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Quantification of Gastrointestinal Liquid Volumes and Distribution Following a 240 mL Dose of Water in the Fasted State

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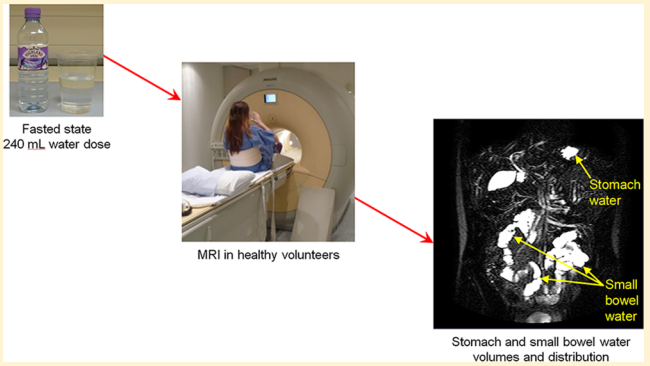
Supporting Information

ABSTRACT: The rate and extent of drug dissolution and absorption from solid oral dosage forms is highly dependent upon the volumes and distribution of gastric and small intestinal water. However, little is known about the time courses and distribution of water volumes *in vivo* in an undisturbed gut. Previous imaging studies offered a snapshot of water distribution in fasted humans and showed that water in the small intestine is distributed in small pockets. This study aimed to quantify the volume and number of water pockets in the upper gut of fasted healthy humans following ingestion of a glass of water (240 mL, as recommended for bioavailability/bioequivalence (BA/BE) studies), using recently validated noninvasive magnetic resonance imaging (MRI) methods. Twelve healthy volunteers underwent upper and lower abdominal MRI scans before drinking 240 mL (8 fluid ounces) of water. After ingesting the water, they were scanned at intervals for 2 h. The drink volume, inclusion criteria, and fasting conditions matched the international standards for BA/BE testing in healthy volunteers. The images were processed for gastric and intestinal total water volumes and for the number and volume of separate intestinal water pockets larger than 0.5 mL. The fasted stomach contained 35 ± 7 mL (mean ± SEM) of resting water. Upon drinking, the gastric fluid rose to 242 ± 9 mL. The gastric water volume declined rapidly after that with a half emptying time ($T_{50\%}$) of 13 ± 1 min. The mean gastric volume returned back to baseline 45 min after the drink. The fasted small bowel contained a total volume of 43 ± 14 mL of resting water. Twelve minutes after ingestion of water, small bowel water content rose to a maximum value of 94 ± 24 mL contained within 15 ± 2 pockets of 6 ± 2 mL each. At 45 min, when the glass of water had emptied completely from the stomach, total intestinal water volume was 77 ± 15 mL distributed into 16 ± 3 pockets of 5 ± 1 mL each. MRI provided unprecedented insights into 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. These data add to our current understanding of gastrointestinal physiology and will help improve physiological relevance of *in vitro* testing methods and *in silico* transport analyses for prediction of bioperformance of oral solid dosage forms, particularly for low solubility Biopharmaceutics Classification System (BCS) Class 2 and Class 4 compounds.



Stomach and small bowel water volumes and distribution

KEYWORDS: *gastric emptying, intestinal water, bioperformance, dissolution, small bowel, MRI*

INTRODUCTION

Solid oral delivery (e.g., tablets and capsules) is the most frequently used route of administration for pharmaceutical drug products due to patient convenience and cost effectiveness. Despite their ubiquity, development of solid oral dosage forms that perform effectively and consistently in patient populations can be challenging due to the complex environment of drug dissolution and absorption in the gastrointestinal (GI) tract. Along with other important physiological parameters such as buffer species, pH, bile salts, gastric emptying rate, motility, and shear rates, the small bowel water content (SBWC) has the potential to influence greatly the rate and extent of drug dissolution and absorption in the GI tract, or "oral

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bioperformance".[1] Using GastroPlus, Sutton, for example, showed that intestinal water volumes had a large impact on the predictive power of simulations of the mean plasma concentration profiles of four solubility-limited compounds.[2] To obtain reasonable predictions of oral bioperformance, scientists must design meaningful *in vitro* dissolution tests and mechanistic drug transport models that capture the range of SBWC in humans.

Of special interest is the SBWC in fasted humans after ingestion of a glass of water (240 mL). This volume of water is typical of relative bioavailability (BA) and bioequivalence (BE) studies performed by innovator or generic pharmaceutical companies who wish to file drug applications with drug regulatory agencies in the United States, Europe, and Japan (FDA, EMA, and MHLW, respectively). These *in vivo* pharmacokinetic studies are designed to demonstrate the rate and extent to which a drug substance is absorbed from a drug product and becomes available at the site of action (BA study) or the absence of a significant difference in the rate or extent of absorption between two different drug products containing the same drug substance (BE study).[3] For BA and BE studies in fasted humans, the U.S. Food and Drug Administration (FDA) guidelines recommend that the drug product be ingested with 240 mL of water (8 U.S. fluid ounces),[3,4] and the EMA and MHLW suggest giving a constant volume of at least 150 mL of water.[5,6] In addition, the solubility class boundary for the Biopharmaceutics Classification System (BCS) Class 2 and Class 4 compounds is defined as the highest dose strength that is soluble in 250 mL of aqueous media over the pH range of 1−7.5.[7,8] Testing solubility in this volume of liquid is likely to be physiologically relevant for a drug that dissolves in the stomach, but it is unclear whether or not it is physiologically relevant for a drug that dissolves primarily in the small intestine.

