



**University of  
Nottingham**

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**NOVEL MAGNETIC RESONANCE IMAGING  
APPROACHES TO STUDY DISEASES OF  
GASTROINTESTINAL FUNCTION IN CHILDREN**

Thesis submitted to the University of Nottingham for the degree of  
Doctor of Philosophy

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## RELATED PUBLICATIONS AND AWARDS

### Publications

1. **H Sharif**, CL Hoad, N Abrehart, PA Gowland, RC Spiller, S Loganathan, M Papadopoulos, M Benninga, D Devadason and L Marciani. Colonic Volume Changes in Paediatric Constipation Compared to Normal Values Measured Using MRI. *Diagnostics* 11, 2021.
2. N Abrehart, K Frost, Young Persons Advisory Group, R Harris, A Wragg, D Stewart, **H Sharif**, R Matthews and L Marciani. A little (PPI) MAGIC can take you a long way: involving children and young people in research from inception of a novel medical device to multi-centre clinical trial. *Res Involv Engagem* in press 2020.
3. **H Sharif**, N Abrehart, CL Hoad, K Murray, AC Perkins, M Smith, PA Gowland, RC Spiller, R Harris, S Loganathan, M Papadopoulos, K Frost, Young Persons Advisory Group, D Devadason and L Marciani. Feasibility study of a new MRI mini-capsule device to measure whole gut transit time in paediatric constipation. *J Pediatr Gastroenterol Nutr* 71: 604-611, 2020.
4. **H Sharif**, D Devadason, N Abrehart, R Stevenson and L Marciani. Imaging measurement of whole gut transit time in paediatric and adult functional gastrointestinal disorders: a systematic review and narrative synthesis. *Diagnostics* 9, 0221, 2019.

## **Conference abstracts**

1. Heat treatment of bovine milk impacts gastric emptying and nutrient appearance. the NZNS Conference December 2022
2. Upright Gastric Emptying. ISMRM-ESMRMB & ISMRT 31st Annual Meeting May 2022
3. Colonic volume changes in paediatric constipation compared to normal values. BSPGHAN Annual Meeting April 2022
4. Colonic volume changes in paediatric constipation compared to normal values. ISMRM MR-Fest September 2021
5. A first-in-child feasibility study of a new mini-capsule medical device to measure whole gut transit in pediatric constipation using MRI (MAGIC). ISMRM & SMRT Virtual Conference & Exhibition August 2020.
6. A first-in-child feasibility study of a new mini-capsule medical device to measure whole gut transit in paediatric constipation using Magnetic Resonance Imaging (MAGIC). Precision Imaging Beacon/BRC imaging event September 2019
7. New MRI measurements of gastrointestinal transit in paediatric constipation: feasibility, acceptability and safety results from young patients and controls. Nottingham Paediatrics research showcase conference June 2019.

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## ABBREVIATIONS

|       |                                                    |
|-------|----------------------------------------------------|
| AC    | Ascending colon                                    |
| ACE   | Antegrade colonic enema                            |
| AE    | Adverse Events                                     |
| BRC   | Biomedical Research Centre                         |
| BSA   | Body surface area                                  |
| CCK   | Cholecystokinin                                    |
| CD    | Crohn's disease                                    |
| CSF   | Cerebral spinal fluid                              |
| CT    | Computed tomography                                |
| DC    | Descending colon                                   |
| DICOM | Digital Imaging and Communications in Medicine     |
| DRAM  | Dual Registration of Abdominal Motion              |
| EC    | Enteroendocrine                                    |
| EEN   | Exclusive Enteral Nutrition                        |
| EPSRC | Engineering and Physical Sciences Research Council |
| FC    | Functional constipation                            |
| FGID  | Functional gastrointestinal disorders              |
| FSE   | Fast spin echo                                     |
| Gd    | Gadolinium                                         |
| GI    | Gastrointestinal                                   |
| HCl   | Hydrochloric acid                                  |
| HRA   | Health Research Authority                          |
| HV    | Healthy volunteer                                  |

