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Assessment of motion of colonic contents in the human colon using MRI tagging.

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ABSTRACT

Background We have previously reported a non-invasive, semi-automated technique to assess motility of the wall of the ascending colon (AC) using Magnetic Resonance Imaging. This study investigated the feasibility of using a tagged MRI technique to visualise and assess the degree of flow within the human ascending colon in healthy subjects and those suffering from constipation.

Methods An open-labelled study of 11 subjects with constipation and 11 subjects without bowel disorders was performed. MRI scans were acquired fasted, then 60 and 120 mins after ingestion of a 500ml macrogol preparation. The amount of free fluid in the small and large bowel was assessed using a heavily T2-weighted MRI sequence. The internal movement of the contents of the AC were visualised using a cine tagged MRI sequence and assessed by a novel analysis technique. Comparisons were made between fasting and postprandial scans within individuals, and between the constipation and control groups.

Key results. Macrogol significantly increased the mobile, MR visible water content of the ascending colon at 60 mins post ingestion compared to fasted data (controls p=0.001, constipated group p=0.0039). The contents of the AC showed increased motion in healthy subjects but not in the constipated group with significant differences between groups at 60 minutes (p<0.002) and 120 minutes (p<0.003).

Conclusions and inferences. This study successfully demonstrated the use of a novel MRI tagging technique to visualise and assess the motion of ascending colon contents following a 500ml macrogol challenge. Significant differences were demonstrated between healthy and constipated subjects.
KEY POINTS:

Manometry techniques provide information about pressure changes that occur when the colon wall contracts. There is little knowledge about how the contents move.

Using an MRI tagging technique we showed differences in the movement of colonic chyme (following a macrogol stimulus) between subjects with constipation and healthy controls.

This non-invasive MRI technique has wide application as a tool to investigate the movement of colonic contents in constipation and diarrhoea to help further our understanding of the physiology of the colon.
INTRODUCTION

Constipation is a common problem worldwide, with estimates of prevalence ranging from 14% to 28% in the USA [1] and associated annual health care costs recently estimated to exceed $230 million [2]. Generally constipation disorders have been categorised in three ways [3]: firstly, constipation arising from disordered or obstructed defecation (OC) (where the primary cause is impaired rectal evacuation) [4], secondly irritable bowel syndrome with constipation (IBS-C) accompanied by pain and bloating, often with transit times within the wide range of normality, and thirdly, functional slow transit constipation (FC). As we have recently shown, although the symptoms overlap substantially, these conditions have different mechanisms of disease [5] and may require different treatments. Distinguishing these conditions based mostly on patient perception leads to trial and error treatments which may explain why 50% of patients are dissatisfied with their treatment [6].

We showed previously that, while baseline measurements do differ, the contrast between patient groups can be enhanced by stressing the colon using an osmotic laxative to distend the ascending colon, so that 1 hour after ingestion of 1 litre of a macrogol drink, colonic volumes become abnormal in 19/20 FC patients [7].

In our previous work, using cine MRI to determine colonic wall motility, it was clear that there was also motion occurring within the colonic contents, probably related to the gross movement and mixing of these contents. MRI tagging is commonly used for the assessment of cardiac function and has been applied previously to monitor small bowel motility [8, 9] and to study movement of the stomach contents following a porridge meal [10].

In this study, we aimed to investigate whether an MRI tagging technique could be used to assess movement within in the human ascending colon and to differentiate between the colonic response to a 500ml macrogol oral stimulus in healthy and constipated subjects.
METHODS

Subjects and study design

The study protocol was approved by the Local Research Ethics Committee (control group: G08052014 SoM NDDC and constipated group: J14082014 SoM NDDC). Twenty two subjects were recruited by advertisement and from a database of subjects who had taken part in previous studies and had agreed to be contacted again. All subjects gave written informed consent and had no contraindications to MRI. There were no adverse events during the studies.

The 11 healthy participants had no history of gastrointestinal disease. There were 4 males and 7 females, aged 28 ± 10 years (mean ± standard deviation) with mean Body Mass Index (BMI) 25 ± 5 kg m⁻². The 11 constipated participants (as defined by Rome III criteria) were 2 males and 9 females aged 36 ± 13 years, with BMI 25 ± 5kg m⁻². Constipated subjects were asked to refrain from taking their usual laxatives for two days before the MRI study day.

