Randomised clinical trial: gut-brain axis dysfunction underlies FODMAP-induced symptom generation in irritable bowel syndrome

Short title: *Gut-Brain responses to fructans in IBS*

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Specific author contributions: JW, IM, JB, HF and LVO conceived the study and designed the protocol. IM and JT recruited participants. JW and IM performed the study procedures. CP, LS & HS performed manual pre-processing of the colon MRI and AB was the second reader for the dynamic small bowel MRI. JW, IM, HF and LVO analyzed the data. The manuscript was written by JW, IM and HF and critically revised by ST, QA, JB, JT and LVO. All authors approved the final version of the manuscript.

Data availability statement: Deidentified individual participant data are available upon reasonable request. Code used for brain, non-brain imaging analysis and sample size calculation is available at https://github.com/labgas/proj-fodmap-fmri.

Summary

Background: FODMAPs produce similar small bowel water and colonic gas in patients with irritable bowel syndrome (IBS) and healthy controls (HC), despite IBS patients reporting increased gastrointestinal (GI) symptoms.

Aim: To unravel the mechanisms underlying FODMAP-induced symptom reporting, we investigated gut and brain responses to fructans administration in IBS patients and HC.

Methods: This randomized, double-blind, cross-over study consisted of three visits where fructans (40g/500mL saline), glucose (40g/500mL saline) or saline (500mL) were infused intragastrically during 1h MR brain scanning; abdominal MRI was performed before, 1h, and 2h post-infusion. Symptoms were rated using validated scales.

Results: In IBS (n=13), fructans induced more cramps, pain, flatulence and nausea compared to glucose ($p=0.03$, 0.001, 0.009 and <0.001, respectively), contrary to HC (n=13) (all $p>0.14$), with between-group differences for cramps and nausea ($p=0.004$ and 0.023). Fructans increased small bowel motility and ascending colonic gas and volume equally in IBS and HC (between-group $p>0.25$). The difference in colonic gas between fructans and saline covaried with differences in bloating and cramps in IBS ($p=0.008$ and 0.035, respectively). Pain-related brain regions responded differentially to fructans in IBS compared to HC, including the cerebellum, supramarginal gyrus, anterior and midcingulate cortex, insula and thalamus ($p_{FWE\text{-}corrected}<0.05$); these brain responses covaried with symptom responses in IBS.

Conclusions: Fructans increased small bowel motility and colon gas and volume similarly in IBS patients and HC. Increased symptom responses to fructans in IBS covary with altered brain responses in pain-related regions, indicating that gut-brain axis dysregulation may drive FODMAP-induced symptom generation in IBS.

Keywords: FODMAP; irritable bowel syndrome; brain response; abdominal imaging

Introduction

Irritable bowel syndrome (IBS) is a prevalent disorder of gut-brain interaction (DGBI) with a high personal and socio-economic burden;[1] treatment is challenging due to variability of symptoms and pathophysiology. Diet is increasingly considered as treatment,[2] specifically a diet low in fermentable oligo-, di- and monosaccharides and polyols (FODMAPs) has shown to be an effective strategy to reduce IBS symptoms.[3] FODMAPs are poorly absorbed short-chain carbohydrates including fructose (in excess of glucose), lactose, polyols, fructans and galacto-oligosaccharides. They are found in a wide variety of foods, and may trigger lower gastrointestinal (GI) symptoms by inducing luminal distention through their osmotic effects in the small intestine and via bacterial fermentation in the colon, resulting in increased gas production.[4]

Recently, a magnetic resonance imaging (MRI) study demonstrated that increases in intestinal water and colonic gas after fructose and inulin were similar in IBS patients and healthy controls (HC), despite higher GI symptom scores in the former.[5] Hence, increased sensitivity to normal FODMAP-induced gas production and/or osmotic activity, rather than excessive gas production and water content, may contribute to FODMAP-induced symptoms in IBS. We demonstrated that IBS patients reported stronger increases in flatulence and cramps compared to HCs as early as 30 minutes after blinded intragastric fructans administration versus glucose. This suggests a potential role of the proximal GI tract in symptom generation in IBS, in addition to colonic hypersensitivity.[6] However, caecal fermentation cannot be ruled out given nutrient delivery was without any nutrient meal and therefore the fructans may have transited the small bowel early. Moreover, together with increased FODMAP-induced non-GI (including psychological) symptoms in our mechanistic study[6] and clinical trials,[7, 8] these findings suggest a role for aberrant central processing of visceral afferent signals in FODMAP-induced GI symptom generation in IBS.

The involvement of the brain-gut-axis in IBS is increasingly being recognized and supported by research.[9] It has been shown that the central processing of viscerosensory information in IBS patients

differs from HC, with lowered perception thresholds to various stimuli in different parts of the GI tract[10, 11] accompanied by increased engagement of brain regions associated with emotional arousal and endogenous pain modulation in IBS compared to HC.[12]

We aimed to investigate brain and gut responses to blinded intragastric administration of one specific FODMAP, fructans, versus glucose (positive control) and saline (negative control), and their association with symptom responses. Specifically, we compared activity in pain-responsive brain regions between IBS patients and HC, as well as small bowel motility, and colon gas and volume. We specifically focused on the ascending colon, as its impaired ability to accommodate post-prandial flow has been observed in IBS.[13] Finally, we assessed the associations between symptom responses and these different gut-brain axis mechanisms. Based on our previous study[6] as well as the abovementioned study[5], we hypothesized that IBS patients compared to HC would show increased symptom responses to fructans compared to both glucose and saline, as well as increased brain responses. Further, we hypothesized gut responses (small bowel motility and colon gas and volume quantified using MRI) to fructans to be similar in IBS patients and HC.

Materials and methods

Study subjects

We recruited female, right-handed or ambidexter healthy subjects and Rome IV IBS patients,[14] aged between 18 and 55 years old (**Supplementary Material S1**). Only females were recruited to avoid confounding sex differences and because females showed less variation in the volume of FODMAP solution consumed in our aforementioned study,[6] where FODMAP and control solutions were intragastrically administered until full satiation. The study was performed at University Hospitals Leuven, Belgium from February 2018 to August 2019. Informed consent was obtained from all subjects before performing any study procedure.

Sample size

In the absence of previous data on brain responses to FODMAP administration, sample size was calculated based on the significantly different increase in cramps after fructans compared to glucose in IBS patients versus HC in our previous study.[6] Assuming a similar effect size for this condition-by-group interaction and within-participant variability over time, a sample size of n=13 per group yields 90% power at α=0.05 as calculated for a linear mixed model with GLIMMPSE version 2.2.8 (Details at https://github.com/labgas/proj-fodmap-fmri/tree/main/sample_size_calculation).[15] It should be noted that we did not correct for the number of symptom outcomes/models in this power calculation, which is not commonly done, but should nevertheless be considered a limitation. However, we did apply stringent multiple testing correction within each model (see below). Power is virtually impossible to calculate for the brain imaging analysis due to lack of previous data, but has arguably higher (within-subject) power compared to the behavioural data analysis given the higher number of repeated measures over time in each condition (49 for brain imaging data versus 7 for GI symptoms).

Study design

This placebo-controlled, randomized, double-blind cross-over trial consisted of three study visits, with a washout period of at least one week. **Figure 1** provides an overview of the procedures performed on each study visit. After an overnight fast, a feeding probe was positioned transnasally into the stomach (50-55 cm); its position was verified by measuring the pH of aspirated gastric fluid using pH strips. The probe was taped to the nose with adhesive tape to prevent displacement after which a 15-minute break was held to avoid any autonomic nervous system influences.[16]

Then, the baseline abdominal MRI scan was acquired, followed by brain scanning using a pharmacological MRI design, starting with a 10 minute pre-infusion baseline. Then, 500mL of solution was intragastrically infused at a constant speed of 60mL/min. The solutions contained fructans (BioCare® Ltd, Birmingham, UK, 40g in 500mL 0.9% saline, degree of polymerisation = 3-10; osmolality = 72 mOsmol/kg), glucose (Glucopur glucose powder, 40g in 500mL 0.9% saline), or 500mL 0.9% saline

only (**Supplementary Table 1**). Functional brain imaging continued for 49 minutes after the start of the infusion; at 1-and 2-hours after the infusion start, abdominal MRI scanning was repeated.

GI symptoms (bloating, fullness, nausea, cramps, pain, flatulence) were scored on a 100mm visual analogue scale (VAS) pre-, and 10, 20, 30, 40, 50, 60, 90, 120, and 150 minutes post-infusion. Emotional state was assessed using the positive and negative affect schedule (PANAS)[17] pre-, and 60, 90, 120 and 150 minutes post-infusion, and the profile of mood states (POMS)[18] (fear, anger, depression, vigor, and fatigue) pre-, and 10, 20, 30, 40 and 50 minutes post-infusion.

The study was approved by the Ethics Committee of the University Hospitals Leuven, Belgium (number: S60607) and was performed in accordance with the declaration of Helsinki and BMJ guidelines. All authors had access to study data and reviewed and approved the final manuscript. The study was registered on www.clinicaltrials.gov as NCT04283487.

Randomization and Blinding

The infusions were given in counterbalanced order with a washout period of at least one week. Allocation using http://www.randomization.com was done by a colleague not involved in the study, who also prepared the solutions; subjects and researchers were both blinded to the test solution. Data were entered prior to unblinding.

Abdominal MR imaging analysis

Abdominal MRI images were acquired using a 1.5T whole-body scanner (Philips Medical Systems, Best, The Netherlands) in the supine position with no oral contrast. Motility was quantified from dynamic balanced turbo field echo (BTFE) images acquired during 20 second breath-hold using GIQuant®, a validated technique based on the dual registration of abdominal motion (DRAM) (Motilent Ltd, Ford, London).[19, 20] Ascending colon gas and volume content were measured on T2-weighted anatomical images. A summary of the data processing pipeline is shown in **Figure 2**. Further details are provided in **Supplementary Material S2, Supplementary Table 2, 3, Supplementary Figure 1-4.**

Brain MR imaging analysis

Functional brain MR images were acquired using a 3.0T Philips Achieva DStream MR system with a 32-channel head coil (Philips Medical Systems, Best, The Netherlands). Data were analyzed using Statistical Parametric Mapping (SPM12, Wellcome Trust Centre for Neuroimaging, UCL, London, UK) implemented in MATLAB R2014b (MathWorks, Natick, MA, USA). Pre-processing, first (i.e. subject) level and second (i.e. group) level analysis were performed. Voxel-level threshold was set at p<0·05 family-wise error (FWE) corrected for multiple testing. Voxel-based analysis was performed within a single mask of a priori pain-responsive regions of interest generated using "pain" as specific term of interest in the automated meta-analytical tool Neurosynth (https://neurosynth.org).[21] Further details are provided in **Supplementary Material S3**.

Statistical analysis of non-brain imaging data

Statistical analysis was performed in Statistical Analysis System (SAS) version 9.4 (SAS Institute, Cary, NC, USA). Data was considered statistically significant when p<0.05. Demographical parameters were compared between IBS patients and HC using two-sample t-tests.

Symptoms, abdominal parameters, and the associations between both were analyzed using marginal linear mixed models. Where the outcome variable or its logarithmic transformation was not normally distributed, the data was ordinalized into tertiles or quartiles and analyzed using generalized linear mixed models with a cumulative logit link function. The variance-covariance structure providing the best fit was chosen based on the minimum Akaike's Information Criterion (AIC).

Comparison between conditions and groups

Condition, time (both within-subject), and group (between-subject) were the categorical independent variables. A main effect of visit (categorical, within-subject) was added to control for putative visit differences. Effects of interest included the main effect of group, testing overall differences between IBS and HC, the group-by-time interaction effect, testing the differences in the time course between

both groups, and the group-by-condition two-way interaction effect, comparing the differences between conditions between both groups. To clarify the latter effect, constituting our main hypothesis test, change from baseline values were compared between conditions (fructans vs glucose and fructans vs saline) within groups as well as between groups within conditions using planned contrast analyses, with stepdown Bonferroni multiple testing correction.

Covariation between abdominal responses and symptoms in IBS patients

Differences in both GI symptoms and gut physiology parameters between conditions (fructans vs glucose and fructans vs saline) at each timepoint were calculated and, in case of colonic parameters, averaged over timepoints. Time and difference in abdominal parameters were added to the model as independent variables with differences in GI symptoms as dependent variable. The main effect of the abdominal parameters constitutes the effect of interest testing covariation between abdominal responses and symptom responses (over all timepoints).

Results

Study population

In total, 14 healthy controls and 14 IBS patients were recruited. Two participants, one in each group, dropped out due to intolerance to the long period of brain MR scanning, resulting in 13 evaluable subjects in each group (Consort flow diagram see **Supplementary Figure 5, 6**). All patients fulfilled the Rome IV criteria for IBS according to the Rome IV questionnaire. 6 IBS patients met criteria for diarrhoea-predominant subtype, 3 for constipation-predominant subtype, and 4 for mixed subtype. Descriptive data are shown in **Table 1**.

GI symptoms

Higher scores for bloating, cramps and flatulence (group main effect: $F_{1,685}$=9.38, p=0.002; $F_{1,684}$=23.55, p<0.001; $F_{1,682}$=8.30, p=0.004, respectively) were reported by IBS patients compared to HC, with increasing differences over time (group-by-time interaction effect: $F=_{10,685}$=2.96, p=0.001; $F_{10,684}$=7.77,

p<0.001; $F_{10,682}$=2.30, p=0.012, respectively) (**Figure 3**). The group by condition interaction effect was observed for cramps and nausea ($F_{2,684}$=5.48, p=0.004; $F_{2,686}$=3.78, p=0.023, respectively). Planned contrast analysis revealed the differences between conditions (fructans vs glucose or fructans vs saline) were seen only in IBS, but not in HC for cramps, pain, flatulence and nausea (**Table 2**). No significant group main effects or group by condition interaction effects were observed for the other GI symptoms (all p>0.12) (**Supplementary Material S4).**

Controlling these analyses for psychological variables (PHQ12, PHQ9, GAD7) did not change the effects of interest, nor were any of the effects of the psychological variables significant (data not shown).

