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Magnetic Resonance Imaging Quantification of Fasted State Colonic Liquid Pockets in Healthy Humans

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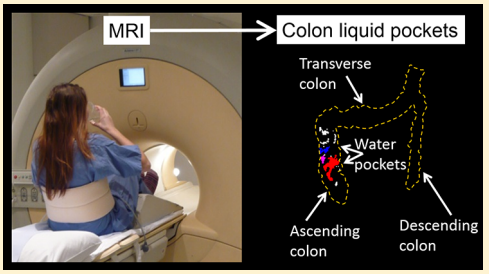
Supporting Information

ABSTRACT: The rate and extent of drug dissolution and absorption from solid oral dosage forms is highly dependent on the volume of liquid in the gastrointestinal tract (GIT). However, little is known about the time course of GIT liquid volumes after drinking a glass of water (8 oz), particularly in the colon, which is a targeted site for both locally and systemically acting drug products. Previous magnetic resonance imaging (MRI) studies offered novel insights on GIT liquid distribution in fasted humans in the stomach and small intestine, and showed that freely mobile liquid in the intestine collects in fairly distinct regions or "pockets". Based on this previous pilot data, we hypothesized that (1) it is possible to quantify the time course of the volume and number of liquid pockets in the undisturbed colon of fasted healthy humans following ingestion of 240 mL, using noninvasive MRI methods; (2) the amount of freely mobile water in the fasted human colon is of the order of only a few milliliters. Twelve healthy volunteers fasted overnight and underwent fasted abdominal MRI scans before drinking 240 mL (~8 fluid ounces) of water. After ingesting the water they were scanned at frequent intervals for 2 h. The images were processed to quantify freely mobile water in the total and regional colon: ascending, transverse, and descending. The fasted colon contained (mean ± SEM) 11 ± 5 pockets of resting liquid with a total volume of 2 ± 1 mL (average). The colonic fluid peaked at 7 ± 4 mL 30 min after the water drink. This peak fluid was distributed in 17 ± 7 separate liquid pockets in the colon. The regional analysis showed that pockets of free fluid were found primarily in the ascending colon. The interindividual variability was very high; the subjects showed a range of number of colonic fluid pockets from 0 to 89 and total colonic freely mobile fluid volume from 0 to 49 mL. This is the first study measuring the time course of the number, regional location, and volume of pockets of freely mobile liquid in the undisturbed colon of fasted humans after ingestion of a glass of water. Novel insights into the colonic fluid environment will be particularly relevant to improve our understanding and design of the *in vivo* performance of controlled release formulations targeted to the colon. The *in vivo* quantitative information presented here can be input into physiologically based mechanistic models of dissolution and absorption, and can be used in the design and set up of novel *in vitro* performance tools predictive of the *in vivo* environment.



KEYWORDS: *intestinal water, bioperformance, dissolution, large bowel, MRI, controlled release, delayed release*

INTRODUCTION

The colonic luminal environment is key for the dissolution of oral drug products specifically aimed at colonic delivery. Colon-specific drug delivery systems (CDDS) have gained momentum in recent years due to their potential for treatment of local diseases as well as for exploitation of the colon as a site of delivery of peptides and proteins, which can degrade in the gastric and small intestine compartments.[1,2] The colon will also play a role in the dissolution of high dose or low permeability drugs that may not have been entirely released and absorbed in the small intestine.[3] Together with other important physiological parameters, such as buffer species, pH, bile salts, and pressure (motility), the amount of fluid available will also

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significantly influence the rate and extent of drug release/dissolution and absorption in the colon and thus bioperformance.

Knowledge of the fluid environment in the upper (stomach and small intestine) gastrointestinal tract (GIT) has considerably improved over the past decade.[4] By contrast, much remains to be learnt about the colonic ("uncharted waters"[5]) environment which has been the subject of only a few studies to date. The colon is a large organ. The ascending, transverse and descending colon together contain an average of 561 ± 321 mL of biomass[6] which is 86% water (e.g., 482 ± 266 mL).[7] However, most of this water in the colonic chyme is not freely available (visible) and is either bound or contained inside bacteria/biomass. Pioneering magnetic resonance imaging (MRI) work by Schiller and colleagues[8] described intestinal free water as separated in a series of scattered pockets and showed that, in the undisturbed colon, there is very little freely mobile, available fluid. Colonic free fluid volumes were estimated at an average of 13 mL with a range of 1−44 mL.[8] Our own previous experience using MRI found 3 mL (range 0−29 mL) in the fasted ascending colon.[9]

Colonoscopy studies allowed direct collection of fluid samples from the cecum[3] and ascending colon.[10] In the cecum, in the fasted state, 5 mL of material were retrieved. The aqueous fraction of this chyme was 70%. In the fasted state, in the ascending colon 22 mL of material were retrieved, of which again 70% were aqueous fraction. While some of the differences between the colonoscopy and MRI values from these studies may reflect sampling difficulties and/or image data processing differences, overall the available literature seems to be in good agreement that there is only a very small amount of free liquid in the colon. Direct collection of colonic fluid samples from colonoscopy, led to the development of biorelevant[11] simulated colonic fluids.[12]

Of particular interest is the fluid distribution in fasted humans after ingestion of a glass of water (~8 oz). This volume of water is typical of relative bioavailability (BA) and bioequivalence (BE) testing in human subjects, to support drug applications with regulatory agencies.[13−16] In our previous study we investigated the time course, number, volume, and location of water pockets in the stomach and small intestine under conditions that represent standard BA/BE studies using validated techniques. However, there are no such data available in the literature for the distribution and time course of free colonic water pockets in the colon.

This study was built on our previous work and was aimed to characterize the time course of the volume and number of colonic freely mobile water pockets of fasted healthy subjects, before and after ingestion of a 240 mL dose of water using MRI. We predicted that the time course of volume and number of colon water pockets following a 240 mL dose of water would reflect the low values previously measured, using different protocols.

