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ORIGINAL ARTICLE

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Mechanisms underlying the laxative effect of lactulose: A randomized placebo-controlled trial showing increased small bowel water and motility unaltered by the 5-HT_3 receptor antagonist, ondansetron

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Abstract

Background: Lactulose is a laxative which accelerates transit and softens stool. Our aim was to investigate its mechanism of action and use this model of diarrhea to investigate the anti-diarrheal actions of ondansetron.

Methods: A double-blind, randomized, placebo-controlled crossover study of the effect of ondansetron 8 mg in 16 healthy volunteers. Serial MRI scans were performed fasted and 6 h after a meal. Participants then received lactulose 13.6 g twice daily and study drug for a further 36 h. On Day 3, they had further serial MRI scans for 4 h. Measurements included small bowel water content (SBWC), colonic volume, colonic gas, small bowel motility, whole gut transit, and ascending colon relaxation time (T1AC), a measure of colonic water content.

Key Results: Lactulose increased area under the curve (AUC) of SBWC from 0 to 240 min, mean difference 14.2 L·min (95% CI 4.1, 24.3), $p = 0.009$, and substantially increased small bowel motility after 4 h (mean (95% CI) 523 (457–646) a.u. to 852 (771–1178) a.u., $p = 0.007$). There were no changes in T1AC after 36 h treatment. Ondansetron did not significantly alter SBWC, small bowel motility, transit, colonic volumes, colonic gas nor T1AC, with or without lactulose.

Conclusion & Inferences: Lactulose increases SBWC and stimulates small bowel motility; however, unexpectedly it did not significantly alter colonic water content, suggesting its laxative effect is not osmotic but due to stimulation of motility. Ondansetron's lack of effect on intestinal water suggests its anti-diarrheal effect is not due to inhibition of secretion but more likely altered colonic motility.

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); $5HT_3RA$, 5-hydroxytryptamine receptor 3 antagonist; ANOVA, analysis of variance; AUC, area under the curve; b.d., twice daily; CI, confidence interval; ECG, electrocardiogram; FODMAP, fermentable oligo-, di-, mono-saccharides and polyols; GPR, G protein coupled receptor; IBS, irritable bowel syndrome; IBS-D, diarrhea-predominant irritable bowel syndrome; IMP, investigational medicinal product; MRI, magnetic resonance imaging; ROI, region of interest; SBWC, small bowel water content; SCFA, short chain fatty acids; t.d.s., three times daily; T1AC, T1 of the ascending colon; WAPS, weighted average position score.

Clinical Trial Registry: clinicaltrials.gov NCT03833999.

KEYWORDS
diarrhea, lactulose, MRI, ondansetron, randomized trial

1 | INTRODUCTION

The British National Formulary describes Lactulose as an "osmotic laxative," a class of laxatives that are thought to soften stool by increasing the amount of water in the large bowel.[1] This conclusion is based on studies which used very large doses (up to 180 g equivalent to 526 mosmoles). These showed that at such high doses, the watery stools contained substantial amounts of lactulose. However at the normal clinical doses of 10–20 g (29–58 mosmoles), it induces laxation with a very low incidence of liquid stools,[2] something which has never been adequately explained.

Lactulose is a synthetic disaccharide, 4-O-β-D-galactopyranosyl-D-fructofuranose, resistant to human digestive enzymes as confirmed by ileostomy studies showing that it passes unaltered through the small intestine.[3] It has a molecular weight of 342, meaning that the usual clinical dose of 10–20 g (29–58 mosmols) exerts an osmotic effect in the permeable small intestine, predicted to require 100–200 mL to create a solution isosmotic to interstitial fluid (290 mosomol/L). In addition, the very low Na^+ content will create a steep electrochemical gradient causing Na^+ and water influx from interstitial fluid into the small bowel increasing the water content still further.[4,5] However, the colon is able to absorb up to 5 L of saline over 24 h,[6] so such a small increase in fluid input into the colon would not be expected to cause laxation unless there was some other effect. Intubation studies show that orally ingested lactulose appears in the cecum within 1 h of oral ingestion. Its cecal concentration peaks at 2 h associated with the appearance of fermentation products including lactic acid and a fall in cecal pH, with maximal effect at 4 h.[7]

Recent studies using magnetic resonance imaging (MRI) have made it possible to demonstrate that, when given as 10 g in 200 mL with no nutrient, it does indeed increase small bowel water content >twofold but its impact on large bowel water content was not assessed.[8]

We wanted to further understand the mode of action of lactulose-induced loose stools as a possible model of irritable bowel syndrome with diarrhea (IBS-D). We used doses of lactulose we had previously used and knew would cause moderate but not unacceptable diarrhea.[2,9,10] We were also interested to see whether the 5-hydroxytryptamine receptor 3 antagonist ($5HT_3$RA) ondansetron would attenuate lactulose's effect, since it is known to benefit IBS-D, reducing urgency and loose stools associated with a slowing of left sided colonic transit.[11]

