Echo-Planar Magnetic Resonance Imaging to Assess Water Volume in the Distal Small Bowel

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Received October 12, 1994; accepted February 20, 1995

Purpose: Assessment of fluid volumes and flow through the small intestine has in the past only been possible by means of invasive intubation studies on human volunteers. Intubation very likely disturbs gut motility and stimulates secretion. *Methods:* The aim of this study was to utilise the new technique of echo-planar magnetic resonance imaging in order to non-invasively visualise the changing volume of water in the small intestinal lumen. 200mls of test solution was ingested and water volume assessed using a multi-slice scanning technique on 3 separate days. The solutions were pure water, pure water plus 2.264 or 10g of mannitol. These were taken on separate days by 8 healthy male volunteers. Regions of interest were constructed in the area of the lower pelvis excluding retroperitoneal structures. *Results:* The water content of the lower small intestine did not change significantly over the 4 hours after the control solution. By contrast after both mannitol solutions there was an increase in the amount of water in the distal intestine as assessed by the area under the curve of the volume time profile (Control 51 ml.h (SD±47); mannitol 2.264g/200ml 72ml.h (SD±57); 10g/200ml mannitol 115ml.h (SD±56)). Page's L Trend test showed that the trend for the volume to increase with increasing mannitol concentration to be statistically significant at the 1% level (L = 108). *Conclusions:* The study highlights the potential of echo-planar magnetic resonance imaging to visualise changes in gastrointestinal physiology in a non-invasive manner.

KEY WORDS: mannitol; small intestinal transit; echo-planar magnetic resonance imaging.

INTRODUCTION

Our knowledge of the control of fluid absorption by the small intestine is largely derived from extrapolation from studies of the absorption of fluids from short perfused study segments (1-3). These have many limitations, for example the intubation procedure has a poor subject tolerability and in order to provide meaningful data a large number of studies need to be carried out, which in practice proves to be extremely difficult. Another major limitation is the fact that the physiologically important parameter is the amount of salt and water absorbed by the entire small intestine not just one segment. Abnormalities in one segment may be compensated for by changes in another. Slow marker infusion techniques assessing flow from the terminal ileum (4) avoid some of these problems, but recent studies have shown that intubation of the gastrointestinal (GI) tract can alter normal gut motility and secretion (5). Gastric emptying was shown to be significantly retarded and colon arrival significantly accelerated in intubated subjects compared to controls. Therefore, values for small intestinal transit (SIT) time were significantly reduced in intubated subjects (5). A procedure that would allow absorption from the small intestine to be quantified non-invasively would clearly be of great value.

Magnetic resonance imaging (MRI) is a non-invasive, non-radioactive quantitative technique which has the capability to perform such an assessment. Conventional MRI which requires repeated sampling over several minutes, of the nuclear magnetic resonance signal, cannot be used for abdominal imaging, owing to image artifacts and blurring of the spatial detail of the organ (6). Echo-planar imaging (EPI) is a development of MRI that will produce complete images in as little as 64ms (7,8). Thus for the first time non-periodic moving organs may be visualised. GI tract imaging has been achieved using the ultrafast, EPI, variant technique MBEST (Modulus Blipped Echo-Planar Single pulse Technique) (9-11). MBEST produces a complete two dimensional image in acquisition times of either 64 or 128 ms for 128 × 64 or 128 × 128 pixel matrix respectively. These times are sufficiently short to "freeze" abdominal motion and thus overcome motional blurring. The signal intensity in MBEST EPI images is chiefly determined by the water proton concentration and mobility (7,12,13). Therefore, this technique is particularly sensitive to free water in the GI lumen, enabling the detection of changes in fluid volume within the small intestine.