Such quantitative information on the time course of freely mobile colonic liquid can be used in conjunction with knowledge of the volume of entrapped liquid, as well as with new insights into the properties and composition of colonic fluid[3,10,12,17] to help develop our understanding of solubility and dissolution of drug products in the fasted colon. When considered as a whole, these different pieces of information can help strengthen the outputs of physiologically based mechanistic modeling of the gastrointestinal tract and guide design of *in vitro* experiments aimed at predicting dosage form bioperformance.

MATERIALS AND METHODS

Study Design. The primary outcome measure was the total volume of freely mobile water in the colon (mL). Secondary outcome measures were the number of water pockets in the colon and their volumes (in mL) as well as their location in the three regional colon segments divided into ascending, transverse, and descending colon. The exploratory outcome was the correlation between small and large bowel liquid pockets.

The study design, participants, and data sets used for this work are from the original MRI study.[18] Briefly, the study had a single-center, one-way, open-label design consisting of a screening visit and one test day. After an initial fasted baseline MRI scan, subjects drank 240 mL of water and were scanned at intervals for up to 2 h post ingestion. Image data was acquired before the 240 mL water drink and afterward at times 2, 4, 8, 12, 16, 20, 24, 28, 32, 45, 60, 75, 90, 105, and 120 min.

The previous study (ClinicalTrials.gov identifier NCT01792453) was approved by the local Medical School Research Ethics Committee and was conducted according to Good Clinical Practice principles. The Ethics approval included additional data analysis work that might have arisen and the volunteers gave written informed consent.

Study Participants. Twelve healthy volunteers took part in the study. They were 4 male and 8 female, 21.3 ± 0.6 years old with body mass index of 22.1 ± 0.6 kg/m^2. The inclusion criteria and lifestyle restrictions were designed to be as close as possible to the US Food and Drug Administration (FDA) guidelines for the assessment of Fasted Treatments in healthy volunteers.[14] They were asked to fast from 10 pm the evening prior to the MRI study day. Water was allowed but not after 7 am of the study day, after which the subjects were asked not to eat or drink anything until arriving at the study site at 8 am.

Magnetic Resonance Imaging. MRI scanning was performed on a research-dedicated 1.5 T Philips Achieva MRI scanner (Philips Healthcare, Eindhoven, The Netherlands). Volunteers were positioned supine with a 16-element parallel imaging receiver coil wrapped around their abdomen. A single shot, fast spin echo sequence (Rapid Acquisition with Relaxation Enhancement, RARE) acquired in a single breath-hold 24 coronal images with in-plane resolution interpolated to 0.78 mm × 0.78 mm and a slice thickness of 7 mm, with no gap between slices (TR = 8000 ms, TE = 320 ms, acquired resolution = 1.56 mm × 2.90 mm). This sequence yields high intensity signals from areas with fluid and little signal from body tissues, and is used to measure small and large intestinal water content. This image set was acquired on a 24 s expiration breath-hold, monitored using a respiratory belt.

Data Analysis. The individual values are plotted providing a full representation of the individual variability. The data are also shown as time course of the mean ± standard error of the mean (SEM) for ease of display, although some of the parameters were not normally distributed. Analyses were carried out using the Per Protocol (PP) population. The colonic water pockets and volumes were assessed using a new extension of the in-house developed program, to determine volumes of freely mobile small bowel water content.[19] The RARE sequence is strongly T2 weighted hence images mostly freely mobile liquid (which appears very bright in the images) while the signal from less mobile liquid and body tissues has decayed (and appears dark in such images). The analysis software then uses the signal

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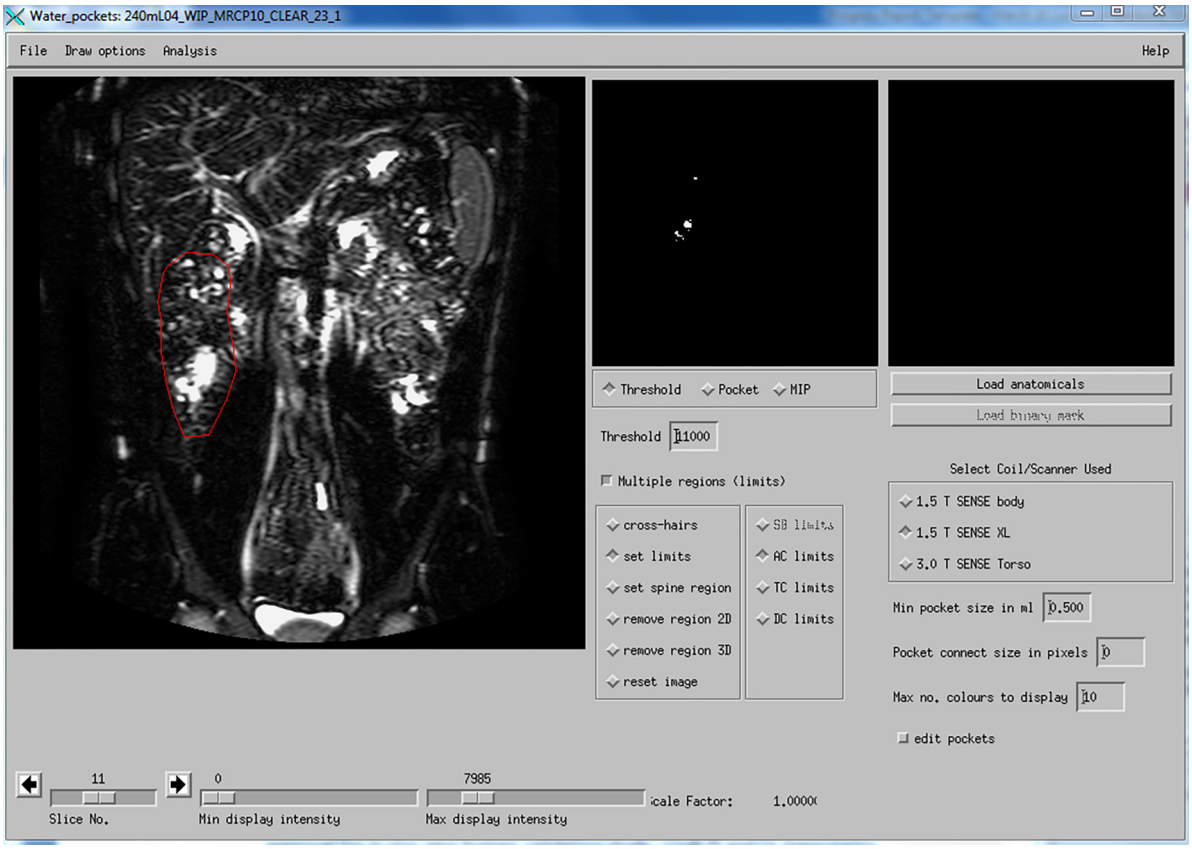