Serotonin (5-HT) mediates intestinal secretion acting via 5-HT_3 receptors in several diarrheic diseases including cholera and rotavirus diarrhea which can be blocked by $5HT_3$RAs.[12] These can also block the meal evoked stimulation of pancreatic secretions.[13] The effect on motility varies by species but human studies suggest 5-HT_3 agonists and antagonists alter both small bowel[14] and colonic motility[15,16] making it unclear whether the benefit of $5HT_3$RAs in IBS-D is due to alterations in motility, sensation, or secretion.

Key Points

- Lactulose, given at the normal therapeutic dose of 13.5 g twice daily, increases small bowel water content and stimulates small bowel motility but does not increase colonic water.
- Thus its laxative effect is most likely due to stimulation of gut motility rather than its osmotic effect.
- Ondansetron 8 mg twice daily did not alter intestinal water in this model of diarrhoea.

The aims of this study were therefore to determine how lactulose induces loose stools, and if ondansetron could inhibit postprandial secretions and/or reduce the laxative effect of lactulose. We tested these hypotheses by means of a randomized, double blinded, placebo-controlled clinical trial of ondansetron 8 mg three times daily (t.d.s.) in healthy subjects who we studied both before and during the consumption of therapeutic doses of lactulose 20 mL twice daily (b.d.).

2 | MATERIALS AND METHODS

2.1 | Trial design

This was a double-blind, two-period, two-treatment crossover trial of ondansetron (8 mg/tablet) versus placebo and lactulose. The trial was prospectively registered on clinicaltrials.gov (NCT03833999), approved by the University of Nottingham Faculty of Medicine & Health Sciences Research Ethics Committee (reference 85-1807), and conducted according to Good Clinical Practice guidelines. There were no changes to the protocol.

2.2 | Healthy volunteers

Healthy volunteers were recruited by general advertisement on social media and University of Nottingham campuses. Eligible participants were aged 18 or older and able to give informed consent. Exclusion criteria were pregnancy or breast feeding; pre-existing gastrointestinal disorder; prior abdominal surgery other than appendectomy or cholecystectomy; congenital long QT syndrome or prolonged QTc on screening ECG; contraindication to MRI scanning; inability to lie flat or exceed scanner limits of weight (120 kg);

inability to stop drugs known to alter GI motility; participation in night shift work in the week prior to the study; being in another trial or being in the opinion of the investigator otherwise unsuitable.

2.3 | Randomization

All participants participated in both study arms, the order of study being randomized using the online program www.randomization.com. Each study day was separated by at least 6 days in order to minimize carryover effects.

2.4 | Interventions

The investigational medicinal product (IMP) was either 8 mg ondansetron (Milpharm) or placebo, both over-encapsulated by the Pharmacy Production Unit at Nottingham University Hospitals NHS Trust so that they were identical in appearance. Lactulose (Teva, UK) was provided as lactulose syrup 13.6 g/20 mL. Participants drank 300 mL of Fortisip (Nutricia, UK) and 150 mL water as a meal substitute. This is a nutritionally complete milkshake style supplement, 300 mL containing 450 kcal, 18 g protein, 55.2 g carbohydrate, and 17.4 g fat similar to meals previously used to stimulate gut motility.[17,18]

2.5 | Study protocol

The study was comprised of two 3-day periods, taking either ondansetron or placebo in random order with a washout period of at least 6 days (see Figure 1). Participants attended visit 1 where informed written consent was obtained and they were screened against the inclusion and exclusion criteria. This included a 12-lead ECG, MRI safety screening questionnaire, height, weight, smoking history, past medical history, and current medications. For 24 h prior to their attendance at visit 2 (Day 1), they were instructed to eat their usual diet but avoid alcohol and beans or pulses, not to engage in strenuous exercise, nor to change their usual smoking habit. On the morning of visit 2 fasted, participants attended the Sir Peter Mansfield Imaging Centre (SPMIC) at the University of Nottingham. Once consent and MRI safety had been re-confirmed, they underwent a fasted scan (see Data S1 for MRI scanning details). Participants were then given one IMP (either placebo or 8 mg ondansetron) with 50 mLs water and a meal comprised of 300 mLs Fortisip and 150 mLs water, then had a second MRI scan ($T = 0$). Further scans occurred 2, 4, and 6 h after the meal. At the end of the study day, participants consumed 20 mLs lactulose and one IMP and were asked to take a further IMP that evening at home. The following day (Day 2), the study was continued in participants' homes. There they consumed their usual meals while following the same dietary restrictions and lifestyle rules as prior to visit 2. Additionally, they also consumed 13.6 g lactulose in 20 mLs twice daily, IMP three times daily and 5 MRI marker pills[15] at 8 p.m. On the third day (Day 3), they returned to SPMIC for visit 3. Compliance and MRI safety was re-confirmed; then, participants had a fasted scan where the position of the transit markers was assessed. A dose of IMP with 50 mLs water, 20 mL lactulose and the same meal as visit 2 was consumed, followed by MRI scans at 0, 120, and 240 min.