Increasing the rate of SIT reduces the time available for absorption and may contribute to impaired uptake of luminal contents (14,15). It has been shown that poorly absorbed solutes, such as mannitol can accelerate SIT and reduce nutrient absorption (16). Until recently the dose of mannitol required to have an effect on SIT was thought to be in the order of at least 100mmol/l (17). However, recent studies carried out at Nottingham, using gamma scintigraphy to measure SIT time have shown that lower concentrations of mannitol in solution (62mmol/l) can significantly reduce intestinal transit time (18,19). In the first of our studies a 2.264g/200ml mannitol solution was shown to have a mean SIT time of 158min compared to a control of 240min, which was a 34% decrease in mean SIT time compared to the control (18). This result was confirmed in a subsequent study which also showed a 34% reduction in SIT time associated with the same concentration of mannitol in solution compared to a control (19). Unabsorbable solutes like mannitol are believed to accelerate SIT by osmotic retention of fluid within the gut lumen (20,21).

The aim of the present study was to investigate the mechanism by which low doses of mannitol accelerate SIT, by visualising the volume of fluid in the distal portion of the

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0724-8741/95/0800-1134$07.50/0

small intestine using MBEST. In conjunction with MRI, the volunteers also provided breath hydrogen samples. On entry of mannitol into the caecum of the subjects unabsorbed disaccharide is fermented by the colonic microflora and generates several products including hydrogen (22). Therefore, the time of entry of the mannitol solutions into the caecum of the volunteers could be determined.

MATERIALS AND METHODS

Subjects

A randomised three way crossover study was carried out in eight, healthy males (23 - 27 years), who were non-smokers and were not on any medication. Volunteers were excluded from the study if they had taken antibiotics in the previous month and if they had more than three bowel movements per day or less than three bowel movements per week. All volunteers were required to have a lactulose breath hydrogen test and those who failed to respond to 10g of lactulose with a greater than 20 part per million rise of breath hydrogen above basal, were excluded. After the procedure was explained thoroughly to the subjects, written consent was obtained for participation in the study as described by a protocol that was previously approved by the Ethics committee of Nottingham University.

Experimental Design

Subjects either took a 200ml test solution containing either 2.264g or 10g of mannitol dissolved in Purified Water B.P. or a 200ml volume of Purified Water B.P. as a control. The night before each study day the volunteers were required to eat a low residue meal, consisting of rice, meat or fish and white bread. This was to ensure that their breath hydrogen at the start of each study day was minimal.

End-alveolar breath samples were collected immediately before administration of the solutions and just prior to each set of images being taken. To avoid dilution of the alveolar hydrogen concentration, each volunteer was carefully instructed not to take a deep breath or hyperventilate prior to sample collection. Four hours post dose the volunteers received a standard lunch.

MR Imaging

Images were obtained on a purpose built echo-planar MR imaging system operating at a proton resonant frequency of 22.03MHz. For data acquisition the MBEST echo-planar sequence was used which yields a single 128 × 128 pixel matrix in 130ms (7,12,13).

Utilising a rapid multislice approach, 40 transaxial slices, each 1cm thick, were taken in 10 seconds to image a volume of approximately 30cm × 30cm × 40cm. Each volunteer was imaged from above the stomach to below the bladder while holding their breath to prevent errors in volume measurement due to changes in diaphragmatic position. These images were then saved on optical disc for analysis at a later stage. Images were acquired every 10 minutes for a period of 4 hours, when lunch was given to the volunteers. One final image was taken 20 minutes post lunch.

DATA ANALYSIS

MR Imaging

Three slices were used out of each set of forty collected at each individual time point. The base of the spine was used as a reference marker, in order to establish that the same three slices in a particular volunteer were being analysed at each time point. The last slice where the spine appeared plus the next two were used to calculate the volume of fluid in the intestine lying within the pelvis, which is for most part the distal ileum.

In order to detect the small changes in water volume within the lower small bowel, it was necessary to exclude pixels corresponding to tissue, i.e., muscle and fat. The signal intensity of tissue is considerably less than that of water, allowing one to distinguish between water and tissue pixels in the final image. Therefore, below a threshold value, any signal received would be produced by the body tissue and would not be included in the final water volume calculation. To calculate this threshold value a background region of interest (ROI) was drawn on the image away from the principal area of interest, the small bowel. The peak pixel value in the background ROI was found for the three slices at each time point. A mean overall value for the background (A) was calculated from all the peak values for background, this value was then utilised as the threshold in the final calculation to find the volume in the lower small bowel at each time point.

