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# Magnetic resonance imaging quantification of gastrointestinal liquid volumes and distribution in the gastro-intestinal tract of children

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#### 25 Abstract

The volume and localisation of fluid in the paediatric gastro-intestinal tract is crucial to inform the design of *in vitro* and *in silico* models that predict the absorption of oral drugs administered to children. Previous studies have used magnetic resonance imaging (MRI) to quantify fluid volumes and localisation in the intestines of adults; this study is the first to undertake similar analysis of paediatric participants.

30 This study quantified the amount and distribution of fluid in fasted and fluid-fed children using MRI data captured during routine clinical assessment.

Data from 32 fasted children (aged 0-16 years) and 23 fluid-fed (aged 8-16 years) was evaluated. The gastric volume ranged from 0-9mL in the fasted and 19-423mL in the fluid-fed state. The small intestinal volume was recorded to be 0-51mL in the fasted and 6-91mL in the fluid-fed state with an average

35 number of 7.7 and 22.4 fluid pockets respectively. The data showed significant differences in gastric volumes and the number of fluid pockets in the small intestine for age-matched fasted and fluid-fed children (p<0.05). Both the number and the volume of pockets reported in children are much lower than those previously reported in adults.</p>

This study is the first to report intestinal volumes and localisation in children and provides new information to inform the design of biorelevant *in vitro* models and real values to update *in silico* models. The availability of data from both fluid-fed and fasted children show the extremes of fluid volumes that are present in the gastro-intestinal tract which is useful to understand the variability associated with drug absorption in children.

Keywords: gastric fluid; intestinal fluid; MRI; children; biorelevant dissolution;

#### Introduction

Oral drugs are often administered as solid dosage forms (e.g. suspensions; tablets or capsules) where disintegration and dissolution are essential prior to absorption. Variable absorption profiles are often observed that are attributed to individual inconsistencies within the gastro-intestinal environment <sup>1</sup>.

- 50 Significant efforts have been made to develop *in vitro* and *in silico* models that replicate the GI environment to better understand the processes of disintegration and dissolution with a view to minimising the variability associated with absorption. Typically pharmacokinetic profiles in children are predicted based on extrapolation from adult data based on body mass or surface area algorithms rather than using physiological data. However, for oral absorption knowledge of the GI fluid is important to
- 55 predict performance in children.

Prior to absorption of orally administered drugs, medicines often have to undergo disintegration and dissolution within the gastro-intestinal tract. The impact of the volume and composition of gastro-intestinal (GI) fluids will influence the processes of disintegration and dissolution and can affect the subsequent absorption profile. The volume of fluid used within *in vitro* testing systems varies from low

60 values (<50mL) in biorelevant testing systems to 900mL for pharmacopoeial methods as limited data exist on relevant values to use. The biopharmaceutics classification system uses fluid volume as a key parameter to classify drugs where the dose number (D<sub>0</sub>) describes the volume in which the maximum dose strength is soluble; this is used to classify medicines as either highly or poorly soluble. The D<sub>0</sub> used in the calculations for determining BCS criteria is determined by the formula:

$$D_0 = \frac{\binom{M}{V}}{C}$$

65 where M is the dose strength of the oral dosage form (mg), V is the volume administered (mL), and C is the drug's solubility (mg/mL).

A dose number value greater than 1 indicates a poorly soluble drug whereas less than 1 is highly soluble. A highly soluble drug is likely to be well absorbed as solubility within the GI environment is not a rate limiting step for absorption. In the USA and Europe a volume of 250mL (representing a glass of water) is

70 used to calculate the dose number, whereas in Japan the volume is lower at 150mL to account for reduced volumes of water co-administered in clinical testing protocols <sup>2</sup>. Extrapolation of adult dose numbers to paediatric populations has been undertaken to obtain age-appropriate fluid volumes

administered using body surface area <sup>3, 4</sup> giving values of 33.6mL for a neonate; 66mL for an infant and 127.6mL for a 7 year old child. Other literature reported a "worst-case" scenario of 25 mL as an