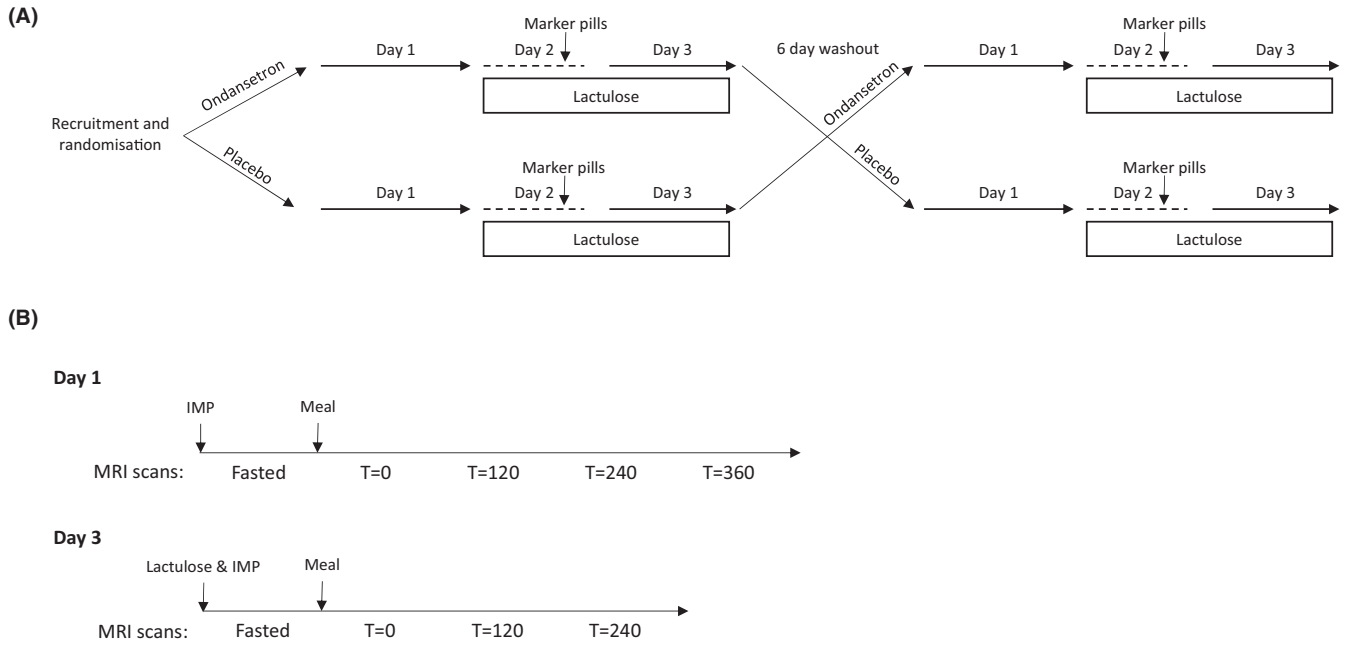

FIGURE 1 Schedule of study events: (A) for the whole study and (B) for each MRI scan day. IMP, investigational medicinal product.

2.6 | Endpoints

The primary endpoint was area under the curve from time 0–240 min ($AUC_{0\text{-}240}$) of small bowel water content (SBWC, mL·min) on visit 3 (Day 3).

Secondary endpoints were $AUC_{0\text{-}240}$ of SBWC on visit 2 (Day 1); T1 relaxation time in the ascending colon (T1AC, s) fasted and at the end of study days; small bowel motility index (arbitrary units, a.u.) 2 and 4 h postprandially; whole gut transit rate on visit 3, assessed by weighted average position score (WAPS)[19]; colonic volumes (mL) fasted and at 4 h; colonic gas (mL) at 4 h; and gastric volume at 2 h to confirm passage of meals into the small bowel.

2.7 | Data analysis

All image analysis was performed blind to the intervention received.

2.8 | Small bowel water content

SBWC was measured as previously validated,[20] using in house software written in IDL (Research Systems Inc. Boulder, Colorado, USA). For each image slice at each time point, a region of interest (ROI) was drawn around the small bowel, and structures such as blood vessels, bladder, and gall bladder were excluded. Any pixel with signal intensity above a calculated threshold (set by the subject's cerebral spinal fluid) in the heavily T2-weighted images was assumed to be filled with free water.