|       |                                                               |
|-------|---------------------------------------------------------------|
| IBD   | Inflammatory bowel diseases                                   |
| IBS-C | Irritable bowel syndrome with predominant constipation        |
| IQR   | Interquartile range                                           |
| JPGN  | Journal of Pediatric Gastroenterology and Nutrition           |
| MIPAV | Medical Image Processing, Analysis and Visualisation software |
| MMCs  | Migrating motor complexes                                     |
| MRI   | Magnetic resonance imaging                                    |
| NDDC  | Nottingham Digestive Diseases Centre                          |
| NHS   | National Health Service                                       |
| NMR   | Nuclear Magnetic Resonance                                    |
| PPI   | Patient and public involvement                                |
| QoL   | Quality of Life                                               |
| RF    | Radiofrequency                                                |
| ROIs  | Regions of interest                                           |
| ROMs  | Radiopaque markers                                            |
| SAEs  | Serious Adverse Events                                        |
| SBWC  | Small bowel water content                                     |
| SC-R  | Sigmoid colon and rectum region                               |
| SEM   | Standard error of the mean                                    |
| SPECT | Single photon emission computed tomography                    |
| SPMIC | Sir Peter Mansfield Imaging Centre                            |
| TC    | Transverse colon                                              |
| TE    | Echo time                                                     |
| TR    | Repetition time                                               |

|      |                        |
|------|------------------------|
| US   | Ultrasonography        |
| VAS  | Visual analogue scales |
| WGTT | Whole gut transit time |

## **ABSTRACT**

**Background:** Gastrointestinal (GI) function in paediatric health and GI disease is a largely unexplored area due to limitations and invasiveness of current techniques. Magnetic resonance imaging (MRI) has been increasingly used to study GI function in adults, but very little has been translated to the paediatric arena to date.

**Aims:** This work aimed therefore to develop and deploy new MRI methods and endpoints to study gastrointestinal function in paediatric health and disease.

**Methods:** The work carried out comprised four main work packages.

1. The translation of existing adult MRI measurement protocols of gastric emptying and small bowel motility to paediatric health and patients with Crohn's disease (CD). The MRI scanning protocol was implemented on a 1.5T MRI scanner and 15 children (13 healthy volunteers and 2 patients with CD) were imaged before and serially after a feeding intervention. Gastric emptying, gallbladder volumes, small bowel water content and small bowel motility were measured. The motility measurements were carried out on a new commercial platform.
2. The evaluation of the safety and feasibility of new MRI methods to measure whole gut transit time (WGTT) in paediatric constipation. This was a first-in-child clinical trial of a new mini-capsule medical device that our team had recently developed was used. Thirty-five children between 7 and 18 years old (19 healthy volunteers and 16 patients with

constipation) were studied. They ingested 24 mini-capsules per day for three days and were then imaged following a common radiopaque markers protocol. Whole gut transit time was determined from the count of the mini-capsules remaining in the gut at days 4 and 7.

3. The development of quantitative measurements of colon volume and colon length in paediatric health and constipation. The images from the 35 participants of the above study were used retrospectively to measure regional and total colon volumes by drawing regions of interest manually. Regional and total colon lengths were subsequently measured using a 3D skeletonization method.
4. The development and validation of a protocol for quantitative MRI measurements of gastric emptying on a 0.5T open-design scanner where the participants could sit upright. An initial development and protocol design phase was carried out first. After this, 6 healthy participants were studied before and serially after ingesting an oral glucose challenge drink whilst concomitant  $^{13}\text{C}$  stable isotope breath samples were collected for validation.

**Key results:** MRI measurements of GI function were successfully carried out in children from the age of 6 to 18 years old, without the need for bowel preparations or general anaesthetic.

The data showed rapid increase in small bowel motility after a meal stimulus.

It was safe and feasible to use the new mini-capsule device to measure WGTT in health and paediatric constipation using MRI. The young patients with constipation had WGTT twice as long as the healthy controls ( $P < 0.0001$ ).

Their colon, corrected for body surface area, had 40% larger volume ( $P = 0.0081$ ) and was 17% longer ( $P = 0.0037$ ) than for the controls, with the sigmoid-rectum area being most affected.

Lastly, a new protocol to measure gastric emptying in the upright position was developed and demonstrated.

**Conclusions:** This work achieved its aims successfully. New methods and endpoints to study gastrointestinal function in paediatric health and disease were developed and deployed. Original data quantifying aspects of paediatric GI function was collected, advancing the field. Such data and methods can help to provide a better understanding of paediatric GI function and dysfunction ultimately helping, in turn, diagnosis and treatment.

# **1 INTRODUCTION**

This work is concerned with developing and using magnetic resonance imaging methods to study non-invasively GI function in children with GI tract disease. This introductory chapter will briefly describe the GI tract anatomy and physiology, and diseases of GI function. Current diagnostic imaging methods to investigate GI function will be described, leading to the main aims and hypotheses of this work.