Psychological state: POMS and PANAS

No significant differences were observed for extra-intestinal symptoms (all p>0.06) (**Supplementary Material S5)**.

Abdominal physiology

Small bowel motility

Fructans induced a higher small bowel motility index compared to both glucose and saline (condition main effect: $F_{2,44}$ =15.39, p<0.001; planned contrasts: fructans vs glucose: t_{44}=5.49, p_{Holm}<0.001; fructans vs saline: t_{44}=3.46, p_{Holm}=0.001). IBS exhibited similar motility indices compared to HC (group main effect: $F_{1,24}$=0.12, p=0.73; group-by-condition interaction effect: $F_{2,24}$=0.57, p=0.57). Planned contrasts confirmed similar differences between conditions in IBS and HC (**Table 2, Figure 4A, B, Supplementary Table 4**).

Ascending colon gas and volume

There were no differences at baseline between IBS and HC (both p>0.21). Fructans infusion induced a greater increase in ascending colon gas (condition main effect: $F_{2,36}$=21.40, p<0.001; planned contrasts: fructans vs glucose: t_{36}=4.96, p_{Holm}<0.001; fructans vs saline: t_{36}=5.43, p_{Holm}<0.001) and volume (condition main effect: $F_{2,36}$=28.47, p<0.001; planned contrasts: fructans vs glucose: t_{36}=6.82,

p_{Holm}<0.001; fructans vs saline: t_{36}=5.65, p_{Holm}<0.001). IBS and HC showed similar increases in gas (group main effect: $F_{1,24}$=0.78, p=0.39; group-by-condition interaction effect: $F_{2,36}$=0.03, p=0.97) and volume (group main effect: $F_{1,24}$=1.39, p=0.25; group-by-condition interaction effect: $F_{2,36}$=0.67, p=0.52). Planned contrasts confirmed significant differences between fructans and both glucose and saline in IBS and HC (**Table 2**).

The increase in ascending colon gas in IBS remained stable between 60 to 120 minutes post infusion, while in HC, gas continued to increase (group-by-time interaction effect, $F_{1,23}$=5.60, p=0.027; planned contrasts: IBS: 120 mins vs 60 mins, t_{23}=-0.43, p_{Holm}=0.90; HC: 120 mins vs 60 mins, t_{23}=2.97, p_{Holm}=0.028). Such effect was not observed for volume ($F_{1,22}$=0.84, p=0.37) (**Figure 4C, D**).

Brain activity

Significant group-by-condition-by-time three-way interaction effects were observed on the blood oxygenation level-dependent (BOLD) signal, relative to pre-infusion baseline.

Comparing fructans and glucose between IBS and HC over time, differential activation patterns were identified in bilateral cerebellum, left and right supramarginal gyrus, bilateral postcentral gyrus, right anterior and midcingulate cortex, left middle and right anterior insula, left rolandic operculum, right putamen, and bilateral thalamus (**Table 3, Figure 5A**).

Furthermore, significant group differences were found following fructans infusion compared to saline over time in bilateral cerebellum, bilateral supramarginal gyrus, right superior temporal gyrus, left superior and right middle frontal gyrus, bilateral anterior cingulate cortex, left midcingulate cortex, bilateral anterior and middle insula, and right rolandic operculum. (**Table 3, Figure 5B**).

Associations between gut physiology and GI symptoms

Due to the limited variability for the differences in GI symptoms between conditions in HC (fructans vs glucose: >48.36% within ±2; fructans vs saline: >39.69% within ±2), associations were analyzed in IBS only.

Small bowel motility

There was no association between the difference in small bowel motility and the difference in GI symptoms, for fructans compared to both glucose and saline, in IBS patients ($p>0.24$ for all main effects).

Ascending colon gas and volume

The difference in ascending colon gas between fructans and saline was associated with the respective difference in bloating (main effect of gas, $F_{1,11}=10.49$, $p=0.008$) and cramps (main effect of gas, $F_{1,11}=5.77$, $p=0.035$) (**Supplementary Figure 7**). No other associations were seen between ascending colon gas and other GI symptoms (fructans vs saline $p>0.40$; fructans vs glucose $p>0.058$ for all main effects of gas).

An association between difference in ascending colon volume and cramps was observed (main effect of volume, $F_{1,11}=6.19$, $p=0.03$) when comparing fructans and saline. No other significant associations were seen between differences in ascending colon volume and in GI symptoms ($p>0.10$ for all main effects of volume).

Associations between brain activity and GI symptoms

Due to the same reason as above, the associations between brain activity and GI symptoms were analyzed in IBS patients only.

The difference in brain response after fructans versus glucose covaried significantly with GI symptoms in bilateral cerebellum, right anterior and left midcingulate cortex, left inferior and superior and right

middle frontal gyrus, bilateral supramarginal gyrus, bilateral postcentral gyrus, right supplementary motor area, bilateral insula, right putamen, right pallidum, and bilateral thalamus (**Supplementary Table 5**).

The difference in brain response after fructans versus saline covaried significantly with GI symptoms in bilateral cerebellum, right anterior and left midcingulate cortex, left superior and right middle frontal gyrus, right supramarginal gyrus, bilateral postcentral gyrus, bilateral insula, left rolandic operculum, right putamen, and bilateral thalamus (**Supplementary Table 6**).

Discussion

Due to the fermentability and osmotic activity of FODMAPs, their ingestion can lead to intestinal distention through increases in luminal gas and water content. In patients with IBS, this process can induce GI symptoms, including bloating, diarrhoea, pain, cramps and flatulence, but the underlying mechanisms remain unclear. In this study, we investigated gut and neural responses to fructans (a key FODMAP), versus glucose and saline via MR-based abdominal and brain scanning. First, we confirmed our previous finding[6] of early (i.e. well within the first hour post-infusion) symptom induction by intragastric fructans infusion in IBS. Further, we demonstrated that fructans increased small bowel motility and ascending colon gas and volume to a similar extent in IBS patients and HC, despite only IBS patients reporting increased levels of cramps, pain, flatulence and nausea, partially confirming earlier results of Major et al.[5] For the first time, we demonstrate differential brain responses to fructans (compared to glucose and saline) were observed between IBS and HC in pain responsive regions including cerebellum, supramarginal gyrus, anterior and midcingulate cortex, insula and thalamus. These brain responses covaried more extensively with GI symptom responses in IBS compared to gut responses (only for colonic gas with bloating and cramps and colonic volume with cramps, and for the fructans versus saline comparison).

As anticipated, IBS patients reported increases in GI symptom ratings after fructans infusion compared to both glucose and saline control conditions, contrary to HC. The stronger increase in cramps in IBS

patients versus HC after fructans is in line with our previous study[6], which remarkably found a rapid increase in symptom ratings, with significance reached at 30 minutes post-infusion. Therefore, one hour of fMRI scanning should allow us to reach peak symptom intensity levels during scanning. In the current study, a significant rise in cramps, pain, flatulence and nausea following fructans compared to glucose was found in IBS, confirming our earlier findings, but it occurred more gradually compared to our previous study and continued to increase towards the end of the measurement. Although both glucose and saline were used as controls, it should be acknowledged that glucose has a calorie effect and can release GI hormones compared to saline, which may account for some of the differences from fructans. The difference in peak symptom intensity between our two FODMAP studies may be due to the higher concentration, as well as lower and fixed volume of the infusion used in the current study (**Supplementary Table 1**). These changes compared to our previous study[6] were implemented to correspond with the study by Major *et al*,[5] but fructans was chosen over inulin as it is more common in the Western diet.[22] This could explain the difference in timing of symptoms, given the average degree of polymerisation (DP) and osmotic activity of fructans (DP = 3-10; 72 mOsmol / kg) is between that of fructose (DP = 1; 462 mOsmol / kg) and inulin (where long chain inulin types have a DP ≥ 23; 36 mOsmol / kg). As lower DP chains are more water soluble, they show more water retention properties than long chains and therefore we can expect greater osmotic effects induced by fructans than inulin in the small bowel. The increased small bowel motility after fructans could also accelerate the transit and prompt the arrival of fructans in the colon.[23] Furthermore, the intragastric infusion of the test solution compared to the oral drinking administration used by Major et al may have also accelerated the arrival of the test solution to the proximal colon. This is supported by the observed increase in signal intensity in the small bowel and ascending colon from 1 hour post infusion of fructans (**Supplementary Figure 8**).

Abnormal GI motility is a hallmark feature of IBS. However, the majority of previous studies have relied upon indirect measures of bowel motility or transit time. We used recent advances in MRI which allow a direct assessment of small bowel motility to show fructans induced a similar increase in small bowel

motility in both IBS and HC. This is consistent with accelerated small bowel transit time following ingestion of a fructose-sorbitol mixture[24] and increased small bowel motility following mannitol solution in HC.[25] The abovementioned supplementary small bowel signal intensity analyses indicate that the increased small bowel motility after fructans may be caused by distention induced by increased small bowel water content (**Supplementary Material S2.3.3, Supplementary Figure 8**). We observed similar fructans-induced increases in gas and volume between IBS and HC up to two hours post-infusion. However, IBS patients showed an initial increase of gas in the ascending colon which remained stable compared to a steadier increase in HC over the 2-hour period. This is contrary to a previous MRI study[13] which showed a *lesser* increase in ascending colon volume in IBS diarrhoea-predominant patients compared to HC following a mixed nutrient challenge in the early post-prandial phase. Supplementary analyses suggest that the increase in colon volume following fructans in our study was driven by gas rather than osmotic effects based on the change in signal intensity (**Supplementary Material S2.3.3, Supplementary Figure 9**).

In IBS patients, we demonstrated that fructans-induced bloating and cramps were associated with changes in colonic gas, but not small bowel motility. This was consistent with the findings by Major et al[5] that colonic gas, but not small bowel water was linked to GI symptom responses. A previous study also showed that symptoms following 20g lactose ingestion in Chinese IBS patients were associated with both presence of rectal hypersensitivity assessed using barostat and gas production measured by a hydrogen breath test.[26] Yang et al found that the likelihood of having GI symptoms was related to the dose of lactose ingestion and hydrogen gas production.[27] Taken together, despite differences in osmotic effects and other properties, all FODMAPs are (variably) poorly absorbed, thereby undergoing colonic fermentation, which in turn leads to gas production playing an important role in symptom generation.

Previous studies showed small differences in symptom generation after oral administration of various FODMAPs despite their differences in osmotic activity and other properties.[28, 29] A common

characteristic of the different FODMAPs is fermentability in the colon leading to gas production, with the latter having been shown to be associated with symptom responses in the abovementioned studies (using hydrogen breath tests). These results are in line with our MRI-based finding of an association between colonic gas production and symptom responses, but our brain results indicate the additional role of visceral hypersensitivity reflected in altered brain response patterns. We also found that colonic volume is associated with cramps, indicating that distension by colonic contents may also be relevant to symptoms.

In addition to increased motility and luminal distension effects, other mechanisms at the level of the gut which cannot be measured using MRI, including mast cells, barrier dysfunction and intestinal microbiota may also be involved in FODMAP-induced symptom generation.[30, 31]

At the brain level, pain is not encoded one specific cortical area. Instead, it is a complex non-linear process involving systems processing homeostatic-afferent information as well as cognitive and affective circuits, and "top-down" descending modulatory pathways that send descending projections from the brainstem areas to the dorsal horn of the spinal cord.[32, 33] Studies using fMRI have reported associations between visceral hypersensitivity and altered brain activation and/or connectivity patterns in IBS.[34-36] The current study, focusing on visceral hypersensitivity following FODMAP intake, showed differential activation patterns in several pain-responsive brain regions between IBS and HC. Compared to glucose, fructans infusion was associated with altered responses in cerebellum, supramarginal gyrus, postcentral gyrus, anterior and midcingulate cortex, anterior and middle insula, rolandic operculum, putamen, and thalamus, in IBS versus HC. Similar results were found for fructans versus saline, including cerebellum, supramarginal gyrus, superior temporal gyrus, superior and middle frontal gyrus, anterior and midcingulate cortex, anterior and middle insula, and rolandic operculum.

These results are partly in line with a meta-analysis of previous studies in IBS showing differential activation in the insula, anterior cingulate cortex and the thalamus following rectal balloon distention in IBS patients compared to HC.[12] Furthermore, fMRI studies showed cerebellar responses to (visceral)

nociceptive stimulation.[37] Notably, these previous studies compare a test stimulus (e.g. rectal balloon distention) to the absence of this stimulus (e.g. deflated balloon), whereas our study compares a test stimulus (fructans infusion) to an "active" control stimulus (e.g. glucose and saline infusion). In addition to being far more ecologically and pathophysiologically valid compared to rectal balloon distension, fructans administration represents an indirect rather than a direct visceral pain stimulus, as the distention resulting from fructans reaching the bowel is thought to cause symptoms, rather than the ingestion of fructans *per se*. Nevertheless, the differential activation patterns in brain areas involved in pain regulation confirms the interaction between colonic distention induced by FODMAP intake and centrally regulated visceral hypersensitivity in IBS, thereby reinforcing the concept of IBS being a DGBI. Our finding that fructans-induced responses in most of these brain areas covaried with levels of virtually all fructans-induced GI symptoms further corroborates this interpretation, and identifies central mechanisms as the main driver of fructans-induced symptom generation in IBS for the first time. However, it should also be noted that visceral sensitivity has peripheral and central components. A limitation of this study is that we didn't measure peripheral sensitivity, at the clinical level (for example by rectal barostat) nor at the molecular level (for example by quantifying peripheral immune responses to food components).