Studies using aspiration, gamma scintigraphy, and magnetic resonance imaging (MRI) show that a dose of water with a volume varying between about 240 and 800 mL empties rapidly from the fasted human stomach, with a gastric half emptying time between approximately 8 and 18 min.[9−14] The liquid emptied from the stomach enters the proximal small bowel, with subsequent absorption into the intestinal membrane or transit down the length of the intestine. Positron emission tomography (PET) and MRI data show that the average SBWC in fasted humans varies between 80 and 300 mL,[15−19] but little data is available postwater consumption, particularly following water ingested immediately prior to imaging.

Recent studies used PET to assess fluid distribution in the gastrointestinal tract of a rat model.[20] A recent MRI study in humans has drawn great attention to the possibility that the liquid in the small intestine exists in discrete liquid "pockets".[15] MRI is the ideal tool to carry out serial and noninvasive imaging of gastrointestinal function[21] and, in particular, liquids in the undisturbed gastrointestinal tract. Gastric emptying measurements have been long established and validated,[22−24] and small bowel water volume measurements have been validated against naso-duodenal infusion.[25]

This study aimed to characterize the time course of volume and distribution of liquid in the stomach and small bowel of healthy subjects before and after ingestion of a 240 mL dose of water using MRI.

MATERIALS AND METHODS

Study Design. This study had a single-center, one-way, open-label design consisting of a screening visit and one test day. The primary outcome measure was the volume of freely mobile small bowel water (SBWC, in mL) with time. The secondary outcome measures were the volume of liquid in the stomach (in mL), the number of water pockets in the small bowel, and their volumes (in mL) with time as well as the location of water pockets by abdominal quadrant.

The subjects arrived at 8:00 am at the site in a fasted state. Eligibility was confirmed. They then consumed the 240 mL water study drink at approximately 9:00 am. Gastric and intestinal water contents were measured using upper and lower MRI scans acquired just before (baseline) and after ingestion of the test drink at predetermined intervals for up to 2 h postingestion as shown in Supporting Information Figure S1. Baseline scans also verified that the stomach of the participants appeared fasted. After this, the scanner bed was moved out of the bore and the participants drank quickly the 240 mL water drink sitting up on the scanner bed, after which they were repositioned supine, and imaging started promptly. The MRI scans were more frequent for the first half an hour when gastric emptying was expected to be faster. During the initial half an hour time the participants remained supine inside the scanner, and after that they were taken out of the scanner and repositioned supine for upper and lower abdominal MRI scans at 15 min intervals. In between scanning, the participants were asked to sit upright in a room next to the scanner. As such, the posture of the participants was standardized in all studies. After the final scan, the subjects were offered some light refreshments (e.g., water, coffee, tea, biscuits) before they were discharged.

This study was approved by the local Medical School Research Ethics Committee and was conducted according to Good Clinical Practice principles. All volunteers gave written informed consent. There were no adverse events during the study. This protocol was registered on ClinicalTrials.gov with identifier NCT01792453.

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 (BMI) of 22.1 ± 0.6 kg/m^2. The inclusion criteria and lifestyle restrictions were designed to be as close as possible to the U.S. Food and Drug Administration (FDA) guidelines for the assessment of Fasted Treatments in healthy volunteers.[4] Inclusion criteria comprised: being healthy; male or female; between 18 and 55 years of age; of normal range BMI,[6] which the UK NHS classifies as having BMI between 18.5 and 24.9 kg/m^2 for the adult population, in agreement with the World Health Organization (WHO) and the U.S. Centers for Disease Control and Prevention (CDC); nonsmokers; without a history of alcohol or drug abuse; with no history of gastrointestinal disorders or gastrointestinal surgery; not on any medication likely to affect gastrointestinal function. In addition the subjects had to be suitable for MRI scanning (e.g., no metal implants). Subjects doing strenuous physical exercise (e.g., competition training) or night shift work were excluded[6] as were excluded subjects who drank more than 21 units of alcohol in a typical week. The subjects were asked to avoid caffeine (tea, coffee, and cola) for 18 h prior to their study day and alcohol for 24 h prior to their study day. They were also asked to take no dietary supplements for 24 h prior to their study day. They were asked to eat an evening meal of similar size to that usually consumed on the night before scanning and to start fasting no later than 10 pm that evening (i.e., at least 10 h fasting). Water was allowed but not after 7 am of the study day after which the subjects are asked not to eat or drink anything until arriving at the study site at 8 am. During the test