All subjects ingested 5 MRI transit marker capsules as described previously [11, 12] 24 hours prior to MRI. Subjects were asked to avoid strenuous exercise and ingesting alcohol and caffeine the day before their attendance for MRI, and to fast from 22:00 hours the previous evening. They were only allowed to consume a small glass of water on waking on the day of the experiment. Participants completed a questionnaire to investigate adherence to the study restrictions before acquiring the baseline, fasted MRI scan. They were then asked to drink a 500ml dose of MOVIPREP® (Norgine Pharmaceuticals Ltd, Harefield, UK) polyethylene glycol (Macrogol 3550) electrolyte solution within 30 minutes. The time the subjects started consuming the test drink was defined as t=0. This was followed by a scan at t= 60 mins and t=120 mins.

Magnetic Resonance Imaging
All MRI scans were carried out using a 3T Philips Achieva scanner (Philips, Best, The Netherlands). The subjects were positioned supine with a 16-channel XL-torso receiver coil wrapped around their abdomen. After the initial set-up scans, the following scans were acquired across the abdomen:

1. A multi-echo mDIXON scan [13] was used to determine the location of the transit pills and calculate a weighted average position score (WAPS) for each subject (a validated measure of whole gut transit time) [11, 12].

2. A T2-weighted single shot (RARE) sequence was used to determine the mobile, MR visible water content of both the small bowel (Small Bowel Water Content, SBWC) and colon [14-17]. This sequence was included to enable monitoring of the progress of the laxative drink through the GI tract.

3. To aid in the positioning of the tagged slice through the AC a high resolution multi-slice bTFE scan was acquired, placed sagitally oblique through the AC [16].

4. Motion of the contents of the AC were visualized using a tagged bTFE sequence [9] centered within the ascending colon (primarily sagittal). This sequence superimposed dark horizontal stripes (tags), 12 mm apart, onto the images. The delay of 250 ms between application of the tag lines and acquisition of the image allowed movement within the colon to be detected (see Fig. 1). This sequence had TR/TE 2.3/1.15 ms, FA 45°, with a single sagittal slice, thickness 15 mm, FOV 222-264 mm (AP), 330 mm (HF) with acquired resolution of 1.5x1.5 mm² and reconstructed to 0.98x0.98 mm², SENSE factor 1.5, and half-scan factor 0.7. In total, 33 dynamic scans were acquired at 600 ms intervals within a single 20 s breath-hold.

All other imaging parameters associated with the different sequences are summarized in Table (1).
The subjects spent 30 minutes per time point inside the scanner and they were asked to spend the rest of the time sitting upright in an adjacent room.

**Data analysis**

An overall assessment of gut transit time (WAPS) was obtained by noting the position of 5 marker capsules ingested 24 hrs prior to scanning as previously reported [11, 12]. The free water in the small bowel (SBWC) and colon were measured using the RARE sequence as previously described [14, 18].

The series of tagged images of the ascending colon provided a method to visualize motion within the colon and were also used to assess the movement of the colon contents. If no movement of colonic contents, or adjacent viscera, occurred during the breath hold (eg due to small respiratory movements or aortic pulsation) then all 33 sequential tagged images would be identical, with all tag lines remaining straight (as seen in Fig 1a). However any movement of the colonic chyme in the delay between the application of the tag and acquisition of the image would change the position of the tag lines on the image. For instance, if laminar flow was present then each frame would show uniform displacement of each tag line within the colon, with the displacement proportional to the flow velocity, 12 mm corresponding to a flow velocity of 4.8 cm/sec in this case. Laminar flow velocities greater than this value would not be detected due to the periodicity of the tag lines. The predominant direction of flow (antegrade or retrograde) would also be revealed from the antegrade (arrowed) or retrograde direction of displacement of the tagged lines (as can be seen in Fig 1b ) Therefore the movement of the tags could, in principle, be used to measure laminar flow velocity. However, as can be seen in Fig. 2, non-laminar flow leads to local variations in the 3D velocity field which can smear the tag and prevent absolute velocity measurement. This
provides a simple method to assess movement of the colonic content, since motion, in any direction, will lead to changes in signal intensity in the tagged colonic contents from frame to frame. It is this variation in intensity which is the basis of the proposed method of analysing the data to assess motion within the colonic contents.

The first 4 frames of each sequence were discarded due to intensity changes occurring as the MRI signal reached steady state. The mean signal intensity (MI(x,y)) and standard deviation (STDEV(x,y)) of each pixel through the remaining 29 sequential cine frames were calculated using IDL® (Research Systems Inc, Boulder, CO) resulting in maps of both mean intensity and standard deviation (Figure 2a and b).

