') under the conditions used both *in vivo* and *in vitro* (online supplemental figure S5).

DISCUSSION

Our study confirms previous data from healthy volunteers and patients with IBS^{3,14} that inulin has little effect in the small bowel but is rapidly fermented in the colon causing a rise in colonic gas and breath hydrogen. Previous studies have shown that psyllium,¹¹ wheat bran and prickly pear cactus fibre (nopai)¹⁷

increase colonic volumes but did not study the impact of coadministered inulin on fermentation. The current study confirmed our hypothesis that both rise in colonic gas and breath hydrogen after inulin can be largely inhibited by concurrent ingestion of psyllium.

We chose inulin as an important example of poorly absorbed dietary polysaccharide commonly found in wheat, onion, banana, garlic, and leeks,²⁸ food types often associated with symptoms in patients with IBS.²⁹ Food manufacturers seeking health benefit claims have sought to use inulin, a storage fructan polymer easily obtained from chicory root, to increase the fibre content of many processed foods. However, its simple linear structure means that compared with other sources of fibre it is rapidly fermented meaning it often causes symptoms. This is particularly true for patients with IBS in whom distension of the colon by fermentation of inulin generates symptoms not seen with healthy volunteers.³

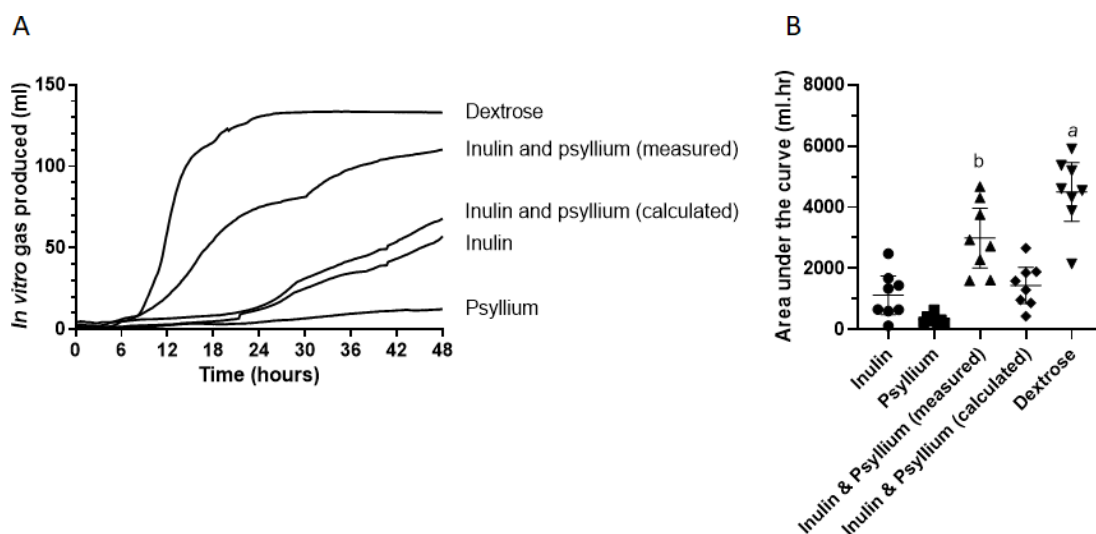


Figure 5 Forty-eight hours of *in vitro* gas production of substrates inoculated using stool from patients who participated in the human MRI study (n=8). (A) Median cumulative gas production showing an early rapid fermentation of dextrose followed by the inulin and psyllium combination. (B) Area under the curve (AUC) of *in vitro* gas production for each test drink (on the x axis). Data shown are mean \pm 95% CI. (A) Dextrose AUC is significantly greater than inulin ($p=0.0008$), psyllium ($p=0.0001$) and calculated inulin and psyllium ($p=0.001$). (B) Inulin and psyllium combination AUC is significantly greater than psyllium ($p=0.002$).

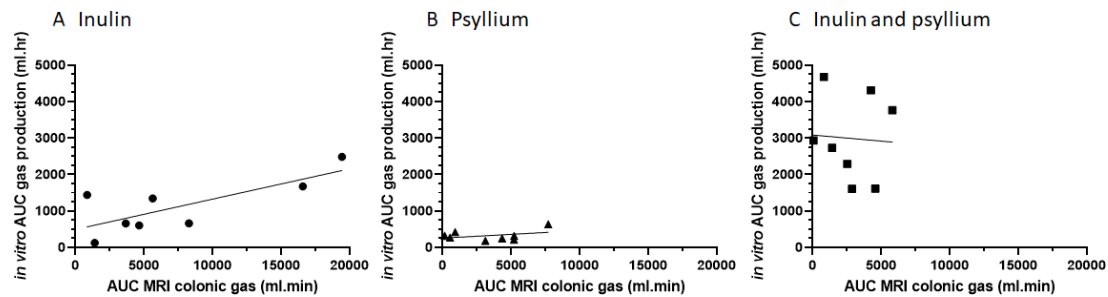


Figure 6 Correlations between *in vitro* area under the curve (AUC) gas production (mL-hour) and *in vivo* AUC colonic gas (mL-min) as assessed by MRI for (A) inulin $r^2=0.58$, $p=0.03$, (B) psyllium $r^2=0.14$, $p=0.35$ and (C) inulin and psyllium in combination $r^2=0.003$, $p=0.89$.