2.9 | Small bowel motility

All motility data were processed as previously described.[21] Briefly, free breathing MRI data were processed to correct for respiratory motion[22] before applying the nonlinear optic flow registration[23] to correct local deformation caused by bowel wall motion and luminal flow. An ROI was then demarcated around any visible small bowel on each coronal image on MATLAB-based software (MathWorks, Natick, MA). For each pixel in the registered dataset, a power spectrum of the intensity changes across the time series (smoothed using a running average of 5 pixels to reduce noise) was calculated and then summed across all frequencies. This metric is termed the total power and is measured in arbitrary units (a.u.) reflecting small bowel motility, both in terms of segmental oscillations and bolus movement of contents. A larger total power motility index represents higher small bowel motility.

2.10 | Whole gut transit rate

The effect of ondansetron on whole gut transit was assessed using the Weighed Average Position Score (WAPS) of the MRI marker capsules. We have previously validated the use of such marker pills to measure transit and found they correlate well with values obtained using the standard radio-opaque marker technique.[19] We modified this method by having the subjects take the marker 12 h rather than the conventional 24 h before the MRI scan because, if transit is rapid as we expected with lactulose, the marker technique can fail if all the markers have left the body by the time the scan takes place. Dosing 12 h before assessing markers has been validated with the radio-opaque marker technique and shown to work well for those with fast transit.[24] From the fasted set of MRI images on visit 3, a transit score was calculated by subdividing the bowel into eight sections and each capsule was scored according to its position in the colon. A weighting factor was calculated for each capsule depending on the difference of the capsule score from the median capsule score as previously described.[19]

2.11 | Colonic volume and gas

Regional colonic volumes were manually drawn on each coronal image slice at each time point using Analyze9™ software (Mayo Foundation, Rochester, MN, USA), building a 3D representation of the colon from which the volume was derived, as previously described.[25] Custom written software (IDL®; Research Systems Inc) was used to assess for colonic gas.[26]

2.12 | Ascending colon T1

T1 is a time constant describing the speed at which protons realign with the static magnetic field after being perturbed by energy from a radiofrequency pulse applied as part of the MR scan sequence.[27] It depends on the physico-chemical makeup of the tissue as well as temperature, pH and the strength of the main static magnetic field. T1 is related to water mobility in a U-shaped curve: liquid water has a long T1 at 3–4 s, and ice also has a long T1 (>4 s),[28] but intermediate biological tissues have a shorter T1 (e.g., fat has a T1 of around 380 ms, liver 810 ms, renal cortex 1150 ms[29]).

The longitudinal relaxation time T1 was measured in the ascending colon using a single slice inversion recovery balanced turbo field echo sequence with a preparatory 180° inversion pulse applied before acquiring the imaging data as previously described.[30] Eight different inversion times were acquired (range 0.1–5 s).

2.13 | Statistical methods

Symmetrical data are represented by mean (SD) and non-symmetrical data by median (IQR). All statistical analysis was performed using Graphpad Prism version 8.2.1 or later for Windows (Graphpad Software, La Jolla California USA). Data were tested for normality using the D'Agostino & Pearson normality test;

then, the paired *t*-test was used for parametric data; and the Wilcoxon test was used for nonparametric data. End point differences between ondansetron and placebo, and placebo with and without lactulose were assessed in this way. Two-way ANOVA was used to test differences in small bowel motility between study arms.

2.14 | Sample size and justification

Previous studies using the same scanning technique have shown the AUC SBWC 0–4 h to be mean (SD) 252(105) L·min after ispaghula 7.5 g. We expect lactulose 20 mL to at least double SBWC as shown by others.[8] Using $n=12$ would give us >99% power to detect the effect of lactulose. The magnitude of the ondansetron effect on SBWC is unknown but it does produce a >50% change in transit time.[11] Using $n=16$ would give us 80% power to detect a change of 79 L·min in AUC SBWC comparing ondansetron to placebo which represents a 31% change, usually taken to be the minimally important difference in many motility parameters. We aimed to recruit up to 20 participants in order to gather at least 16 complete data sets.

3 | RESULTS

Sixteen participants completed the study, 11 female, mean age 22 (range 20–33), BMI 23 kg/m^2 (SD 3.3). Although there was some increase in reported flatulence and loose stools, no subject had to reduce their lactulose dose.