- 75 administration volume for all paediatric subgroups <sup>5</sup>. Normalisation of the residual gastric fluid volume using extrapolation from adults on a mL per Kg basis gave gastric fluid volume values of 14.9mL in neonates; 29.8mL in a 6 month old infant and 86.8mL in a 7 year old child <sup>6</sup>. A recent study on minipigs showed that gastric fluid volume was linearly related to the weight of the pig which suggests that weight is a suitable measure to use to extrapolate gastric fluid volumes <sup>7</sup>.
- 80 Knowledge of the volume and composition of fluids in the gastro-intestinal environment are critical to prediction of absorption as they provide an understanding of the media for disintegration and dissolution which can be replicated in *in vitro* and *in silico* models. Significant efforts have been made to measure both volume <sup>8-10</sup> and composition of gastro-intestinal fluids in adults <sup>11-14</sup> and to a more limited extent children <sup>15</sup>. Fluid within the adult small intestine has been reported to be present in pockets
- 85 rather than homogenously distributed along the GI tract. This distribution pattern will further complicate the absorption profile and adds to variability observed in pharmacokinetic profiles from orally absorbed medicines <sup>16</sup>. The comparative gastric and small intestinal volume values in children are likely to have a substantial impact on the rate and extent of drug absorption following oral administration which provides a more physiological approach to extrapolation of data.
- 90 The impact of gastro-intestinal fluid volume on predictions of pharmacokinetic profiles has been shown to be significant for poorly soluble drugs in adult populations <sup>17</sup>. Therefore, it is critical that representative fluid volumes are used to predict absorption for all populations. The range of fluid volumes from the fasted to fed state is also of interest as the variation can impact upon the absorption of medicines. Therefore, knowledge of the fluid volumes in the extremes of fasted and fed states is of interest.

Previous studies conducted in adults demonstrated the value of magnetic resonance imaging (MRI) to accurately measure the volume and localisation of fluid within the gastro-intestinal tract <sup>8-10, 16</sup>. Many children undergo MRI as part of their clinical care where their abdomen is visible. This study sought ethical approval to use data from clinical MRI procedures in children to measure fluid volumes and

100 localisation in the stomach and small intestine of these paediatric patients. The primary outcome measure was to quantify the mean and range of fluid volumes within the stomach and small intestine of children. Secondary outcome measures were to quantify the mean (and range) number of fluid pockets

distributed within the GI tract of children and to record their location (duodenum, jejunum and ileum). The study also explored the difference in these outcomes from fluid-fed and fasted participants.

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#### **Materials and Methods**

This study was an observational, retrospective study with non- probability sampling. The project sites were two major teaching Hospitals in the West Midlands (University Hospitals Coventry and Warwickshire NHS Trust and Birmingham Children's Hospital). The project was ethically approved REC reference: 18/EM/0251 (IRAS 237159 MRI: Fluid volumes and localisation in paediatric GI tract).

#### **Study Participants**

Anonymised MRI data sets were provided to the researchers from children (from 0-16 years) who required MRI for clinical purposes where the abdomen (stomach and small intestine) were clearly

115 visible. The two sites involved had different protocols for the MRI procedure: one required children to ingest 500mL Oral Klean Prep (a macrogol solution) in the 60 minutes prior to their MRI (hereon referred to as fluid-fed children); the other site required children to fast overnight prior to the MRI.

In order to ensure that the patient population was as close to "normal-healthy" as possible, the following exclusion criteria were used, patients with: acute abdomen (appendicitis or perforated

120 viscous); malignant bowel disease; surgery (bowel section, excluding appendicectomy); bowels wall thickening/ stricture/ fistula/ abcess.

#### Magnetic resonance Imaging (MRI)

MRI scanning was performed at two sites using different apparatus, the methods of subsequent analysis accounted for these differences in equipment used to acquire images. This imaging process provided clear images where fluids can be clearly distinguished.

The fluid fed participants were scanned at University Hospital Coventry and Warwickshire using either a 1.5T MR imaging unit (Optima MR450w, GE Healthcare, Chicago, USA) with a 48-channel body coil where the MRI protocol used was a coronal balanced steady-state gradient echo sequence (FIESTA) (slice thickness= 4.0 mm; echo train length = 1; intersection gap = 5.0mm; matrix size, 512 x 512; field of

view, a x b cm; TR/TE, 5.7/1.9 ms). Or the scan was conducted using a 1.5T MR imaging unit (Aera,

Siemens Healthcare, Erlingen, Germany) using a body coil and the MRI protocol used was a coronal balanced steady-state gradient echo sequence (True FISP) (slice thickness = 6.0 mm; echo train length = 1; intersection gap = 3.0mm; matrix size, 256 x 256; field of view, a x b cm; TR/TE, 652.8/2.1 ms).