Whether fermentation produces gas depends on complex cross-feeding networks in which some bacteria degrade inulin, while others such as *Bifidobacteria* use the products of degradation to generate short chain fatty acids (SCFAs) including butyrate.³⁰ The production of H_2 , CH_4 and CO_2 after ingestion of large amounts of inulin reflects its rapid fermentation in an anaerobic environment. It is possible that slowing fermentation would allow more efficient metabolism with transfer of reducing equivalents to acetate and other SCFAs rather than gas. We have previously shown both *in vitro* and *in vivo* that psyllium is poorly fermented and produces a much smaller rise in breath hydrogen than equal doses of more readily fermentable fibres including wheat bran and the prickly pear cactus fibre, nopal.¹⁷ It follows therefore that adding psyllium to inulin would not be expected to increase the gas burden but why it reduces colonic gas requires further explanation.

Our *in vitro* studies were designed to explore the possibility that combining inulin and psyllium would inhibit fermentation directly. However, this actually enhanced gas production in the model where complete mixing was facilitated by the low viscosity of the concentrations used. Thus, the effect we observed *in vivo* was not due to any direct inhibition of colonic fermentation by psyllium. Furthermore, we showed that the inulin and psyllium combination had the same high viscosity seen with psyllium alone which did not per se inhibit fermentation. We hypothesise therefore that the *in vivo* effect is due to the increase in chyme viscosity leading to both a reduction in the rate of delivery of inulin to the colon together with restriction of mixing of colonic microbiota with the inulin bolus.

This hypothesis is supported in part by our observation in subjects that produced hydrogen, that oro-caecal transit time (OCTT) was delayed by around 23% by psyllium. However, this needs further study using a method of measuring OCTT which does not depend on fermentation, since in this study, using the breath hydrogen technique, it was only possible to measure OCTT in 8/19 subjects coadministered psyllium. Considerations of subject comfort and inconvenience meant that our study was limited to 8 hours so we cannot be certain that the total hydrogen production over 12–24 hours might not have been similar. Nevertheless, we can be certain that the rate of fermentation in the first 6 hours was significantly reduced.

When we simulated *in vitro* the concentrations of psyllium likely to occur in the colon we found a high viscosity, 10 Pa-s, which is likely to impede mixing in the colon and thereby delay access of colonic bacteria to the inulin. Previous MRI studies show that chyme passing through the ileocaecal valve, which coming from the small bowel has a low bacterial count, is distributed largely in the middle of the colon.¹¹ Tagging studies show that within the haustra, close to the colonic wall, there is much less movement and mixing which may favour anaerobic

fermentation.³¹ We hypothesise that psyllium impairs movement from the main stream into the haustral pockets and hence delays fermentation.

It is worth pointing out that MRI gas measurements are based on selecting a minimum of five adjacent voxels with signal intensity below a threshold (defined from an area outside the patient) which occupy volumes greater than 0.1 mL, so will miss smaller, more homogeneously dispersed gas. The values therefore are the minimum amount of gas present. The technique measures all gases equally. The large interindividual variation in gas volumes can in part be explained by our observation that large gas pockets can rapidly move through the colonic regions and be expelled, causing large differences in gas volumes seen on MRI from one scan to the next. This is in keeping with earlier studies which used a ventilated tent and a smaller dose of poorly absorbed carbohydrate and showed that a substantial amount of hydrogen was expelled per rectum.³²

We had intended to measure methane as well as hydrogen but technical failure prevented this. Methane producers do produce less hydrogen when exposed to poorly absorbed carbohydrate,³³ however they are found in only a third³⁴ or a fifth³⁵ of study populations, so with our small numbers we would have been unlikely to be able to show this. Furthermore, since each individual acted as their own control it is unlikely to have altered our findings.

While the 20 g dose of inulin we used substantially exceeds the UK average daily intake which is around 4 g, the dose is less than the 25–30 g recommended as daily intake of total dietary fibre so for a mechanistic study it is not unphysiological. Ileostomy³⁶ and intubation studies⁹ show that >85% of ingested inulin enters the colon intact where it is largely completely metabolised since very little is excreted in stool.³⁷ Inulin supplements in humans selectively increase *Bifidobacterium* and *Anaerostipes*,^{38,39} genera which comprise a quarter of the colonic bacteria and in concert with other bacteria are capable of rapidly fermenting inulin and producing large amounts of SCFAs, especially butyrate which is thought to underlie its potential health benefits. The findings that both inulin and inulin plus psyllium resulted in more gas and pain in IBS-D also support the idea that the faster oro-caecal transit, noted in previous MRI studies,⁴⁰ would increase the rate of delivery of substrate to the caecal microbiota. We hypothesise that this would overwhelm the microbiota's ability to use the excess reducing equivalents generated, which would therefore be disposed of as hydrogen or methane rather than used to generate SCFAs. However, to confirm or refute this hypothesis requires more detailed study.

Our mechanistic study was powered on MRI gas volume and was not adequately powered for symptoms, however the fact that the greatest colonic volume was seen with the psyllium and inulin coadministration with no apparent worsening

of symptoms suggests that other factors are important. Previous studies have indicated that increased tone is a more important determinant of pain during distension than volume alone.⁴¹ Psyllium, by slowing fermentation, reduces not only gas but also other products of fermentation such as SCFAs. These activate enteroendocrine cells and potentially stimulate motility and tone.⁴² Thus, reducing these products may reduce symptoms such as pain despite the greater volume. Future studies which measure both volume and microbial metabolites would help resolve these questions.