3.1 | Effect of lactulose

Prefeeding lactulose for 2 days did not significantly increase fasting small bowel water (Day 1 vs. Day 3) in participants taking placebo. The mean difference (Day 3 - Day 1 values) between fasting SBWC was 27 mL (95% CI −4, 57), $p=0.057$. However, lactulose did significantly increase postprandial SBWC $AUC_{0\text{-}240}$ on study Day 3 compared to Day 1 (mean difference 14.2 L·min (95% CI 4.1, 24.3), $p=0.009$; see Figure 2). This equates to an increase on average of 58 mL which represents an approximately 60% increase over fasting values. Small bowel motility was also significantly increased after lactulose, from mean 523 a.u. (95% CI 457–646) at baseline to 852 a.u. (95% CI 771–1178) at 4 h ($p=0.007$). Two-way ANOVA demonstrated a significant effect of lactulose (DF = 1, $F=16.0$, $p=0.0001$) but not time (DF = 2, $F=1.4$, $p=0.26$) on small bowel motility (see Figures 3 and 4). Despite increased SBWC and motility there was no effect on fasted T1AC (mean difference 0.07 s (95% CI −0.16, 0.31), $p=0.72$, see Figure 5), colonic gas, ascending colonic volume nor total colonic volume (see Table 1).

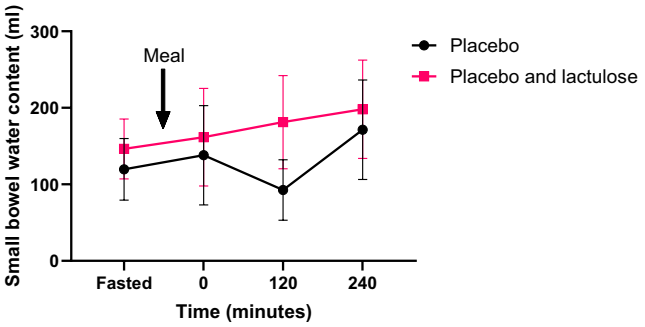

FIGURE 2 Effect of lactulose on postprandial small bowel water content (SBWC). Area under the curve analysis demonstrates a significantly greater SBWC for placebo with lactulose than placebo alone (43.3 [25.0] L·min vs. 29.7 [16.9] L·min, $p=0.0078$).

3.2 | Effect of ondansetron

Colonic volume, SBWC, and T1AC were unchanged by ondansetron compared to placebo ($p=0.9$, 0.71 and 0.37, respectively; see Table 2). There was no evidence of alteration in gastric emptying, gastric volumes at 2 h did not differ ($p=0.37$), and small bowel motility at 2 h and 4 h likewise was not different ($p=0.9$ and 0.34 respectively). Colonic gas, for both ondansetron and placebo, was negligible at less than 5 mLs.

Similarly, after 36 h of lactulose and ondansetron or placebo (Day 3), ondansetron did not significantly alter the primary endpoint SBWC nor any of the secondary endpoints (T1AC; gastric volume, small bowel motility nor whole gut transit as assessed by WAPS). Mean total colonic volumes and colonic gas were lower on ondansetron but owing to wide variability these differences could have been due to chance (see Table 3).

3.3 | Effect on colonic motility

Although we did not acquire full motility sequences for the colon, some of the cine images taken for small bowel motility also captured mass movements of the transverse and descending colon shortly after ingestion of the test meal and placebo with lactulose (see Videos S1 and S2).

4 | DISCUSSION

Our study differs from previous studies examining the role of osmotic forces in lactulose-induced diarrhea in using clinically relevant doses, which produce stool softening but not profuse watery stools. As others have reported using 10 g of lactulose in 200 mL of water,[7] small bowel water content increased modestly. The osmotic load we gave of 40 mosmoles would be predicted to require 138 mL to generate a solution isotonic to the interstitial fluid in the gut mucosa. Additionally, we would expect the low Na^+ concentration to

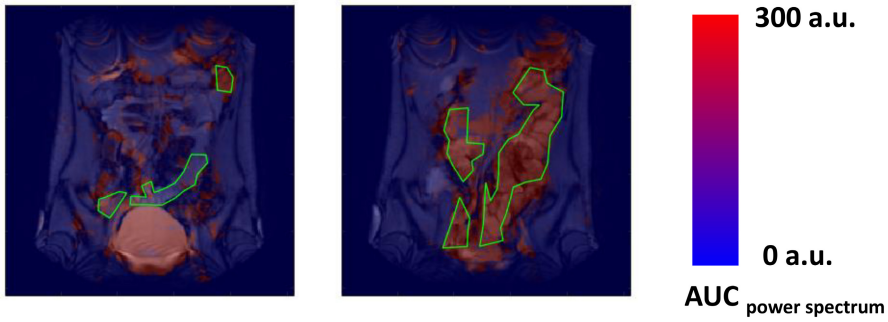

FIGURE 3 Comparison of small bowel motility 240 min after a meal, having taken (A) placebo only, and (B) placebo and 20 mL lactulose three times daily for 36 h. In (A), most areas of small bowel remain blue while in (B) small bowel is shaded in red, indicating increased power measured in arbitrary units [a.u.] signifying movement throughout the small bowel. Motility method is sensitive to flow of fluid and wall motion. This can be seen in the bladder on image A where flow is produced by urine entering the bladder from the ureter.[17]