The signal intensity in MBEST EPI images is chiefly determined by the water proton concentration and mobility (7,12,13). Images are displayed on the grey scale, with a value of zero assigned to a pixel where no signal was received, and a value of 255 was given to a white pixel. A water phantom was included in the acquisition of all the image sets. This contained the brightest pixels in the image, corresponding to a volume of tissue entirely full of water. A ROI was drawn inside the phantom so that the mean pixel value could be found and this value was then used to standardise the pixel values for each image.

A further ROI was drawn around the small intestine for each subject, which remained constant in size and shape for each volunteer on all three study days. Figure 1 shows representative images from one volunteer following administration of the 10g/200ml mannitol solution. Two time points are illustrated, one immediately post-dose and the second during a rise in relative water volume in the distal small bowel acquired at 0.88 hours after dosing. The ROI shown on the images was constructed to exclude the strong signals obtained from the aorta and the internal ileac arteries. A histogram (Figure 2) showing the number of pixels with each value on the grey scale was constructed from the ROI.

Pixel values below the background level, 'A' were assumed to be due to background tissue signal and were therefore excluded. Pixel values above 'A' corresponded to areas containing water in the ROI. These pixels could therefore be used to calculate a volume of water in the ROI. For all three slices for each time point a histogram was constructed from the ROI. To calculate the water volume in each of the three slices the histogram was linearly scaled from zero at zero on the grey scale to unity at the mean phantom value 'B'. The

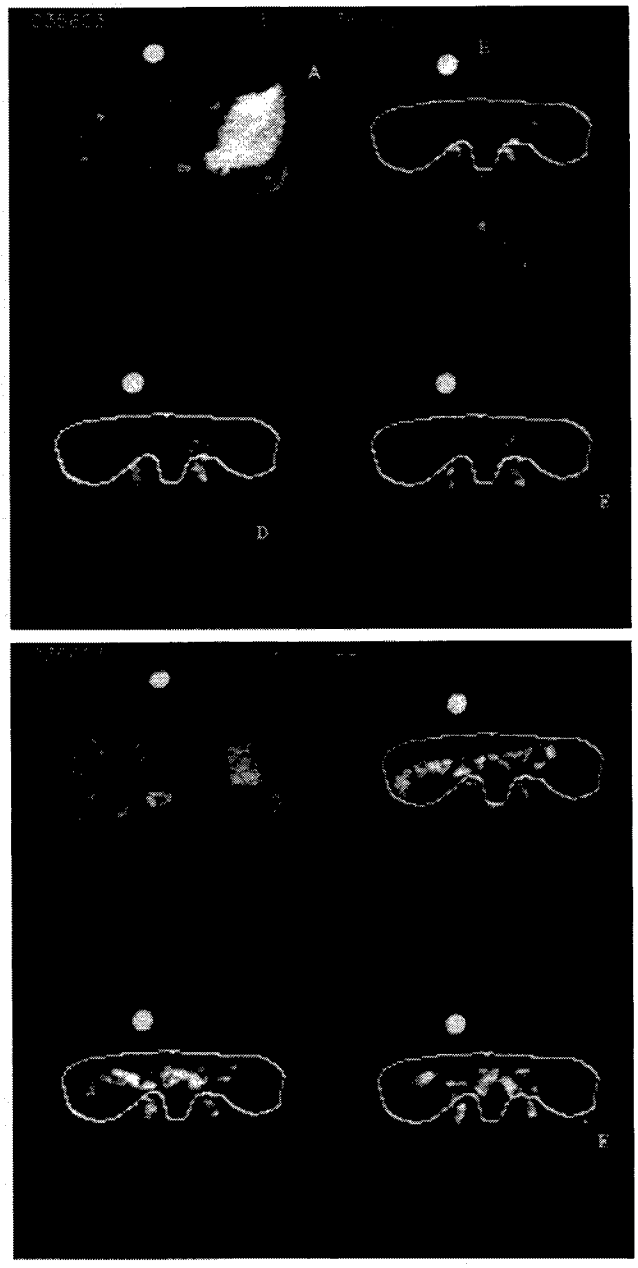

Fig. 1. MR images obtained from a volunteer after administration of 10g/200ml mannitol solution. T = 0.03 hours (top) are images produced immediately post administration, showing a full stomach (A), the water phantom (B), the spinal column (C), the background noise (D) produced by the tissue and the region of interest, enclosed by the white line (E). T = 0.88 (bottom) hours are images when a peak in the water volume in the region of interest (E), the lower small bowel can be seen, compared to the 0.07 hours images.

