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Contrasting effects of viscous and particulate fiber on colonic fermentation *in vitro* and *in vivo,* and their impact on intestinal water studied by magnetic resonance imaging in a randomized trial

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Short running head: Fermentation of fibers and effect on colonic water

Abbreviations

ANOVA – Analysis of variance

AUC – area under the curve

FODMAP - fermentable oligo-, di-, mono-saccharides and polyols

MRI – magnetic resonance imaging

SBWC – small bowel water content

SCFA – short chain fatty acids

T1AC – T1 of the ascending colon

Clinical Trial Registry: clinicaltrials.gov NCT03263065

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Abstract

Background: Wheat bran, nopal and psyllium are examples of particulate, viscous and particulate, and viscous fibers respectively, with laxative properties yet contrasting fermentability.

Objective: To assess these fibers' fermentability *in vitro* and effect on intestinal function relevant to laxation *in vivo* using magnetic resonance imaging (MRI).

Methods: Each fiber was predigested prior to measuring gas production *in vitro* during 48 hours anaerobic incubation with healthy fecal samples. We performed a randomized, three-way crossover trial in 14 healthy volunteers who ingested 7.5 g fiber twice on the day prior to study and once with the study test meal. Serial MRI scans, fasting and hourly for 4 hours following meal ingestion, assessed small bowel water content (SBWC), colonic volumes and T1 of the ascending colon (T1AC) as a measure of colonic water. Breath samples for hydrogen analysis were obtained fasted and every 30 minutes for 4 hours.

Results: Mean (SD). *In vitro*, the onset of gas production was significantly delayed with psyllium versus wheat bran, 14(5) vs 6(2)hours, p=0.003; associated with a smaller total gas volume (p=0.01). 24 hours of pre-feeding of all three fibers was associated with an increased fasting T1AC (over 75% of values >90th centile of the normal range). There was a further rise during the 4 hours after psyllium, +0.3(0.3)s p=0.009, fall with wheat bran, -0.2(0.2)s p=0.02, but unchanged by nopal, 0.0(0.1)s p=0.2. SBWC increased for all fibers; nopal stimulated more water than wheat bran (AUC mean (95% CI) difference 7.1 (0.6,13.8) L.min, p=0.03).

Breath hydrogen rose significantly after wheat bran and nopal but not after psyllium (p<0.0001).

Conclusion: Both viscous and particulate fibers are equally effective at increasing colonic T1 over 24 hours. Mechanisms include water trapping in the small bowel by viscous fibers and delivery of substrates to the colonic microbiota by more fermentable particulate fiber.

Keywords: fiber, bran, nopal, psyllium, MRI, intestine, colon, water

Introduction

The underlying physico-chemical and functional properties of dietary fibers vary widely. Gel forming fibers such as psyllium have evolved as mucilage plant polymers with extremely high water-holding capacity which despite their large molecular weight (in excess of 1MDa) are easily able to hydrate. Such fibers form highly viscous solutions and gels when dissolved in water. In contrast, some fibers such as wheat bran, which has a large particle size (>100 µM), have very limited solubility and do not form a gel nor contribute significantly to viscosity in the bowel(1).

Clinical evidence shows that some viscous, gel-forming fibers (e.g. psyllium) benefit patients with irritable bowel syndrome whereas fiber from different sources (e.g. bran) may worsen symptoms(2), suggesting that the differing physico-chemical properties impact on mode of action in the gut, although this has to date not been studied in detail.

Psyllium fiber is a hemicellulose rich mucilage comprising highly branched arabinoxylan, composed of a xylan polymer densely decorated with arabinose and xylose sidechains(3). Although this is poorly fermented it facilitates water-holding in the small bowel causing an increase in both small bowel and colonic water content as well as colonic volume assessed by magnetic resonance imaging (MRI)(4). Constipated patients have lower colonic water content which can be normalized by therapeutic doses of psyllium (7g three times daily)(4), which are widely used to treat constipation.

The main fiber component of wheat bran is also an arabinoxylan, which comprises the majority of the cell wall in wheat but, unlike the arabinoxylan in psyllium, is highly fermentable(5). Wheat bran acts as a substrate for the colonic microbiota and is fermented to produce significant amounts of short-chain fatty acids (SCFA)(6,7). Several studies have

shown that wheat bran also accelerates oro-cecal transit(8) and increases small bowel water content (SBWC)(9).

Nopal fiber is an extract from the nopal cactus *Opuntia ficus-indica*. In contrast with psyllium, nopal fiber is primarily a pectic mucilage comprising a complex mixture of galacturonan, rhamnogalactans and rhamnogalacturonans as well as arabinoxylans, which are gel forming(10–12) but readily fermentable. Nopal has been traditionally used as a laxative in North Africa and Mexico(13) but its effects on human gut microbiota and function have yet to be examined.

The three fibers used in this study, with the contrasting physico-chemical properties described above, can be expected to be associated with different physiological behavior in the gastrointestinal tract but these have not previously been directly compared in humans.

Our aim was to compare equal doses of wheat bran, nopal and psyllium fibers on gas production by microbial fermentation *in vitro;* and their dynamic effects on SBWC, colonic volume and water content of the chyme in the ascending colon *in vivo* using MRI in healthy human volunteers.