## **1.1 Gastrointestinal tract anatomy and physiology**

The digestive system is composed of a long muscular tube dedicated to storage, propulsion, mixing, digestion and absorption of foods and drinks. Several accessory organs produce fluids and regulate the production of enzymes that aid digestion. The entire length of the GI tract (Figure 1.1) from the mouth to the anus is of the order of 9 meters [1]. The length also varies depending on the muscle tone of the digestive tract, age and even the race of the individual. From this, the small intestine reaches up to 4 to 6 meters in length and around 2.5 cm in diameter while the large intestine amounts to about 1.5 meters long and about 7.6 cm in diameter [2].

Food digestion starts at the mouth, where foods are macerated by the teeth and mixed with saliva in the oral cavity. But before this, the perception of the food through sight or smell already activates the first phase of digestion, which is the cephalic phase [3]. With this, there is a significant increase in saliva secretion from the salivary glands including the parotid, submandibular and sublingual glands. From here on, the act of chewing the food initiates mechanical digestion that gradually incorporates salivary amylase in the food,

which commences chemical digestion of the carbohydrate component of the food lingual lipase action. Thus, both mechanical and chemical digestion of the food occurs as early as the food is being chewed in the mouth. This process forms a ball of food matter known as bolus, which is then swallowed after chewing. The food bolus enters the stomach through the oesophagus.

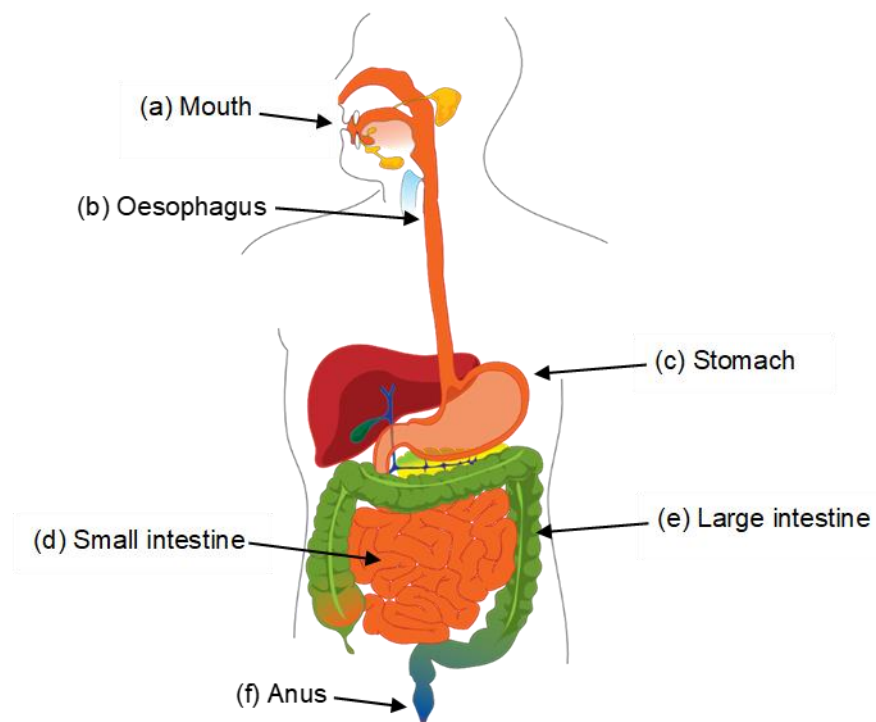


**Figure 1.1:** Diagram illustrating the gastrointestinal tract: **(a)** mouth **(b)** oesophagus **(c)** stomach **(d)** small intestine **(e)** large intestine **(f)** anus [4].

### 1.1.1 Oesophagus

The oesophagus, which connects the oral cavity to the stomach, reaches about 25 centimetres in length, with its width ranging from 1.5 to 2 centimetres [5]. The food enters the cardiac sphincter which regulates the entry of food and drink to the stomach. This is also part of the muscular tube that is

responsible for vomiting, or anti-peristalsis, whenever a toxic substance or a surplus of food is perceived by the digestive system.

### **1.1.2 Stomach**

When the bolus gets to the stomach reservoir, it is mixed with gastric juices which chemically breakdown the food. With its three layers of muscles, namely the longitudinal, circular, and oblique layers, the stomach efficiently alternates contraction and relaxation throughout digestion. This continuous churning of the stomach also contributes to mechanical digestion of the bolus.