Interestingly, fructans infusion did not only increase activity in pain-responsive brain regions compared to glucose and saline in IBS, but also reduced activation in several of these brain regions over the post-infusion timecourse. Remarkably, consistently opposite patterns from those seen in HC were observed: where fructans induced higher brain activation compared to glucose and saline in HC, reduced activation following fructans compared to glucose and saline was seen in IBS, and vice versa. The decreased activation of the right anterior cingulate cortex following fructans infusion in IBS can be associated with reduced pain coping.[38] Alternatively, the decreased activation of the anterior cingulate cortex in IBS patients can be interpreted as dysfunction of opioid-rich descending modulatory pain pathways, which has been linked to pain chronification.[39] Further, imaging studies have shown that descending pain pathways can be altered by emotional state or negative emotion.[40] Hence, there

might be a link between psychological state, reduced activity in the anterior cingulate cortex and increased symptom responses in IBS. However, further research to verify this interpretation is warranted. Interpretation of these differences in the direction of the brain responses between IBS patients and HC is complicated, since it remains unknown whether the brain activity observed in the current study is driven by inhibitory or excitatory neurons. Finally, it should also be noted here that, due to the very different nature of the stimuli used in the current versus previous studies (single nutrient infusion versus repeated phasic balloon distension), different fMRI design and analysis methods are required (pharmacological MRI versus classic block- or event-related designs), further increasing difficulties with direct comparisons of studies.

The anterior cingulate cortex and the anterior insula are often jointly activated.[41, 42] Accordingly, a reduced activation of the left anterior and middle insula following fructans infusion compared to glucose and saline was found in IBS, whereas opposite activation patterns were seen in HC. The insula plays an important role in processing and integrating viscerosensory and, more broadly, interoceptive information.[42, 43] Therefore, reduced activation in IBS might indicate a disturbed processing of interoceptive information. However, our results do not allow us to identify whether the underlying mechanisms lie in the brain, or in the gut, in the absence of measurements of peripheral mechanisms which may be driving aberrant interoceptive signaling, such as localized immune responses which have recently been shown to play a role in postinfectious IBS.[44] On the other hand, IBS patients displayed increased activation of the right insula, which is involved in the integration of the emotional and interoceptive state. Asymmetric activation of the insula has been reported previously, with increased activation of the left insula in positive emotional conditions and activation of the right insula in negative emotional conditions.[43, 45] Again, these findings support the link between emotional state, symptom perception and brain responses.

Additionally, increased activation of the right supramarginal gyrus was observed in IBS patients. Considering the role of the supramarginal gyrus in pain regulation and specifically attention to pain,[46]

we can speculate that IBS patients are more attentive to symptom responses, which may in turn lead to increased symptom reporting.

Some limitations of our study should be addressed. *First,* only female subjects were included in this study. Although IBS is a disorder with female predominance, our results cannot be generalized to both sexes. *Second*, based on the results of the current study and the previous study by Major *et al.*,[5] it is possible that the peak of symptom intensity lies outside the time frame of brain scanning and consequently, larger differences in activity of pain-responsive brain regions may have been missed in the current study protocol. Nevertheless, we did observe within- and between-group differences in symptom and brain responses after fructans well within 1 hour post-infusion. While a longer fMRI measurement would have been desirable, it was practically and methodologically not feasible. Longer scanning periods challenge the subjects' tolerance, inevitably leading to artefacts, and are seldom used in pharmacological MRI studies to avoid excessive influence of scanner drift. Importantly, to acquire the abdominal scans, brain scanning needed to end as pharmacological MRI analysis does not allow interruption of brain scanning. Hence, our paradigm represents the best compromise to capture both gut and brain responses (both pre- and post-infusion) within subjects' tolerance range. *Third*, we acknowledge there might be putative differences between IBS subtypes.[47] However, IBS diarrhoea-predominant patients do not show a more favorable response to a low FODMAP diet compared to IBS constipation-predominant patients in published clinical trials[48, 49] and a meta-analysis,[50] so we opted for a mixed sample to increase generalizability of our findings towards the entire IBS patient population. Our study was not adequately powered to analyse IBS subtypes groups separately. Studies in large sample sizes are warranted to address this issue. *Fourth,* the sample size of our study was rather small, but adequately powered for the hypothesized group-by-condition interaction effect based on our previous data. Nevertheless, our findings should be confirmed in a larger sample. *Finally,* given that a big dose of fructans was chosen in our study, our findings cannot be generalized to smaller doses of dietary fructans.

To conclude, we demonstrated increased symptom responses to fructans infusion in IBS. Fructans increased small bowel motility and ascending colon gas and volume to the same extent in IBS and HC. Colonic gas responses to fructans versus saline (but not versus glucose) were associated with bloating and cramps in IBS. No associations were found with small bowel motility responses. Differential responses to fructans infusion (versus glucose and saline) were found in IBS patients versus HC in pain-responsive brain regions, including the cerebellum, supramarginal gyrus, anterior and midcingulate cortex, insula and thalamus. Associations between these brain responses and virtually all GI symptoms were found in IBS. These fMRI observations provide direct evidence that dysfunction of the gut-brain axis may underlie FODMAP-induced symptom generation in IBS, more specifically by showing a strong link between aberrant brain responses and fructans-induced symptom generation; they may represent the central readout of hypersensitivity to normal FODMAP-induced gut responses. Future research should further elaborate on these findings to unravel the exact implications of these findings for IBS pathophysiology and treatment, but targeting hypersensitivity by pharmacological, dietary and/or psychological therapies (for example exposure-based cognitive behavioural therapy) may constitute promising avenues.

References

1. Ford AC, Sperber AD, Corsetti M, et al. Irritable bowel syndrome. Lancet 2020;396:1675-1688.
2. Simren M, Tack J. New treatments and therapeutic targets for IBS and other functional bowel disorders. Nat Rev Gastroenterol Hepatol 2018;15:589-605.
3. Black CJ, Staudacher HM, Ford AC. Efficacy of a low FODMAP diet in irritable bowel syndrome: systematic review and network meta-analysis. Gut 2021.
4. Staudacher HM, Whelan K. The low FODMAP diet: recent advances in understanding its mechanisms and efficacy in IBS. Gut 2017;66:1517-1527.
5. Major G, Pritchard S, Murray K, et al. Colon Hypersensitivity to Distension, Rather Than Excessive Gas Production, Produces Carbohydrate-Related Symptoms in Individuals With Irritable Bowel Syndrome. Gastroenterology 2017;152:124-133 e2.
6. Masuy I, Van Oudenhove L, Tack J, et al. Effect of intragastric FODMAP infusion on upper gastrointestinal motility, gastrointestinal, and psychological symptoms in irritable bowel syndrome vs healthy controls. Neurogastroenterol Motil 2018;30.
7. Goyal O, Batta S, Nohria S, et al. Low fermentable oligosaccharide, disaccharide, monosaccharide, and polyol diet in patients with diarrhea-predominant irritable bowel syndrome: A prospective, randomized trial. J Gastroenterol Hepatol 2021.
8. Eswaran S, Chey WD, Jackson K, et al. A Diet Low in Fermentable Oligo-, Di-, and Monosaccharides and Polyols Improves Quality of Life and Reduces Activity Impairment in Patients With Irritable Bowel Syndrome and Diarrhea. Clin Gastroenterol Hepatol 2017;15:1890-1899 e3.
9. Drossman DA. Functional Gastrointestinal Disorders: History, Pathophysiology, Clinical Features and Rome IV. Gastroenterology 2016.
10. Simren M, Mansson A, Langkilde AM, et al. Food-related gastrointestinal symptoms in the irritable bowel syndrome. Digestion 2001;63:108-15.
11. Simrén M, Törnblom H, Palsson O, et al. Visceral hypersensitivity is associated with GI symptom severity in functional GI disorders: consistent findings from five different patient cohorts. Gut 2018;67.
12. Tillisch K, Mayer EA, Labus JS. Quantitative meta-analysis identifies brain regions activated during rectal distension in irritable bowel syndrome. Gastroenterology 2011;140:91-100.
13. Pritchard SE, Marciani L, Garsed KC, et al. Fasting and postprandial volumes of the undisturbed colon: normal values and changes in diarrhea-predominant irritable bowel syndrome measured using serial MRI. Neurogastroenterol Motil 2014;26:124-30.
14. Mearin F, Lacy BE, Chang L, et al. Bowel Disorders. Gastroenterology 2016.
15. Kreidler SM, Muller KE, Grunwald GK, et al. GLIMMPSE: Online Power Computation for Linear Models with and without a Baseline Covariate. J Stat Softw 2013;54.
16. Farmer AD, Coen SJ, Kano M, et al. Psychological traits influence autonomic nervous system recovery following esophageal intubation in health and functional chest pain. Neurogastroenterology & Motility 2013;25:950-e772.
17. Crawford JR, Henry JD. The positive and negative affect schedule (PANAS): construct validity, measurement properties and normative data in a large non-clinical sample. Br J Clin Psychol 2004;43:245-65.
18. Wyrwich KW, Yu H. Validation of POMS questionnaire in postmenopausal women. Qual Life Res 2011;20:1111-21.
19. Menys A, Taylor SA, Emmanuel A, et al. Global small bowel motility: assessment with dynamic MR imaging. Radiology 2013;269:443-50.
20. Menys A, Hamy V, Makanyanga J, et al. Dual registration of abdominal motion for motility assessment in free-breathing data sets acquired using dynamic MRI. Phys Med Biol 2014;59:4603-19.

21. Yarkoni T, Poldrack RA, Nichols TE, et al. Large-scale automated synthesis of human functional neuroimaging data. Nat Methods 2011;8:665-70.
22. Shepherd SJ, Lomer MC, Gibson PR. Short-chain carbohydrates and functional gastrointestinal disorders. Am J Gastroenterol 2013;108:707-17.
23. Staudacher HM, Irving PM, Lomer MC, et al. Mechanisms and efficacy of dietary FODMAP restriction in IBS. Nat Rev Gastroenterol Hepatol 2014;11:256-66.
24. Madsen JL, Linnet J, Rumessen JJ. Effect of nonabsorbed amounts of a fructose-sorbitol mixture on small intestinal transit in healthy volunteers. Dig Dis Sci 2006;51:147-53.
25. de Jonge CS, Menys A, van Rijn KL, et al. Detecting the effects of a standardized meal challenge on small bowel motility with MRI in prepared and unprepared bowel. Neurogastroenterol Motil 2019;31:e13506.
26. Zhu Y, Zheng X, Cong Y, et al. Bloating and distention in irritable bowel syndrome: the role of gas production and visceral sensation after lactose ingestion in a population with lactase deficiency. Am J Gastroenterol 2013;108:1516-25.
27. Yang J, Deng Y, Chu H, et al. Prevalence and presentation of lactose intolerance and effects on dairy product intake in healthy subjects and patients with irritable bowel syndrome. Clin Gastroenterol Hepatol 2013;11:262-268 e1.
28. Shepherd SJ, Parker FC, Muir JG, et al. Dietary triggers of abdominal symptoms in patients with irritable bowel syndrome: randomized placebo-controlled evidence. Clin Gastroenterol Hepatol 2008;6:765-71.
29. Zhao J, Fox M, Cong Y, et al. Lactose intolerance in patients with chronic functional diarrhoea: the role of small intestinal bacterial overgrowth. Aliment Pharmacol Ther 2010;31:892-900.
30. Kamphuis JBJ, Guiard B, Leveque M, et al. Lactose and Fructo-oligosaccharides Increase Visceral Sensitivity in Mice via Glycation Processes, Increasing Mast Cell Density in Colonic Mucosa. Gastroenterology 2020;158:652-663 e6.
31. Vervier K, Moss S, Kumar N, et al. Two microbiota subtypes identified in irritable bowel syndrome with distinct responses to the low FODMAP diet. Gut 2021.
32. Van Oudenhove L, Crowell MD, Drossman DA, et al. Biopsychosocial Aspects of Functional Gastrointestinal Disorders. Gastroenterology 2016.
33. Mayer EA, Tillisch K. The brain-gut axis in abdominal pain syndromes. Annu Rev Med 2011;62:381-96.
34. Larsson MB, Tillisch K, Craig AD, et al. Brain responses to visceral stimuli reflect visceral sensitivity thresholds in patients with irritable bowel syndrome. Gastroenterology 2012;142:463-472 e3.
35. Lowen MB, Mayer E, Tillisch K, et al. Deficient habituation to repeated rectal distensions in irritable bowel syndrome patients with visceral hypersensitivity. Neurogastroenterol Motil 2015;27:646-55.
36. Icenhour A, Witt ST, Elsenbruch S, et al. Brain functional connectivity is associated with visceral sensitivity in women with Irritable Bowel Syndrome. Neuroimage Clin 2017;15:449-457.
37. Van Oudenhove L, Kragel PA, Dupont P, et al. Common and distinct neural representations of aversive somatic and visceral stimulation in healthy individuals. Nat Commun 2020;11:5939.
38. Emmert K, Breimhorst M, Bauermann T, et al. Active pain coping is associated with the response in real-time fMRI neurofeedback during pain. Brain Imaging Behav 2017;11:712-721.
39. Ossipov MH, Morimura K, Porreca F. Descending pain modulation and chronification of pain. Curr Opin Support Palliat Care 2014;8:143-51.
40. Crofford LJ. Chronic Pain: Where the Body Meets the Brain. Trans Am Clin Climatol Assoc 2015;126:167-83.
41. Craig AD. How do you feel--now? The anterior insula and human awareness. Nat Rev Neurosci 2009;10:59-70.
42. Strigo IA, Craig AD. Interoception, homeostatic emotions and sympathovagal balance. Philos Trans R Soc Lond B Biol Sci 2016;371.

43. Duerden EG, Arsalidou M, Lee M, et al. Lateralization of affective processing in the insula. Neuroimage 2013;78:159-75.
44. Aguilera-Lizarraga J, Florens MV, Viola MF, et al. Local immune response to food antigens drives meal-induced abdominal pain. Nature 2021;590:151-156.
45. Dixon ML, Thiruchselvam R, Todd R, et al. Emotion and the prefrontal cortex: An integrative review. Psychol Bull 2017;143:1033-1081.
46. Christidi F, Karavasilis E, Michels L, et al. Dimensions of pain catastrophising and specific structural and functional alterations in patients with chronic pain: Evidence in medication-overuse headache. World J Biol Psychiatry 2019:1-13.
47. Gunn D, Abbas Z, Harris HC, et al. Psyllium reduces inulin-induced colonic gas production in IBS: MRI and in vitro fermentation studies. Gut 2021.
48. Patcharatrakul T, Juntrapirat A, Lakananurak N, et al. Effect of Structural Individual Low-FODMAP Dietary Advice vs. Brief Advice on a Commonly Recommended Diet on IBS Symptoms and Intestinal Gas Production. Nutrients 2019;11.
49. Bohn L, Storsrud S, Liljebo T, et al. Diet low in FODMAPs reduces symptoms of irritable bowel syndrome as well as traditional dietary advice: a randomized controlled trial. Gastroenterology 2015;149:1399-1407 e2.
50. van Lanen AS, de Bree A, Greyling A. Efficacy of a low-FODMAP diet in adult irritable bowel syndrome: a systematic review and meta-analysis. Eur J Nutr 2021;60:3505-3522.