Methods

Two studies were performed; the first examined the effect of the three fibers in a laboratory model of colonic fermentation (*in vitro* fermentation study) and the second examined the effect of ingesting the three fibers for two days on healthy subjects' SBWC, colonic volume and colonic water content using MRI and breath hydrogen (human MRI study).

The fibers used for both the *in vitro* fermentation study and the human MRI study were; coarse wheat bran (Holland and Barrett, Hinckley, UK), nopal provided as dehydrated cactus leaf (OroVerde Nopal Cactus Green Leaf Powder, Cuernavaca, Mexico) and psyllium husk (98%, Supernutrients, Bath, UK). Their composition is shown in **Table 1,** analyzed by Medallion Labs (Minneapolis, MN, USA) using standard AOAC methods and by Quadram Institute Biosciences (Norwich, UK) using Megazyme Fructan HK enzymatic assay kit (Megazyme, Bray, IE) according to manufacture recommendations (14), see **Supplementary Methods** for details.

In vitro fermentation study

In vitro fermentations for the three test fibers were carried out using a well-established model of the human colon seeded with microbiota obtained from healthy human volunteers(15–18). Prior to the fermentation, wheat bran and nopal underwent *in vitro* digestion using the INFOGEST, a validated international consensus method(19) that mimics small intestinal digestion and absorption of non-fiber carbohydrates that would otherwise be fermented in the *in vitro* fermentation model. Digestions were performed using the INFOGEST digestion method(19) with the addition of amyloglucosidase (final concentration 3 U/mL) at the intestinal phase. On completion, pre-digested fiber samples were frozen and lyophilized for 6 days. Once dry, samples were washed with absolute ethanol to release

unbound sugars. Ethanol was added at the concentration of 20mL ethanol / g dried substrate, the sample mixed and incubated at room temperature for approximately 90 minutes. Samples were centrifuged to allow excess ethanol to be removed, and the remainder evaporated through for three days. Once complete the final mass of substrate was recorded. Psyllium did not undergo digestion as it is 98% dietary fiber.

Gas production from the three fibers was measured using single stage anaerobic colon models(20). In brief, per 125mL vessel; 0.5g of pre-digested wheat bran, pre-digested nopal or psyllium were mixed with 76mL of media, as described by Williams *et al.*(20), kept anaerobic under a constant stream of CO_2. Once sealed, bottles were injected with 5mL of a vitamin and buffer solution, 1mL of the reducing solution(20) and pre-warmed overnight at 37°C.

Fecal samples were obtained from five healthy individuals who had no history of gastrointestinal disease nor antibiotic use in the previous three months, and who were non-smokers. Ethical approval for collection of stool samples from healthy people was obtained from London - Westminster Research Ethics Committee (REC) (15/LO/2169). Individual fecal samples were diluted in pre-reduced phosphate buffered saline (10% w/v) and strained to remove particulate. Each fiber substrate was fermented in triplicate per volunteer fecal sample. Each vessel was inoculated with 3mL of slurry by injection and incubated at 37°C for 10 days. Gas production was measured at regular intervals using a pressure transducer (Omega USB-H transducer, Omega Engineering, Manchester, UK) and syringe. At each time point, the pressure in the bottle was recorded with the transducer and the volume measured by removing gas with a syringe to bring the pressure in the bottle to atmospheric pressure. Data are reported as cumulative gas volume produced during fermentation,

averaged from five volunteers measured in triplicate per fiber, a total of 15 fermentation studies per fiber.

Human MRI study

This was a single center, randomized, three-treatment crossover study of wheat bran, nopal and psyllium's effects on SBWC, colonic volume and water content of the chyme in the ascending colon assessed by MRI, and on exhaled breath hydrogen breath concentration. The study followed the principles of Good Clinical Practice in accordance with the Declaration of Helsinki and was approved by the University of Nottingham Medical School Ethics Committee (51-1707). The study was completed between September 2017 and March 2018 and prospectively registered on www.clinicaltrials.gov (NCT03263065). There were no changes to the protocol or endpoints.

Participants

Healthy volunteers were recruited by poster advertisement on University of Nottingham campuses and gave written informed consent. Participants were eligible for inclusion if they age 18 years or older and were able to give informed consent. The exclusion criteria were pregnancy, history of pre-existing gastrointestinal disorder including irritable bowel syndrome, previous intestinal resection, any serious medical condition, contraindications to MRI scanning, and inability to stop medications known to alter intestinal motility. All subjects assessed completed the study protocol (see **Supplementary Figure 1**).

Test fibers and controlled diet

The wheat bran, nopal and psyllium consumed in the study were identical to those used in the *in vitro* fermentation except that wheat bran and nopal did not undergo pre-digestion. All fibers were stored in a sealed container in a cool, dry and dark environment. Doses were

standardized to provide approximately 7.5 g of total fiber per dose so participants received 20.6 g wheat bran, 14.8 g nopal and 8.4 g psyllium per dose (see Table 1 for nutritional composition). The pre-weighed test fiber was mixed with 300mL of water and taken with breakfast and lunch the day before the study visit and then again at the research center on the day of the study visit (therefore three doses in total over 24-hour period). Participants consumed the three fibers in random order with study days separated by at least 6 days to ensure adequate washout.