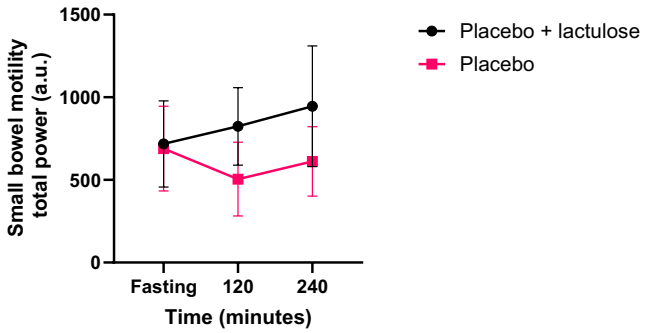

FIGURE 4 Effect of lactulose on postprandial small bowel motility (in total power, arbitrary units [a.u.]). Two-way ANOVA demonstrated a significant effect of lactulose (DF = 1, $F = 16.0$, $p = 0.0001$) but not time (DF = 2, $F = 1.4$, p = 0.26) on motility.

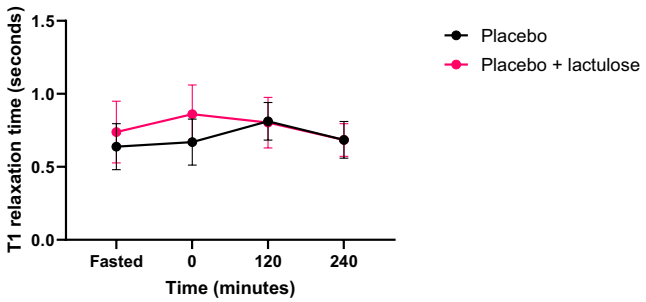

FIGURE 5 Relative time courses of T1 (in seconds) in the ascending colon (mean 95% CI) fasting and after taking the placebo following 36 h of lactulose, demonstrating no significant difference.

cause intestinal secretion down the electrochemical gradient.[4] We actually observed that 13.6 g lactulose increases SBWC $AUC_{0\text{-}240}$ by 14.2 L·min representing an average increase of 59 mL, considerably less than the 291 mL for IBS patients and the 145 mL increase for healthy controls reported by Undseth.[8] This difference most likely is due to the fact that Undseth gave lactulose alone while we wanted to study the effect of lactulose when given as it is in clinical practice, that is, combined with normal food intake. The Fortisip we used is a simple mixed nutrient meal which includes simple sugars and proteins whose rapid digestion and absorption would stimulate water absorption and thus reduce SBWC. The increase observed would be on its own unlikely to cause symptoms although the much larger increases (mean of 236 mL) which have been observed after fructose 40 g[31] did correlate with symptoms of gas, bloating, discomfort, and diarrhea. It is worth noting that patients with irritable bowel syndrome and diarrhea who have accelerated small bowel transit have reduced postprandial SBWC,[32] so the modest increase we observed with lactulose may in part reflect an acceleration of transit with transfer of content into the colon which would tend to lower SBWC.

The persistent 60% increase in small bowel motility after lactulose from baseline to 4 h compared with the fall for placebo arm is distinct from other studies which show a shorter lived increase immediately after meal intake followed by a fall to baseline by 4 h.[21] It should be noted that different scanners and field strength used by Khalaf et al.[21] mean that the numerical values cannot be directly compared.

What causes this persistent increase in small bowel motility with lactulose is uncertain. Although it could be a response to bowel distension, known to produce propulsive motility in both humans[33] and animals,[34] the increase in SBWC seen with lactulose is very modest compared to the fourfold increase seen after the osmotic laxative, Moviprep.[35] It cannot be excluded that the stimulation is due to more direct stimulation of motility by lactulose or, given the abundance of facultative anaerobes in the distal small intestine, its fermentation products such as short chain fatty acids.[36] Our measurement cannot distinguish between antegrade and retrograde movements but given the known acceleration of transit induced by lactulose in previous studies[9] using similar dosage it seems likely that antegrade pressure waves will be increased.

Human enterocytes lack the ability to hydrolyze lactulose so it is not normally absorbed in the small bowel[3] and most passes unchanged into the ascending colon along with the osmotically "trapped" small bowel water. Once it enters the colon, prior studies have shown it is rapidly anaerobically fermented with evolution of hydrogen within 10 min[37] and short chain fatty acids within 60 min.[7] The novelty of our observation is that we have showed that the speed of fermentation means that lactulose did not significantly alter colonic water content as assessed by T1AC, neither acutely nor

TABLE 1 Effect of lactulose 20 mLs twice daily for 36 h on small bowel water content, small bowel motility and colonic volumes (Day 1 vs. Day 3 on placebo).