The fasted participants were scanned at Birmingham Children's Hospital using either a 1.5T MR imaging unit (Siemens MAGNETOM Avanto 1.5T MRI System, USA) with a 16-element parallel imaging receiver coil where the MRI protocol used was a T2 SPACE coronal sequence (no gap between slices; slice thickness= 0.90 mm; matrix size = 0.8 x 0.8mm; field of view = 250; TR/TE = 1700/98 ms). Alternatively a 1.5T MR imaging unit (Aera, Siemens Healthcare, Erlingen, Germany) using a 16-element parallel imaging receiver coil was used where the MRI protocol used was a coronal T2 SPACE sequence (no gap

between slices; slice thickness, 0.9 mm; matrix size = 0.8 x 0.8 mm; field of view = 400; TR/TE = 2000/241 ms).

T2 SPACE is a variant of a three dimensional (3D) turbo spin echo sequence. Compared to a conventional turbo spin echo sequence, T2 SPACE uses non-selective, short refocusing pulse trains that consist of radiofrequency pulses with variable flip angles. This allows for very high turbo factors (> 100)

145 and high sampling efficiency. A SPACE sequence produces high resolution isotropic images which can be reconstructed in multiple planes. SPACE sequences are less sensitive to susceptibility, flow and chemical shift artifacts which make it superior over the conventional turbo spin echo. By using a SPACE sequence, it was possible to produce isotropic 0.9mm 3D T2 contrast images in less than 7 minutes.

#### 150 Image analysis

Horos software was used to visualise the MRI date sets. Horos is a free and open source code software (FOSS) program that is distributed free of charge under the LGPL license at Horosproject.org and sponsored by Nimble Co LLC d/b/a Purview in Annapolis, MD USA. The fluid within images was identified using the cerebrospinal fluid as a reference point for each data set; this approach acts as an internal

- 155 control for each data set and represents the "free" water present (<sup>18, 19</sup>). This value was used to provide a threshold to enable the fluid in the MRI data set to be clearly visualised. In brief, the image slice that most clearly showed the CSF within the spinal canal was selected to generate the threshold value. A rectangle was drawn entirely within the CRF and HOROS software was used to calculate the signal intensity of this area to set the limit for free fluid within the MRI data set. In this study, signals from the
- 160 stomach and the small intestine were manually identified whereas the kidneys, gallbladder, bladder and

visible vessels were manually excluded from subsequent analysis. Representative images are shown in Figure 1. The gastro-intestinal fluid was identified in each slice manually and the area of each fluid pocket was calculated following transfer of the image into imageJ <sup>20</sup>. The volume of each pocket was determined by multiplication of the slice thickness by the area measured as previously described <sup>16</sup>.

- 165 Using each patient's individual data, it was possible to create 3D MRI images (using Horos software) that assisted in mapping the GI anatomy which was particularly useful in the determination of the location of the identified fluid pockets. The 3D images were used to identify fluid pockets that overlapped into adjacent slices to better calculate the volume of each continuous pocket. For each participant, the total number of pockets was recorded as well as the volume and location of each pocket. Location of pockets
- 170 within the small intestine was based on the visual distinction of small and large bowel, based on anatomical knowledge and observed differences after thresholding the slices using HOROS software. It was challenging to identify where the duodenum, jejunum and ileum start and finish, as there is no specific small bowel length for all subjects. Approximation of the location of fluid pockets was undertaken by dividing the total small bowel into two parts; the upper left hand corner in an X,Y coronal
- 175 plane was used as the starting point and moving in a diagonal line the upper left 2/5 were designated to be the duodenum and jejunum; the lower right hand 3/5 of the image represented the ileum; this was used to then determine where the fluid pockets were located.

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All analysis was undertaken by a single operator initially with a sub-sample of 10% of all participants being checked by a second operator to ensure that there was consistency ( $\pm 10\%$ ) in the measurements obtained.



Figure 1. Example MRI data from two participants; the images show a sequence of slices in the coronal
 plane moving from the front to the back (spine) of the subject; the red areas are the fluid areas
 thresholded and included in further analysis. The upper image set is from a fluid-fed 16 year old and the
 lower imaged from a fasted 8 year old.