Tables

Table 1. Demographical data of the study population.

		HC (n=13)	IBS (n=13)	p-value
Age	y, mean (range)	31 (20-53)	28 (20-41)	0.42
Height	cm, mean ± s.d.	167 ± 7	170 ± 6	0.33
Weight	kg, mean ± s.d.	62.46 ± 7.3	69.12 ± 13.6	0.13
BMI	kg/m^2, mean ± s.d.	22.31 ± 1.74	23.94 ± 4.23	0.20
PHQ12*	mean ± s.d.	1.74 ± 1.01	9.18 ± 2.82	<0.001
PHQ9°	mean ± s.d.	0.46 ± 0.88	4.38 ± 3.04	<0.001
GAD7$^{\Delta}$	mean ± s.d.	0.08 ± 0.28	4.00 ± 2.86	<0.001

* somatic symptom severity subscale: 0-3 minimal, 4-7 low, 8-12 medium, 13-24 high.

° depression subscale: 0-4 none, 5-9 mild, 10-14 moderate, 15-19 moderately severe, 20-27 severe.

$^{\Delta}$ generalized anxiety subscale: 0-4 none, 5-9 mild, 10-14 moderate, >15 severe.

Table 2. Results of within-group planned contrast analyses.

		p-value								
		Bloating	**Fullness**	**Nausea**	**Cramps**	**Pain**	**Flatulence**	**Small bowel motility**	**Δ AC gas**	**Δ AC volume**
HC	**Fructans vs glucose**	0.96	1.00	1.00	0.48	0.14	0.74	**0.006**	**0.002**	**<0.001**
	Fructans vs saline	0.98	1.00	0.18	0.48	0.69	0.74	0.09	**<0.001**	**<0.001**
IBS	**Fructans vs glucose**	0.11	1.00	**<0.001**	**0.03**	**0.001**	**0.009**	**<0.001**	**0.002**	**<0.001**
	Fructans vs saline	0.57	1.00	1.00	**<0.001**	0.08	0.24	**0.006**	**0.002**	**0.001**

Significant p-values are bold and underlined. All p values adjusted by stepdown Bonferroni correction for multiple comparisons. HC, Healthy Control; IBS, Irritable Bowel Syndrome; Δ, change from baseline; AC, Ascending Colon.

Table 3. Brain regions in which responses to fructans versus glucose and saline differ significantly over time between IBS patients and healthy controls.

Region	Subregion	Side	Peak coordinates			F-value	Cluster volume	F-values within groups		Direction of response	
			X	Y	Z			HC	IBS	HC	IBS
fructans vs glucose											
Cerebellum	crus 2	left	-44	-60	-48	8.19	24	3.61	4.84	↑	↓
	vermis 3	right	6	-42	-16	6.27	41	7.09	/	↑	=
Supramarginal gyrus		left	-66	-36	30	7.38	75	3.77	3.77	↑	↓
		right	66	-20	20	5.70	48	2.31	3.55	↓	↑
Postcentral gyrus		left	-48	-24	26	9.45	206	2.46	7.92	↓	↑
		right	30	-30	60	6.35	82	2.43	4.05	↓	ns
		right	48	-20	32	4.04	43	/	4.06	=	↑
Cingulate cortex	anterior	right	2	32	18	9.09	179	6.30	3.14	↑	↓
	anterior	right	4	30	-4	6.74	27	1.77	5.60	↑	↓
	middle	right	2	-14	40	5.58	116	7.27	/	↑	↓
Insula	middle	left	-38	-2	-10	8.49	209	4.20	4.40	↑	↓
	anterior	right	38	16	-4	4.63	151	/	5.48	=	↑
Rolandic operculum		left	-42	-22	24	6.37	206	3.11	3.35	↓	↑
Putamen		right	22	14	2	7.28	51	7.17	1.35	↑	↓
Thalamus		left	-10	-14	14	7.06	318	2.69	4.98	↑	↓
		right	6	-4	10	5.57	57	/	6.94	↑	↓
fructans vs saline											
Cerebellum	crus 2	left	-44	-60	-48	5.03	318	2.09	3.19	↑	↓
	cerebellum 9	right	8	-60	-42	19.48	24	13.35	6.87	↓	↑
Supramarginal gyrus		left	-66	-36	30	14.04	91	11.61	4.02	↑	↓
		right	52	-24	30	4.25	279	/	5.80	=	↑
Temporal gyrus	superior	right	52	-20	12	5.22	279	1.71	3.79	↓	↑
Frontal gyrus	superior	left	-22	46	24	4.40	51	/	4.22	=	↓
	middle	right	42	48	8	3.22	22	/	4.64	=	↑
Cingulate cortex	anterior	left	-12	8	34	3.48	38	3.45	/	↑	=
	anterior	right	6	16	26	6.22	46	4.21	2.37	↑	↓
	middle	left	0	-2	30	4.24	48	/	6.73	=	↓
Insula	anterior	left	-32	24	4	6.73	137	4.20	3.05	↑	↓
	anterior	right	36	10	6	3.57	58	/	3.61	↓	↑
	middle	left	-36	-4	-10	4.88	83	1.35	3.92	↑	↓
	middle	right	40	4	-8	3.31	43	4.26	/	↑	↑
Rolandic operculum		right	46	-14	10	3.79	279	/	2.31	↓	↑

Higher, lower or equal brain responses in fructans compared to glucose, fructans compared to saline and glucose compared to saline are indicated with ↑, ↓ and =, respectively. No response is indicated by 'ns'.

Voxel level threshold p_{FWE}-corrected<0.05, cluster extent threshold k>=20.

IBS, Irritable Bowel Syndrome; HC, Healthy Control; FWE, family wise error.

Figure legends

Figure 1. Overview of procedures performed on one study visit.

VAS, visual analogue scale; GI, Gastrointestinal; POMS, profile of mood states; PANAS, positive and negative affect

Figure 2. Overview of the abdominal MRI data processing pipeline.

Figure 3. GI symptom responses to infusion of fructans, glucose, and saline in IBS patients and healthy controls.

(A) Bloating; (B) Fullness; (C) Nausea; (D) Cramps; (E) Pain; (F) Flatulence.

GI, Gastrointestinal; HC, Healthy Control; IBS, Irritable Bowel Syndrome

Figure 4. Abdominal physiology responses to infusion of fructans, glucose, and saline in IBS patients and healthy controls.

(A) Small bowel motility 1-hour post infusion in HC; (B) Small bowel motility 1-hour post infusion in IBS; (C) Ascending colon gas (D) Ascending colon volume, after infusion of the three solutions, over time.

HC, Healthy Control; IBS, Irritable Bowel Syndrome

Figure 5. Comparison of brain responses (change from baseline) between IBS patients and healthy controls over time.

(A) following fructans vs glucose; (B) following fructans vs saline.

Voxel level threshold p_{FWE}-corrected<0.05, extent threshold k >= 20. Colour scale reflects F-values.

IBS, Irritable Bowel Syndrome; HC, Healthy control; FWE, family wise error

Supplementary Material

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S1. Study subjects

IBS patients had to meet the Rome IV criteria for IBS, defined by the presence of abdominal pain at least 1 day per week over the last 3 months, related to changes in bowel habits.[1] All subjects had a stable body weight over the last 3 months. Specific exclusion criteria for healthy subjects were the presence of symptoms or a history of gastrointestinal diseases or disorders or other significant diseases. General criteria for exclusion were the use of non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, or other immunosuppressive drugs in the preceding 6 months. IBS patients who took medication affecting the central nervous (antidepressants, opiate analgesics, etc) or GI system were excluded. In addition, subjects presenting with endocrine or neurological disorders, psychiatric disorders, significant heart, lung, liver or kidney dysfunction, hypertension, food or drug allergies, anaemia or claustrophobia were not eligible for participation. Subjects who showed abnormal eating behavior or had followed a gluten-free or low-FODMAP diet previously were excluded from the study, as were subjects with current or previous drug or alcohol abuse. All subjects were asked to adhere to a low FODMAP diet for the preceding 24 hours and undergo at least 12-hour overnight fasting before each study visit. Eating instructions were standardized between subjects to minimize any previous meal residue (especially any fermentable foods) and also to minimize variability between subjects and conditions. IBS patients who had an overlap with functional dyspepsia were also excluded from the study to exclude any potentially confounding effects of upper GI physiology. Pregnant or breastfeeding women or subjects with conditions that could interfere with magnetic resonance imaging (MRI), including but not limited to cochlear implants, metal fragments or metal implants in the body, pacemaker or a neural stimulator, were excluded from study participation. Healthy subjects were recruited from an existing mailing list of subjects who previously participated in clinical studies within our research group. IBS patients were recruited via the (neuro)gastroenterology outpatient clinics at the University Hospitals Leuven and through advertising.

S2. Abdominal MRI

2.1. Acquisition

Anatomical images were acquired within a single 20 second breath hold (coronal) or over three successive breath-holds (axial). Dynamic images were acquired for 90 seconds, while participants were instructed to hold their breath for 20 seconds then slowly breathe out and continue with shallow breathing for the remainder of the acquisition. Two dimensional (2D) dynamic images were oriented to cover the terminal ileum or if not identified, to provide the most coverage of the small bowel. The three dimensional (3D) dynamic acquisition covered the whole abdominal region. Abdominal MRI acquisition parameters are provided in **Supplementary Table 2.**

2.2. Data processing

Abdominal imaging data was de-identified before exporting from the scanner and reviewed in Horos (version 3.3.5; horosproject.org, Annapolis, MD USA). Dynamic images were evaluated by a single reader with 5 years' experience of gastrointestinal MRI (HF) blinded to the infusion. Due to the collapsed nature of the bowel, baseline small bowel motility could not be assessed consistently in several participants, so analysis was restricted to 1-hour post infusion. Images were reviewed for image artifacts, excessive respiratory motion and to ensure the selected imaging slice had adequate coverage of the small bowel. Where multiple 2D slices had been acquired with equivalent scan quality and/or bowel coverage, the best slice was selected in consensus with a second reader (AB). Where 2D dynamic imaging was not suitable for analysis at ≥1 visit, a single representative slice was extracted from 3D dynamic imaging for all three visits to control for differences in image quality.

The first 20 seconds of each dynamic time-series acquired during breath-hold was processed using GIQuant® (Motilent Ltd, Ford, London).[2, 3] This validated image registration algorithm for quantifying small bowel motility produces one reference image and one 'motility map'. The motility map represents the standard deviation of the fractional change in area of each pixel over time, multiplied by 1000. This can be summarised within a region of interest as the mean motility index (GIQuant®

score) measured in arbitrary units (a.u).[4] A summary of the final dataset used in the analysis is shown in **Supplementary Table 3**.

2.3. Data analysis

2.3.1. Small bowel motility

Small bowel motility was quantified from a single slice position by two independent readers (HF & AB), blinded to the nature of the infusion and the motility maps. ROIs were placed on all visible small bowel on the reference image on a single slice. Agreement in ROI placement between readers was assessed using the Dice similarity coefficient (DSC) which measures twice the area of overlap between two readers' ROIs over the number of pixels in both regions of interest. For example, Readers A and B:

$$DSC = (2*(A \cap B))/(A+B)$$

A DSC of 0 indicates no overlap and DSC of 1 indicates perfect agreement with the threshold of 0.7 for 'good' overlap in medical imaging.[5] The mean motility index (GIQuant® score, **Supplementary Figure 1**) within the regions of interest was extracted using this ROI mask and agreement between the two readers was quantified as the bias and Bland-Altman limits of agreement (**Supplementary Figure 2**).[6] The average of the two readers was used in the final analysis.

2.3.2. Ascending colon gas and volume

All region of interest (ROI) based analysis was performed by readers blinded to the infusion using Horos (Version 3.3.5; horosproject.org, Annapolis, MD USA). Ascending colon volumes were segmented on the T2-weighted coronal images using the freehand ROI tool (HF, CP, HS & LS). The region of interest included the caecum and terminated at the hepatic flexure (**Supplementary Figure 3**). The consistency of this landmark was verified by a single reader (HF) for each participant by comparing ROI placement on the scans between visits and timepoints.

Gas within the ascending colon was segmented by a single reader (HF) using a region growing tool with a signal intensity threshold of <250 (**Supplementary Figure 3**). In 13/78 visits where a different coil was used due to equipment malfunction, this threshold was lowered to 150 to account for reduced contrast between gas and colon contents. These thresholds were based on the distribution of pixel signal intensities within an index region on a clearly defined pocket of gas in each image. This approach was chosen over the more objective threshold of < mean+2 S.D. used by Major et al,[7] which overestimated gas volume in the lower quality scans compared to manual segmentation.

Consort flow diagram of abdominal MRI analysis is shown in **Supplementary Figure 4.**

2.3.3. Signal intensity and volume

We did not acquire abdominal images to specifically quantify water content,[8] but we did observe a marked increase in signal intensity on balanced-turbo field echo images in the small bowel and T2-weighted images of the ascending colon following fructans. To corroborate our visual assessment, we used signal intensity as a proxy measure of water content in the bowel. To control for differences in coil loading, we normalised the signal intensity in the ascending colon against the right psoas muscle. As the small bowel images were acquired in a single slice, there was no structure in the field of view which could consistently be used to normalise the signal against, so absolute signal intensity values were used. Although we acknowledge that signal intensity is a crude measure, we used this to perform an exploratory analysis of the relationship between signal intensity in the small bowel; signal intensity in the ascending colon; and relationship between small bowel signal intensity and motility; relationship between ascending colon signal intensity and volume; relationship between ascending colon gas and volume.