The 20 g dose of psyllium we used would be expected to transit through the small intestine largely intact and be distributed within the ascending and transverse colon, a volume of 400–600 mL²⁷. The unusually high side-chain density of psyllium limits bacterial access so that it is poorly fermented in the colon compared with other viscous fibres,^{43 44} producing little gas as the current study confirmed. Although time constraints on scanning prevented study of events later in the day, from other studies we can expect the fermentation to continue slowly during transit through the distal colon. Further benefits from delaying fermentation in the proximal colon would include shifting SCFA production and particularly butyrate production more distally, where the highest cancer risk lies. Exactly how this will alter colonic physiology is uncertain but increases in butyrate can stimulate enteric nerve growth⁴⁵ and potentially sensitise the colon.⁴⁶ Whether our psyllium intervention would achieve a big enough change to produce such changes should be further investigated.

Possibly more relevant are the studies which have shown that adding wheat bran fibre reduced carcinogenesis in rats fed resistant starch from raw potato,⁴⁷ thought to be mediated by changes in fermentation though the precise mechanism was unclear. Subsequent studies showed that fermentation of resistant starch in pigs was delayed and shifted distally by coadministration of wheat bran,⁴⁸ a phenomenon confirmed in humans.⁴⁹ Such changes in fermentation throw new light on the epidemiological associations between colorectal cancer and fibre intake and suggest future studies will need to take in account the type of fibre and not just its amount.

While our studies suggest ways of relieving symptoms without restricting prebiotic intake, it is worth pointing out that evidence for a significant health benefit from prebiotics is largely based on the strong epidemiological associations between fibre intake and disease with relatively few direct intervention studies.⁵⁰ These show significant changes in microbiota and their metabolites but more long-term studies will be needed to confirm that these translate into significant health benefits.

The water trapping softens stool leading to increased stool frequency in both healthy and constipated subjects¹¹ without however changing transit, despite an increase in colonic volume. This lack of effect on transit is unexplained since distension of the colon by balloon⁵¹ or osmotic laxatives such as macrogols⁵² stimulates propulsive colonic contractions. A possible explanation is that psyllium dampens the response to distension by reducing access to colonic sensors of luminal stimulants such as bile acids and faecal proteases. A recent study using xyloglucan supports the idea that enhancing the barrier between lumen and mucosa by dietary means has promise in the treatment of IBS.⁵³ Further studies are plainly warranted of this simple and safe method of alleviating symptoms in patients with IBS.

CONCLUSION

Our small mechanistic study has demonstrated that adding psyllium to inulin reduces gas production in patients with IBS and suggests that by choosing diets with adequate amounts of viscous fibre, patients may be able to obtain the prebiotic health benefits of high-fibre diets without exacerbating their IBS symptoms, particularly flatulence. Larger clinical trials are now indicated to confirm the clinical value of such mechanistic insights.

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Contributors RCS, KW and FJW: designed the study; GM, DG, CH, LM, JMRT, SKG and MR: contributed to study design; DG, ZA and HCH: conducted the study and performed the statistical analyses; DG: wrote the manuscript with primary responsibility for the final content and all authors: read and approved the final manuscript.

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Disclaimer The views expressed in this study are those of the author(s) and not necessarily those of the Medical Research Council, Consejo Nacional de Ciencia y Tecnología, Biotechnology and Biological Sciences Research Council, NHS, the NIHR or the Department of Health and Social Care.

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SUPPLEMENTAL MATERIAL

Supplemental Tables

Supplemental Table S1a: Nutritional composition of standardised meal consumed the evening prior to MRI scans

	Energy (kcal)	Carbohydrate (g)	Protein (g)	Fat (g)	Fibre (g)
Lean corned beef (Princes) 200g	194	1.0	25	10	-
Half of 300g tinned whole carrots in water (Sainsbury's)	25	4.3	0.5	0.3	1.9
Steamed basmati plain rice (Tilda) 250g	358	70.4	7.2	4.8	1.8
2 Highland All Butter Shortbread finger biscuits (Sainsbury's)	208	23.8	2	11.6	1
Total kcal= 950.6	785	398	138.8	240.3	9.4

Supplemental Table S1b: Nutritional information of meals consumed during the study day

	Energy (kcal)	Carbohydrate (g)	Protein (g)	Fat (g)	Fibre (g)
220 g Sainsbury's creamed rice pudding	211	37	6.8	3.7	<0.5
34g Sainsbury's seedless raspberry jam	85	21	<0.5	<0.5	0.9
100 mL Sainsbury's pure orange juice from concentrate	42	8.6	0.6	<0.5	<0.5
Total calories	338	266.4	29.6	33.3	1.8

Supplemental Table S2: Exploratory analysis of endpoints divided by IBS subtype. P value relates to testing for differences between the subtypes using Mann-Whitney tests or unpaired t-tests as appropriate. AUC, area under the curve; IBS-C, constipation-predominant irritable bowel syndrome; IBS-D, diarrhoea-predominant irritable bowel syndrome.