water volume was calculated for each slice by finding the area under the scaled histogram between points 'A' and 'B'. The sum over the three slices then gives a representation of the volume of water present at that time point. This value was then multiplied by the pixel volume, typically 2.5 × 2.5 × 10mm, to calculate the relative volume of water in the lower small bowel.

There is no published research utilising MR techniques for measuring water volume in the distal small bowel and

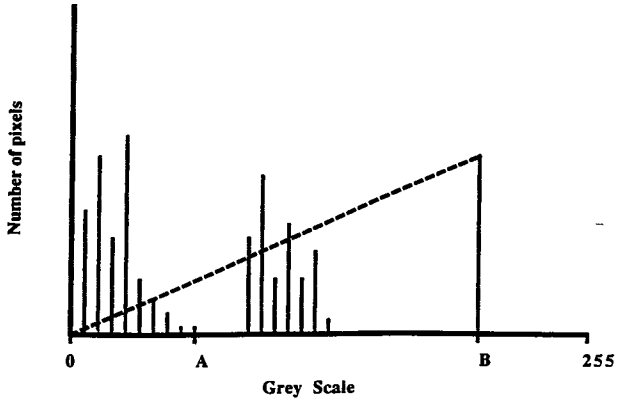

Fig. 2. A histogram produced by sampling the region of interest and linear scaling of the histogram in order to calculate the final volume in the small bowel.

therefore the approach for analysis of the data has been derived from established principles in MRI, however, there are three main assumptions implicit in this method;

(1) All the pixels in the small bowel below the background level (A) contain no water. This is clearly not the case, as although most correspond to tissue, a minority will correspond to pixels containing a very low amount of water. However, this assumption will contribute only a small error in the final calculation.

(2) The signal intensity is proportional to the water content of a corresponding pixel. This is a reasonable assumption, although magnetic susceptibility effects at the interfaces between different media (air/water) which are common in the small bowel, will lead to some signal attenuation. This will lead to a slight underestimation of the water volume in the lower small bowel.

(3) The slice profile is square. This effect is significantly reduced by using the reference water phantom, however, a slight underestimation of the volume may still be possible.

Data were represented by constructing relative volume profiles and calculating the area under the curve (AUC) for the three solutions for all eight subjects.

Breath Hydrogen

The hydrogen concentration in the samples was analysed using an exhaled hydrogen monitor (GMI, Renfrew, Scotland). This incorporates a gas-sensitive polarographic cell, enabling the partial pressure of hydrogen in the same to measured. Breath hydrogen profiles were constructed for each of the three solutions administered to all eight subjects.

Statistical Analysis

Statistical analysis was performed on the AUC values for the individual relative volume data using the Wilcoxon Signed Rank test and Page's L Trend test.

RESULTS

The water volume calculated at each time point is termed the relative water volume because we could not prove that the actual volume values were the exact volumes present in the lumen of the distal small bowel. Therefore, it

was decided to compare relative volumes, and monitor the changes in volume that resulted from administration of the various solutions. The mean relative water volume profiles produced from all eight subjects are shown in Figure 3. After administration of the Purified Water B.P. the volunteers showed no real increase in relative water volume from the baseline value at time point zero. After administration of the 2.264g/200ml solution to the subjects, the relative water volume profiles showed considerable intersubject variation, but no significant peaks in the mean relative volume profile. The 10g/200ml mannitol solution showed the most consistent change in the relative volume in the lower small bowel. All the volunteers showed definite peaks in the volume profiles, but the time at which these peaks occurred post administration varied between individuals. The AUC was calculated for each of the solution profiles and these values are presented in Table I.