Fructans was associated with the highest small bowel signal intensity (main effect of condition: $F_{2,44}$=36.79, p<0.001; planned contrasts: fructans vs glucose, t_{44}=8.56, p_{Holm}<0.001; fructans vs saline, t_{44}=4.49, p_{Holm}<0.001). No differences were seen between IBS and HC in small bowel signal intensity (main effect of group: $F_{1,24}$=0.06, p=0.81). The condition-by-group interaction effect was significant

($F_{2,44}$=3.28, p=0.047). Planned contrast analyses showed a significant difference between fructans and glucose in both groups, with the difference between fructans and saline reaching significance in IBS patients only (HC: fructans vs glucose, t_{44}=5.59, p_{Holm}<0.001; HC: fructans vs saline, t_{44}=1.48, p_{Holm}=0.145; IBS: fructans vs glucose, t_{44}=6.44, p_{Holm}<0.001; IBS: fructans vs saline, t_{44}=4.95, p_{Holm}<0.001) (**Supplementary Figure 8A, B**). In sum, these results show a very similar pattern to the small bowel motility results.

We also investigated the correlation between small bowel motility and signal intensity. The differences in small bowel motility between fructans and saline were associated with the respective differences in small bowel signal intensity in both IBS patients (r=0.6, p=0.03) and HC (r=0.81, p=0.001). These results are consistent with an association between small bowel motility and distension from poorly absorbed carbohydrates,[9, 10] although they should be interpreted with caution.

Fructans induced the highest signal intensity in the ascending colon (main effect of condition: $F_{2,44}$=27.90, p<0.001; planned contrasts: fructans vs glucose, t_{44}=7.47, p_{Holm}<0.001; fructans vs saline, t_{44}=3.61, p_{Holm}=0.001). The main effect of group (IBS vs HC: $F_{1,24}$=6.91, p=0.015) and condition-by-group interaction effect ($F_{2,44}$=4.14, p=0.023) were also significant. Planned contrast analyses showed a significant difference between fructans and glucose in both groups, with the difference between fructans and saline reaching significance in IBS patients only (HC: fructans vs glucose, t_{44}=3.29, p_{Holm}=0.006; HC: fructans vs saline, t_{44}=1.85, p_{Holm}=0.07; IBS: fructans vs glucose, t_{44}=7.27, p_{Holm}<0.001; IBS: fructans vs saline, t_{44}=3.26, p_{Holm}=0.006) (**Supplementary Figure 8C**).

The change in gas volume was associated with the change in ascending colon volume in both IBS and HC (all p <0.015, **Supplementary Figure 9**) while there was no association between the change in signal intensity and ascending colon volume (all p >0.20) suggesting that the increase in colon volume was mostly driven by the increase in gas following fructans.

S3. Brain MRI

3.1. Acquisition

Functional brain MR images were acquired using a 3.0T MR system (Philips Medical Systems, Best, The Netherlands). In total, 1416 functional volumes were acquired for an examination period of 59 min, including a 10-minute pre-infusion baseline scan. Each scanning procedure started with acquiring a T1-weighted structural scan (46 slices of 3mm thick, slice gap=0.3mm, repetition time=9.6msec, echo time=4.6msec, flip angle=90°, voxel size=0.98x0.98x1.20mm³) to co-register with the functional images and to exclude anatomical abnormalities. Functional T2*-weighted volumes were acquired as previously described.[11] A gradient echo planar imaging (EPI) sequence with blood oxygenation level-dependent (BOLD) contrast was used (46 slices of 2.70mm thick, slice gap=0.3 mm, repetition time=2500msec, echo time=30msec, flip angle=90°, voxel size=2.40x2.39x2.70mm³), covering the whole brain including the cerebellum. A 32-channel head-coil was used for radio frequency transmission and reception.

Data were analyzed using Statistical Parametric Mapping (SPM12, Wellcome Trust Centre for Neuroimaging, UCL, London, UK) implemented in MATLAB R2014b (The MathWorks Inc., Natick, MA, USA).

3.2. Data pre-processing

Pre-processing (realignment, co-registration, spatial normalization, and smoothing) was performed using the CONN toolbox implemented in Statistical Parametric Mapping (SPM12, Wellcome Trust Centre for Neuroimaging, UCL, London, UK) (https://web.conn-toolbox.org/). Spatial realignment was performed to correct for small movements during scanning. Further, co-registration of each functional image to the structural image of each subject and segmentation of the structural image were performed. The structural image was used for each participant as reference for the spatial normalization to the EPI template image supplied with SPM12, based on information obtained during the segmentation step. Spatial smoothing using an 8×8×8mm^3 Gaussian smoothing kernel was applied

to the normalized images to improve the signal-to-noise ratio and to correct for residual inter-individual differences in anatomy after normalization. A denoising procedure[12] was then performed by linear regression of potential confounding effects including noise components from white matter and cerebrospinal areas (the first computed as the average BOLD signal, and the next four computed as the first four components in a principal component analysis of the covariance within the subspace orthogonal to the average BOLD signal and all other potential confounding effects), 12 realignment parameters (3 translation and 3 rotation parameters and their derivatives) and outliers scrubbing (one for each identified outlier scan; outliers were defined by 97th percentiles in normative sample with global signal z-value threshold set at 5 and subject motion threshold set at 0.9 mm, which is the default in the CONN toolbox).

3.3. First level analysis

First (i.e. subject) level analysis was performed according to a previously described pharmacological fMRI analysis method.[13, 14] For each condition and each subject, pre-infusion brain volumes were considered as baseline. Post-infusion volumes were divided into 1-min time bins, reflecting the change in brain activity compared to baseline. For each of the 49 post-infusion time bins, a t-contrast was calculated within the general linear model framework to compare brain responses (relative to pre-infusion baseline period) between the three conditions (fructans, glucose and saline) in two pairwise comparisons of interest (fructans vs. glucose, fructans vs. saline). This resulted in 49 first-level contrast images per subject, corresponding to the difference in BOLD signal between the test solutions in each time bin.

3.4. Second level analysis

Second (i.e. group) level analysis was performed within a single mask of a priori pain-responsive regions of interest generated using “pain” as specific term of interest in the automated meta-analytical tool Neurosynth (https://neurosynth.org).[15] A factorial model was applied to compare the difference in signal change from baseline between groups and between conditions over time bins on

the 49 individual first-level contrast images, with the group-by-condition-by-time interaction effect being the effect of interest. Voxel-level threshold was set at $p < 0.05$ family-wise error corrected in the single mask consisting of pain-responsive regions of interest derived from Neurosynth.

3.5. Covariation analysis between brain activity and GI symptoms

To test whether the difference in GI symptoms between conditions covaried with the difference in brain response, the differences in GI symptom ratings were used to weigh the first level contrasts. For this purpose, GI symptom ratings were linearly interpolated to match the number of time bins in the first level analysis.

S4. GI symptoms

4.1. Bloating

At pre-infusion baseline, patients reported higher scores for bloating (t_{685}=2.24, p=0.025). Overall, higher bloating scores were reported by patients compared to HC, with increasing differences over time (group main effect: $F_{1,685}$=9.38, p=0.002, group-by-time interaction effect: $F=_{10,685}$=2.96, p=0.001). The difference between HC and IBS was present within each condition (group-by-condition interaction effect: $F_{2,685}$=0.70, p=0.50) and conditions did not differ within groups. Furthermore, over both groups, no differences were observed between conditions (condition main effect: $F_{2,70}$=2.38, p=0.10).

4.2. Fullness

No significant differences in baseline scores were found for fullness (t_{688}=0.20, p=0.84). None of the effects in the mixed model were significant (p>0.28 for all main effects and interaction effects).

4.3. Nausea

No significant differences in baseline scores were found for nausea (t_{686}=0.27, p=0.79). Overall, nausea did not differ between groups (group main effect: $F_{1,686}$=2.37, p=0.12), but more fluctuation in nausea scores in patients resulted in a significant group-by-time interaction effect ($F_{10,686}$=4.02, p<0.001).

Further, nausea was significantly higher in the fructans condition compared to glucose (condition main effect: $F_{2,686}$=6.69, p=0.001). This was driven by a significant difference in IBS patients only (group-by-condition interaction effect: $F_{2,686}$=3.78, p=0.023; planned contrast: fructans vs glucose, t_{686}=4.05, p_{Holm}<0.001).

4.4. Cramps

No significant differences in baseline scores were found for cramps (t_{684}=1.31, p=.19). Overall, IBS patients reported higher cramp scores compared to HC (group main effect: $F_{1,684}$=23.55, p<0.001), with an increasing difference towards the end of the measurements (group-by-time interaction effect: $F_{10,684}$=7.77, p<0.001). Over both groups, there was a significant difference in cramp scores between conditions (condition main effect: $F_{2,684}$=3.80, p=0.023), with increased differences towards the end of the measurement (condition-by-time interaction effect: $F_{20,684}$=1.73, p=0.025). The difference between conditions was larger in IBS compared to HC (group-by-condition interaction effect: $F_{2,684}$=5.48, p=0.004). Planned contrast analysis showed no significant differences between conditions within HC, but higher cramp scores for fructans compared to both glucose (t_{684}=2.57, p_{Holm}=0.03) and saline (t_{684}=4.06, p_{Holm} <0.001) in IBS.

4.5. Pain

No significant differences in baseline scores were found for pain (t_{683}=0.97, p=0.33). Over both groups, pain scores differed significantly between conditions, with higher pain scores reported in the fructans condition compared to glucose (condition main effect: F=$_{2,70}$=7.70, p<0.001; fructans vs glucose: t_{70}=3.88, p_{Holm}<0.001). Neither the main group effect ($F_{1,683}$=2.47, p=0.12) nor the group-by-condition interaction effect ($F_{2,683}$=1.09, p=0.34) were significant, but planned contrast analysis showed a significant fructans vs glucose difference in IBS patients (t_{683}=3.60, p_{Holm}=0.001), but not in HC.

4.6. Flatulence

No significant differences in baseline scores were found for flatulence (t_{682}=1.15, p=0.25). Flatulence was significantly higher in IBS compared to HC (group main effect: $F_{1,682}$=8.30, p=0.004), with increased differences towards the end of the measurement (group-by-time interaction effect: $F_{10,682}$=2.30, p=0.012). Over both groups, no significant difference was found between conditions (condition main effect: $F_{2,70}$=2.69, p=0.075). The group-by-condition interaction effect was not significant ($F_{2,682}$=0.28, p=0.76), but planned contrast analysis revealed a significant difference between fructans and glucose in IBS patients (t_{682}=3.08, p_{Holm}=0.009), contrary to HC.

S5. Extra-intestinal symptoms

5.1. POMS

There was too little variability in scores for fear, anger, depression due to too strong zero-inflation (>76.5% of the scores were zero) rendering these variables improper for further analysis.

No significant differences were found for baseline vigor (t_{119}=0.65, p=0.52) or post-infusion vigor scores between groups or between conditions (condition main effect: $F_{2,47}$=0.33, p=0.72; group main effect: $F_{1,24}$=0.36, p=0.55; condition-by-time interaction effect: $F_{10,213}$=0.43, p=0.93; group-by-time interaction effect: $F_{5,119}$=0.42, p=0.84; group-by-condition interaction effect: $F_{2,47}$=0.83, p=0.44).

No significant differences were found for baseline fatigue (t_{120}=1.76, p=0.081) or post-infusion fatigue scores between groups or between conditions (condition main effect: $F_{2,47}$=0.52, p=0.60; group main effect: $F_{1,24}$=3.65, p=0.068; condition-by-time interaction effect: $F_{10,219}$=0.38, p=0.96; group-by-time interaction effect: $F_{5,120}$=0.28, p=0.92; group-by-condition interaction effect: $F_{2,47}$=0.28, p=0.76).

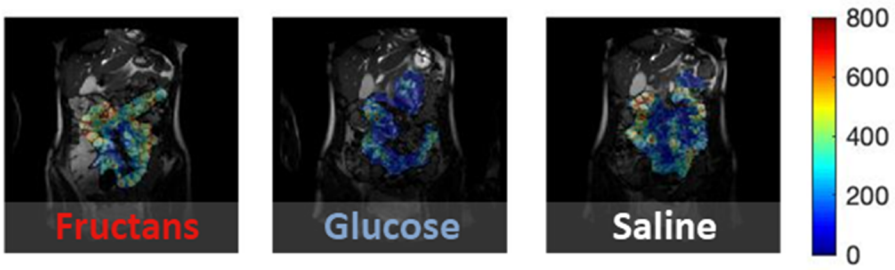
5.2. PANAS

There was too little variability in scores for negative affect (64.1% of the scores were the minimal score of 10) rendering it improper for further analysis.

No differences were found in baseline positive affect scores (t_{96}=0.37, p=0.71) or post-infusion positive affect scores between groups or between conditions (condition main effect: $F_{2,48}$=1.27, p=0.29; group main effect: $F_{1,24}$=0.01, p=0.91; condition-by-time interaction effect: $F_{8,189}$=0.88, p=0.54; group-by-time interaction effect: $F_{4,96}$=0.77, p=0.55; group-by-condition interaction effect: $F_{2,48}$=0.21, p=0.81).