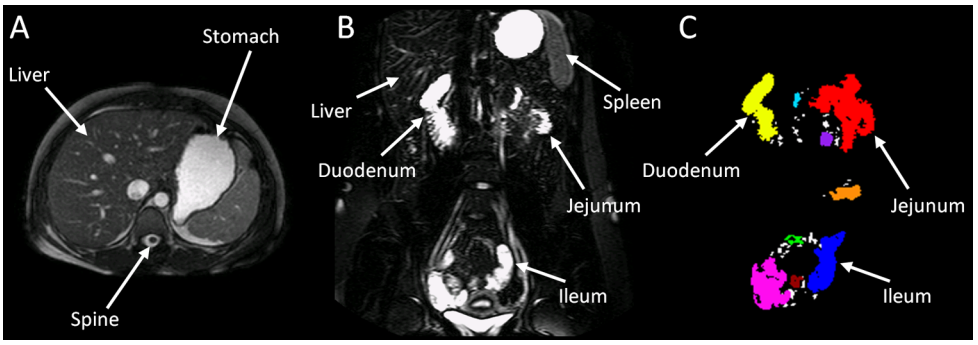

Figure 1. Examples of MRI images. Representative examples of MRI images acquired after a 240 mL dose of water in a healthy volunteer. (A) One axial image of the water drink inside the stomach. (B) One heavily T2 weighted coronal image of the abdomen. In this kind of MRI sequence the signal from tissue has mostly decayed and only bright signal from freely mobile water is seen. (C) The maximum intensity projection image of all the individual small bowel water pockets, color coded and extracted from the entire three-dimensional set of panel B and manually segmented.

day, they were not allowed to eat or drink anything other than the test water drink until the study was finished and were asked to minimize activity.

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 the abdomen. Two MRI sequences were used to image the abdomen at each time point. A balanced turbo field echo (TrueFISP or bTFE) sequence with in-plane resolution 2.0 × 1.77 mm^2, field of view FOV 400 × 320 mm^2, echo time T_E/repetition time T_R 1.5/3.0 ms, acceleration factor 2.0, and flip angle = 80° acquired 50 multislice contiguous, 5 mm, transverse slices in a single breath-hold. This was used to measure gastric volumes. A single shot, fast spin echo sequence (rapid acquisition with relaxation enhancement, RARE) was 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 (T_R = 8000 ms, T_E = 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 bowel water content. Each image set was acquired on an expiration breath-hold with duration of 13 and 24 s, respectively, monitored using a respiratory belt.

Data Analysis. The data are shown as mean ± standard error of the mean (SEM). Analyses were carried out using the Per Protocol (PP) population as planned. Volumes of gastric contents were measured by a single, experienced operator using an intensity based, semiautomatic method, which defined the bright liquid stomach contents on each image slice[26] using in-house software on an IDL platform (IDL 6.4; Research Systems Inc., Boulder, CO, USA). Plots of volume against time (up to 24 min) were created and used to determine the time to half empty the initial volume $T_{50\%}$. $T_{50\%}$ is referred to as the time needed for the stomach to empty 50% of its initial content. This was calculated by fitting the individual gastric emptying curves to a standard model.[26,27] Since the stomach may start emptying while ingesting the drink, the initial volume V_0 in the gastric emptying model was not set as fasting volume + drink volume but fitted from the data, improving accuracy. Only a couple of subjects showed a brief lag time after drink ingestion, and for these, the standard model parameters were constrained. The volumes of freely mobile small bowel water (SBWC) were assessed from the images acquired using the RARE MRI sequence as previously described and validated.[25] Briefly, regions of interest were drawn manually around the small bowel on each image excluding regions such as the stomach, gall bladder, and blood vessels using in-house software on an IDL platform (IDL 6.4; Research Systems Inc., Boulder, CO, USA). The software then identified (in the 3-dimensional multislice data set) all small bowel regions containing freely mobile water (e.g., water with intensity above a threshold, determined from the subject's cerebrospinal fluid). The sum of the volumes of all these small bowel regions provided the total SBWC. To define the small bowel water pockets, a mask was generated from the small bowel 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, from a volume of a single imaging voxel up to a volume <0.5 mL. The other bins were predefined in the ranges 0.5−2.5, 2.5−5, 5−10, 10−20, and >20 mL. As the water pockets are derived from the total SBWC image segmentation, it follows that the sum of all the pockets' volumes corresponds to the total SBWC value at any given time point. Where indicated, the results for mean number of water pockets and for mean volume of pockets exclude the smallest "bin" of <0.5 mL. This action was taken since their contribution to the total volume is very small and they confuse the display and interpretation of data due to their large number. The analysis software also allowed the operator to divide the abdomen into four anatomical quadrants by placing, in the coronal view, a crosshair in the middle of the intervertebral disc just below the kidneys (Supporting Information Figure S2). The quadrants were named: upper right (containing mostly the proximal duodenum), upper left (containing mostly distal duodenum and proximal jejunum), lower left (containing mostly distal jejunum and proximal ileum), and lower right (containing mostly distal ileum). The software then produced an additional output assigning the number of liquid pockets and volumes into these quadrants, dependent on their center of mass.

Power and Statistical Analysis. The primary outcome was SBWC so the study was powered to an acceptable error in the mean estimates of small bowel water volume. Our previous studies pooled together indicated that the fasting SBWC in healthy volunteers was 99 mL ± SD of 69 mL, n = 151 subjects. Using this information it was predicted that, with n = 12 subjects, SBWC could be estimated with a SEM of ±20 mL with a 90% power and α = 0.05.