The mean value for the AUC of the 2.264g/200ml mannitol solution showed an increase compared to the control. However, this increase was not statistically significant ($P=0.05$). The 10g/200ml mannitol solution showed a statistically significant increase in the individual AUC data when compared to the control for each volunteer ($p=0.05$). Analysis of the group data by Page's L Trend test showed the trend for the AUC value to increase with increasing mannitol concentration to be statistically significant at the 1% level ($L=108$).

An increase in the breath hydrogen concentration of 3ppm was taken to be the time of the onset of caecal filling (23). Only volunteers 1,5,6 and 7 showed an increase of 3ppm after administration of the 2.264g/200ml solution of mannitol. The failure of all volunteers to show an increase in breath hydrogen output was not surprising, since the concentrations of unabsorbed mannitol reaching the caecum, after administration of the 2.264g/200ml mannitol solution would be very low. The rate of delivery of the mannitol solution to the caecum would also be slow. No peaks in breath hydrogen were seen after administration of the Purified Water B.P. The mean breath hydrogen response to ingestion of the three solutions is shown in Figure 4. All volunteers showed an increase in breath hydrogen concentration after administration of the 10g/200ml mannitol solution. The time to reach this rise in breath hydrogen concentration is provided in Table II.

Table I. Area Under the Curve Values (ml.h) for the Volume-Time Profile following Administration of the Three Solutions to Eight Male Volunteers

Volunteer number	Purified water B.P.	2.264 g/200ml Mannitol solution	10 g/200ml Mannitol solution
1	162	44	170
2	28	114	201
3	34	20	127
4	44	195	15
5	28	36	66
6	26	57	77
7	60	50	61
8	24	57	61
Mean	51	72	115
SD	47	57	56

DISCUSSION

From intubation studies, the mechanism by which large doses of mannitol in excess of 150 mmol/l, accelerate SIT was shown to be osmotic inhibition of fluid absorption by the unabsorbed solute, causing an increase in the volume of the luminal contents (22). This in turn stimulated peristalsis and hence propulsion of the luminal contents (25). The aim of the present study was to verify that low doses of mannitol such as 62 mmol/l cause a reduction in SIT time by the same mechanism as the larger doses of mannitol used in the intubation studies, by utilising EPI to allow non-invasive monitoring of the volume of water retained in the small intestine,

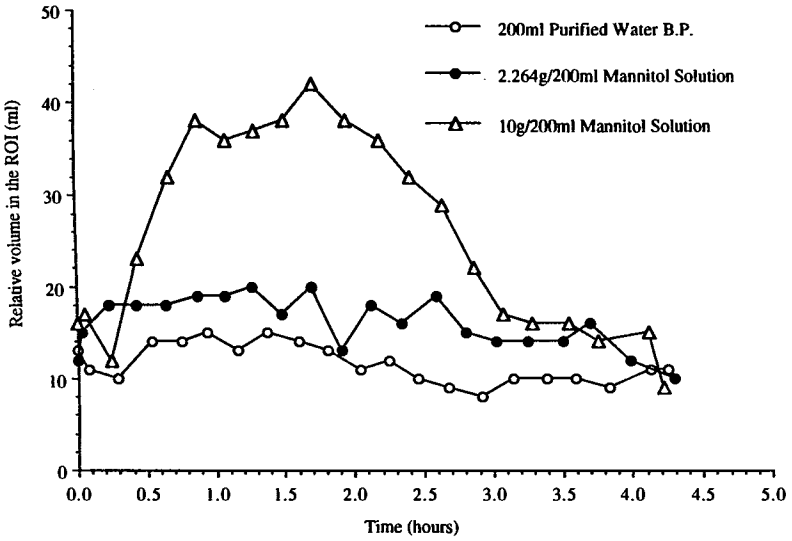

Fig. 3. Mean volume of fluid in the distal small bowel region of interest following administration of different solutions to eight male volunteers.