In the standard deviation maps those voxels whose intensity is changed during the 20 seconds of data acquisition (either by the movement of structures such as the colon wall, or by the movement or smearing of the null tagged lines) have a larger standard deviation (Fig. 2b) whereas static structures (liver, muscle and fat) or motionless colonic contents have a value close to zero (Fig. 2a). The resulting map thus highlights any motion of the colonic contents, even where the net displacement over 20 seconds could be zero. This standard deviation map also reveals where the motion is concentrated eg if it is the whole colon that has moved then all the contents will show a high standard deviation whereas movement in a narrow ‘jet’ will lead to a smaller region of high intensity within the colon. In order to derive a single parameter that easily summarises this motion of the colonic contents a region encompassing the AC was drawn on the mean intensity map, as shown in figure 2, and the average mean intensity (MI_R) and average STDEV (STDEV_R) within that region was calculated (using Analyze9™, Mayo Foundation, Rochester, MN, USA). The average coefficient of variation (%COV) for the tagged scan is then estimated from
%COV = 100x STDEV_R / MI_R

Statistics
Normality of the data was tested using D’Agostino Pearson’s normality test and the data are expressed as mean, (standard deviation) for normally distributed data and median values (with the IQR indicated in brackets), for non-normally distributed data. Statistical analysis was carried out using Prism 6 (GraphPad Software Inc.). Comparisons within group were performed using a paired t-test (for normally distributed data) or Wilcoxon’s matched-pairs signed rank test, for data non-normally distributed. Comparisons between groups were performed using an unpaired t-test (with Welch’s correction for normally distributed data) or Mann-Whitney rank sum test for non-normally distributed data. Multiple comparisons of data were Bonferroni corrected, with comparison between baseline and 60, baseline and 120, for both cohorts and at all times between groups resulting in a corrected significant p-value of < 0.0071

RESULTS
All subjects completed the study day. MRI scans were not available for two constipated subjects at t=60 mins, one healthy volunteer at t=60 mins and one healthy volunteer at t=120 mins due to equipment problems.

Whole Gut Transit
At baseline the WAPS measured using the MR markers were significantly different between groups: controls 0.6 (0-1) and constipated group 2.6 (1.4-3.6), p=0.0011 Mann-Whitney rank sum test.

Free water in the small bowel and ascending colon
The amount of mobile, free water in the small bowel (SBWC) is shown in Fig. 3a. There was no significant difference in the fasted baseline values of these groups (controls 33 (22-89) ml, constipated group 66 (42-124) ml p=0.11). Sixty minutes after macrogol ingestion, the SBWC was significantly increased in both groups, (controls 372 (220-566) ml p=0.001, constipated group 472 (337-708) ml p=0.0039; Wilcoxon ranked pairs test). By t=120 minutes SBWC had dropped in both groups (controls 231 (157-322) ml, constipated group 406 (215-517) ml), although the change from baseline remained significant for both groups (controls p=0.0068, constipated group p=0.001 Wilcoxon ranked pairs test), when multiple comparisons were taken into account. However there were no significant differences in SBWC between groups at 60 (p=0.41) and 120 mins (p=0.09) (Mann-Whitney rank sum test).

The variation in free water content in the ascending colon is shown in Fig. 3b. There was no significant difference in the very low baseline values between groups (controls 2 (0-7) ml, constipated group 11 (1-29) ml p=0.16). By 60 mins post ingestion the amount of mobile, free water was significantly increased in the healthy control groups, (140 (104-347) ml, p=0.001), and in the constipated group 228 (91-259) ml, p=0.0039 (Wilcoxon ranked pairs test) showing that the macrogol solution had entered the ascending colon in both groups. By 120 minutes post ingestion the amount of mobile, free water in the ascending colon had only dropped in the constipated group (controls 146 (32-227) ml, constipated group 84 (3-195) ml) although the change from baseline was still significant in both groups, (controls p=0.002, constipated group p=0.0039) when corrected for multiple comparisons. Again there were no significant differences in the free water in the ascending colon between groups at 60 (p=0.66) or at 120 mins (p=0.24) (Mann-Whitney rank sum test).