The stomach is anatomically divided into four regions: cardia, fundus, body, and pylorus. The interior of the stomach is composed of multiple folds and ridges called gastric rugae that increase its surface. The stomach walls can relax and expand the stomach volume upon increased food intake. In addition to mechanical digestion, chemical digestion plays a central role in the digestion within the gastric environment. Gastric juices, with a pH as low as 2 to 3, are secreted by the lining of the lumen and are composed of pepsin, hydrochloric acid (HCl) and mucus. Within the duration of this process, the antrum of the fed stomach rhythmically contracts to ensure efficient mechanical breakdown of food and mixing. This takes about 2.5 to 3 hours of continuous churning and will gradually release its stomach contents through the pyloric sphincter. It can take about 4 to 5 hours to fully empty the stomach [6] depending on food characteristic and nutrient content. This process known as gastric emptying. From here, the resulting food matter is already at a viscous state called chyme and is poured from the stomach into the small intestines.

### **1.1.3 Small intestine**

Once food particles are broken down to smaller sizes, a few millimetres in diameter, the chyme is emptied into the small bowel (Figure 1.2). Emptying is regulated by various factors with caloric content being the dominant one. Chyme is released at a rate of approximately 4 kcal per minute into the segment of small intestines [7]. The duodenum is the first segment of the small intestine. It is the shortest segment, yet the bulk of chemical digestion and absorption occurs here. From here on, the goal of the digestive system is to increase the pH of the intestinal contents for the enzymes to function. If the food matter remains acidic, the enzymes, being made of proteins, will denature, and would be rendered incapable of metabolism. To accomplish this, the pancreas produces bicarbonate ions in the pancreatic juice.

Once the environmental conditions within the intestine are appropriate for further enzymatic digestion, chemical digestion takes place at a micro scale to convert larger molecules into units small enough for them to be transported/absorbed through the small intestine walls. Different enzymes operate on different types of nutrients, with a particularly important role of pancreatic juice enzymes for digestion. The second part of the duodenum is where the ampulla of Vater introduces the bile to the intestinal tract, though in some individuals the common bile duct is directly inserted into the duodenum. The bile fluid helps to emulsify the fats in the chyme for it to be effectively accessible to the intestinal enzymes. Enzymes are also produced in the lining of the small intestines.

Progressing more distally from the duodenum, the rest of the digestion takes place in the subsequent sections of the small intestine, known as jejunum and

ileum. These segments are intraperitoneal and, because of their lengthy structure, are secured to the posterior abdominal wall through the mesentery. A periodic muscular movement known as peristalsis allows the small intestine to continue to mix and move along its contents to assure interaction between the enzymes and macromolecules from the food. Through the finger-like the lining of the intestinal lumen comprises projections called villi, where the macromolecules in their most basic form are absorbed and delivered to the blood vessels for circulation to the rest of the body.

After these processes, the remaining chyme heads toward the large intestine from the terminal ileum into the cecum. This intersection forms the ileocecal valve which prevents reverse transport of caecal contents back to ileum.