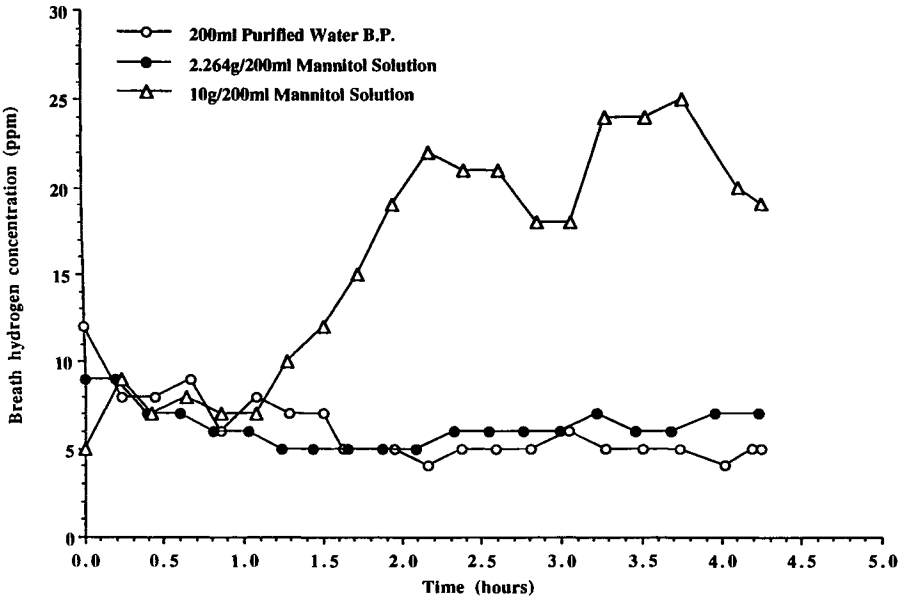

Fig. 4. Mean breath hydrogen data collected after administration of different solutions to eight male volunteers.

thereby overcoming the problems associated with the intubation technique.

The lack of any increase in relative water volume in the distal small bowel associated with administration of the Purified Water B.P. was an expected result since the 200ml of water would have been rapidly absorbed. Volunteer 1 and 7 had a much greater relative water volume in the distal small bowel following administration of the Purified Water B.P. compared to the other subjects. The reason for these large values of 162 and 60 for volunteer 1 and 7 respectively is that their baseline relative water volume values taken before administration of the water were much greater than those from the other subjects. The relative water volume remained elevated throughout the study day, therefore leading to much greater AUC values for volunteer 1 and 7 compared to the controls.

The 2.264g/200ml mannitol solution did not show a statistically significant increase in relative water volume in the distal small bowel compared to the control, unlike the 10g mannitol solution. However, the trend for the AUC value to increase with increasing mannitol concentration was statistically significant. Volunteer 4 did not follow this trend since the 10g/200ml mannitol solution had an AUC value of 158 compared to 195 for the 2.264g/200ml solution. This anomaly was due to the high baseline value obtained at the start of the study day, therefore resulting in an AUC value higher than expected.

We could not demonstrate any direct correlation between the breath hydrogen data and the results obtained from the magnetic resonance images for each subject. This is likely to be due to the fact that the magnetic resonance images merely show the presence of the solution in the small bowel and pelvis. This is not precisely localised and could include both proximal and distal ileum as well as on occasions some part of the jejunum. Reliable identification of the caecum proved difficult and it is the arrival of mannitol in the caecum which we would expect to find a correlation with the breath hydrogen.

The use of EPI has demonstrated in a non-invasive way the mechanism by which low concentrations of mannitol cause an acceleration of intestinal transit. Our study has also demonstrated the potential application of this new technique for studying various areas of the GI tract. The technique could be extended to study the large bowel and the volume of water contained in the ascending colon which is clearly important when trying to understand colonic drug delivery.

Table II. Breath Hydrogen Data for the 10g/200ml Mannitol Solution After Administration to Eight Male Volunteers

Volunteer number	Mean baseline value (ppm)	Peak value (ppm)	Peak above baseline (ppm)	Time of 3ppm increase (hours)
1	17	64	47	2.93
2	12	47	35	1.53
3	6	20	14	0.72
4	3	15	12	1.82
5	2	34	32	1.14
6	6	12	6	1.79
7	4	64	60	1.20
8	5	8	3	1.35
Mean	7	33	26	1.56
SD	5	23	21	0.70

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