The order of fiber consumption was determined by random sequence generated using the online program www.randomization.com. The researchers were not blinded to the order of fiber allocation as they prepared the supplement and water mix on the day. Although participants were not informed about the order of fiber allocation, the differing appearance, taste and texture of the fiber supplement meant that they could not be formally blinded to the fiber consumed that day. However, all study MRI and other data were link-anonymized via a study ID and MRI analysis was done blind to the intervention.

Whilst consuming the fiber supplements (i.e. the day before and the day of the study visit), participants were instructed to avoid caffeine, alcohol and strenuous exercise and provided with a standardized controlled diet (see **Supplementary Table 1**), that was low in fermentable carbohydrates (low FODMAP diet, known to reduce the symptoms of bloating(21)) and otherwise low in fiber. This aimed to reduce the intra- and inter-individual variability in colonic fermentation due to background diet and effects on gastrointestinal motility.

Protocol

On the day prior to the study day, the allocated test fiber was provided at two meals (breakfast and lunch). All food was provided as low fiber, low FODMAP meals (see **Supplementary Methods**), including a supervised and standardized breakfast and lunch, and a standardized dinner was provided for participants to consume in the evening at home. Participants arrived the following morning at the Sir Peter Mansfield Imaging Centre at the University of Nottingham after an overnight fast and verbally confirmed compliance with dietary restrictions. MRI safety questionnaires were completed with the radiographer. Participants underwent a fasted MRI scan (see **Supplementary Methods** for details) and measurement of breath hydrogen by exhaling into a gas analyzer (GastroCH4eck, Bedfont, UK). Participants then consumed the same meal and fiber supplement as was taken for lunch the previous day. MRI scans were performed immediately after the meal and then hourly for four hours with hydrogen breath tests every half hour (see **Figure 1** for study schematic).

Abdominal MRI was performed on a 3.0 T Philips Achieva scanner (Best, The Netherlands) using a parallel imaging SENSE 16-element torso coil. Images were acquired with an expiration breath-hold between 13 and 24 seconds, with participants spending approximately 15 minutes inside the magnet at any one time. MRI parameters included SBWC, colonic volume and T1 of the chyme in the ascending colon (T1AC). T1 is the time constant for the water hydrogen protons to return to their equilibrium state following radiofrequency excitation. More watery chyme has a longer T1 relaxation time, and the T1 of the descending colon has been recently shown to correlate with stool water content(4).

The primary outcome was the T1AC 4 hours post-meal ingestion, measured by MRI. Secondary outcomes included the fasting T1AC and change in small bowel water content, colonic volume, and breath hydrogen over the same time period (0-4 hours). We also compared fasting values to our normal range for T1AC and colonic volumes. There were no changes to the pre-specified endpoints during the course of the study.

Statistical considerations

Sample size determination

Our previous studies of psyllium showed a mean (SD) increase in T1AC of 0.35 (0.42) s (unpublished data on file) after therapeutic doses of psyllium which is a mild laxative and this increase represents a minimal clinically significant difference. Using the PS Power and Sample Size Calculations program, version 3.0.43 with a false discovery rate of 0.05 and power of 80% we calculated that we would need 13 subjects in order to demonstrate such a difference.

Statistical analysis

Symmetrical data are presented as mean (SD) and non-symmetrical data as median (interquartile range). All statistical analysis was performed using Graphpad Prism version 8.2.1 for Windows (GraphPad Software, La Jolla California USA). Repeated measures one-way ANOVA followed by Tukey's multiple comparisons test was performed for area under the curve (AUC) volume versus time for *in vitro* gas production, comparison of time to onset, T1AC, SBWC and total colonic volumes. Equal variance was not assumed, the Geisser-Greenhouse correction was used, and normality of the distributions was assessed by the D'Agostino & Pearson test. Friedman's test followed by Dunn's multiple comparisons test was used to assess non-symmetrical breath hydrogen data at 4 hours. We have assessed

multiple MRI endpoints but have not corrected the p values for this. While we can be confident that our primary outcome result is not due to chance, secondary endpoints need confirming in further studies. Onset of fermentation was assessed from the inflection point of the volume versus time plot.

Results

In vitro fermentation study

AUC total gas production over 48 hours was significantly different between fibers (one-way ANOVA F=9.07, p=0.01), with a significantly greater AUC for wheat bran compared with psyllium, mean (95% CI) difference 370.4 (76.8, 664.0) mL.hr, p=0.02, but not nopal, mean (95% CI) difference 164 (-117.6, 446.4) mL.hr, p=0.2 (see **Figure 2**). Onset to gas production was significantly longer for psyllium than wheat bran, mean (95% CI) difference 8.4 (2.9, 13.9) hours, p=0.004 and compared to nopal 10.1 (4.6, 15.6) hours, p=0.001.

Human MRI study

Fourteen participants completed the human MRI study (64% female, aged median [interquartile range, IQR] 20 [20-22] years with BMI median [IQR] 22.8 [21.1-25.8]). All participants consumed the allocated fibers with no adverse effects. Due to equipment failure 11 complete data sets were available for analysis for SBWC and for breath hydrogen (see **Supplementary Figure 1**).

Primary outcome

As **Table 2** and **Figure 3** show, fasting values of T1AC were similar for the three fibers despite receiving 2 doses the previous day. However, over the study day T1AC rose significantly with psyllium but not wheat bran or nopal so that the differences were greatest

at the end of the study day. T1AC at 4 hours showed a significant difference between the fibers (one-way ANOVA F=23.2, $p<0.0001$) and Tukey's multiple comparisons showed a significant T1 increase for psyllium compared to both wheat bran and nopal, mean (95% CI) difference 0.439 (0.207, 0.672)s, $p=0.0007$, and 0.338 (0.17, 0.505)s, $p=0.0004$, respectively.