	Test drink	IBS-C	IBS-D	p value
MRI colonic gas change from fasting AUC (ml.min)	Inulin, median (IQR)	1080 (282-3717)	5728 (2172-16772)	0.01
	Psyllium, median (IQR)	-12 (-429-1255)	1402 (-660-4418)	0.24
	Inulin and Psyllium, median (IQR)	212 (-1400-1063)	2057 (198-6742)	0.03
	Dextrose, mean±SD	416±1186	3063±4750	0.12
Colonic volume AUC (L.min)	Inulin, mean±SD	319.1±88.9	375.8±76.2	0.15
	Psyllium, mean±SD	296.5±105.7	376.4±72.5	0.08
	Inulin and psyllium, mean±SD	330.6±108.3	384.2±105.1	0.28
	Dextrose, mean±SD	275.5±80.3	322.6±56.4	0.15
Small bowel water content AUC (l.min)	Inulin, mean±SD	46.7±28.2	47.6±18.6	0.45
	Psyllium, mean±SD	112.3±55.9	94.0±43.2	0.43
	Inulin and Psyllium, mean±SD	94.2±50.0	80.2±36.5	0.32
	Dextrose, mean±SD	41.9±32.7	42.8±12.3	0.94
Breath hydrogen AUC (ppm.hr)	Inulin, mean±SD	12432±9354	12623±12331	0.97
	Psyllium, median (IQR)	900 (390-1080)	615 (300-949)	0.33
	Inulin and Psyllium, median (IQR)	1290 (510-6330)	1133 (338-4913)	0.62
	Dextrose, median (IQR)	1065 (653-2055)	608 (41-1958)	0.32
Flatulence score AUC (arbitrary unit.min)	Inulin, median (IQR)	150 (79-443)	281 (203-773)	0.16
	Psyllium, median (IQR)	135 (64-315)	206 (62-319)	0.92
	Inulin and Psyllium, median (IQR)	105 (41-341)	270 (113-465)	0.29
	Dextrose, mean±SD	253±278	406±364	0.32
Bloating score AUC (arbitrary unit.min)	Inulin, median (IQR)	15 (8-420)	379 (219-958)	0.04
	Psyllium, mean±SD	262±268	407±321	0.30
	Inulin and Psyllium, median (IQR)	173 (49-338)	450 (191-840)	0.08
	Dextrose, median (IQR)	98 (0-236)	278 (81-435)	0.11
	Inulin, median (IQR)	30 (0-64)	184 (98-939)	0.01

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Abdominal pain score AUC (arbitrary unit.min)	Psyllium, median (IQR)	38 (8-184)	218 (43-572)	0.09
	Inulin and Psyllium, median (IQR)	0 (0-143)	289 (73-600)	0.03
	Dextrose, median (IQR)	0 (0-53)	120 (6-373)	0.14

Supplementary Table S3: Area under the curve of colon volumes (in L.min, mean \pm SD) for the ascending, transverse, descending and sigmoid colon for each test drink. P value relates to testing for differences using analysis of variance between the test drinks for each region of the colon.

	Inulin	Inulin and Psyllium	Psyllium	Dextrose	P value
Ascending colon AUC (L.min)	121 \pm 32 ^a	134 \pm 42 ^c	124 \pm 38	107 \pm 30	0.0004
Transverse colon AUC (L.min)	117 \pm 34 ^a	124 \pm 51 ^a	106 \pm 40	100 \pm 35	0.03
Descending colon AUC (L.min)	68 \pm 26	66 \pm 32	68 \pm 27	59 \pm 20	0.2
Sigmoid colon AUC (L.min)	44 \pm 24 ^b	38 \pm 17	41 \pm 20	34 \pm 17	0.05

^a significantly greater than dextrose, $p < 0.05$

^b significantly greater than dextrose, $p < 0.005$

^c significantly greater than dextrose, $p < 0.0005$

MRI endpoints and methods

MRI data analysts were blinded to the intervention received. A range of MRI sequences were used to image the abdomen to obtain the various endpoints including:

- 1) Colonic gas was assessed as previously published¹ using a dual echo gradient echo sequence (TR 175 ms, TE1= 2ms, TE2 = 4.3 ms, FA 80°, ASSET 2) to acquire 24 coronal images with a slice thickness 7mm (no gap) and reconstructed in plane resolution of 1.76 x 1.76 mm². This sequence was used to measure colonic volumes as well and was acquired during a breathhold. An additional identical sequence was also acquired with the R.F. power set to zero to acquire the noise distribution across the images for gas measurements.
- 2) Small bowel water content was measured as previously reported² using a single shot fast spin echo (SSFSE) sequence with fat saturation (TE_{eff} = 325 ms, Echo spacing 5ms) to acquire 32 coronal images with a 7mm slice thickness (no gap) and reconstructed in plane resolution of 0.78 x 0.78 mm².
- 3) Colonic volumes were measured from the dual echo images as previously reported³ using MIPAV software⁴ to segment the different colonic regions.

In vitro fermentation study details

Gas production from the fermentation of the test substrates was measured using the ANKOM RF gas production system (ANKOM Technology, Macedon, NY, USA). Briefly, per 125ml bottle, 0.5g of psyllium, inulin, or dextrose were added to each fermentation bottle. Additionally, 0.5g of psyllium and 0.5g of inulin were also added to bottles to test the impact of the substrates combined on gas production (inulin + psyllium). To remove any discrepancies in gas produced from sources other than the test substrates, a non-substrate blank was used. To each fermentation bottle, 76ml of media, 5ml of a vitamin and phosphate buffer solution, and 1ml of the reducing solution⁵ were added under a constant stream of CO₂. Once sealed, the substrates were allowed to hydrate, and bottles pre-warmed overnight at 37°C.