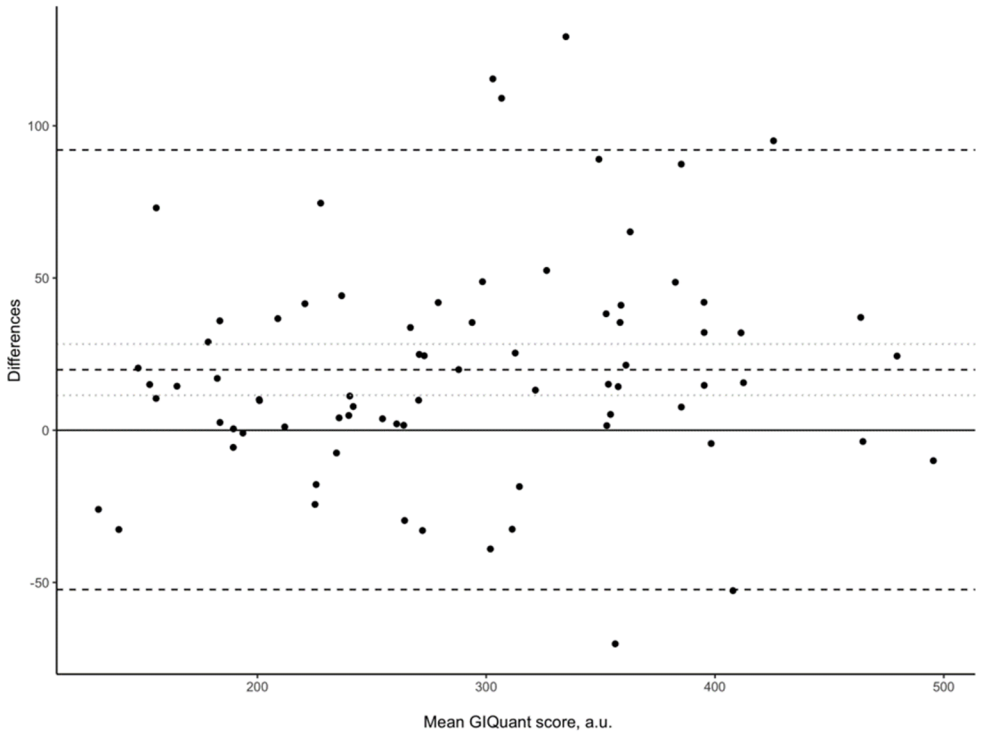
S6. Supplementary Figures

Supplementary Figure 1. Quantification of small bowel motility from dynamic 'cine' MRI.



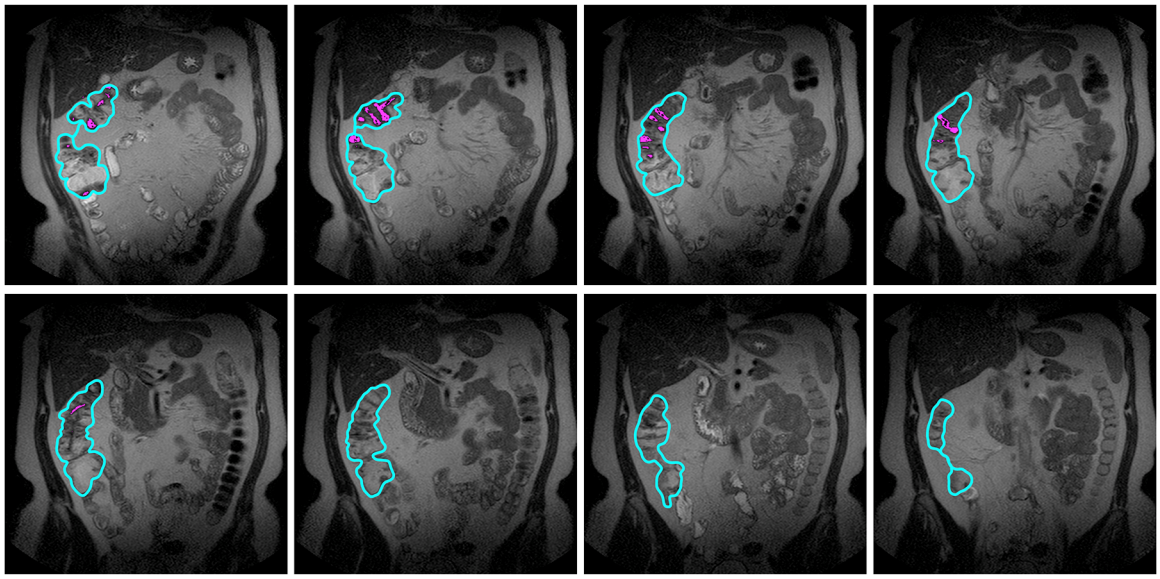
Representative Reference Images are shown with the parametric Motility Map on the segmented small bowel 60-minutes after each test solution was intragastrically administered. Mean motility index within the small bowel for this IBS patient was 383 a.u. 60 minutes after fructans; 177 a.u. after glucose; and 274 a.u. after saline.

Supplementary Figure 2. Bland-Altman limits of agreement for small bowel motility index.



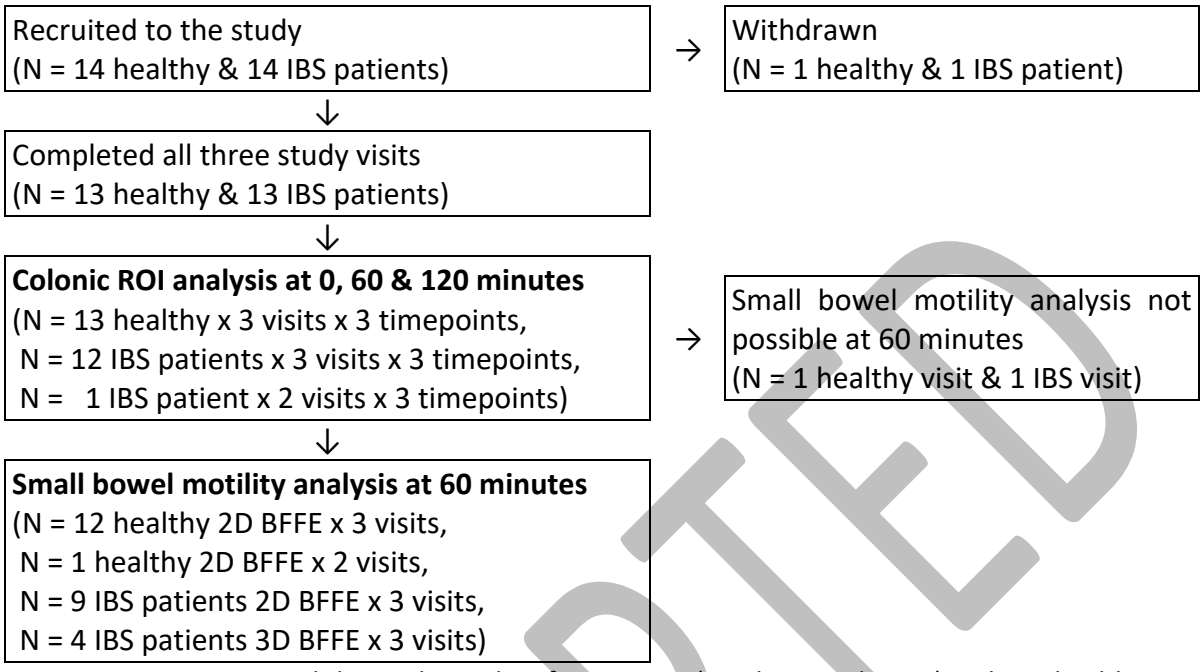
The small bowel was segmented by two independent readers and motility quantified as the mean motility index within the region. Reader 1 has a positive bias of 19.9 (95% CI: 11.4 to 28.3) ±72.2 arbitrary units (a.u.) for mean motility within the small bowel region of interest.

Supplementary Figure 3. Segmentation of the ascending colon and gas.



The ascending colon was manually segmented (cyan) and a region growing tool used to delineate gas (magenta). Ascending colon volume for the IBS patient shown was 482 cm^3 60 minutes after fructans including 42 cm^3 of gas.

Supplementary Figure 4. Consort diagram of the number of datasets available for abdominal MRI analysis.

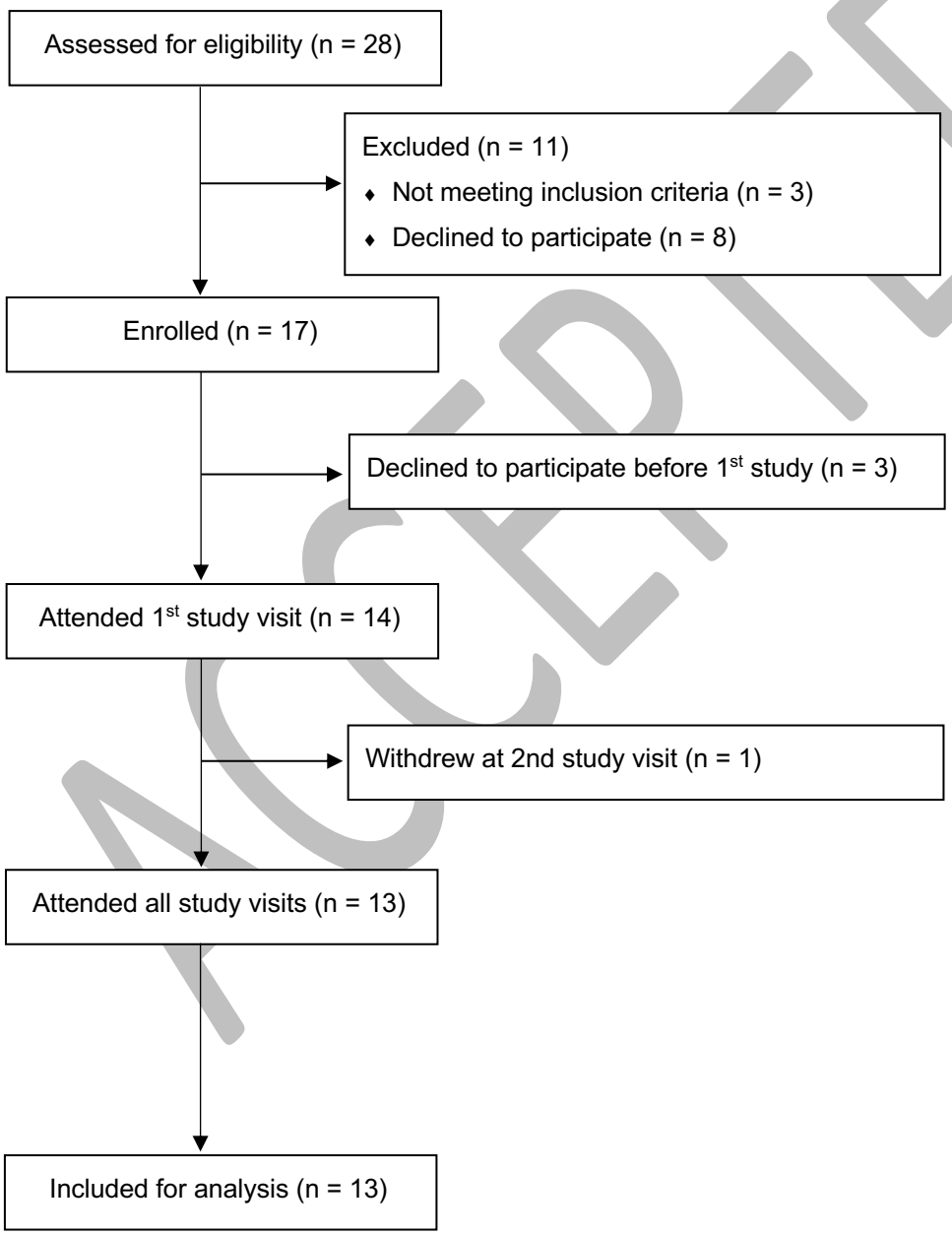


One patient was missing abdominal MRI data for one visit (condition = glucose) and two healthy participants were missing one timepoint at one visit.

Supplementary Figure 5. Consort flow diagram for healthy controls.

CONSORT
TRANSPARENT REPORTING of TRIALS

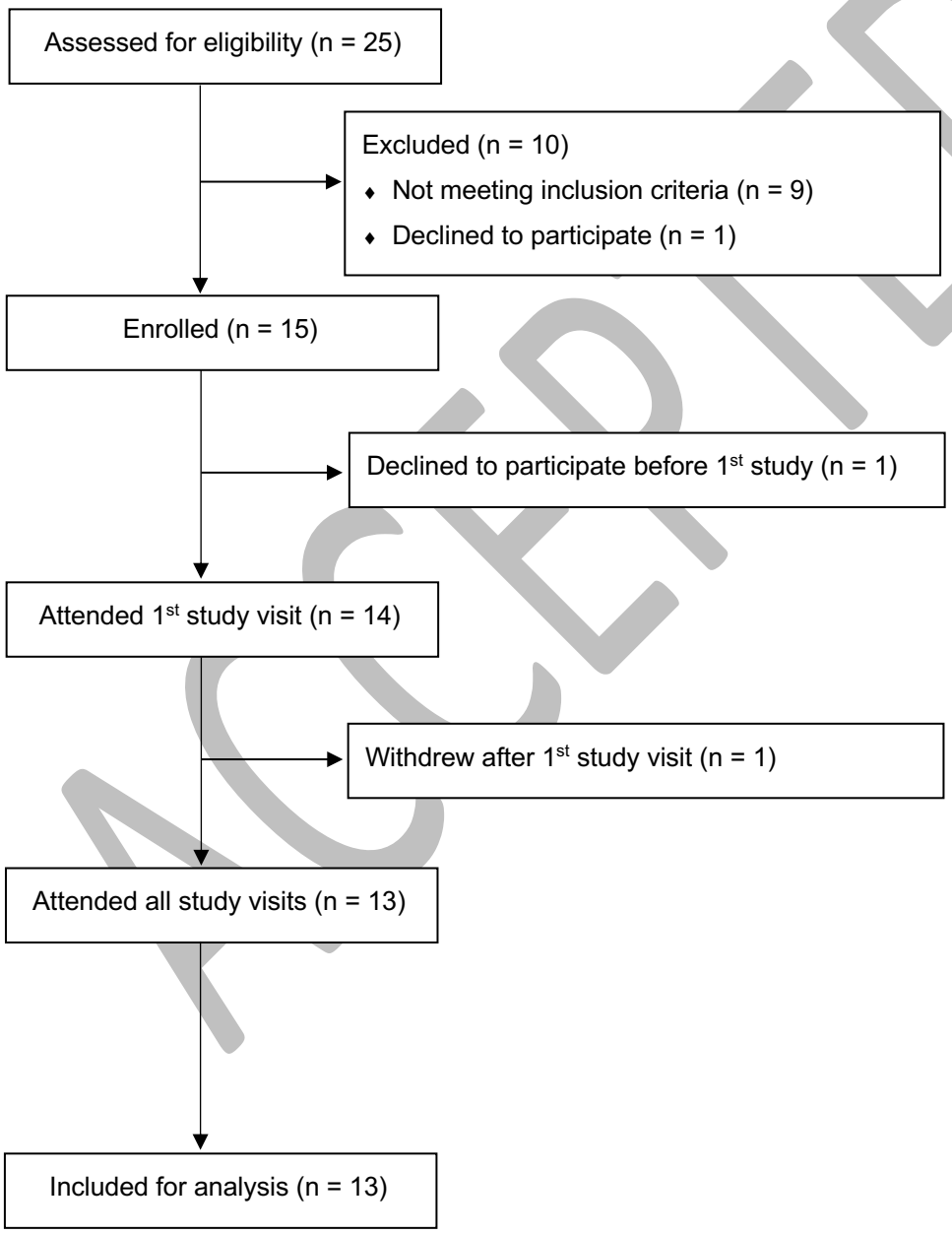
CONSORT Flow Diagram: Healthy Controls Group