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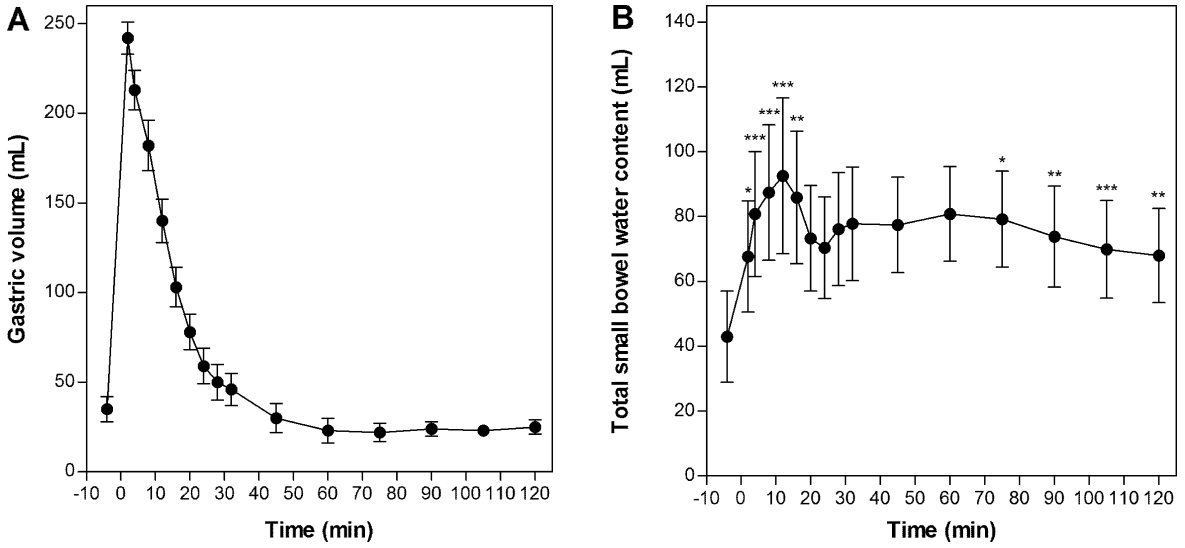

Figure 2. (A) Mean gastric volume and (B) mean total small bowel water content before and after ingestion of a 240 mL dose of water given at $t = 0$ min. $n = 12$ healthy volunteers. Error bars represent ± SEM. Dunn's multiple comparison test versus baseline value * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Error bars represent ± SEM.

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. Friedman's test was used to assess the significance of differences over time, followed by Dunn's multiple comparison test versus baseline values. Differences between the mean number of pockets divided by bin size were assessed using nonparametric Friedman's test followed by Dunn's ntest. A p-value < 0.05 was considered to be statistically significant.

RESULTS

The study procedures were very well tolerated and good quality images were obtained from all subjects. The liquid contents of the stomach (Figure 1A) and of the small bowel (Figure 1B) were clearly seen in the MRI images of each subject.

Gastric Volume and Emptying. The fasted stomach contained 35 ± 7 mL of resting liquid. Upon drinking, the gastric liquid volume rose to 242 ± 9 mL after 2 min. The liquid in the stomach appeared as a single continuous water pocket. Its volume declined rapidly (Figure 2A) with very brief lag time observed only in a couple of subjects (Supporting Information Figure S3). The first part of the individual gastric emptying curves was mostly exponential with a mean fitted half emptying time $T_{50\%}$ of 13 ± 1 min. For about half of the subjects, the second part of the gastric emptying curves deviated from the initial single exponential with the mean gastric volume returning back to baseline values 45 min after the water dose.

Total Small Bowel Water Content (SBWC). The fasting small bowel contained a total of 43 ± 14 mL water (range of 5−159 mL). After ingestion of the dose of water, the total SBWC increased significantly from baseline (Friedman's $P = 0.002$) as shown in Figure 2B. Volumes peaked at 92 ± 24 mL (range of 15−264 mL) at 12 min after dosing. At 45 min, when the stomach had completely emptied the ingested water, the total intestinal liquid volume was 77 ± 15 mL (range of 15−172 mL), and after this time, the total small SBWC remained fairly constant around this value. The individual time curves (Supporting Information Figure S4) showed marked interindividual variation.

Small Bowel Water Pockets. The fasting small bowel water was distributed in 8 ± 1 pockets of 4 ± 1 mL on average each. After water ingestion, the total number of small bowel liquid pockets increased significantly from baseline (Friedman's $P = 0.0332$) as shown in Figure 3A. The individual time courses showed a certain degree of interindividual variation (Supporting Information Figure S5). At 12 min when the peak total volume was reached, the number of liquid pockets had risen to 15 ± 1. At 45 min, when the ingested water had emptied from the stomach completely, the number of pockets was 16 ± 3, and after this time, the total number of pockets gradually decreased to values that are statistically not different from the baseline value. The distribution in the number of liquid pockets on the basis of pocket size bin (Supporting Information Figure S6) showed that the majority of pockets are small (0.5−2.5 mL size bin). Before and at all times subsequent to drinking the dose of water, the percentage of the number of pockets in the size bin 0.5−2.5 mL was greater than 60%, and the percentage of the number of pockets in the size bin >20 mL was less than 10%. At all time points there was a significant effect of bin size on number of pockets (Friedman's $p < 0.0001$).