Tagged Images
Baseline sequences showed intact tags (as shown in Figs. 1a, 2a and supplementary information video 1) indicating very little motion within the colon in both groups. After 60 mins displacement and smearing of the tags (as shown in Fig. 1b) was observed in some subjects (mainly healthy volunteers). Fig. 4a shows the presence of forward and backward tag displacement occurring simultaneously in the central regions of the ascending colon. Fig. 4b demonstrates the presence of a fast (>4.8 cm/sec) retrograde central ‘jet’, which was also observed (supplementary information video 2). Tag smearing resulting from non-1D laminar flow can be seen as a reduction of tag intensity in Fig. 4c. Increased movement in the central regions of the ascending colon, compared to that adjacent to the colon walls, (seen in all parts of Fig. 4) was observed throughout the data sets. Such motion was still visible at t=120 minutes, although predominantly in the healthy subjects rather than the constipated group.

The calculated average coefficient of variation (%COV) for the all the completed tagged scans of the ascending colon is shown in Fig. 5. As expected there is no significant difference in the low baseline values between groups (controls 20% (14-23), constipated group 12% (11-20); p=0.1). By 60 mins post ingestion the %COV was significantly increased in the control group only (30% (26-35), p=0.002; constipated group 17% (13-23), p=0.57; Wilcoxon ranked pairs test). By 120 minutes the %COV had dropped in both groups but the change from baseline remained significant only for controls (controls 25% (18-36), p=0.002; constipated group 13.% (12-18), p=0.76). This led to a significant difference in the %COV between groups at 60 mins (p=0.002) and 120 mins (p=0.003) (Mann-Whitney rank sum test).

DISCUSSION

MRI tagging and our proposed analysis technique provide a new method to visualize movement of the colonic contents and a novel means of quantifying this. The technique has
been used to demonstrate differences between healthy and constipated subjects in response to a 500ml macrogol challenge.

MRI is an ideal tool to study the physiology of the bowel, allowing assessment of changes in whole gut transit, contractile activity, and fluid distribution both in the small bowel [9, 14, 15, 19-30] and the colon [11, 12, 21, 22, 31-33] during interventional studies. The whole bowel (small and large) can be assessed in a single scanning session and, since MRI is non-invasive, repeated studies are possible. The additional information gained from the tagging sequence provides further insights into how the chyme moves within the large bowel. Specifically the proposed %COV measure relates to the movement and blurring of the tags and therefore mixing related motion. More sophisticated analysis of this tagged data might allow more detailed assessment of the velocity fields within the colon. Additional blurring of the tag lines could arise from motion occurring during the readout phase of the acquisition and will have contributed to the variance measured here. In future alternative methods of analysis providing more quantitative measures will be investigated. Phase contrast MRI (PC-MRI) is the conventional method of measuring flow but is less suited to measuring flow in the colon for two reasons. Firstly, tagging is less sensitive to overall subject motion than PC-MRI since the tag lines are inherently sensitive to local motion, not bulk motion. Secondly, tagging will lose sensitivity if the $T_1$ of the material being scanned is too short as the lines will recover (and hence disappear) in the delay period. PC-MRI will lose sensitivity if the $T_2$ of the sample is short as the signal will decay during the phase encoding period. In practice the MRI properties of normal colonic contents are likely to favour the use of tagging over PC-MRI. Finally, tagging provides an immediate assessment of the motion which would be useful for non-expert sites.

A central channel of increased displacement was seen on the majority of healthy volunteers’ data, with the haustra probably impeding flow near the walls of the colon. This may be a
biological mechanism to reduce the impact of the axial fluid flow on the anaerobicity of the more static chyme adjacent to the colon walls (where anaerobic microbiota may escape poisoning by oxygenated ileal contents entering the colon), thus preserving the mixture of aerobic and anaerobic microbiota essential for health.

This study demonstrated clear changes in SBWC, ascending colon water and motion of the chyme in the ascending colon of 11 healthy volunteers at 60 mins and 120 mins following the 500 ml macrogol challenge drink, a smaller stimulus than has previously been used [34]. This indicates that relatively moderate quantities of fluid passing through the small bowel and arriving in the ascending colon can trigger wall contractions and movement of the colon contents in health. However, although the SBWC and ascending colon water was also increased in the constipated group at 60 mins and 120 mins following the challenge, the motion of the contents in the ascending colon (as assessed by %COV) was not increased in this group post-ingestion. This lack of motion led to a significant difference in % COV being observed between groups at both 60 and 120 mins post-ingestion. This suggests that this parameter may successfully discriminate between healthy and constipated subjects when the ascending colon is challenged appropriately.