Bottles were seeded with faecal samples from eight of the IBS individuals from the human MRI study (four IBS-C, four IBS-D). Faecal samples were frozen at -80°C, therefore prior to testing, each faecal sample was defrosted at room temperature. Once defrosted they were diluted in pre-reduced PBS (10% wt/vol), homogenised in a stomacher and strained to remove particulates. Each substrate was fermented in triplicate per volunteer faecal sample. Each bottle was inoculated with 3ml of slurry, sealed, and incubated at 37°C in a shaking water bath (80 rpm) for five days.

Using the ANKOM RF system, the gas pressure was automatically measured every 15 minutes. Gas production from fibre was calculated using previous methods⁶. Data are reported as cumulative gas volume produced during fermentation, averaged from eight IBS individuals and measured in triplicate per individual/substrate type, thus a total of 24 individual fermentations were performed per substrate.

Stool collection instructions

1. Use the complete kit provided. It contains:
 - Cardboard tray
 - 1 Plastic sample tube with spoon attached to lid
 - Larger opaque plastic bag
 - 1 pair rubber gloves
 - Ice/frozen gel packs
 - Cooler bag/container
2. If possible, urinate before stool collection to avoid mixing urine and stool as this may affect the sample.

THEN PLEASE WASH HANDS YOUR HANDS

3. Place or hold the cardboard tray to collect the stool as passed.

THEN PLEASE WASH HANDS YOUR HANDS

4. After opening your bowels, put on the gloves provided (ensure your hands are dry or these will be difficult to put on).
5. Place a large amount of the stool passed into the tube so that it is at least 75% full. To do this, use the spoon attached to the tube as seen in the image below.



6. Place the spoon and stool sample into the tube and lightly secure the lid. **DO NOT SCREW THE TOP ON TIGHTLY.**
7. After stool collection the remaining stool sample should be flushed away. The rubber gloves and cardboard tray should be placed in a plastic bag and disposed of in your bin as normal rubbish.
8. Place the tube with stool samples into the transparent plastic bag and seal the bag tightly.

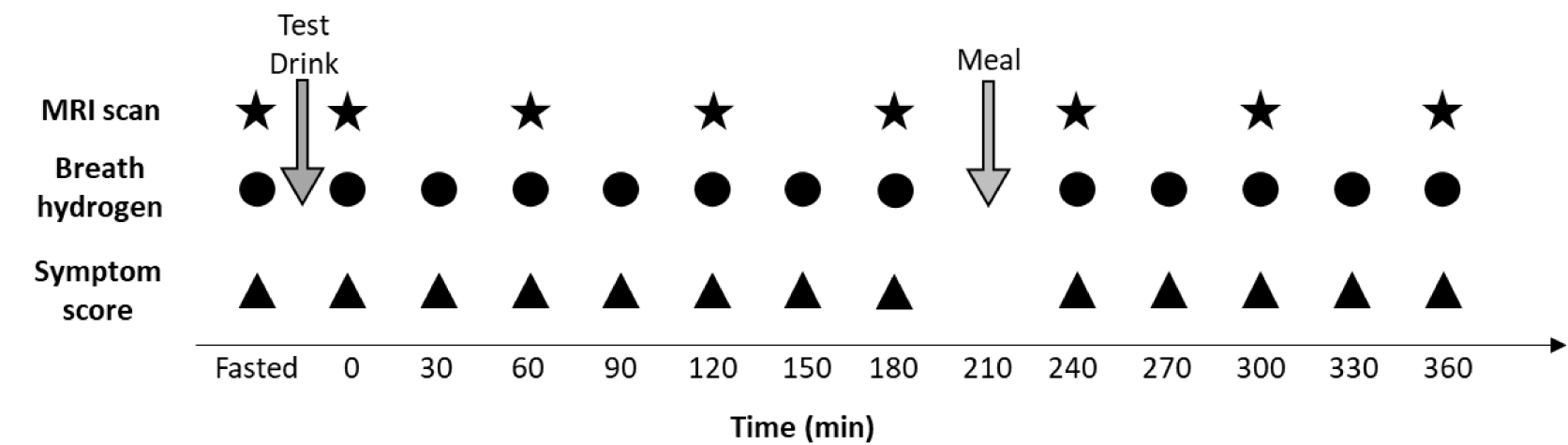
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9. Place the transparent plastic bag and the ice packs inside the cooler bag/ container provided. This should be sealed and placed in your freezer.
10. Ideally the stool sample should be returned to us as soon as possible (within a few days). Please keep the sample frozen. When you are ready to take the sample to the research site, the cooler bag can be removed from the freezer and taken to your next appointment.