Post hoc analysis showed that the number of liquid pockets in the smaller 0.5−2.5 mL size bin was significantly higher than all the larger bin sizes and that there was no significant difference between the remaining larger bin sizes.

The fasting small bowel water pockets had a volume of 4 ± 1 mL on average each. The mean pocket volume had a trend to increase with time to about 7 mL (Figure 3B; individual time courses in Supporting Information Figure S7) after water ingestion, but differences between means were not statistically significant (Friedman's $P = 0.4251$). Despite the greatest number of pockets being in the smallest size bin (0.5−2.5 mL) as mentioned above, most of the total volume of liquid in the small intestine was found in fewer pockets in the size range >20 mL. At all times before and after ingestion of water, the liquid pockets in this size range accounted for over 45% of the total volume. The actual volumes and numbers of pockets varied greatly between individuals as shown in Supporting Information Figures S5 and S7. Figure 3C shows a comparison between the mean pocket volume distributions on a number versus a

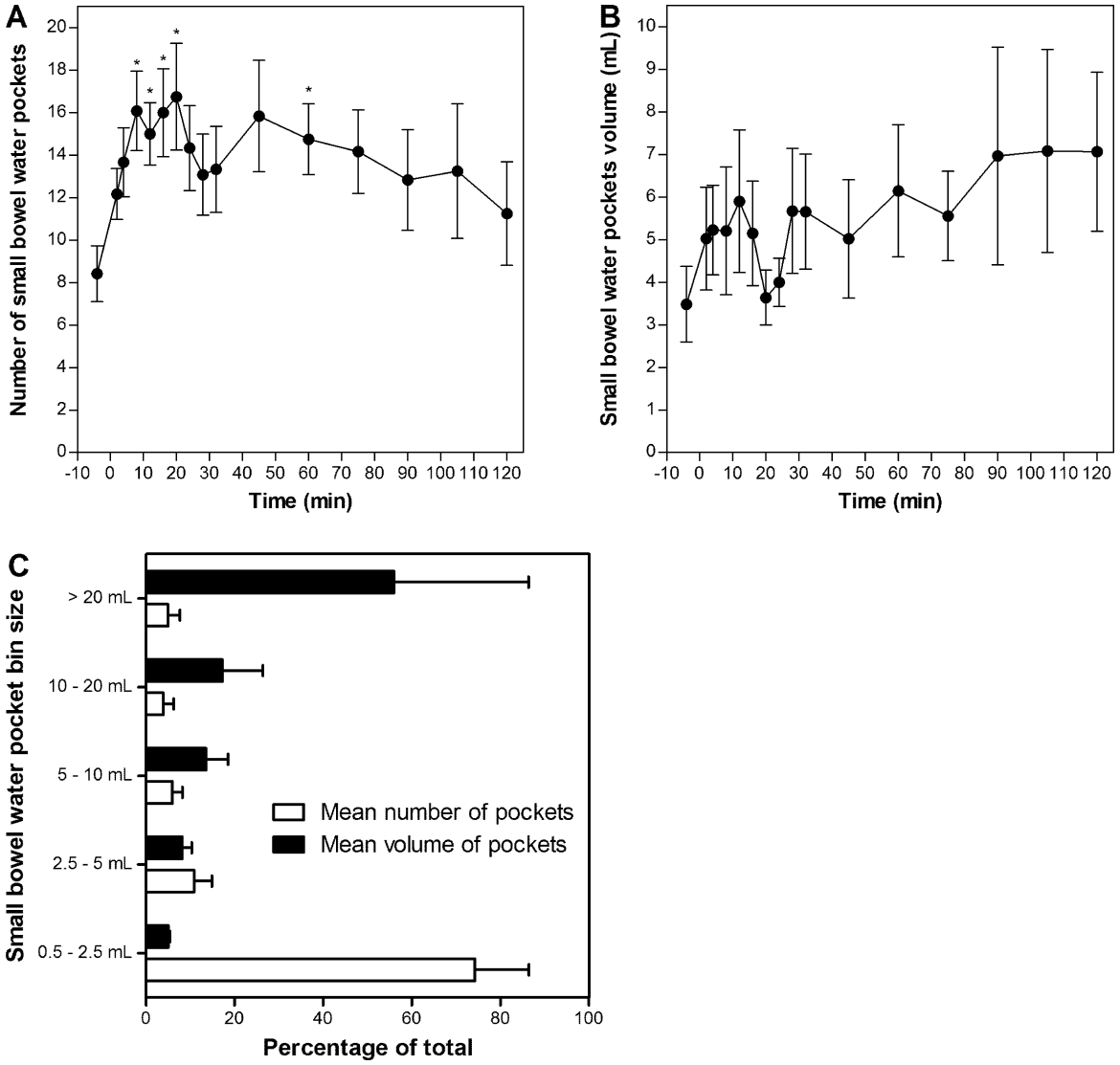

Figure 3. Small bowel water pockets before and after ingestion of a 240 mL water dose administered at t = 0 min (excluding all pockets in the smallest range <0.5 mL). (A) Mean total number of small bowel water pockets. (B) Mean volume of small bowel water pockets. (C) Comparison between the percentage of the total number of pockets and the percentage of the total volume of each pocket in each volume range at fasting baseline (before administration of the study drink of water). n = 12 healthy volunteers. Error bars represent ± SEM. Dunn's multiple comparison test versus baseline value * $P < 0.05$.