Secondary outcomes

Fasting T1AC

24 hours of fiber pre-feeding resulted in at least 75% of fasting T1AC values lying above the 90th centile of the normal range with no significant differences between the three fibers (repeated measures one-way ANOVA F=0.05, $p=0.93$, see **Figure 3B**).

Small bowel water content

There was a significant increase in SBWC for all fibers from fasting to 4 hours (see **Figure 4**). AUC analysis demonstrated a significant difference between fibers (repeated measures one-way ANOVA F=4.8, $p=0.02$); nopal stimulating significantly more small bowel water than wheat bran (mean (95% CI) difference 7.1 (0.6, 13.8) L.min, $p=0.03$).

Colonic Volume

There were significant differences in the fasting colonic volume between fibers after 24 hours of pre-feeding (repeated measures one-way ANOVA F=20.5, $p<0.0001$); participants pre-fed with psyllium for 24 hours had larger fasting total colonic volumes than both nopal (mean (95% CI) difference 128 (71, 185) mL, $p=0.0001$) and wheat bran (mean (95% CI) difference 129 (53, 205) mL, $p=0.002$) with no significant difference between nopal and wheat bran. AUC for the study duration was significantly different (F=40, $p<0.0001$); psyllium was greater than nopal (mean (95% CI) difference 36.0 (24.1, 47.8) L.min,

246 p<0.0001) and wheat bran (mean (95% CI) difference 45.8 (31.1, 60.4) L.min, p<0.0001),
247 with no difference between nopal and wheat bran (**Figure 5**).

248 ## Breath hydrogen

249 Fasting breath hydrogen concentrations were not different, however after 4 hours there
250 was a significant difference between the fibers (Friedman's test, p<0.0001). Breath
251 hydrogen concentration was significantly higher for both wheat bran and nopal *versus*
252 psyllium (mean difference (SD) 56.1 (42.8)ppm, p=0.0003 and 32.3 (32.4)ppm, p=0.04,
253 respectively), with no difference between wheat bran and nopal (**Figure 6**).

Discussion

The laxative effect of the many and various dietary fibers is well recognized but the individual underlying mechanisms have until recently been unclear. Our study utilizes three very different fibers and shows that all three increase colonic water but by different mechanisms. We confirmed the results from our previous study(4) by showing that psyllium is highly effective in acutely trapping water in the small bowel, which rose steadily in the hours after meal ingestion. It should be noted that without fiber supplementation postprandial SBWC between 180-240 minutes has been shown to average under 100mls(9) whereas in our study it was 178mls. The *in vitro* fermentation studies, showing more rapid fermentation of wheat bran and nopal fiber compared to psyllium, match the earlier and more substantial rise in breath hydrogen seen *in vivo*. Psyllium is only very slowly fermented which will ensure a prolonged “trapping” of water in the colon. The larger colonic volume after psyllium may also reflect a lack of stimulation of colonic motility compared to the other more fermentable fibers. Psyllium would be predicted to produce fewer short chain fatty acids, which are known to stimulate 5-HT release from colonic enteroendocrine cells(22) which is known to have a prokinetic effect.

Previous publications show that postprandial SBWC is strongly influenced by nutrient absorption and osmotic factors. Glucose(9), bread or rice meals(23) lead to rapid falls in SBWC over the next 1-2 hours as glucose and sodium are actively absorbed by small intestinal transporters with accompanying passive water absorption. Psyllium slows nutrient absorption(24), possibly by increasing viscosity and reducing the mixing which is essential to allow access of luminal contents to the mucosa. Psyllium potently retains water within its complex network making it unavailable for absorption. We have shown in the current study

277 that repeated doses of psyllium lead to an increase in colonic volumes and water content as
278 assessed by the MRI parameter, T1. The rise in colonic volume may be due to the fact that,
279 unlike wheat bran(25), psyllium does not significantly accelerate whole colonic transit(4,26),
280 a feature which would reduce colonic volumes by increasing the frequency of defecation.

281 Wheat bran by contrast, being less viscous, cannot trap water like psyllium but does
282 however produce a similar increase in SBWC. Previous studies(8) had showed that 15g of
283 both wheat bran and plastic particles caused similar acceleration of meal transit suggesting
284 that this is driven by mechanical rather than chemical stimulation of the mucosa. Earlier
285 studies have shown that stroking intestinal mucosa activates neurogenic secretion(27)
286 which could accelerate transit. More recently, it has been shown that a subpopulation of
287 enterochromaffin cells express mechanosensitive piezo-2 ion channels(28).
288 Enterochromaffin cells are stimulated by shear forces to release serotonin(29) which
289 stimulates crypt secretions. This may be an important mechanism to dilute luminal contents
290 if they become too viscous and threaten to cause intestinal obstruction(9,30). Another
291 potential mechanism through which particulate fiber can increase postprandial water is
292 inhibiting amylase digestion of starch in the rice meal through adsorption of amylase to the
293 particle surface(31). Wheat bran is also known to increase fecal bacterial mass, a factor that
294 accounts for a substantial proportion of stool mass(32) and may thus exert a laxative effect.
295 Given that both viscous and particulate fiber increase small bowel water content but by
296 different mechanisms, it is perhaps not unexpected that nopal, which contains both
297 mucilage and particulate fiber, had a greater effect on small bowel water than either
298 psyllium or wheat bran alone.