We appreciate that some participants may not have access to a freezer in your own home, and if this is the case please inform us so we can make alternative arrangements

Supplemental figures

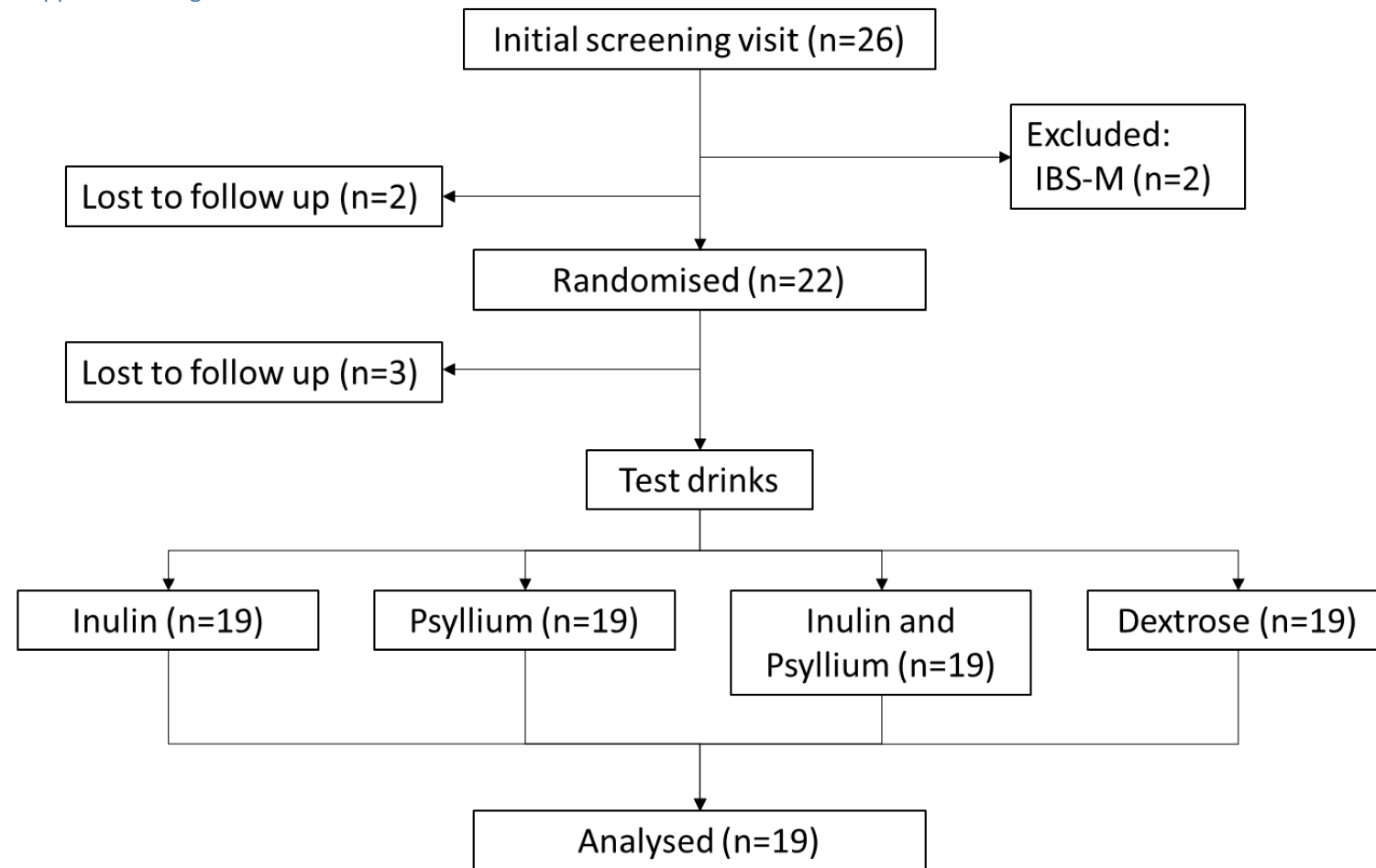
Supplemental Figure S1



Supplemental figure S1: Schematic of the study day. MRI scans were performed fasted, immediately after the test drink and at hourly intervals while breath hydrogen and symptom scores were obtained at 30 minute intervals. Test drinks were consumed before time 0 scan and the standardised meal given at 210 minutes.