volume basis calculated again excluding the smallest pocket bin of <0.5 mL volume in keeping with the previous figures. For completeness the corresponding figure including the smallest pocket bin of <0.5 mL volume is displayed in Supporting Information Figure S8. This illustrates that the smallest small bowel water pockets (<0.5 mL in volume each) constitute only 0.4% of the total small bowel water content, which is the reason why they have been excluded from some of the figures in the article for clarity as their number is high but the percentage of the total volume very small; hence, their functional contribution to drug dissolution and absorption will be limited.

Splitting the analysis by abdominal quadrant showed that the larger amount of small bowel water was consistently located in the lower quadrants, particularly the lower left (distal jejunum and proximal ileum) with also higher numbers of water pockets. The upper right quadrant (proximal duodenum) showed little water content even during gastric emptying, indicating that liquid here was absorbed and/or moved forward rapidly. Liquid volumes increased rapidly from baseline in the upper left (distal duodenum and proximal jejunum) and then lower left (distal jejunum and proximal ileum) quadrants followed by a slow rise in the lower right quadrant (distal ileum). Liquid pocket volumes were very variable between quadrants making it difficult to determine trends. The analysis by quadrant is presented in Supporting Information Figures S9 and S10.

■ DISCUSSION

Knowledge of the volume and distribution of liquid in the fasted human small intestine as a function of time after ingestion of 240 mL of water (conditions representing standard bioequivalence and bioavailability studies) is of great importance to the pharmaceutical industry. This information is needed to develop physiologically relevant *in vitro* tests and *in silico* predictions of solid oral drug bioperformance, which can be used to design robust drug products that perform successfully during human clinical pharmacokinetic BA and BE studies. For the current study, the water drink volume, inclusion criteria, and the fasting conditions were chosen to

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match the international standards for fasted state drug testing in healthy volunteers as far as possible, in order to increase the relevance of this study to current practices. Although a later EMA document allows inclusion of overweight subjects (BMI up to 30 kg/m^2[5]), we felt the original inclusion of normal range only would be easier to map on all the various guidelines. The subjects had to follow an overnight fasting of at least 10 h, in accordance with the FDA fasting guidance.[4] Water was allowed, but it was forbidden for 1 h before the study start.[4] The EMA guidelines of overnight fasting[6] of at least 8 h before testing are also in line with the FDA guidance and the current study.[5]

The results for gastric liquid volume and emptying are in line with a previous study conducted using a similar volume of water drink, 300 mL. Steingoetter and co-workers measured liquid volume in the stomach before and after administration of 300 mL of water using MRI.[14] They found the resting volume to be 28 mL (range of 18−54) prior to water administration, which agrees with our measured volume of 35 ± 7 mL. They found gastric liquid volume to be 296 mL (range of 279−323) immediately after ingestion of 300 mL of water. This volume is equal to about 90% of the expected volume (resting liquid volume + administered volume of water), which is in line with our measured value of 242 ± 9 mL measured after administering a 240 mL volume of water. The 300 mL water drink in their study emptied immediately (without a lag phase) and in an exponential pattern from the proximal and distal regions of the stomach, with a half emptying time of 16 min (range of 11−17). Our data confirmed this overall picture with an initial, rapid exponential emptying and only a couple of individuals showing a very brief lag phase before emptying. Yamashita and colleagues used a smaller initial volume of 150 mL of water and used nasogastric aspirates to calculate rate constants for both emptying and secretion of fluid.[28] The half-life of gastric emptying in that study (4.2 min) was shorter than our values possibly due to differences in initial volume and experimental set up. Their study highlighted the importance of oro-gastric secretion in determining gastric volumes. We did not have to estimate this here as one of the advantages of MRI is that it measures directly the actual volume of liquid in the stomach, as a balance of input and output, at any given time point. The participants spent the first part of the study supine due to the MRI scanner configuration. Posture and meal structure may have an effect on gastric emptying,[29] particularly for liquid meals that layer in the stomach such as mixtures of fat and water.[22] However, in the case of a water drink, it has been shown that even radical changes in body position do not affect gastric emptying rate and gastric emptying half times.[14]