### **Statistical analysis**

The individual volumes and number of fluid pockets were plotted to provide data on individual variability. The data are also shown as the median, interquartile values and full range.

Statistical tests were performed using SPSS. An independent samples t-test was used to compare the volume of fluid and number of fluid pockets between age-matched fluid-fed and fasted children. Where multiple age-groups were compared ANOVA tests were undertaken.

## 195 **Results**

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MRI data sets were available from 32 fasted children and 23 fluid-fed children. The demographics of the participants are shown in Table 1. The youngest child was one week old and the oldest 16 years old. The gender split was approximately even with 52% (12/23) of the fluid-fed participants and 59% (19/32) of the fasted participants being female. All data sets were of sufficient quality to allow subsequent analysis.

Number of data sets available

Age range	Fasted Children	Fluid-fed Children
<2 years (newborn/infant/toddler)	10	
2-5 years (pre-school children)	13	
6-11 years (school age children)	8	6
12-16 years (adolescents)	1	17

200 **Table 1.** Demographics of the participants included in the study.

#### **Gastric fluid volume**

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The gastric fluid was either present as a single pocket or absent. Residual gastric fluid was absent in 65.6% (21/32) of fasted children and present in all fluid-fed children. A comparison of the total volume of gastric fluid present in fasted vs fluid-fed children is shown in Figure 2. The difference in gastric fluid present in fluid-fed versus fasted children was statistically significant (p<0.05).



**Figure 2.** Comparison of gastric volumes in fluid-fed (n=23) and fasted (n=32) children. Data shows median; Q1; Q3 and range.

The age of the child had no impact on the volume of gastric fluid present in either fasted or fluid-fed children (p>0.05); this data is shown in the supporting information (Figure S1).

#### Number of pockets in the small intestine

The total number of fluid pockets identified in the small intestine of fasted patients ranged from 0-16;

whereas for fluid-fed patients it ranged from 11-40, as shown in Figure 3.



220 **Figure 3.** Comparison of the number of fluid pockets identified in fluid-fed (n=23) (shown as black circles) and fasted (n=32) (shown as white circles) children. Data is stratified by age group as well as the fluid-fed and fasted state.

As the fluid-fed children were typically older than the fasted children the impact of age on the number of fluid pockets was explored. Participants were stratified into groups based on those <2 years; 2-5

225 years; 6-11 years and 12-16 years to determine whether there was a difference in the number of fluid pockets identified. The number of pockets in aged-matched (school age children) was significantly different (p = 0.011) for fluid-fed (n=6) and fasted (n=8) children. Pooled analysis of all participants showed that there were significant differences in the number of pockets identified based on age for the overall population (p<0.05); see supplementary material. However, there was no statistically significant difference (p= 0.420) for the number of pockets in fasted children based on their age.

#### Location of fluid pockets

In both the fluid-fed and fasted populations the majority of fluid pockets were located in the jejunum; 86% in fluid-fed children and 93% in fasted children, all remaining pockets were located in the ileum.

### 235 Small intestinal fluid volume

The overall volume of fluid in the small intestine was compared by both age range and the fluid-fed/fasted state. The results are shown in Figure 4. The mean total small intestinal volumes were 30  $\pm$ 24mL and 7  $\pm$ 10mL in the fluid-fed and fasted state respectively.



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**Figure 4.** Total small intestinal volume presented as a function of age range and the fluid-fed/fasted state.

- 245 Statistical analysis showed that there is not a statistically significant difference in the total volume of fluid in the small intestine in school age children between the fluid-fed and fasted state (p=0.101); although there is an outlier with one fasted participant with a small intestinal fluid volume of 50mL which has skewed the results; elimination of this outlier resulted in a significant difference in the values (p<0.05). Age had no significant effect on the total volume for those in the fluid-fed or the fasted state</p>
- sub-populations.

The proportion of pockets that were less than 1mL was 66.0% (306/464) in fluid-fed children and 76.5% (202/264) in fasted children. These small pockets contributed to 21% of the total fluid found in the small intestine of the fluid-fed participants and 27% for the fasted participants. Figure 5 shows (a) the relative number and (b) proportion of fluid present based on pocket size in the intestine of the fluid-fed and

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(a)

260 (b)



**Figure 5.** Comparison of (a) the percentage of fluid pockets by each volume per sub-population and (b) the total volume occupied by these pockets by each sub-population.