Figure 1. Screenshot of the colonic liquid pockets data analysis software. The operator loads the single shot, fast spin echo sequence (Rapid Acquisition with Relaxation Enhancement, RARE) images as shown on the left-hand side. In this kind of MRI sequence, freely mobile liquid appears very bright and less mobile liquid and body tissues appear dark. The operator can draw a specific region of interest and assign it to a given region (in this example the ascending colon). The software then locates and measures the liquid pockets whose signal is above a signal threshold for freely mobile liquid, calculated from the cerebrospinal fluid of the subject. Anatomical reference images can be loaded in the right side panel to aid segmentation.

distribution of the cerebrospinal fluid, which is freely mobile, to determine the threshold (cutoff) above which a bright signal in the image belongs to freely mobile fluid. The exact threshold point has been determined from a validation intubation study[19] whereby known amounts of poorly absorbable fluid were instilled in the small bowel and measured by MRI. Without this internal reference and thresholding method the determination of fluid volumes would be inaccurate. The user graphic interface is shown in Figure 1.

Separate regions of interest (ROIs) were drawn manually around the visible regions of the ascending, transverse, and descending colon on each coronal image slice using in-house software on an IDL platform (IDL 6.4; Research Systems Inc., Boulder, CO, USA). These ROIs excluded regions, such as the stomach, gall bladder, small bowel, and blood vessels. The software then identified (in the 3-dimensional multislice data set) all signal regions within the colonic ROIs with intensity above a threshold, determined from the subject's cerebrospinal fluid. The sum of all those colonic regions provided the total colon water content. To define the colonic free water pockets, a mask was generated from the large intestinal regions identified and a region-growing algorithm was used to determine the size of each connected region. The software recoded the number of pockets and their volume. The pockets were categorized by volume into predefined pocket "bins". The first bin contained all the smallest water pockets, with volumes ranging from a single imaging voxel up to <0.5 mL. The other bins corresponded to the ranges 0.5−2.5, 2.5−5, 5−10, 10−20, and >20 mL. As the water pockets are derived from the segmentation of all the colon in the images, it follows that the sum of all the pockets' volumes corresponds to the total colon value at any given time point. For the colon water pocket analysis all water pockets were included. This is different from the previous small bowel analysis whereby very small water pockets contributed only to a small percentage of the total small bowel water volume.

Power and Statistical Analysis. This was a posthoc analysis hence no power calculation was available. Statistical analysis was carried out using Prism 5 (GraphPad Software Inc., La Jolla, CA). The data were tested for normality using the Shapiro-Wilk Test. Pearson's test was used to assess the correlation between peak colon liquid volumes and lower right quadrant small bowel liquid volumes. A p-value < 0.05 was considered to be statistically significant.

RESULTS

Good quality images were obtained from all subjects and the software was successfully used to assess the colonic free water pockets (Figure 2).

Colon Liquid Volume as a Function of Time. This was the primary outcome of this study. The individual colon freely mobile liquid volume data as a function of time are shown in Figure 3A and the corresponding mean data in Figure 3B. The fasted colon contained a small amount of resting liquid with a

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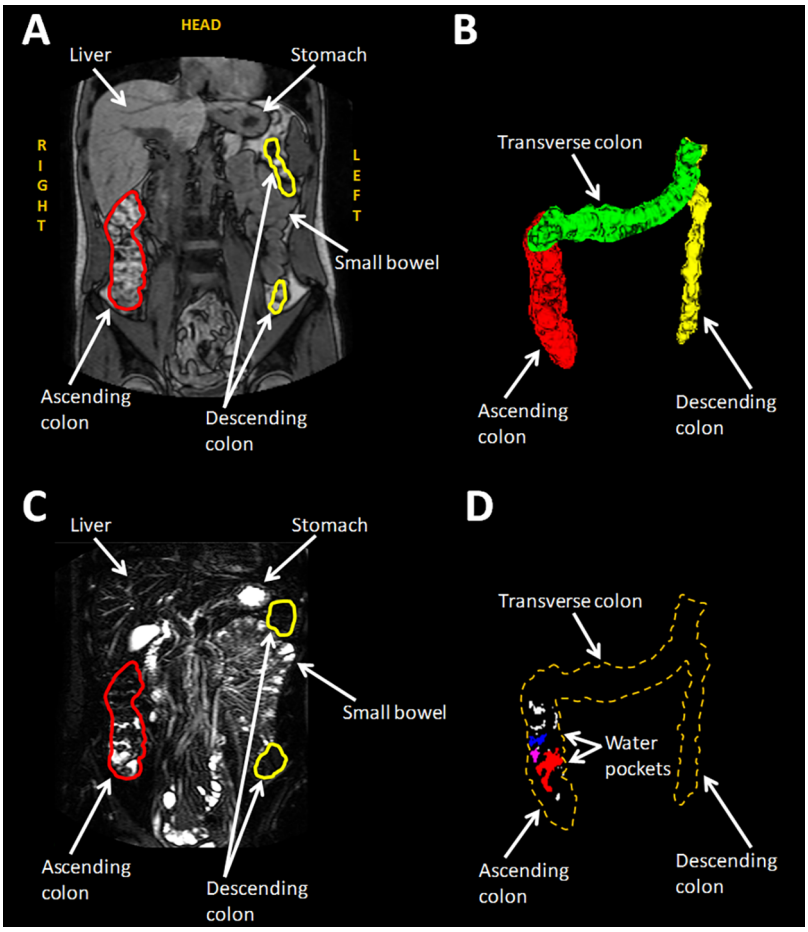