A major objective of the current study was to confirm the existence of water in discontinuous pockets throughout the small intestine after administration of a glass of water. Schiller and co-workers first described intestinal water as separated in a series of pockets and provided an initial overall quantification.[15] Our study builds on that work using a validated method to quantify small bowel water using MRI and also provides a detailed breakdown of liquid pocket size and location in the small bowel. Another advantage of our study is that, to our knowledge, it is the first quantifying total volume of water in the stomach and small intestine under conditions representing BA/BE studies. In addition, previous studies characterizing the volume of water in the small intestine have been conducted without administration of water immediately prior to imaging. Therefore, the question whether small intestinal water volume would indeed be increased and by how much following drinking of water remained unanswered. Schiller and co-workers administered water at intervals leading up to the initial MRI scan, starting as early as 7 h before and as late as 2 h prior to the scan, and determined a mean total small intestinal liquid volume of 105 mL.[15] Previous studies from our group for which volunteers were fasted overnight with no access to water for up to 8 h before MRI or were allowed a small glass of water but only 2 h before imaging showed average small intestinal water volumes of about 80−100 mL.[17,19,30] Our study (in which 240 mL of water was given 2 min before imaging and SBWC was assessed serially for up to 2 h after) confirmed liquid volumes ranging from 67 ± 17 to 93 ± 24 mL, with a range in individual values of 10−264 mL, despite recent water administration. The initial average resting value of 43 ± 14 mL (range of 5−158 mL) was somewhat lower than anticipated from previous studies, probably due to chance given the small number of participants. The maximum average value of 93 ± 24 mL occurred at the point when just under half of the ingested water had been emptied from the stomach.

Before ingestion of water, there were on average 8 pockets of 4 mL volume each. Shortly after ingestion of water, both the number and mean volume of pockets roughly doubled (although the increase in mean pocket volume was not statistically significant), before returning to baseline values after the dose of water has been completely emptied from the stomach. At 2 h after ingestion of water an average of 11 ± 2 pockets of 7 ± 2 mL each were present. In their study, Schiller and co-workers reported a median number of pockets of 4 with a median volume of 12 mL each for water administered approximately 2 h earlier, which is roughly half the number of pockets with twice the volume as we found.

When thinking about the time frame for dissolution and absorption of a rapidly dissolving immediate release dosage form, the time period after administration of the dose up to about 45 min (when the entire contents of the stomach have been emptied) is critical. During this time frame it was found that the largest amount of liquid (~60% of the volume) is contained in a small number of large pockets, while the number of smaller pockets (2.5−20 mL) contains about 40% of the total volume. The smallest pockets analyzed in detail (0.5−2.5 mL) account for less than 5% of the total liquid volume, whereas the smallest pocket bin (<0.5 mL), represents less the 0.5% of available water. The MRI sequence used is strongly T2 weighted so possible motion and relaxation artifacts are unlikely to have created artificial false-positive bright pixels counted as small pockets of water. There is a possibility that turbulent flow artifacts would make a large water pocket appear as two (or more) separate water pockets disconnected. This is, however, usually associated with a combination of larger volumes and motion as seen sometimes in the stomach, and we have no indication that this was the case here for the larger range small bowel water pockets. The water in the small intestine was found to be mostly in the lower regions of the small intestine (the distal jejunum and ileum) in keeping with previous observations.[15] Any water associated with mucus on the surface of the intestinal epithelium was not taken into account in this study, as it would not give a measurable signal on the strongly T2 weighted sequence used in this study, due to its limited mobility. As it is assumed that the majority of drug particles dissolve in the freely mobile water, any water contained in the mucus layer should have a potentially small relative effect on bulk dissolution rate. While water contained in the mucus layer and within pockets of <0.5 mL may be important to consider in

some cases, complete characterization of these regions was not the focus of this study.

The time course of liquid volumes in the stomach and small intestine following administration of 240 mL of water has important implications for predicting the oral bioperformance of solid dosage forms using both *in vitro* testing as well as computational methods. For instance, gastric and intestinal water volume can significantly affect dissolution, the amount of drug dissolved, precipitation, and absorption kinetics and therefore the rate of appearance of poorly soluble compounds in the bloodstream. The information regarding total liquid volume in the stomach and small intestine and distribution into individual pockets as determined in this study can be used to develop *in vitro* biopredictive dissolution tests, estimate the potential percentage of a dose soluble in the stomach and small intestine, and be used as a starting point for development of a physiological model describing transport and distribution of liquid throughout the gastrointestinal tract.

The time course of gastric water volume is an important parameter that can affect the dissolution and precipitation kinetics of numerous drug substances that dissolve in the stomach. Knowledge of gastric volume is important for the purpose of evaluating the maximum percentage of the dose soluble in the stomach *in vivo* under conditions representing BA/BE studies. This study confirms that it would be reasonable to experimentally determine the fraction of the dose soluble in a volume of 240−250 mL *in vitro*, and on the basis of work by Kalantzi and co-workers, a buffer consisting of 0.01 N HCl or other media simulating gastric liquid could be used for the analysis.[31−34] An understanding of the gastric emptying rate and pattern is important when conducting *in vitro* physiological dissolution testing of numerous compounds, such as those compounds for which gastric emptying may be a rate-limiting step to systemic availability or, for low solubility, weakly basic compounds, which may dissolve extensively in the stomach and precipitate in the small intestine.[35,36] This work suggests that when conducting *in vitro* experiments in an apparatus such as the Artificial Stomach and Duodenum, which includes a separate stomach compartment,[37] or predicting oral bioperformance computationally, that an average first-order gastric emptying rate coefficient of 3.2 h^{-1} ($T_{50\%}$ of 13 min), with a range of 2.2−6.0 h^{-1}, would be a good starting point for the analysis.