Towards the second half of the 4-hour study, small bowel contents would be expected to start entering the ascending colon and hence increase T1AC. At this point psyllium seemed to be most effective. This may reflect the slow breakdown and fermentation rate of psyllium's highly branched structure, demonstrated by the delayed onset *in vitro* of gas production in our fermentation study and the virtual absence of a rise in breath hydrogen in the MRI study. The undegraded psyllium will continue to trap water, making it unavailable for absorption, hence increasing colonic volumes. Wheat bran, with a particulate structure, a less branched arabinoxylan and a small amount of fructans, is more rapidly fermented *in vitro* and shows a clear rise in breath hydrogen *in vivo*. This rapid fermentation would increase bacterial mass and produce SCFA that stimulate sodium and water absorption(33). Fermentation products may also stimulate motility and accelerate transit thereby reducing colonic volumes though direct evidence of the impact of SCFA on motility is contradictory, with some studies suggesting stimulation(34) and others not(35). More recently it has been shown that SCFA stimulate colonic motility in rats via the release of 5-hydroxytryptamine (5-HT)(36) that stimulates colonic peristalsis. Using germ free and mice colonized with human microbiota it has been shown that the presence of colonic microbiota increases serotonin synthesis and release by enteroendocrine cells(37), providing a mechanism whereby dietary fiber modulation of colonic microbiota could accelerate transit.

We assessed fasting values of T1AC after two doses of fiber the day before to understand the longer-term effects. Despite the greater rise in T1AC soon (2-4 hours) after acute ingestion of psyllium compared to the other fibers, by 24 hours their effects were similar; all three fibers increasing T1AC to the upper limit of our normal range. While both wheat bran and nopal increase small bowel water, this does not appear to increase colon volumes in the

short term. This may be because, as shown by the greater breath hydrogen response, the more readily fermentable components of both wheat bran and nopal are rapidly fermented and absorbed thus limiting any increase in colonic volume. Alternatively, it may reflect greater stimulation of motility by wheat bran and nopal which would also reduce colonic volumes but demonstrating this would require further studies. Previous studies have shown a link between increased colonic volumes and the sense of distension and bloating(38) that may limit the use of psyllium in constipated patients.

Conclusion

In summary, both viscous and particulate fiber stimulate an increase in postprandial small bowel water content and an increase in colonic T1. Possible mechanisms include inhibiting absorption of both water and nutrients or stimulating intestinal secretion. Psyllium appears more effective in trapping small bowel water and its slow metabolism means that colon volumes remain increased over at least 24 hours. Nopal and wheat bran, despite not being viscous, also increase small bowel water but are rapidly fermented in the colon and do not lead to colonic distension. Whether this will translate into greater efficacy and tolerability in the treatment of constipation remains to be seen when clinical trials, currently under way, are completed.

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Tables

Table 1. Composition of the three test fibers

Test	Wheat Bran	Nopal	Psyllium
Resistant Starch	<2%	<2%	<2%
Total Dietary Fiber	41.3%	50.8%	88.9%
- Soluble Fiber	6.2%	13.2%	23.5%
Total Fructans[1]	1.2%	0.1%	-
Total Sugars	4.4%	4.9%	-
Mannitol	trace	0.1%	-
Glucose	2.0%	1.4%	-
Fructose	0.7%	2.2%	-
Sucrose	ND	1.2%	-
Raffinose	0.1%	trace	-
1-Kestose	0.1%	trace	-
Maltose	1.42%	0.1%	-
Nystose	ND	trace	-
Kestopentose	ND	ND	-

[1]quantified using high-performance anion exchange chromatography with pulsed amperometric detection. ND: not detectable

Table 2. T1 (in seconds) in the ascending colon of participants, fasted and 4 hours postprandially, indicating a more watery colonic chyme for psyllium than nopal and wheat bran after ingestion

	Ascending colon T1, mean (SD)	
Fiber	Fasting	4 hours after meal and fiber
Wheat bran, mean (SD)	0.98 (0.19)	0.82 (0.18)
Nopal, mean (SD)	0.97 (0.16)	0.92 (0.16)
Psyllium, mean (SD)	0.99 (0.17)	1.26 (0.29)[1]

Repeated measures one-way ANOVA shows a significant difference between the fibers 4 hours after meal ingestion (n=14, F=23.2), $p<0.0001$. [1]Tukey's multiple comparisons demonstrated a significantly longer T1 for psyllium than wheat bran and nopal, $p<0.005$.

Legends for figures

Figure 1 - Schematic of events during the human MRI study. MRI scans are represented by  , hydrogen breath tests by  and test meal ingestion by ↓. Test meals comprised 7.5g total fiber with a low fiber, low FODMAP meal. Each scan day was separated by a washout period of at least 6 days.