Figure 2. (A) Example of a MRI coronal roadmap image. Regions of interest are drawn on the segments of the colon that are visible on this image plane. (B) Three-dimensional reconstruction of the colon of this subject. The ascending colon is rendered in red, the transverse colon in green, and the descending colon in yellow. (C) Highly T2 weighted image from a plane approximately corresponding to (A). In this MRI sequence freely mobile liquid appears white and most of the other tissues and poorly mobile or bound liquid appear dark. (D) Maximum intensity projection (MIP) image of all the freely mobile liquid pockets in the colon after thresholding. The colon outline is sketched for anatomical reference using a dashed orange line. In the MIP shown for this subject, 60 min after ingesting the 240 mL water dose, 25 small water pockets were located for a total colon freely mobile liquid volume of 11.1 mL. The liquid pockets were all located in the ascending colon and no water pocket was found in the transverse and descending colon.

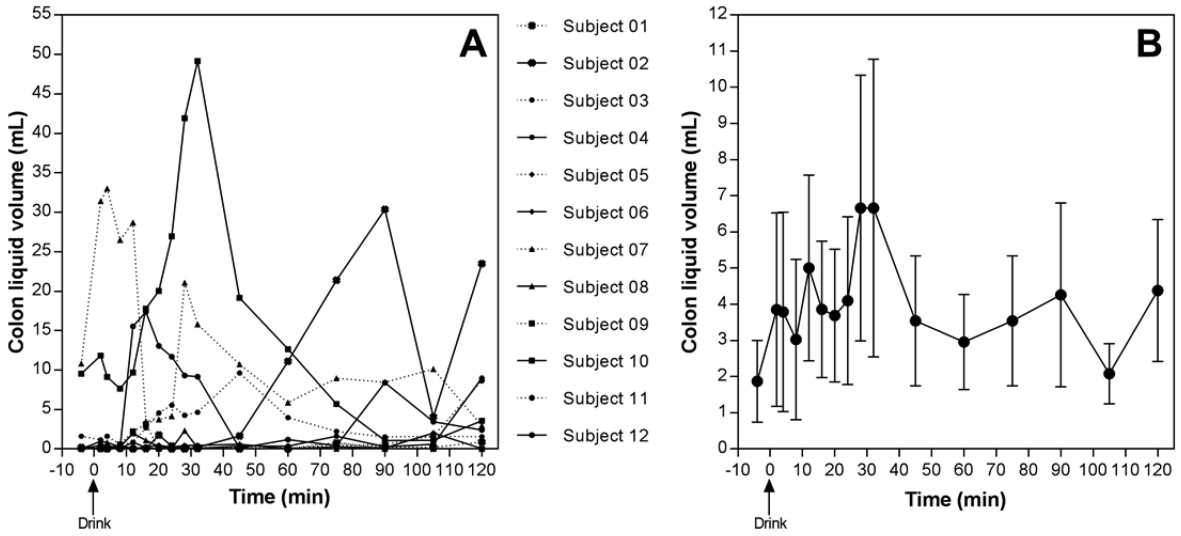

Figure 3. (A) Individual time course of the volume of freely mobile liquid in the colon. Average time course (mean ± SEM) of the volume of freely mobile liquid in the colon. The data are from n = 12 fasting healthy volunteers who ingested a 240 mL (~8 ounces) drink of water.

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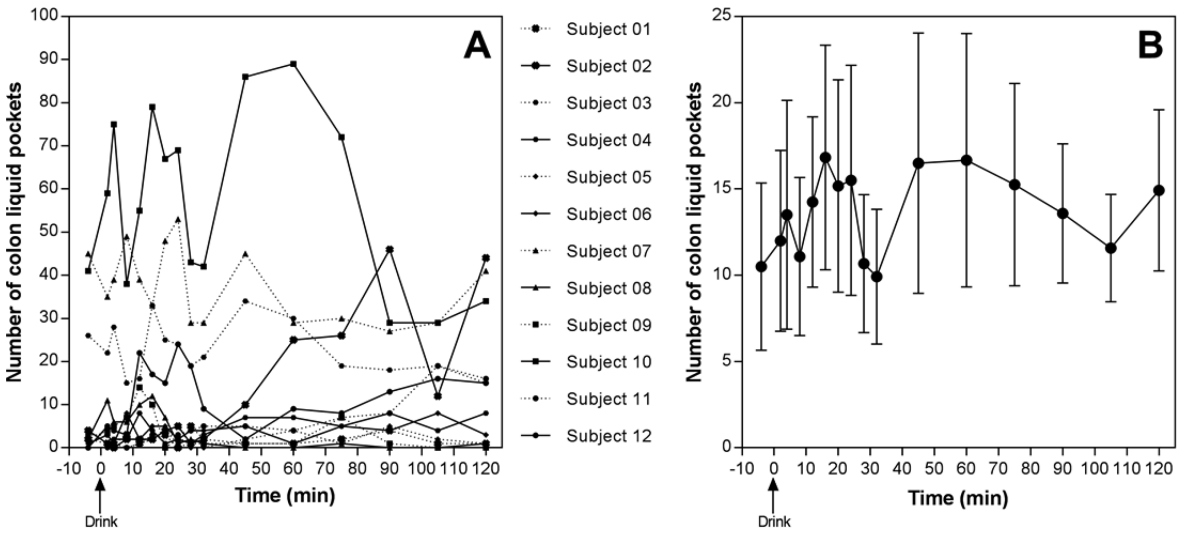

Figure 4. (A) Individual time course of the number of freely mobile liquid pockets in the colon. (B) Average time course (mean ± SEM) of the number of the freely mobile liquid pockets in the colon. The data are from n = 12 fasting healthy volunteers who ingested a 240 mL (~8 ounces) drink of water.

total volume of 2 ± 1 mL. The colonic fluid peaked 30 min after the water drink to 7 ± 4 mL (Figure 3B) and declined toward 3−4 mL mean 60 min after the drink. The individual variability was very high, with subjects having freely mobile fluid volume ranging from 0 to 49 mL (Figure 3A). A volume peak is often seen after the drink but its timing varies greatly too. Figure S1 in Supporting Information shows the different order of magnitude of freely mobile liquid volumes in stomach, small bowel (using the data from the previous publication), and colon.