In vitro dissolution testing is often conducted using large volumes of dissolution medium, often with the assumption that intestinal liquid volumes are indeed large, or that a large volume of dissolution medium is a reasonable substitute to describe *in vivo* dissolution of a drug into a smaller volume of medium with rapid absorption of that drug into the intestinal membrane. In many cases, such as for some BCS II and IV compounds with high dose numbers,[38] it is important to maintain a physiological volume of water, which this study would suggest to be about 50−100 mL on average, and include a separate medium (such as 1-octanol) or compartment in the dissolution test to remove dissolved drug from the bulk aqueous medium at a physiologically relevant rate, as can be accomplished using a two-phase dissolution apparatus.[39]

While, for practical reasons, using a continuous volume of aqueous buffer *in vitro* is convenient, the presence of a series of separate, smaller volumes of water contained within different regions of the small bowel has significant implications. One possible scenario to *in vitro* dissolution testing or computational transport modeling would be to assume the dose is equally distributed among the liquid pockets. However, it is conceivable that a significant portion of the total dose may be confined to only a few of the liquid pockets. These different scenarios could quite possibly result in substantially different absorption rates due to the significantly different areas available for absorption, especially for a poorly soluble drug substance. Also, when examining the information on number and volume within one individual rather than on an average basis, one can observe large variability between subjects, as illustrated in Figure 4. This phenomenon is likely due to differing fasting

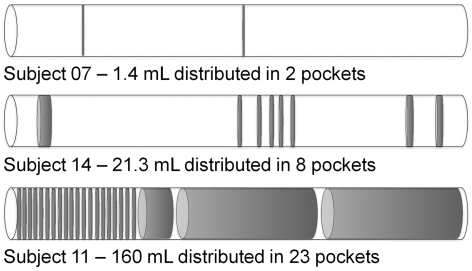

Figure 4. Graphical representation of number and volume of small bowel water pockets for three subjects. The small intestine is represented as a cylinder. Shaded areas represent liquid pockets. The minimum (1.4 mL, Subject 14), near median (21.3 mL, Subject 07), and maximum (160.1 mL, Subject 11) small bowel water volumes at fasting baseline (before administration of the study dose of water) are shown to illustrate schematically the high interindividual variability.

motility, which is highly variable with episodes of intense activity, the "migrating motor complex" (MMC), followed by long periods of quiescence. The MMC frequency is known to be affected by many factors including psychological stressors.[40]

The volume and number of liquid pockets also has implications for estimation of the mass of a dose soluble in the small intestine *in vivo* under conditions representing BA/BE studies. As mentioned above, 250 mL is a logical volume for assessment of the mass of a dose of drug soluble in the stomach. However, as evidenced by the total volume of liquid and the distribution of liquid into pockets, this volume may be too large for estimation of the mass of a dose soluble in the small intestine. A more reasonable approach may be to use a volume of about 80−100 mL for an assessment.

The information on distribution of liquid down the length of the intestinal tract is also of interest. Many high permeability drugs are assumed to be absorbed mainly within the proximal small intestine, namely, the duodenum and upper jejunum, and as such, many physiological measurements such as pH and bile salt concentration are performed within this region. There is also known segmental permeation of some drugs due to the pH of the intestinal fluid within a specific region of the intestinal tract and/or the presence of different types of transporters.[41,42] The amount of water available in the intestine will also be relevant for unconventional or enterically coated tablets. According to this study the highest percentage of fluid exists within the distal regions of the small intestine, such as the distal duodenum and proximal and distal ileum. Therefore, it may be that for drugs to absorb in the duodenum and proximal jejunum they must be in solution upon entry into the small intestine or dissolve rapidly in the upper small intestine and be subsequently rapidly absorbed into the membrane.

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CONCLUSIONS

This study is the first to quantify total volume and distribution of liquid in the stomach and small intestine under conditions representing BA/BE studies using a validated MRI methodology. Results confirmed the existence of discontinuous liquid pockets within the small intestinal tract, a phenomenon recently suggested by Schiller and co-workers.[15] Here, gastric emptying rate, volumes, numbers, and locations of liquid pockets present in the upper gastrointestinal tract were determined as a function of time after administration of a standard testing volume of 240 mL of water in 12 healthy volunteers in the fasted state. The information collected has important implications for the rates and extents of dissolution, precipitation, and absorption of oral dosage forms *in vivo* in the gastrointestinal tract and can be used to develop biorelevant dissolution methodologies, as well as mechanistic computational transport analyses for oral bioperformance prediction.

ASSOCIATED CONTENT

Supporting Information

Study day diagram, individual data, comparison between percentage number and volume, and abdominal quadrants division. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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. and S.E.P. analyzed the image data. C.L.H. designed the image analysis software. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

bTFE, balanced turbo field echo; GI, gastrointestinal; MRI, magnetic resonance imaging; PET, positron emission tomography; RARE, rapid acquisition with relaxation enhancement; SBWC, small bowel water content; T_E, echo time; T_R, repetition time; $T_{50\%}$, time to half empty the initial gastric volume

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