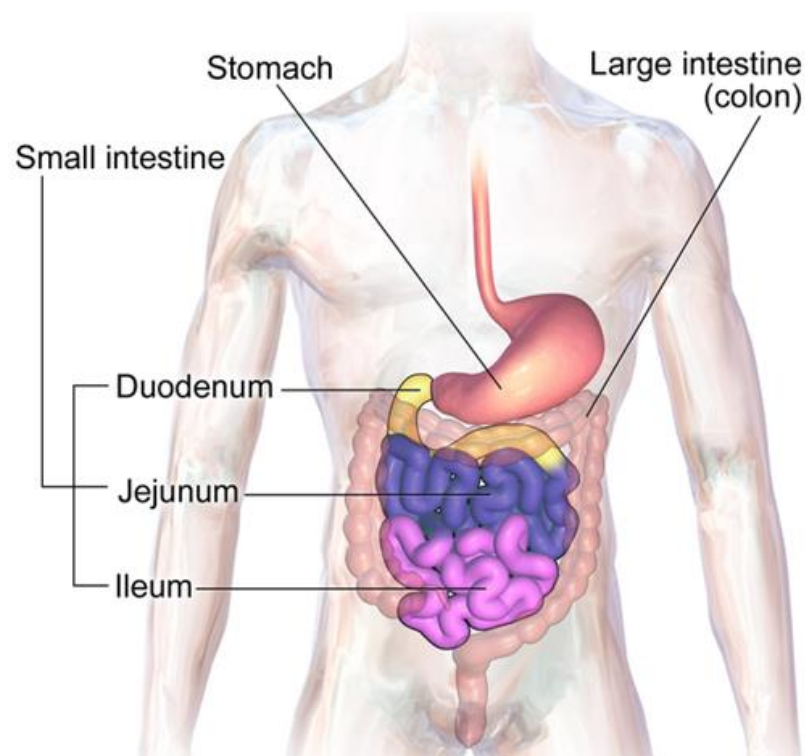


**Figure 1.2:** The small intestine comprised of Duodenum, Jejunum, and Ileum [8].

#### **1.1.4 Colon**

The large intestine (Figure 1.3) is divided into 4 regions: ascending colon, transverse colon, descending colon, and sigmoid colon and rectum region. The colonic muscle is divided into two layers the outer, longitudinal layer and inner, circular layer, which drive peristaltic movements in terms of mixing contractions and the propagating contractions. The mixing contractions allow the chyme to move gradually to the haustra of the colon to ensure proper water absorption. Microbiota deconjugate and dehydrogenate primary bile salts to produce secondary bile acids thereby promoting their reabsorption.

The propagating contractions propel the chyme distally with substantial (mass) movements. Water in the chyme is progressively absorbed making the stool more compact [9].

The ascending colon absorbs most of the excess water in the chyme, although almost 90% of the water has already been absorbed by the small intestine. Alongside water, the colon also absorbs vitamins and electrolytes from the luminal contents. The descending colon serves as a reservoir for the by-product of digestion, consisting one-fifth of the length of the GI tract to ensure that it is adequate for storage. This segment also harbours intestinal bacteria that break down the remaining contents.

The sigmoid colon then pushes the faeces forward to the rectal vault [10]. The rectum is the terminal segment of the luminal structure and is continuous with the posterior opening, which is the anus, where the faecal matter exits the GI tract. In between meals, the migrating motor complex initiates periodic patterns of motility waves within the GI tract to facilitate movement of chyme and

undigested materials. All of these digestive mechanisms are regulated by gastrointestinal peptides.

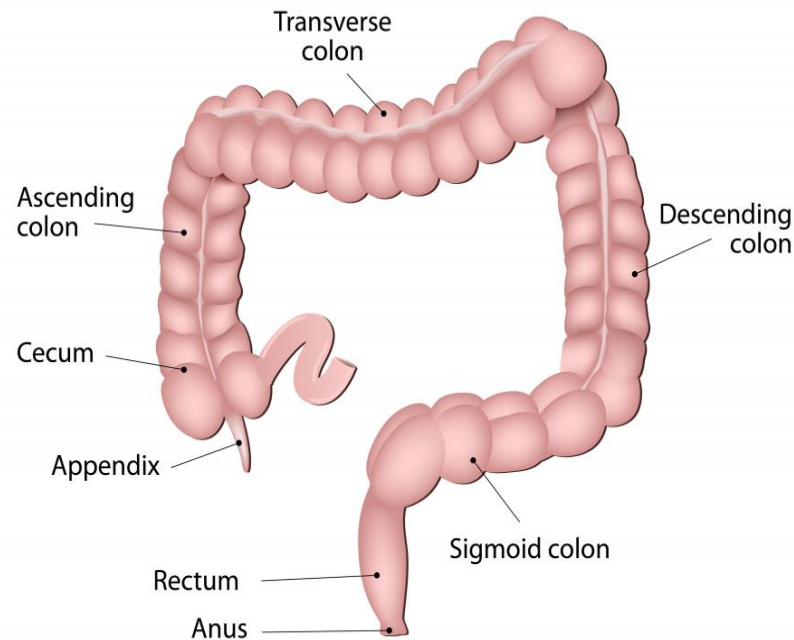


**Figure 1.3:** The large intestine extends from the cecum to the rectum. It is comprised of the ascending, transverse, descending and sigmoid colon. Licensed to TeachMeSeries Ltd

The principal functions of the gastrointestinal tract are to store food and drinks, digest and absorb ingested nutrients, and to excrete waste products of digestion. Most nutrients are ingested in a complex form that are ready for absorption or not directly soluble, and therefore require mechanical and chemical breakdown before absorption.

#### **1.1.5 Gastrointestinal responses to feeding.**

Food ingestion induces a complex response from the digestive system. The upper digestive system first reacts to break down and extracts meal components. Later, leftover food that was not absorbed in the small intestine travels to the colon where it stimulates the metabolism of the local microbiota

[11]. Following ingestion of food, the proximal part of the stomach relaxes and serves as a reservoir for the food. Following that, a tonic contraction will redistribute the food forwards the distal part of the stomach and periodic contractions of the antrum will facilitate the breakdown and mixing the contents with gastric juices [7]. Subsequently, the process of mechanical digestion will start by pushing the food against a closed pylorus through gastric muscles. Food contents will exit the antrum of the stomach through the pylorus into the duodenum. This process is known as gastric emptying and is controlled by neurohormonal factors [7, 12].