#### Discussion

- 265 Knowledge of the volume and distribution of gastric and small intestinal fluid in children in both the fluid-fed and fasted state is of great interest to the pharmaceutical industry. This information will inform the design of biorelevant *in vitro* and *in silico* methods that will predict the performance of oral solid dosage forms which can, in turn, aid in prediction of the pharmacokinetics of a drug. In the current study two populations were available; those who were fasted and those who were fluid-fed having ingested
- 270 up to 500mL of KleanPrep in the 60 minutes prior to data collection. These two populations enable the impact of fluid on overall intestinal volumes to be explored although the data does not exactly replicate clinical testing as has been undertaken in adult studies due to ethical constraints surrounding paediatric clinical testing <sup>10</sup>.

The MRI protocol for fluid-fed participants required children to consume 500mL Oral Klean Prep (a
macrogol solution) in the 60 minutes prior to their scan yet this was not monitored thus there is likely to be variability in the total amount consumed and the time frame for consumption prior to data capture. Data is available that details the time that the fluid-fed participants started the study and the time the MRI was conducted which ranged from 14 minutes to 2 hours 35 minutes with a mean value of 68 minutes. There was no correlation with time compared to volume in the small intestine (correlation showed R<sup>2</sup>=0.0006) (Shown in Figure S2 of supporting information).

Our results showed that fasted children had gastric volumes ranging from 0 to 8mL with a mean volume of 1.3 mL. The fasted gastric volume in children is very much lower than that reported in adults (see Figure 6) which may have consequences on the disintegration and dissolution of medicines given to children in a fasted state. The inter-individual variability in adult fasted gastric fluid volume has previously been reported as having a relative standard deviation of 31% with intra-individual variability relative standard deviation of 23% <sup>21</sup>. A comparison of the gastric volume reported in this study compared to previous values from adults is shown in Figure 6.





Figure 6. Comparison of the mean (± standard deviation) reported for fasted gastric volumes in adult
 studies compared to the current paediatric study. [Steingoetter study only reported a range not a mean and standard deviation]. Note that several studies included sub-populations hence multiple data from the same source.

The fasted gastric volume reported here is very much lower than that previously shown in adults.
Fasting gastric volumes previously reported in children were 0.4-0.56 ml/Kg <sup>22, 23</sup>. The data in this study
would provide an estimated value would be 0.08mL/Kg (based on a mean age of 4 years with an approximate weight of 16Kg). This much lower fasting gastric volume has implications in dosing to children where it is important to highlight the need to dose with water. Oral suspensions are a

commonly used dosage form in children where drug dissolution is a rate limiting step for absorption. Previous work has demonstrated that larger gastric fluid volumes enhance dissolution of poorly soluble

- drugs <sup>24</sup>, therefore low gastric volumes may impact the onset of drug action. Medicines administration in children is often not accompanied by an excess of water, particularly for liquid medications <sup>25</sup>.
   Furthermore, there is evidence that children are often on fluid restricted diets prior to night-time dosing which may lead to slow onset of action for medicines given at night <sup>26</sup>. The lowest fasted gastric volume previously reported in adults was 1mL and the highest was 95mL <sup>8</sup>; our results showed that almost two-
- 305 thirds of fasted children had no measureable gastric fluid volume.

Previous work that has looked at the impact of gastric volumes following ingestion of water have shown increased gastric volumes of 296mL immediately after ingestion of 300mL water <sup>27</sup> and 242mL following ingestion of 240mL water <sup>10</sup>. Our study collected data from children who had ingested up to 500mL Kleanprep in the 60 minutes prior to the MRI; the data showed increased gastric volume yet the data is

310 not directly comparable to that found in adults due to the differences in protocols used. Ethical restrictions limit the extent of experimentation that can be conducted using MRI with paediatric populations.

This study has confirmed that, as previously seen in adults, fluid is present in discontinuous pockets in the small intestine of children in both the fluid-fed and fasted state. Discontinuous pockets of fluid in the

315 small intestine of adults was first reported in 2005 by Schiller et al. The total number of pockets identified in our study was 8 ±4 and 22 ±4 in the fasted and fluid-fed state respectively. This data is similar to that reported by Mudie et al where 8 pockets were reported in the fasted state in adults and 16 in the fed state (in adults) <sup>10</sup>.