Parameter	Placebo	Placebo and lactulose	*p* value
Fasted SBWC (median (IQR), mL)	92 (70–124)	133 (81–203)	0.057
SBWC AUC 0–240 min (L·min)	30 ± 17	43 ± 25	**0.0078**
Fasted T1AC (median (IQR), s)	0.55 (0.47–0.79)	0.66 (0.37–1.08)	0.72
Fasted ascending colon volume (median (IQR), mL)	202 (144–323)	211 (156–260)	0.33
Fasted total colonic volume (median (IQR), mL)	592 (474–671)	597 (438–775)	0.12
Colonic gas at 4 h (mL)	4 ± 3	5 ± 8	0.60
Small bowel motility at 2 h (a.u.)	506 ± 241	794 ± 225	**0.02**
Small bowel motility at 4 h (median (IQR), a.u.)	523 (457–646)	852 (771–1178)	**0.007**

Note: Data are represented as mean ± SD unless otherwise stated. *p* values <0.05 highlighted in bold.

Abbreviations: a.u., arbitrary units; AUC, area under the curve; SBWC, small bowel water content; T1AC, T1 of the ascending colon.

TABLE 2 Effect of ondansetron versus placebo without lactulose (Day 1).

Parameter	Ondansetron	Placebo	*p* value
Gastric volume at 2 h (mL)	94 ± 32	107 ± 64	0.37
SBWC AUC 0–240 min (L·min)	28 ± 24	30 ± 16	0.71
Small bowel motility at 2 h (a.u.)	528 ± 234	505 ± 223	0.48
Small bowel motility at 4 h (a.u.)	738 ± 380	630 ± 226	0.34
T1AC at 6 h (s)	0.51 ± 0.14	0.59 ± 0.27	0.37
Total colonic volume at 4 h (mL)	498 ± 195	503 ± 219	0.90

Note: Data are represented as mean ± SD.

Abbreviations: a.u., arbitrary units; AUC, area under the curve; SBWC, small bowel water content; T1AC, T1 of the ascending colon.

TABLE 3 Effect of ondansetron versus placebo while taking lactulose (Day 3).

Parameter	Ondansetron	Placebo	*p* value
Gastric volume at 2 h (mL)	100 ± 44	84 ± 38	0.26
SBWC AUC 0–240 min (L·min)	40 ± 28	43 ± 25	0.63
Small bowel motility at 4 h (a.u.)	910 ± 306	997 ± 466	0.62
T1AC fasted (median (IQR), s)	0.64(0.46–0.91)	0.66(0.37–1.08)	0.84
T1AC at 4 h (s)	0.66 ± 0.21	0.68 ± 0.21	0.76
WAPS	2.5 ± 2.4	2.7 ± 2.7	0.63
Colonic gas (mL)	5 ± 3	7 ± 9	0.43
Total colonic volume fasted (mL)	507 ± 301	644 ± 299	0.11
Total colonic volume at 4 h (mL)	502 ± 173	616 ± 340	0.29

Note: Data are represented as mean ± SD.

Abbreviations: a.u., arbitrary units; AUC, area under the curve; SBWC, small bowel water content; T1AC, T1 of the ascending colon; WAPS, weighted average position score of MRI markers.

after repeated dosing. This is not due to lack of sensitivity of T1AC since we have been able to show that another laxative psyllium, given in therapeutic doses does increase this parameter.[38] Short chain fatty acids are known to be rapidly absorbed, co-transported with Na^+ which would tend to reduce colonic water content.[39] This suggests that its undoubted laxative effect may be due to the stimulatory effects of products of fermentation on the small bowel and colon rather than increasing colonic water. We found no evidence that lactulose, when given with a nutrient meal, increased colonic gas though it would undoubtably have increased breath hydrogen. Earlier studies have shown that after 15 g of lactulose around 65% of hydrogen is excreted in breath with the remained being excreted as flatus.[40] The lack of increase in colonic gas we presume reflects both efficient absorption and excretion in the breath of the hydrogen generated together with an accelerated excretion as flatus, though we did not measure this.

Lactulose is also a prebiotic, stimulating the growth of a range of bacteria including Bifidobacteria[41] which will also contribute to

its laxative effect though the relative importance of increased water versus bacterial mass has not been evaluated.

It is worth noting that patients with IBS-D have faster underlying transit and hence inadequate time to ferment poorly absorbed small molecules such as fructose, so in such patients increased colonic water driven by osmotic load might contribute to loose stools. This could be a fruitful area for future research.