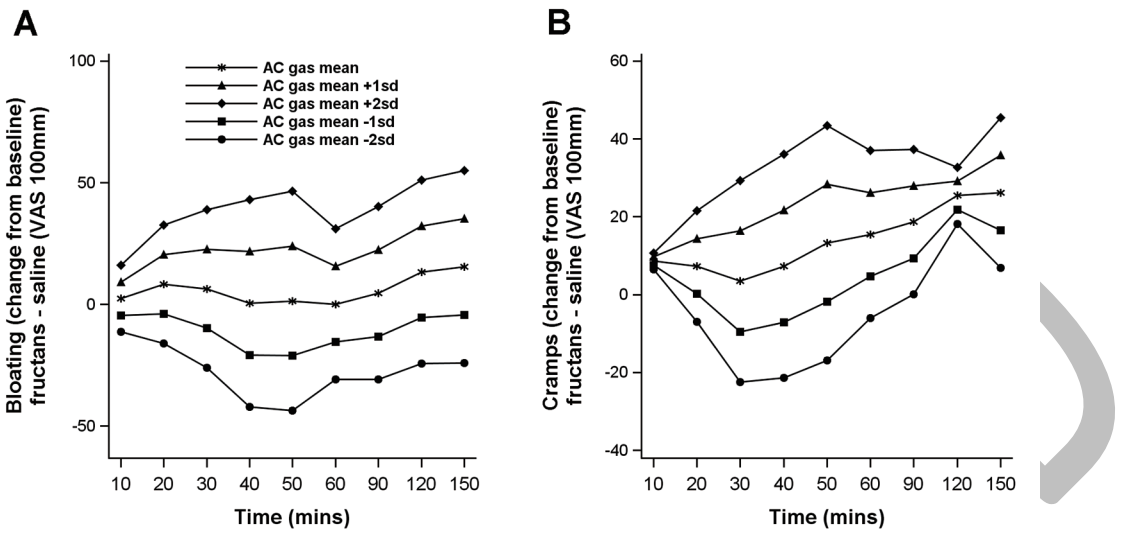
Supplementary Figure 6. Consort flow diagram for IBS patients.

CONSORT
TRANSPARENT REPORTING of TRIALS

CONSORT Flow Diagram: IBS Patients Group



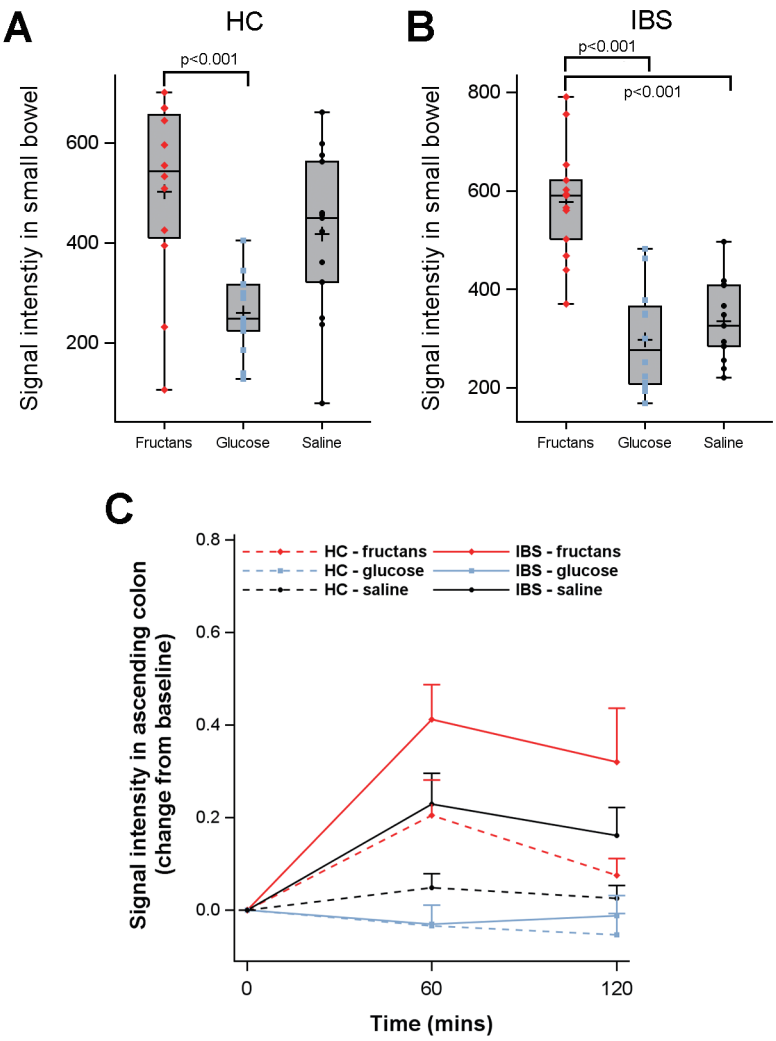
Supplementary Figure 7. Associations between differences in ascending colon gas and differences in GI symptoms when comparing fructans to saline in IBS patients.



Differences in symptoms (change from baseline) are plotted for different levels of differences in ascending colon gas (change from baseline, z-scored). (A) Bloating; (B) Cramps.

AC, ascending colon

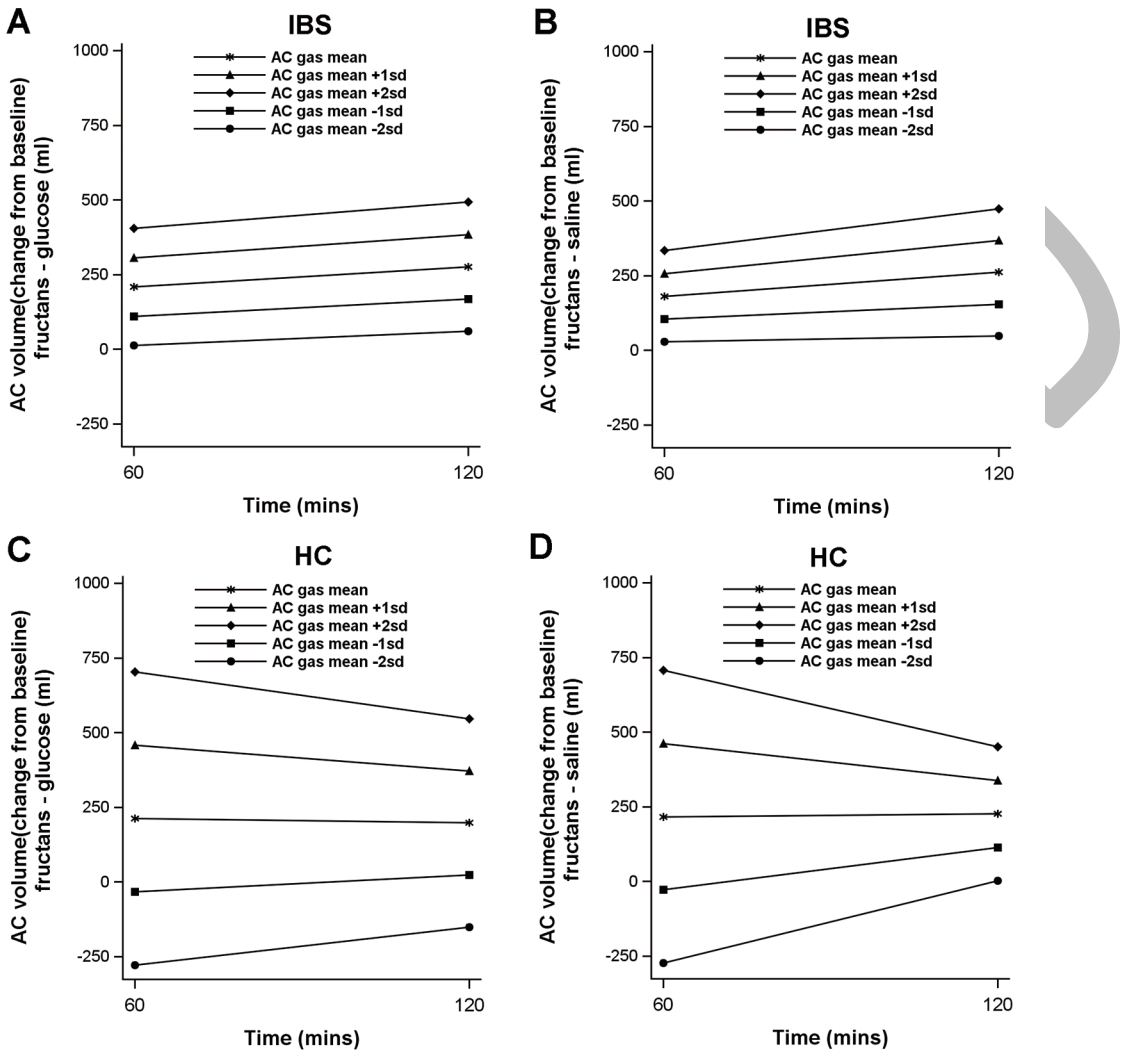
Supplementary Figure 8. Signal intensity following intragastric infusion of fructans, glucose, and saline in IBS patients and healthy controls.



(A) Small bowel signal intensity 1-hour post infusion in HC; (B) Small bowel signal intensity 1-hour post infusion in IBS; (C) Ascending colon signal intensity (change from baseline, normalised against the right psoas muscle) after infusion of the three solutions, over time.

HC, Healthy Control; IBS, Irritable Bowel Syndrome

Supplementary Figure 9. Associations between differences in ascending colon volume and differences in ascending colon gas when comparing fructans to glucose or saline in IBS patients and healthy controls.



Differences in ascending colon volume (change from baseline) are plotted for different levels of differences in ascending colon gas (change from baseline, z-scored) (A) when comparing fructans to glucose in IBS; (B) when comparing fructans to saline in IBS; (C) when comparing fructans to glucose in HC; (D) when comparing fructans to saline in HC.

HC, Healthy Control; IBS, Irritable Bowel Syndrome; AC, ascending colon

S7. Supplementary Tables

Supplementary Table 1. Composition of test solutions and key findings of previous MRI studies compared to the data presented here (far right).

Study	Murry et al[16]	Major et al[7]	Gunn et al[17]	Masuy et al[18]	Current study
Participants	N = 16 Healthy	N = 29 IBS N = 29 Healthy	N = 9 IBS-C N = 10 IBS-D	N = 20 IBS N = 20 Healthy	N = 13 IBS N = 13 Healthy
FODMAP challenge solution	**Polysaccharide (DP ≥23)**			**Oligosaccharide (DP 3-10)**	
	Inulin, 40g in water & lemon juice	Inulin, 40g in water & lemon juice	Inulin, 20 g in water	FOS 19g in water	FOS 40g in saline
Caloric value, kcal / 500 mL	60	60	30	29	29
Osmolality, mOsmol / kg	36	36	36	72	72
Volume, ml	500	500	500	~900 (IBS);~1,400 (HC) §	500
Other test solutions	Glucose, Fructose, Glucose + Fructose	Glucose, Fructose	Inulin + Psyllium, Psyllium, Glucose	Glucose §§	Glucose, Saline (0.9%)
Administration (duration, mins)	Drink (<15 mins)	Drink (<15 mins)	Drink 4 x 125 ml (5 min)	Intragastric infusion, 60 mL / min	Intragastric infusion, 60 mL / min (10 mins)
Effect of FODMAP on primary symptomatic endpoint	-- Symptoms	↑ CSS (IBS vs healthy) (inulin vs glucose)	↑ flatulence (inulin vs psyllium) -- flatulence (inulin vs inulin + psyllium)	↑ cramps (IBS vs healthy)	↑ cramps (FOS vs both)
Effect of FODMAP challenge on GI parameters	↑ Breath H2 -- SBWC ↑ total colonic gas (inulin vs glucose)	↑ Breath H2 -- SBWC ↑ total colonic volume (inulin vs glucose)	↑ Breath H2 -- SBWC (inulin vs glucose) -- ascending colonic volume (inulin vs glucose) ↓ ascending colonic volume (inulin < psyllium < psyllium + inulin)	↑ IGP >30mins (FOS vs glucose)	↑ small bowel motility (FOS vs both) @ 1hr ↑ small bowel signal intensity (FOS vs both) @ 1hr ↑ ascending colon gas & volume (FOS vs both) @ 1 & 2 hr

DP, average degree of polymerization; FOS, Fructooligosaccharide; SBWC, small bowel water content; CSS, composite symptom score; IGP, intragastric pressure; § infused until maximal satiation, IBS patients tolerated less than healthy participants but the difference was not significant (p=0.17); §§ Healthy participants also consumed a mixed FODMAP and fructose solution

Supplementary Table 2. Abdominal MRI acquisition parameters.