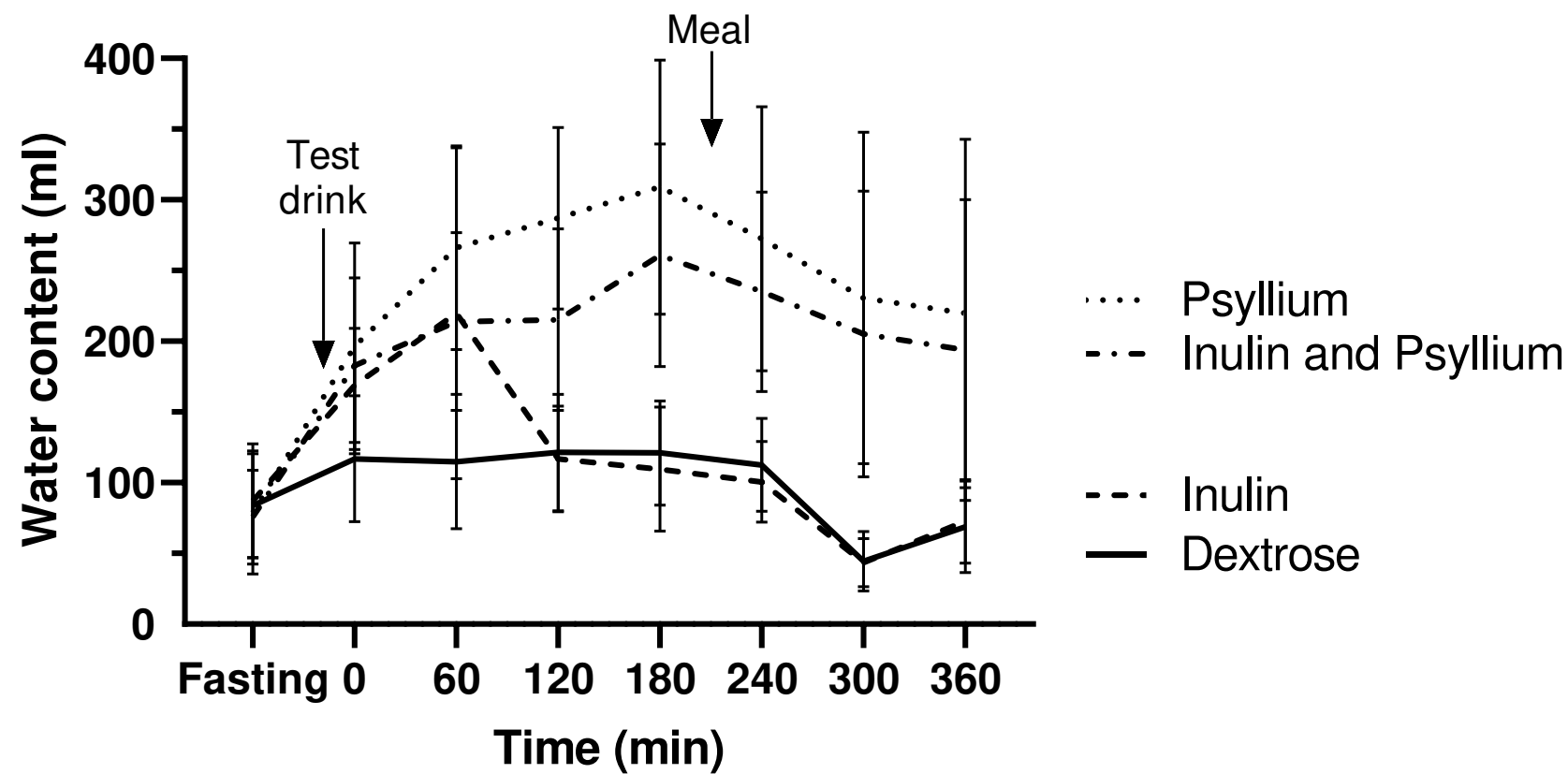
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Supplemental Figure S2



Supplemental Figure S2: CONSORT diagram of the study. IBS-M, Mixed-type irritable bowel syndrome.

Supplemental Figure S3



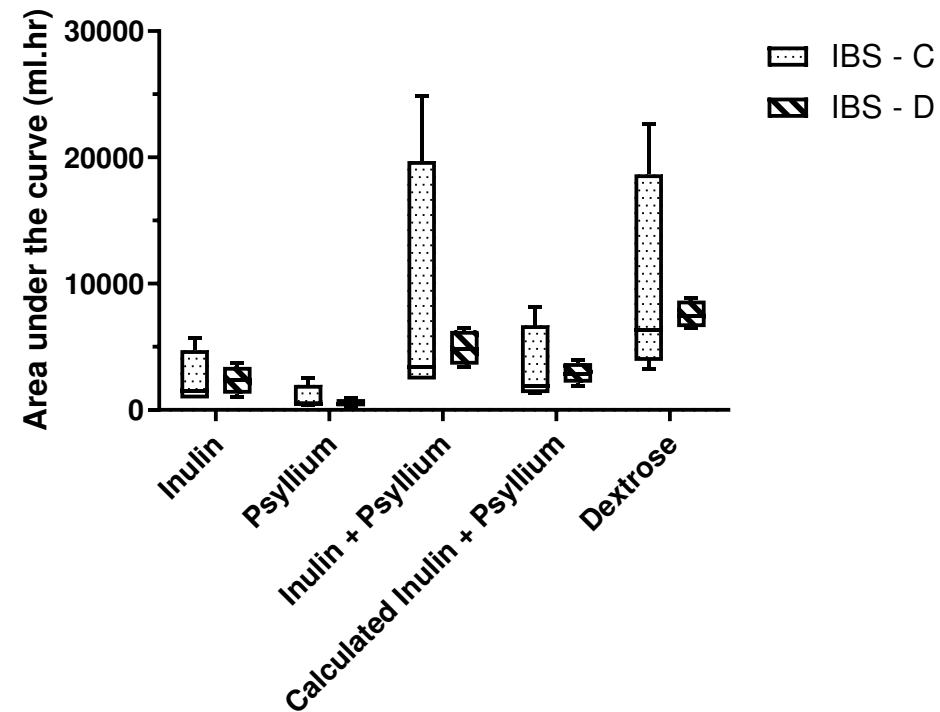
Supplemental Figure S3: Small bowel water content (SBWC) rose after test drinks containing psyllium but hardly at all after dextrose. Comparison of areas under the curve (AUCs) showed psyllium was associated with the highest values, significantly greater than inulin plus

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psyllium, $p=0.03$. Adding psyllium to inulin produced a significant rise in SBWC, $p=0.0007$. Inulin and dextrose were not significantly different. Data shown are mean \pm 95% CI.