Number of Freely Mobile Colon Liquid Pockets as a Function of Time. The individual numbers of colon freely mobile liquid pockets as a function of time are shown in Figure 4A and the corresponding mean data in Figure 4B. The fasted colon contained 11 ± 5 pockets of resting liquid. The number of pockets peaked in a similar fashion and timing to the colonic liquid volume, around 30 min after the drink at 17 ± 7 separate liquid pockets. After a short dip, the number of pockets increases again to 17 pockets around 60 min after the drink. The interindividual variability was very high, with subjects showing a range of number of colonic fluid pockets from 0 to 89.

Size of Colon Liquid Pockets as a Function of Time. The size (volume) of freely mobile liquid pockets in the colon as a function of time are shown for each individual in Figure 5A. The corresponding mean data as a function of time are shown in Figure 5B. A breakdown by bin size shows that the majority of freely mobile water pockets in the colon are small (Figure 5C).

Regional Analyses of Colon Water Pocket Volumes and Numbers. The regional analysis of liquid volumes showed that the colonic liquid volume was found primarily in the ascending colon (Figure 6). The same applies to the regional analysis by number of liquid pockets which is dominated by the ascending colon. The individual data for the ascending colon (Figure 7A) and the regional means (Figure 7B) are shown.

Correlation Analyses. Having obtained the colon liquid volume data, we performed exploratory *post hoc* analyses, at an individual level, to assess if changes in colon water would correlate with changes in small bowel liquid volume as the data for the latter are available from our previous study. In Figure 8 the peak colon liquid volume for each participant is plotted against the corresponding lower left quadrant (mostly ileal) small bowel liquid volume at the same time point. There was a positive and significant correlation between peak colon liquid volumes and lower left (ileal) small bowel liquid volumes (r = 0.78, p = 0.0030).

DISCUSSION

This initial study achieved its aims and the data confirmed our prediction. MRI provided unprecedented insights on the fasting time course, number, and volume of liquid pockets in the undisturbed human colon under conditions that represent standard BA/BE studies. The baseline, fasting colon contained only a few milliliters of freely mobile water, distributed in small pockets. Thirty minutes after ingestion of the 240 mL water drink an influx of fluid increased the number and size of the colonic liquid pockets; these differences were not significant, possibly due to large variability. One hour after the drink, the colonic liquid pockets size returned to the small baseline size but in higher numbers and total volume. The water pockets are primarily very small, Figure 5C shows that most are <0.5 mL size. The regional analysis showed that the colonic liquid pockets were located primarily in the ascending colon region only.

In some subjects we observed a rise in the mean total volume of liquid shortly after the drink was ingested. This could reflect gastro-colonic reflex, moving ileal residue into the colon. However, it could also represent the arrival of the "head of the drink" in the colon whereby initial aliquots of the ingested liquid can exit the stomach before any feedback signaling can be established and travel quickly the length of the small bowel. Breath test studies can indeed show a rise in the signal from the colonic label shortly after ingestion.

In Shiller's study,[8] the colon fluid volumes in the fasted state were similarly variable from 1 to 44 mL and the fluid pockets were detected mainly in the cecum, the ascending and the descending colon. On the other hand, in the fed state the colon fluid volumes ranged from 2 to 97 mL and there was a significant increase in the number of fluid pockets, although the

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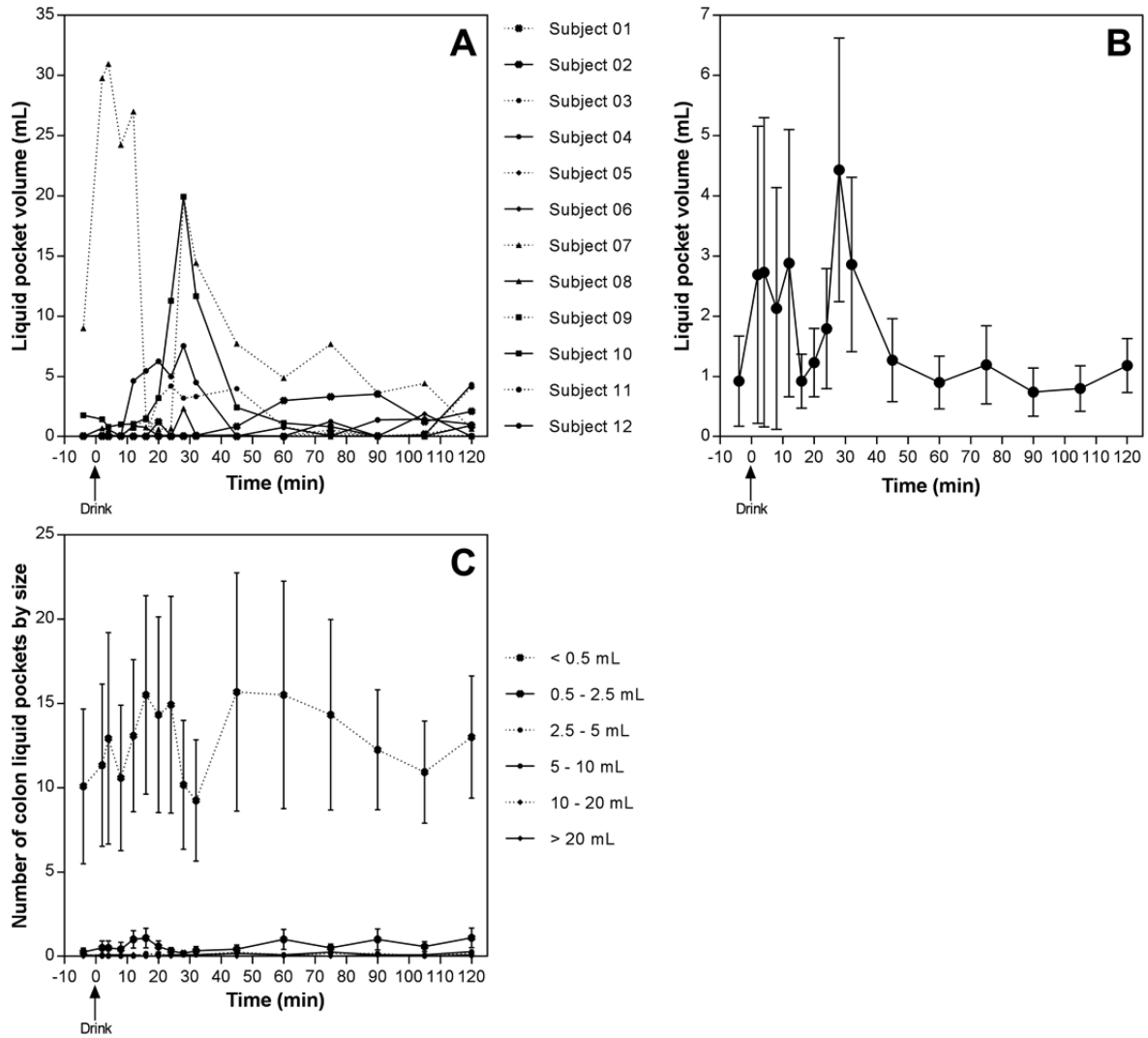