A total mean small intestinal volume of 105 mL (range 45-319mL) was reported in the fasted state in a study conducted by Schiller et al in 2005. A lower total volume of 43mL (range 5-158mL) was reported by Mudie et al (2014) <sup>10</sup> which was similar to a value of 54 ±37mL reported by Grimm et al (2018) <sup>9</sup>. Our study reported a fasted mean small intestinal volume of 7.4mL (range 0-50.58 mL) in children which is much lower than values previously reported in adults. The forest plot in Figure 7 compares the volumes of fasted small intestinal fluid reported in the literature to the values found in our study.



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**Figure 7.** Comparison of reported volumes of fasted small intestinal fluid in adult studies compared to the current paediatric study (data shows mean and range except for Grimm (2018b) study where mean ±standard deviation is shown as range data was not available).

- This lower volume in children is even smaller than values previously used in representative paediatric dissolution testing. Conventional dissolution testing of pharmaceutical dosage forms typically involves large volumes of fluid (500-900mL). The appropriate volume to be used to represent the paediatric intestinal media has previously been suggested to be 50mL for neonates; 100mL for infants and 200mL for pre-school children <sup>28</sup>. This study suggests that volumes of 7-40mL may be more appropriate in pre-school and school aged children. However, it is important to consider that this volume is not present as a single homogenous pocket but is distributed into several small pockets along the intestinal tract.
  - Figure 8 shows a cartoon bubble plot that represents the distribution and relative size of fluid pockets in fluid-fed and fasted children.



**Figure 8.** Representation of fluid pockets in fluid-fed children (blue) and fasted children (red); each bubble represents one pocket and the bubble size the relative volume. The data is presented from left to right in order of age for each sub-population.

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Limitations of our data set need to be considered. The presented data were all collected from children undergoing MRI for clinical reasons, therefore the protocol for ingestion of fluid was not tightly controlled and the actual volume and timing of ingestion of the recommended 500mL of Oral Klean Prep was not accurately recorded for the fluid-fed participants. Adherence to the protocol for fasted children was also not checked where children were asked to fast overnight prior to the MRI. It is recognised that using Klean Prep (an osmotic laxative) to represent fluid fed children is not fully representative of a typical fluid fed child. The mechanism of action of this product is to draw water into the intestines which maximises the fluid content increasing the water content and volume of stools in the bowel. The Klean

355 prep was administered within 60 minutes of imaging thus the fluid present in the intestine was observed prior to defecation. This provided a fluid fed scenario to allow comparisons to the fluid fasted children. The age ranges of the children who were fasted and fluid-fed are not matched and additional efforts are ongoing to try to match the demographics of fluid-fed and fasted children.

### 360 **Conclusion**

This study is the first to quantify the total volume and distribution of fluid in the stomach and small intestine of children in the fasted and in a fluid-fed state. Fluid in the small intestine is present in discontinuous pockets with a mean volume of 7.4mL in the fasted state and 30.4mL in the fluid-fed state. There are a significantly higher number of pockets in the fluid-fed state with a significantly higher

365 overall volume. The information presented has implications to inform the design of biorelevant *in vitro* and *in silico* methods to predict drug absorption in children.

The very low volumes observed in childrens' intestines compared to previous studies in adults raises concerns about the direct extrapolation of *in vitro* dissolution data based on adult physiology to predict performance of medicines in paediatric populations.

# **Author Information**

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Author Contributions: J.M, C.P, A.O, M.T and E.P-S and H.K.B. designed the study. E.P-S. analyzed the
image data. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

The authors declare no competing financial interest.

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# **Abbreviations**

GI, gastrointestinal; MRI, magnetic resonance imaging; CSF, cerebrospinal fluid; PBPK models, Physiologically based Pharmacokinetic models; UHCW, University Hospitals Coventry and Warwickshire; BCH; Birmingham Children's Hospital.

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# **Supporting Information is Available**

- Comparison of gastric volume vs age for fluid-fed and fasted participants
- Relationship between the total small intestinal volume measured and the time lapsed from instruction to consume fluid to the time of MRI data collection for the fluid-fed participants

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