The motility of the descending and sigmoid colon has been previously studied using manometric techniques and these have demonstrated differences in contractile activity present in IBS-C and in chronic slow transit constipation [33, 35]. IBS-C patients have normal, or even excess, antegrade and retrograde contractile activity (but this activity fails to move the contents through the colon) whereas FC patients frequently have much lower levels of contractions. However, manometry is invasive and often does not monitor the entire colonic region (particularly the proximal ascending colon where much of the mixing processes occur), and as a result is limited clinically to extreme cases, particularly for
paediatric populations. Manometry cannot be used to study movement of the contents of a fluid filled colon as the pressure measurements become less accurate when the colonic contents become less viscous [36] and tracking of contents is not possible so that manometry cannot directly detect mixing.

The main limitations of this study were the small numbers in each group. In addition, the smaller macrogol challenge drink may not have stimulated the same type of motion seen previously with a larger challenge drink. This smaller fluid stimulus was also less likely to reach the more distal parts of the colon (descending and sigmoid) and hence may be less suitable to differentiate between subgroups of constipation patients.

As the motion and mixing observed in this study was due to the stimulus of the laxative drink, data on the reproducibility of the measurement would be extremely useful and work is underway to obtain this data. Further work is required to fully explore the potential of MRI tagging for assessment of mixing and transportation of colonic chyme including other possible physiological challenges such as a high fat meal. This potential ability to monitor non-invasively both antegrade and retrograde flow patterns could be used to assess the efficiency with which the ascending colon mixes and transports the contents and has applications in functional disorders of both constipation and diarrhoea. Allowing for data acquisition during free breathing, and using image registration techniques to remove the effects of respiration, could extend the application of this technique and allow for more sporadic motion to be investigated. Combining this technique with existing motility and volume measurements could provide increased discrimination between healthy subjects, subjects whose contractions are ineffectual at mixing and propelling the colonic contents, and
subjects in whom colonic contractions are absent, potentially allowing stratification of IBS type disorders from FC.

In conclusion this study has demonstrated the use of MR tagging and a novel analysis method to study movement of the colonic content and has used this to demonstrate significant differences in the transport and mixing of colonic chyme between healthy volunteers and constipation subjects following a macrogol challenge drink.

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COMPETING INTERESTS

RCS has received research funding from Lesaffre and Ironwood and free drugs for clinical trial from Norgine. He has also acted on Advisory Boards for Almirall, Astellas, Ibsen and Danone. All other authors have no competing interests.

AUTHOR CONTRIBUTIONS
Research was designed by CLH, LM, GM, PAG, and RCS and performed by JP, GM, and CLH. It was analyzed by SEP, JP, CLH and the paper was written by SEP, CLH, GM, LM, PAG and RCS. All authors read and approved the final manuscript.
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‡ Two 3D blocks were positioned coronally with a 30 mm overlap.

Table (1) Imaging parameters
FIGURE LEGENDS

Figure 1. Tagging applied to the ascending colon. ‘Tags’ are the dark stripes across the image. There is a small shift in position of these tag lines in regions of predominately fat tissue compared to water tissue (grey arrow) (a) Typical sagittal image showing no movement within the colonic chyme - tags are straight and intact (white arrow). (b) Typical sagittal image showing movement within the colonic chyme. Both tag distortion (white arrow) and smearing and reduction of tag intensity (red arrow) due to movement are highlighted.

Figure 2. Processed images from tagged cine data showing (a) little motion (b) visible motion of colonic contents: (i) Mean pixel intensity map (calculated over 29 dynamic images). (ii) Corresponding pixel standard deviation map (calculated over 29 dynamic images). The displayed intensity scale for the standard deviation map is 5 times smaller than the mean pixel intensity map. The ascending colon region for each %COV calculation is outlined in red.

Figure 3. (a) Individual data for MR free water in the small bowel (SBWC) at baseline, 60 and 120 minutes post ingestion. (b) Individual data for MR free water in the ascending colon at baseline, 60 and 120 minutes post ingestion. Statistically significant differences from baseline are shown (corrected for multiple comparisons).

Figure 4. Tagged images showing: (a) Simultaneous antegrade (red arrow) and retrograde flow (white arrow) in ascending colon. (b) The presence of a fast moving central retrograde ‘jet’ (grey arrow). (c) The reduction in tag intensity (red arrow) and complex flow in hepatic flexure of ascending colon (white arrow).
Figure 5. Individual data for the calculated % COV at baseline, 60 and 120 minutes post ingestion. Statistically significant differences from baseline and between groups are shown (corrected for multiple comparisons).
FIGURES

1a

1b
SUPPLEMENTARY INFORMATION

Video 1 An example of tagged images showing little motion in the fasted ascending colon.

Video 2 An example of tagged images demonstrating motion in the fluid filled ascending colon.