Figure 5. (A) Individual time course of the number of freely mobile liquid pockets in the colon. (B) Average time course (mean ± SEM) of the number of the freely mobile liquid pockets in the colon. (C) Average time course (mean ± SEM) of the number of freely mobile liquid pockets in the colon sorted into 6 different bin sizes (i.e., bands). The data are from n = 12 fasting healthy volunteers who ingested a 240 mL (~8 ounces) drink of water.

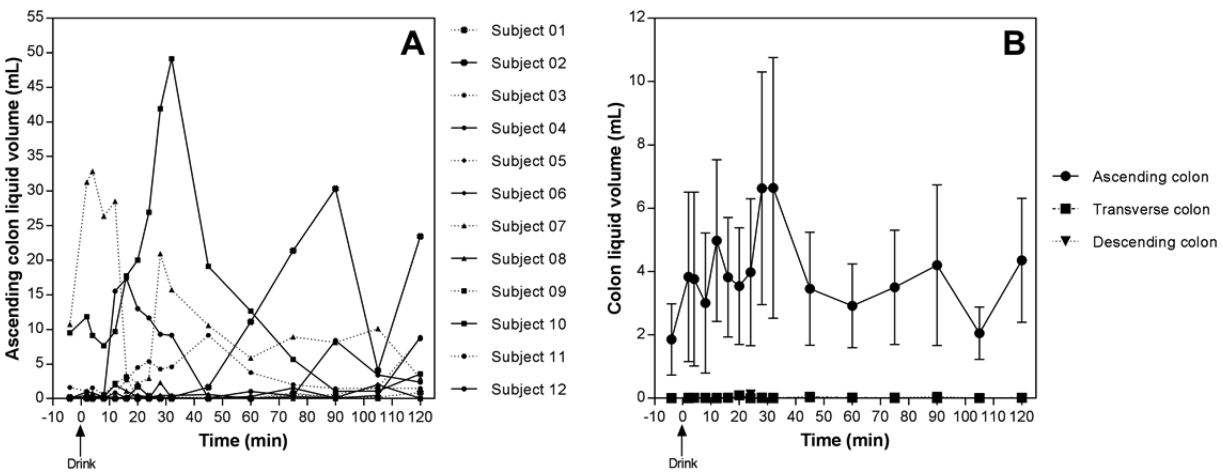

Figure 6. Individual time course of the volume of freely mobile liquid in (A) the ascending colon. (B) Average time course (mean ± SEM) of the number of the freely mobile liquid pockets in the colon sorted by region for the ascending, transverse, and descending colon. The data are from n = 12 fasting healthy volunteers who ingested a 240 mL (~8 ounces) drink of water.

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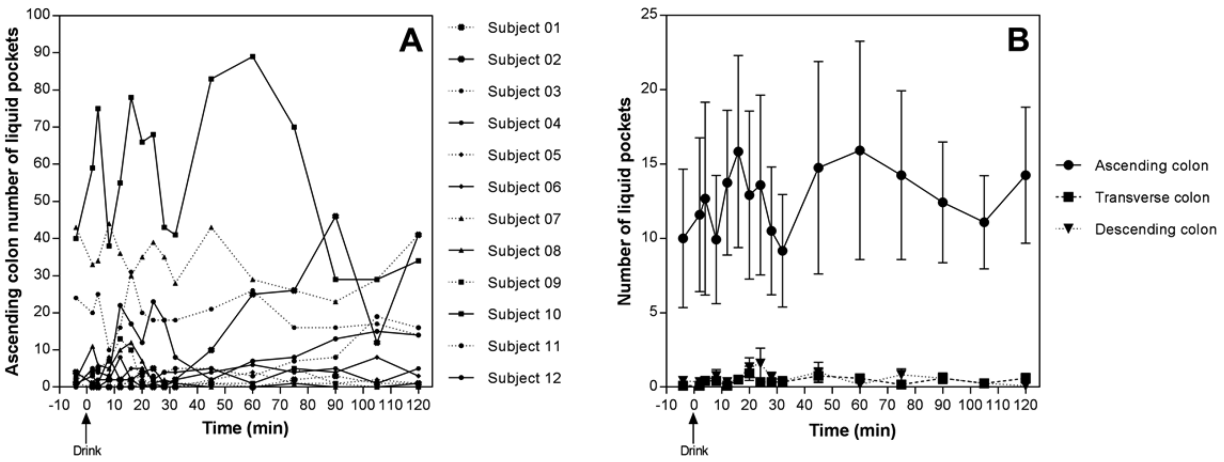

Figure 7. (A) Individual time course of the number of freely mobile liquid in the ascending colon. (B) Average time course (mean ± SEM) of the number of the freely mobile liquid pockets in the colon sorted by region for the ascending, transverse and descending colon. The data are from n = 12 fasting healthy volunteers who ingested a 240 mL (~8 ounces) drink of water.