We have previously performed a study using a true osmotic laxative, mannitol with half the molecular weight of lactulose (182 daltons) in which we gave 17 g of mannitol providing 93 mosmol, over twice the 40 mosmol of lactulose in the current study. The larger mannitol stimulus without the Fortisip meal increased SBWC to over 400 mL, and at this larger dose, we did see an increase in ascending colonic water content indicating that there is a threshold of fluid delivered to the ascending colon which, if exceeded, will increase colonic water.[42]

Although our scanning sequences were not designed to systematically detect mass movements, we did observe some after lactulose (see Videos S1 and S2). These infrequent but substantial movements are unlike the normal mixing movements seen in the colon and represent movement of the entire colon contents distally "en mass." These are well described in earlier radiographic literature[43] and captured using radio-isotopic labeled colonic content. Using such a technique, we were able to document the increase in mass movements induced by lactulose[9] and their moderation by antispasmodic, mebeverine.[44] This stimulation of propulsive colonic motor patterns seems mostly likely to mediate the laxative effect observed, though more frequent scanning would be needed in future studies to prove this. The main focus of this study was the underlying mechanisms, and we did not record bowel symptoms after lactulose in which healthy subjects tend to be minimal and quite different from IBS patients.

The key products of lactulose fermentation in humans are acetate and lactic acid whose production leads to marked acidification of cecal contents with a 10-fold rise in hydrogen ion concentration.[7] Whether this process can occur to a significant amount in the distal ileum is uncertain. Prior studies had suggested around 10^6 organisms per mL[45] but this may have reflected contamination during sample collection since more recent studies suggest lower levels 10^2–10^4 with only a minority (14%) harboring colonic organisms.[46] Most are oral facultative anaerobic organisms which are capable of producing SCFAs though oral concentrations are much less than colonic.[42,47] Several studies have indicated that SCFAs stimulate propulsive motility in the terminal ileum[36] while in the colon they may both inhibit and stimulate propulsive motility depending on the type and concentration.[48] SCFAs act by activating the G protein coupled receptors (GPRs), GPR41 and GPR43 which are expressed on Peptide YY containing enteroendocrine cells in rats[49] and humans.[50] Human studies infusing solutions of SCFAs showed no obvious change in colonic motility patterns.[51] However several animal studies indicate that SCFAs stimulate colonic motility via mechanisms involving serotonin release.[52,53] More recently, it has been shown that the GLP-1 containing enteroendocrine cells appear to be the most responsive to microbial metabolites and that the resulting GLP-1 release activates serotonin secretion from neighboring enterochromaffin cells.[54]

Despite this possible link between acidification of the ascending colon by fermentation of lactulose and serotonin release, we were unable to show that this could be altered by the $5HT_3$RA ondansetron though this might be because other receptors, 5-HT1, 4 or 7 are involved. There may be species differences in these effects since, despite animal studies suggesting $5HT_3$RAs could block postprandial pancreatic secretion,[13] we could not see any change in postprandial small bowel water content which is markedly influenced by pancreatic secretions and strikingly increased by high fat meals.[55]

Earlier studies in healthy volunteers suggested that ondansetron inhibited the normal increase in left sided colonic tone in response to feeding.[15] Ondansetron also slows colonic transit in both healthy volunteers and IBS-D patients in whom the main effect was on the descending and sigmoid colon.[11] Unlike the ascending and transverse colon, these are regions where the rate of absorption is usually less, suggesting that ondansetron's anti-diarrheal effect may be primarily due to altered colonic motility rather than enhanced absorption or inhibition of secretion. Ondansetron does not reduce ascending colon water content (as assessed by T1) nor significantly reduce colonic volumes. Future studies should examine in detail the impact of ondansetron on left sided colonic motor patterns, particularly examining its impact on retrograde motor patterns as recently described.[56] Alosetron, a potent $5\text{-}HT_3$ antagonist, stimulates rectosigmoid motility,[16] a paradox which could be resolved if it was proven that it was the retrograde motility that was increased, since this would delay transit of both liquid and gas colonic content and inhibit defecation.

Our study has thrown new light on the mode of action of lactulose and ondansetron. The major finding is that at normal therapeutic doses the osmotic effect of lactulose seems less important for its laxative effect compared to its prokinetic effect. This effect is mostly likely mediated via the SCFA or other products of fermentation. Defining these more precisely may allow the production of more potent prokinetic agents.

AUTHOR CONTRIBUTIONS

The authors' responsibilities were as follows: RS designed the study; DG, CH, AM, PG, and LM contributed to study design; DG, CY, and AM conducted the study; DG and CY performed the statistical analyses; DG and RS wrote the manuscript with primary responsibility for the final content; and all authors read and approved the final manuscript.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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