Figure 2 – Data are presented as mean and 95% confidence intervals, n=5 (in triplicate). A) *in vitro* onset of gas production (in hours) when combined with the study fibers, demonstrating significantly longer onset time (defined by the inflection point in the time versus volume curve) for psyllium than wheat bran (14 (5) hours vs 6 (2) hours, p=0.0031) and nopal (4 (2), p=0.0011). B) *in vitro* stool sample gas production when combined with the pre-digested fibers over 48 hours. AUCs were significantly different between fibers (F=9.07, p=0.0109), with a significantly greater AUC for wheat bran compared with psyllium, mean (95% CI) difference 370.4 (76.8, 664.0) mL.hr, p=0.02, but not nopal, mean (95% CI) difference 164 (-117.6, 446.4) mL.hr, p=0.2.

Figure 3 – A) Time course of T1 relaxation in the ascending colon (T1AC) (mean, 95%CI) following fiber ingestion on the MR imaging day (n=14). 4 hours after ingestion there was a significant difference between the fibers (p<0.0001) and Tukey's multiple comparisons showed a significant T1 increase for psyllium, corresponding to a more watery chyme, compared to both wheat bran and nopal (mean (95% CI) difference 0.439 (0.207, 0.672)s, p=0.0007, and 0.338 (0.17, 0.505)s, p=0.0004, respectively). Ingestion of the test meal is designated by ↓. B) Fasting T1AC (mean, 95%CI) after 24 hours of fiber pre-feeding (n=14),

demonstrating at least 75% of values lying above the 90th centile of the normal range with no significant differences between the three fibers (*p*=0.93). Normal values for T1AC after an 8-hour fasting period have previously been obtained from 29 healthy volunteers from previous studies, published(39) and unpublished, on the same 3.0 T Philips Achieva MRI scanner, and are shown as the median and 10th- 90th centiles.

Figure 4 – Time course of Small Bowel Water Content (SBWC) (mean, 95%CI) following fiber ingestion on the MR imaging day (n=11). AUC analysis demonstrated a significant difference between fibers (*p*=0.02); nopal stimulating significantly more small bowel water than wheat bran (mean (95% CI) difference 7.1 (0.6, 13.8) L.min, *p*=0.03). Ingestion of the test meal is designated by ↓.

Figure 5 - Time course of total colonic volumes (mean, 95%CI) following fiber ingestion on the MR imaging day (n=14). AUC for the study duration was significantly different *p*<0.0001); psyllium was greater than nopal (mean (95% CI) difference 36.0 (24.1, 47.8) L.min, *p*<0.0001) and wheat bran (45.8 (31.1, 60.4) L.min, *p*<0.0001), with no difference between nopal and wheat bran. Normal colonic volumes after an 8-hour fasting period (mean, SD) have been obtained from 34 healthy volunteers from a previous study(40) on a 1.5T Philips Achieva MRI scanner and are demonstrated in grey. Ingestion of the test meal is designated by ↓.

Figure 6 - Time course of breath hydrogen concentration (mean, 95% CI) following fiber ingestion on the MR imaging day (n=10). After 4 hours there was a significant difference between the fibers (*p*<0.0001); breath hydrogen concentration was significantly higher for both wheat bran and nopal *versus* psyllium (mean difference (SD) 56.1 (42.8)ppm, *p*=0.0003

and 32.3 (32.4)ppm, p=0.04, respectively), with no difference between wheat bran and nopal. Ingestion of the test meal is designated by ↓.

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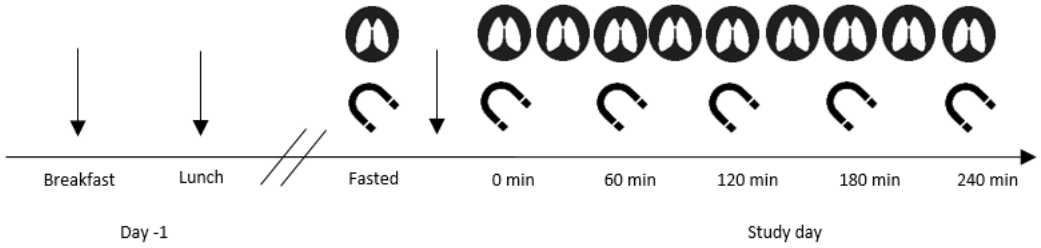
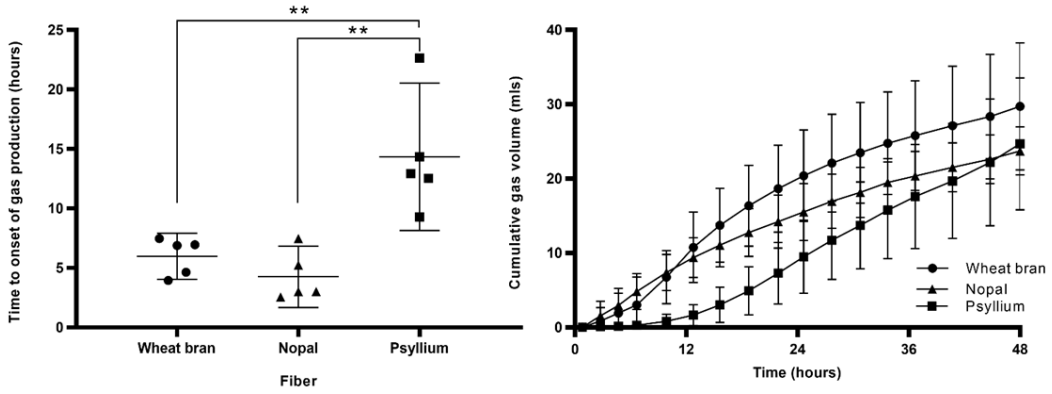
Figure 1