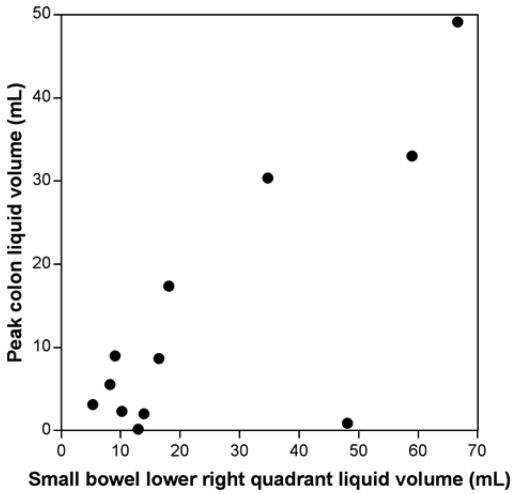

Figure 8. Plot of peak colon liquid volume for each participant against the corresponding lower left quadrant (mostly ileal) small bowel liquid volume taken from ref 18. The data are from n = 12 fasting healthy volunteers who ingested a 240 mL (~8 ounces) drink of water.

fluid volume per pockets was not significantly changed (median: fasting 2 mL and fed 1 mL).

Here we referred to the regions of freely mobile fluid in the colon as "pockets" in keeping with previous literature. It is worth noting that the concept of pockets (or packets) of fluid exiting the stomach and populating the small bowel is easier to picture than within the colon environment, where contractility does not tend to create the small isolated spaces one would associate with a pocket. In the colon the pockets represent more regions of freely mobile water within the chyme and their boundaries are susceptible to changes depending on the threshold calculated by the software in relation to the cerebrospinal fluid.

This study had some limitations. The subjects were studied for only 2 h while modified release formulations act for a much longer time, hence future studies should image the colon for longer than 2 h. The MRI quantification method has been validated using naso-duodenal intubations.[19] The validation work consisted in intubating healthy volunteers naso-duodenally and instilling rapidly known volume boluses of poorly absorbable fluid. A complete abdominal image data set was acquired immediately after each bolus was instilled in the bowel therefore allowing calibration of the signal distribution versus the infused volumes. While the translation of such methods to the colon is straightforward, it should be noted that the quantification of liquid volumes in mL in the colon has not yet been directly validated. The imaging voxels that contain some freely mobile water and some gas/tissue/more solid component suffer from partial volume effects and may be excluded by the calculated liquid intensity threshold. As a result, the total amount of freely mobile water may be slightly underestimated. The MRI method used here has a long echo time TE and strong T2 weighting hence images only the hydrogen protons of freely mobile, high tumbling, long relaxation time water molecules. The signal from less mobile, more viscous fractions, such as mucous or unstirred layers adjacent to the mucosa, will have decayed and therefore are not captured. Another parameter that was not investigated is intrasubject variability, a further test-retest study is needed.

There are indeed other MRI methods that can provide information on the water environment of the colon chyme. We have used the longitudinal relaxation time T1 following dosing with oral polyethylene glycol[20] and the transverse relaxation time T2 following a mannitol model of diarrhea and dosing with loperamide,[9] both can be informative but do not quantitate volumes. Quantitative magnetization transfer ratio is another method but the magnetization transfer dependence on the physicochemical environment such as chemical exchange, presence of macromolecules, dipolar interactions and water diffusion is very complex and quantifying volumes from this difficult.

Our study is also first in trying to correlate the amounts of water in the small bowel and in the colon. This was an exploratory *post hoc* analysis. It was somehow difficult to predict the direction of the correlation, if any. Larger amounts of water in the small bowel could allow larger transfers to the colon hence higher volumes of colonic water. On the other hand, increased motility could reduce the amount of water in the small bowel and increase it in the colon as seen for example in patients with irritable bowel syndrome with diarrhea (IBS-D).[21,22] We found here a positive and significant correlation between peak colon liquid volumes and distal (lower right quadrant) bowel liquid volume at corresponding time points. This study was not initially aimed or powered to look at such

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correlations hence caution in interpreting them is needed. These preliminary findings require further investigation.

The colon is critical for release and subsequent absorption of controlled release formulations designed to release their drug contents between 6 and 24 h. Solubility and dissolution have been suggested to be more restricted in the colon than in the upper small intestine due to several factors, lower water content being one of them.[23] Tannergren and co-workers collected regional absorption data of 42 drugs and obtained or calculated the relative bioavailability after administration directly to the colon compared to after oral dosing (abbreviated as $Frel_{colon}$). They found the majority of BCS I drugs (high solubility and high permeability) administered as solutions, granules, or suspensions to be well absorbed in the colon, with a measured or calculated $Frel_{colon} > 70\%$. It can be inferred that the amount of total water, whether it be freely mobile or entrapped in colonic chyme/biomass, was enough to maintain an adequate amount of dissolved drug to be transported to the intestinal wall via the convective transport responsible for water reabsorption as well as likely via diffusive transport for these high solubility compounds. In the same study, it was documented that some BCS II drugs (low solubility and high permeability) administered to the colon as solutions had lower absorption than would be expected if one assumed the entire dose of drug remained in solution in the colon. This result could have been due to potential precipitation in the colon as a result of low water content.