	Anatomical		Dynamic	
Sequence name	T2 SENSE	T2 SENSE	2D BTFE	3D BTFE
Plane	Coronal	Axial	Coronal	Coronal
Participant position	Supine	Supine	Supine	Supine
Duration (secs)	14	54	91	99
Breath hold (secs)	$20_{inspiration}$	$20_{inspiration}$	$20_{inspiration}$	$20_{inspiration}$
No of slices	32	90	1	20
Repetition time (TR, msec)	417	5982	3	2
Echo time (TE, msec)	60	40	1.5	1.6
Image matrix	252 x 207	168 x 149	232 x 232	180 x 179
Slice thickness (mm)	5	1.8	8	10
Slice gap (mm)	5	1.8	NA	5
In-plane resolution (mm)	0.98 x 0.98	0.94 x 0.94	0.97 x 0.97	1.5 x 1.5
Averages	1	1	1	1
Flip angle	90	90	55	50
Temporal resolution (images/sec)	NA	NA	1.5	2

SENSE, SENSitivity Encoding; BTFE, balanced-turbo field echo.

Supplementary Table 3. Overview of the quality review for data used in the colonic volume and small bowel analysis.

Patients				Volunteers			
		Colon 0, 60 & 120 mins	**Small bowel** 60 mins only			**Colon** 0, 60 & 120 mins	**Small bowel** 60 mins only
ID	**Visit**	Gas threshold, signal intensity	Slice agreement (before consensus)	**ID**	**Visit**	Gas threshold, signal intensity	Slice agreement (before consensus)
P02	**SV1**	250	Yes	**V01**	**SV1**	250	No
P02	**SV2**	250	Yes	**V01**	**SV2**	250	Yes
P02	**SV3**	250	Yes	**V01**	**SV3**	250	Yes
P03	**SV1**	150	Yes	**V02**	**SV1**	250	Yes
P03	**SV2**	150	No	**V02**	**SV2**	250	No
P03	**SV3**	150	No	**V02**	**SV3**	250	Yes
P04	**SV1**	150	Yes	**V03**	**SV1**	250	No
P04	**SV2**	150	Yes	**V03**	**SV2**	250	No
P04	**SV3**	150	Yes	**V03**	**SV3**	250	No
P05	**SV1**	250	Yes	**V05**	**SV1**	250	Yes
P05	**SV2**	250	No	**V05**	**SV2**	250	No
P05	**SV3**	250	Yes	**V05**	**SV3**	250	Yes
P06	**SV1**	150	Yes	**V06**	**SV1**	250	Yes
P06	**SV2**	150	Yes	**V06**	**SV2**	250	Yes
P06	**SV3**	150	Yes	**V06**	**SV3**	250	No
P07	**SV1**	150	Yes	**V07**	**SV1**	250	Yes
P07	**SV2**	150	Yes	**V07**	**SV2**	250	Yes
P07	**SV3**	150	No	**V07**	**SV3**	250	Yes
P08	**SV1**	150	No	**V08**	**SV1**	250	No
P08	**SV2**	250	No	**V08**	**SV2**	250	Yes
P08	**SV3**	250	Yes	**V08**	**SV3**	250	Yes
P09	**SV1**	250	Yes	**V09**	**SV1**	250	Yes
P09	**SV2**	250	No	**V09**	**SV2**	250	Yes
P09	**SV3**	250	Yes	**V09**	**SV3**	250	Yes
P10	**SV1**	250	No	**V10**	**SV1**	250 *	MISSING DATA
P10	**SV2**	250	No	**V10**	**SV2**	250	No
P10	**SV3**	250	Yes	**V10**	**SV3**	250	Yes
P11	**SV1**	250	No	**V12**	**SV1**	250	No
P11	**SV2**	250	Yes	**V12**	**SV2**	250	No
P11	**SV3**	250	Yes	**V12**	**SV3**	250 **	Yes
P12	**SV1**	250	No	**V13**	**SV1**	250	Yes
P12	**SV2**	250	No	**V13**	**SV2**	250	Yes
P12	**SV3**	250	Yes	**V13**	**SV3**	250	Yes
P13	**SV1**	250	Yes	**V14**	**SV1**	250	Yes
P13	**SV2**	250	Yes	**V14**	**SV2**	250	No
P13	**SV3**	250	Yes	**V14**	**SV3**	250	No
P14	**SV1**	MISSING DATA	MISSING DATA	**V16**	**SV1**	250	No
P14	**SV2**	250	Yes	**V16**	**SV2**	250	No
P14	**SV3**	250	Yes	**V16**	**SV3**	250	Yes§
		12 Complete cases	**26 Agreed 12 Disagreed**			**13 Complete cases**	**24 Agreed 14 Disagreed**

* missing T1 data at 1-hr post infusion; ** missing data at 2-hrs post infusion.

Supplementary Table 4. Between-group planned contrast analyses of small bowel motility assessed with GIQuant®.

	Mean motility index ± SD, a.u.		Difference
	HC	IBS	p-value
Fructans	348± 76	366± 94	1.00
Glucose	246± 65	228± 64	1.00
Saline	289± 81	268± 78	1.00

Mean motility index in arbitrary units (a.u.) is shown with adjusted p-values by stepdown Bonferroni correction for multiple comparisons.

Supplementary Table 5. Overview of regions in which brain responses to fructans versus glucose covaried with GI symptom responses in IBS patients.

Region	Subregion	Side	Bloating	Cramps	Flatulence	Fullness	Nausea	Pain
Cerebellum	vermis 3	left	[0;-42;-22]	[0;-42;-22]	[0;-42;-22]		[0;-42;-22]	[-2;-44;-20]
	crus 2	left	[-46;-40;-48]	[-46;-60;-48]	[-46;-62;-50]		[-26;-80;-44]	
	cerebellum 8	left	[-28;-50;-48]	[-28;-58;-54]	[-28;-58;-56]			
	cerebellum 9	left	[-14;-60;-48]	[-16;-60;-48]			[-14;-60;-48]	[-12;-60;-52]
	cerebellum 9	right	[4;-62;-48]	[12;-50;-60]	[12;-52;-58]		[10;-60;-40]	
Cingulate cortex	anterior	right	[4;28;-2]	[4;28;-2]	[4;28;-2]	[4;30;-4]	[6;34;-6]	[6;30;-4]
	middle	left	[-2;20;30]	[-2;20;28]			[-2;-12;42]	[-4;-16;38]
Frontal gyrus	inferior	left	[-38;22;8]	[-38;22;8]	[-40;22;8]	[-36;22;8]	[-38;22;8]	[-40;22;8]
	middle	right	[48;44;8]	[50;46;10]	[48;46;8]	[44;46;10]	[42;44;10]	[50;44;10]
	superior	left	[-24;46;20]	[-18;54;22]	[-20;52;20]	[-28;52;22]	[-18;52;22]	[-20;52;20]
Supramarginal gyrus		left	[-66;-38;30]	[-64;-22;28]	[-64;-22;28]	[-50;-24;28]	[-58;-18;-16]	[-66-22;20]
		right			[68;-34;24]	[66;-22;22]		
Postcentral gyrus		left	[-62;-24;18]	[-32;-32;52]	[-34;-34;54]	[-50;-24;28]		[-32;-32;54]
		right	[48;-20;32]	[48;-28;56]	[46;-30;56]	[28;-34;62]		[48;-20;32]
Supplementary motor area		right	[12;14;56]	[6;18;58]	[6;18;58]		[10;14;56]	[12;14;56]
Insula		left	[-36;-14;22]	[-32;-8;12]	[-32;-8;12]	[-28;-18;6]	[-32;-8;12]	[-32;-8;12]
		right	[38;6;-10]	[38;8;-12]	[38;8;-12]	[40;8;-14]	[52;-18;16]	[38;10;-12]
Putamen		right				[28;14;-2]	[18;8;2]	[32;0;12]
Pallidum		right		[16;10;-2]				[16;8;-2]
Thalamus		left	[-8;-16;14]	[-16;-22;10]	[-8;-8;6]	[-6;-16;14]		[-8;-16;16]
		right		[14;-20;6]			[6;-8;-4]	

Voxel level threshold p_{FWE}-corrected<0.05, cluster extent threshold k>=20.
GI, Gastrointestinal; IBS, Irritable Bowel Syndrome; FWE, family wise error.

Supplementary Table 6. Overview of regions in which brain responses to fructans versus saline covaried with GI symptom responses in IBS patients.

Region	Subregion	Side	Bloating	Cramps	Flatulence	Fullness	Nausea	Pain
Cerebellum	vermis 3	left	[0;-42;-22]	[0;-32;-22]	[-2;-36;-22]			
	crus 2	right		[44;-50;-40]				[38;-54;-42]
	cerebellum 4_5	left	[-12;-36;-28]	[-14;-36;-28]	[-14;-36;-28]		[-14;-42;-26]	[-6;-60;-14]
	cerebellum 8	left	[-14;-62;-50]	[-16;-60;-48]	[-16;-60;-48]		[-10;-56;-58]	[-18;-64;-48]
Cingulate cortex	anterior	right	[6;36;-4]	[6;36;-4]	[6;32;-4]		[6;34;-6]	[6;32;-6]
	middle	left	[-6;0;32]	[-4;0;34]	[-4;0;34]	[-6;-2;32]		[-6;0;32]
Frontal gyrus	middle	right	[48;46;4]	[46;48;6]	[46;44;6]	[44;48;6]		[44;46;8]
	superior	left	[-20;50;24]	[-18;52;22]	[-18;-54;22]		[-22;32;26]	[-18;50;22]
Supramarginal gyrus		right	[52;-26;32]	[52;-24;30]	[52;-24;30]	[52;-28;32]		[50;-22;30]
Postcentral gyrus		left	[-38;-28;56]	[-36;-28;56]	[-36;-32;54]	[-52;-18;38]		[-36;-30;56]
		right	[20;-36;58]	[20;-38;60]	[20;-36;60]	[12;-38;70]	[52;-16;38]	[20;-36;58]
Insula		left	[-38;-4;-10]	[-28;20;4]	[-32;-8;10]	[-30;-18;2]	[-32;0;16]	[-32;-12;10]
		right	[52;-2;10]	[40;6;-14]	[34;-14;10]		[44;-4;-10]	[32;2;10]
Rolandic operculum		left		[-38;6;14]	[-40;-4;-12]			
Putamen		right		[30;14;2]			[30;0;12]	[20;10;0]
Thalamus		left	[-4;-8;16]	[-6;-6;10]	[-4;-8;8]	[-16;-18;12]		[-8;-12;14]
		right	[8;-6;2]		[6;-12;-2]			[10;-24;0]

Voxel level threshold p_{FWE}-corrected<0.05, cluster extent threshold k>=20.
GI, Gastrointestinal; IBS, Irritable Bowel Syndrome; FWE, family wise error.

S8. References

1. Mearin F, Lacy BE, Chang L, et al. Bowel Disorders. Gastroenterology 2016.
2. de Jonge CS, Gollifer RM, Nederveen AJ, et al. Dynamic MRI for bowel motility imaging-how fast and how long? Br J Radiol 2018;91:20170845.
3. Menys A, Taylor SA, Emmanuel A, et al. Global small bowel motility: assessment with dynamic MR imaging. Radiology 2013;269:443-50.
4. Menys A, Makanyanga J, Plumb A, et al. Aberrant Motility in Unaffected Small Bowel is Linked to Inflammatory Burden and Patient Symptoms in Crohn's Disease. Inflamm Bowel Dis 2016;22:424-32.
5. Zou KH, Warfield SK, Bharatha A, et al. Statistical validation of image segmentation quality based on a spatial overlap index. Acad Radiol 2004;11:178-89.
6. Altman DG, Bland JM. Measurement in medicine: the analysis of method comparison studies. Journal of the Royal Statistical Society: Series D (The Statistician) 1983;32:307-317.
7. Major G, Pritchard S, Murray K, et al. Colon Hypersensitivity to Distension, Rather Than Excessive Gas Production, Produces Carbohydrate-Related Symptoms in Individuals With Irritable Bowel Syndrome. Gastroenterology 2017;152:124-133 e2.
8. Hoad CL, Marciani L, Foley S, et al. Non-invasive quantification of small bowel water content by MRI: a validation study. Phys Med Biol 2007;52:6909-22.
9. de Jonge CS, Menys A, van Rijn KL, et al. Detecting the effects of a standardized meal challenge on small bowel motility with MRI in prepared and unprepared bowel. Neurogastroenterol Motil 2019;31:e13506.
10. Marciani L, Cox EF, Hoad CL, et al. Postprandial changes in small bowel water content in healthy subjects and patients with irritable bowel syndrome. Gastroenterology 2010;138:469-77, 477 e1.
11. Iven J, Biesiekierski JR, Zhao D, et al. Intragastric quinine administration decreases hedonic eating in healthy women through peptide-mediated gut-brain signaling mechanisms. Nutr Neurosci 2019;22:850-862.
12. Whitfield-Gabrieli S, Nieto-Castanon A. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. Brain Connect 2012;2:125-41.
13. Iven J, Biesiekierski JR, Zhao D, et al. Intragastric fructose administration interacts with emotional state in homeostatic and hedonic brain regions. Nutr Neurosci 2020:1-12.
14. Van Oudenhove L, McKie S, Lassman D, et al. Fatty acid-induced gut-brain signaling attenuates neural and behavioral effects of sad emotion in humans. J Clin Invest 2011;121:3094-9.
15. Yarkoni T, Poldrack RA, Nichols TE, et al. Large-scale automated synthesis of human functional neuroimaging data. Nat Methods 2011;8:665-70.
16. Murray K, Wilkinson-Smith V, Hoad C, et al. Differential effects of FODMAPs (fermentable oligo-, di-, mono-saccharides and polyols) on small and large intestinal contents in healthy subjects shown by MRI. Am J Gastroenterol 2014;109:110-9.
17. Gunn D, Abbas Z, Harris HC, et al. Psyllium reduces inulin-induced colonic gas production in IBS: MRI and in vitro fermentation studies. Gut 2021.
18. Masuy I, Van Oudenhove L, Tack J, et al. Effect of intragastric FODMAP infusion on upper gastrointestinal motility, gastrointestinal, and psychological symptoms in irritable bowel syndrome vs healthy controls. Neurogastroenterol Motil 2018;30.