Breakfast
Lunch
Fasted
0 min
60 min
120 min
180 min
240 min
Day -1
Study day

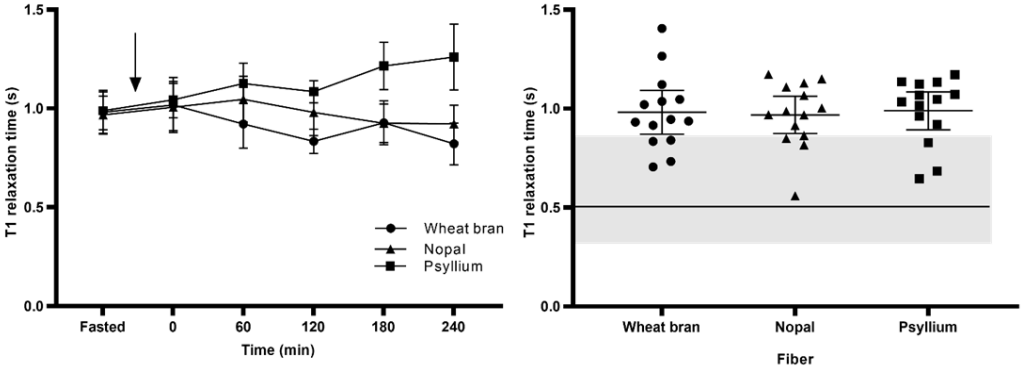
364

Figure 2


**
**
Time to onset of gas production (hours)
25
20
15
10
5
0
Wheat bran
Nopal
Psyllium
Fiber
Cumulative gas volume (mls)
40
30
20
10
0
0
12
24
36
48
Time (hours)
Wheat bran
Nopal
Psyllium

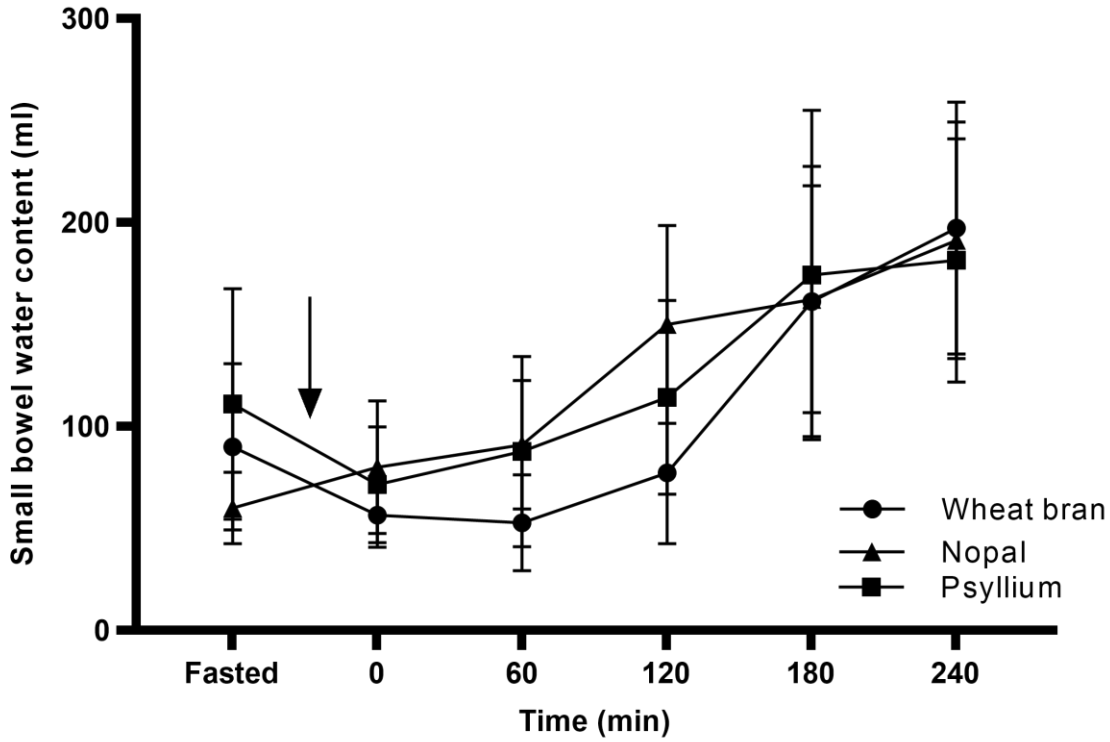
365

Figure 3


1.5
1.0
0.5
0.0
T1 relaxation time (s)
Fasted
0
60
120
180
240
Time (min)
Wheat bran
Nopal
Psyllium
Wheat bran
Nopal
Psyllium
Fiber