While it is reasonable to infer that both entrapped and freely mobile water would have the ability to maintain a drug in solution provided a high enough drug solubility, the question of at what rate and to what extent drug solids could dissolve in entrapped water and be transported to the colonic mucosal membrane is less straightforward to answer due to a lack of direct measurements. It is likely that dissolution and transport of drug particles is slowed in the presence of biomass compared to in freely mobile water as a result of multiple factors. For example, a decreased extent of stirring in the colonic lumen due to obstruction from solid materials would be expected to decrease flux from the solid particle surface for particles above a diameter of approximately 20 μm.[24] In addition, increased tortuosity/viscosity in the presence of biomass likely hinders diffusive and convective transport of solid particles from the colonic lumen to the colonic mucosa. While the data set of Tannergren and co-workers was insufficient to assess the impact of low solubility and slow dissolution rate on colonic absorption, it was shown that for two compounds administered to the colon both as solutions and suspensions, C_{max} was diminished for the suspension compared to the solution. The documented dose/solubility ratio was 213 mL for one compound ("AZ6") and 375 mL for the other (Dexloxiglumide). Assuming the documented solubility reflected the solubility in the human colonic fluid, an average entrapped water content of 482 mL (i.e., higher than the dose/solubility ratio) would be needed to dissolve these compounds such that C_{max} matched that of the solution, if the volume of entrapped versus freely mobile water were the more relevant parameter to consider.

Recently Georgaka and co-workers[25] conducted a study designed to evaluate the impact of "solid particles" contained within dissolution medium designed to simulate properties and composition in the fasted ascending colon (FaSSCoF). To simulate solids they added microcrystalline cellulose spheres to the medium and compared dissolution results in the same medium in the absence of solid particles. In the first stage of the 2-stage dissolution test, the dosage form was placed in a mini dissolution vessel filled with 40 mL of medium representative of the distal ileum (no solid particles) and stirred for 2 h (Stage 1). A second solution was then added to the vessel to achieve ~200 mL of FaSSCoF with or without microcrystalline cellulose spheres and stirred for an additional 160 min (Stage 2). To ensure reproducible sampling in the presence of solid particles, contents were stirred along the length of the mini dissolution vessel at 100 rpm using an in-house paddle system consisting of five blades distributed along a shaft. For experiments conducted in the absence of solid particles, a Distek (Distek, Inc., New Brunswick, NJ) single blade, mini paddle stirred at 75 rpm (ileal portion, Stage 1) and 40 rpm (colonic portion, Stage 2) was used. Results demonstrated nearly identical concentration−time profiles in FaSSCoF during the colonic portion of the test in the presence and absence of solid particles for sulfasalazine (Azulfidine 500 mg immediate release tablet) and mesalamine (Asacol 400 mg colon targeting tablet). These results demonstrate that the simple presence of solid material in the fasted simulated fluids coupled with potential differences in hydrodynamics between the two paddles and stirring speeds did not alter apparent drug concentration versus time profiles for these two dosage forms. The paddle stirring mechanism used in Stage 2 of the *in vitro* test is different from colonic stirring *in vivo*. For example, *in vitro*, the paddle blades are positioned within the bed of solid particles, which presumably results in some radial as well as axial movement within the mini dissolution vessel. *In vivo*, the colon contents move mainly axially along the length of the intestine with a central channel of increased displacement with reduced flow near the walls of the colon, probably impeded by the haustra.[26] Due to these differences in stirring, it is difficult to judge how accurately the *in vitro* test set up used by Georgaka and co-workers can assess the impact of actual *in vivo* solid particles/biomass on the rate and extent of dissolution of Azulfidine or Asacol tablets. For example, if the particle size of disintegrated drug particles of sulfasalazine administered as Azulfidine (immediate release) and/or mesalamine administered as Asacol (pH-triggered release via a coating designed to dissolve at pH > 7) are > ~ 20 μm, then rate of stirring (e.g., shear and convection) could affect dissolution rate. If, however, drug particles are mostly <20 μm, then stirring may not significantly affect release. In addition, the transport of solid drug particles to the colonic mucosal membrane *in vivo* is likely influenced by the stirring rate/mechanism of solid particles/biomass *in vivo*, which was not a factor studied in the *in vitro* test.

Taken together with knowledge of the entrapped water content and physicochemical properties of colonic fluid, the time course of freely mobile liquid volumes in the colon has significant implications for designing and predicting the oral bioperformance of solid dosage forms, particularly those with low aqueous solubility (e.g., BCS II or IV) in small intestine and/or colon, and which may be in the form of relatively large solid particles upon entry to the colon. Intestinal water volume can significantly affect dosage form release, dissolution, and absorption kinetics and therefore the rate of appearance of compounds in the bloodstream. The information regarding total liquid volume in the colon and distribution into individual pockets as determined in this study can be used to inform *in vitro* biopredictive dissolution tests. It can also be used as a starting point for the development of novel computational

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models describing transport and distribution of liquid as well as drug release and dissolution throughout the gastrointestinal tract.

CONCLUSION

This study is the first to quantify the time course of volume and distribution of freely mobile water in the colon under conditions representing BA/BE studies using MRI methodology. The information collected will have important implications for the rates and extents of dosage form release, dissolution, precipitation, and absorption when present in the human colon, and can be used to develop biorelevant dissolution methodologies, as well as mechanistic computational transport analyses for oral bioperformance prediction.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.molpharmaceut.7b00095.

Comparison of the average time course of freely mobile liquid volume liquid in the stomach, small bowel, and colon (PDF)

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D.M.M., G.E.A., G.L.A., and L.M. designed the study. K.M. and L.M. performed all the experiments. K.M. analyzed the colon water image data. C.L.H. designed the image analysis software. All authors contributed to data interpretation. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

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ABBREVIATIONS

AUC, area under the curve; BA, bioavailability; BCS, biopharmaceutics classification system; BE, bioequivalence; CDDS, colon-specific drug delivery systems; C_{max}, maximum plasma concentration; FaSSCoF, fasted state simulated colonic fluid; FDA, the U.S. Food and Drug Administration; $Frel_{colon}$, bioavailability after administration directly to the colon compared to after oral dosing; GIT, gastrointestinal tract; MIP, maximum intensity projection; MRI, magnetic resonance imaging; NDDC, Nottingham Digestive Diseases Centre; RARE, rapid acquisition with relaxation enhancement; ROI, region of interest; TE, echo time; TR, repetition time

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