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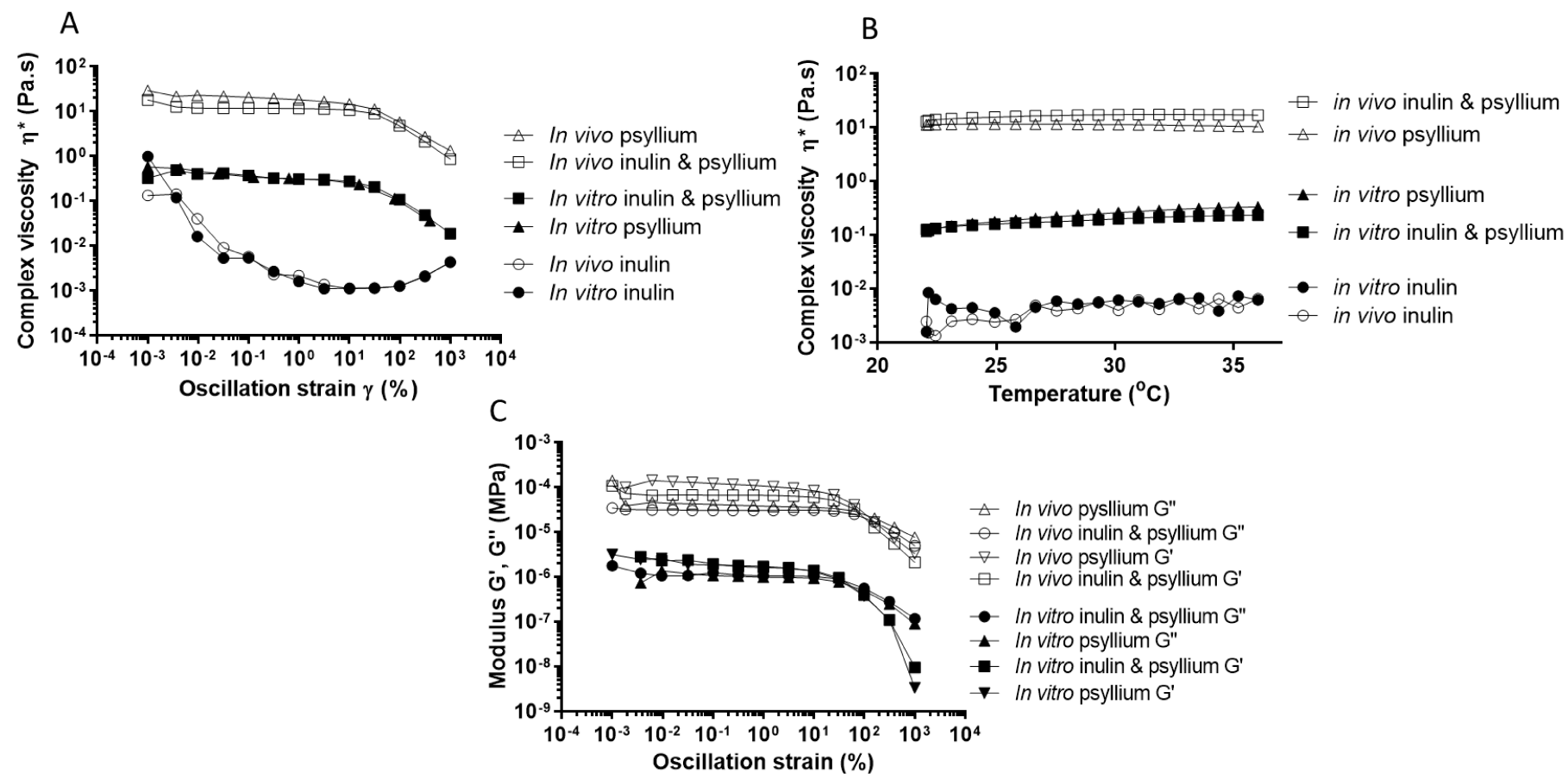
Supplemental Figure S4



Supplemental Figure S4: Tukey box and whiskers plot of area under the curve (ml.hr) after 48 hours of *in vitro* gas production, divided by IBS subtype (n=4 for each). No significant differences were found between subtypes. IBS-C, constipation predominant irritable bowel syndrome; IBS-D, diarrhoea predominant irritable bowel syndrome.

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Supplemental Figure S5



Supplemental Figure S5: Rheometric analysis of substrates tested. Samples labelled *in vitro* were 0.5% solution while those labelled *in vivo* were 4% solution, this being the estimated concentration *in vivo* assuming 20g of ingested psyllium is diluted in 500ml of colonic water, colonic

volumes being based on MRI assessment by Pritchard *et al*³. Analysis was performed with the AR-G2 magnetic bearing rheometer (TA instruments) with a cup (diameter: 30 mm) and vane (bob diameter 28 mm). Inulin was prepared by solubilising in boiling water and storing at 4°C overnight. Psyllium was mixed with the water or the inulin solution immediately prior to analysis. Parameters for data within oscillation strain sweeps ranging from 10⁻³ to 10³ at 37°C and a frequency of 6.28 rad/s (A, C).

Data shows that the addition of inulin to the psyllium did not alter the viscosity compared to psyllium alone (A). Varying the temperature from room to body temperature did not affect results (B). The 4% solution was more viscous than the 0.5% solution, but both solutions yielded gel structures as demonstrated by an increase in G' (storage modulus) compared to the G'' (loss modulus). These gels also behaved the same whereby they lost their structure, becoming a liquid at the same oscillation strain (C).

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