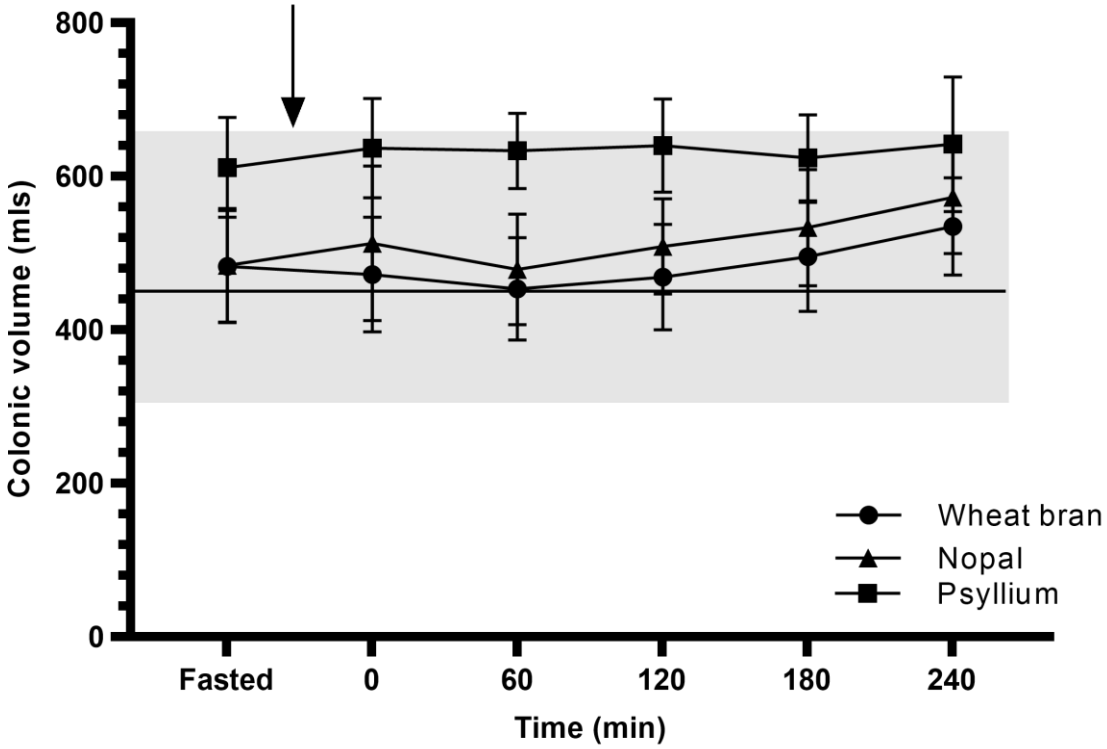
366

Figure 4


300
200
100
0
Small bowel water content (ml)
Fasted
0
60
120
180
240
Time (min)
Wheat bran
Nopal
Psyllium

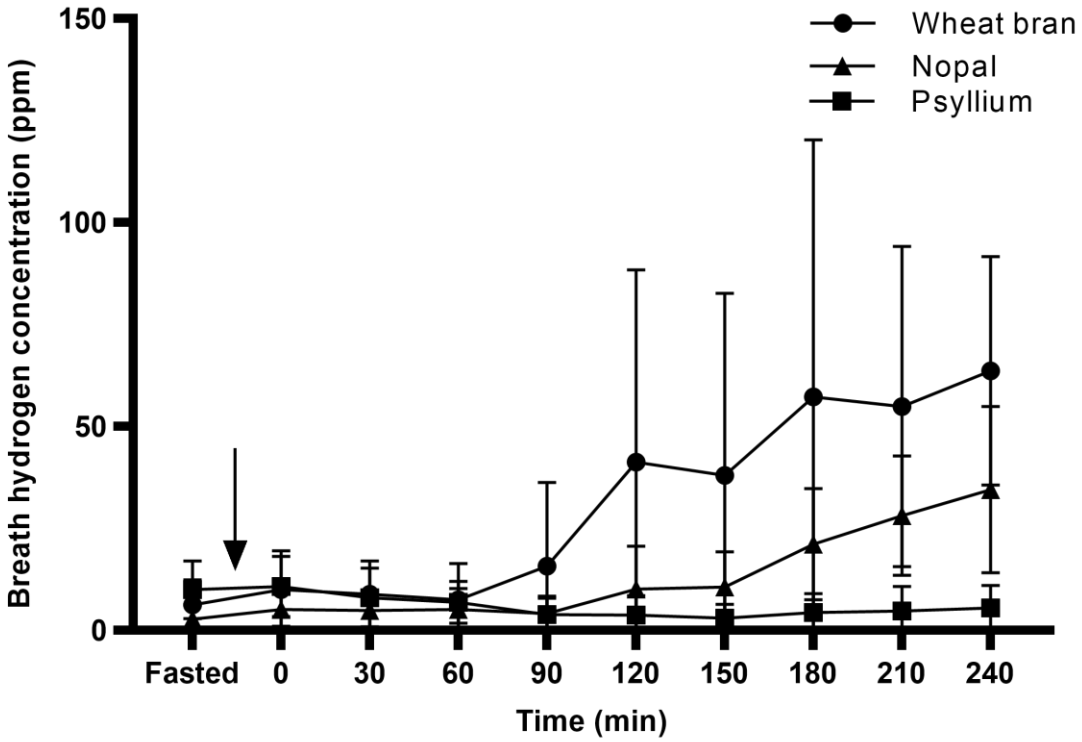
367

Figure 5


800
600
400
200
0
Colonic volume (mls)
Fasted
0
60
120
180
240
Time (min)
Wheat bran
Nopal
Psyllium

368

Figure 6


Wheat bran
Nopal
Psyllium
Breath hydrogen concentration (ppm)
150
100
50
0
Fasted
0
30
60
90
120
150
180
210
240
Time (min)