#### **1.1.6 Gastrointestinal peptides**

Gastrointestinal peptides are produced by the stomach and duodenum in order to guide the rest of the GI tract with their function. These GI peptides aid in the transit of contents within the GI tract, enzymatic secretion, and absorption of nutrients. Gastrin, cholecystokinin (CCK), somatostatin, and ghrelin are some of them. Gastrin stimulates the release of stomach acid, whereas CCK enhances the production of the exocrine pancreas and gallbladder, so greatly slowing down gastric emptying. Somatostatin controls local cell activity and lessens the three gastrointestinal peptides' stimulatory properties [13].

#### **1.1.7 Gastrointestinal motility**

Gastrointestinal motility is characterised by different types of waves and contractions that control mixing and moving the chyme through the gut. It has two forms of motility patterns: the fasting (starvation) pattern and the fed pattern [14]. Throughout fasting, small bowel motility is determined by a type

of waves called the migrating motor complexes (MMCs), which consist of three different phases, repeated approximately every 2 hours and coordinated by neural and hormonal factors [15]. The MMC force the chyme to travel down the intestine during the fasting state. Upon feeding, the MMC is replaced by the postprandial (fed) pattern. The postprandial intestinal motility is triggered by the luminal distention and is modulated by the luminal nutrients [16]. Fed pattern is the stationary segmenting contractions which help in mixing and pushing the chyme.

#### **1.1.8 Gastrointestinal transit**

Gastrointestinal transit is the time it takes for food to leave the stomach and travel through the intestines. Transit time can be affected by many factors, including medications, diet, prior surgeries, gender, level of physical activity and stress level, as well as any chronic or acute illnesses that affect the gastrointestinal tract. It takes varying amounts of time for food to pass through the different areas of the GI tract [17].

## **1.2 Impaired gastrointestinal tract function**

Normal gastrointestinal physiology can be disturbed in many ways. Gastrointestinal disease can be organic (e.g. neuropathies, Crohn's disease) or functional (e.g. irritable bowel syndrome, functional constipation). This work focuses on the disturbances of motor function of the gut with a particular emphasis on children.

### **1.2.1 Crohn's disease**

Crohn's disease is a recurring disorder of the GI system, characterised by pockets of inflammation across the length of GI tract. It is one of the two primary manifestations of inflammatory bowel diseases (IBD). CD inflammation may present anywhere along the length of the GI tract and across all layers of the gut, though it most commonly affects the ileum and/or the colon. Upon colonoscopy, CD shows features such as patchy inflammation and lesions, where normal mucosa occurs in between inflamed sites. Ulcers in CD are also usually longitudinal and impart a cobblestone-like appearance to the ileum or colon; other hallmarks of the disease include fistulous orifices and strictures [18].

### **Aetiology**

IBD and CD in particular, poses a medical challenge, especially in children. CD affects at least 115,000 individuals in the United Kingdom, and about 33% of cases are diagnosed before the age of 21 [19]. According to recent data, the incidence of Crohn disease in children and adolescents is increasing [20, 21]. In children, CD is the most common type of IBD, comprising 59% to 73% of all paediatric IBD cases. While the majority will develop the symptoms in

their 20s or 30s, around 5% to 10% will get diagnosed before adulthood [22]. It is known as an early-onset CD, though the exact age for cut off varies greatly and is more of a function of local guidelines. Regardless of these heterogeneities in standards, there is a consensus that age at disease onset affects the clinical features and course of CD. Crohn disease behaves differently in children than in adults. Children are more prone to disease extension than elderly adults [22]. Several studies have also shown a high incidence of psychological morbidity in children and young people as well as adults with CD. This problem has a strong negative effect on the patients' quality of life and significantly increases the cost of treating CD for the NHS.

### **Symptoms**

Crohn's disease is a very heterogeneous disease, and its symptoms in children include perianal infections, abdominal pain, diarrhoea, fatigue, loss of appetite, and the presence of blood in the stool. Abdominal tenderness and palpable masses are also relatively common. Weight loss, growth failures, and delays in puberty are all similarly warning signs of CD in children and should be monitored closely [23]. Poor nutrition is common among children with CD and appears to be an active driver for such impairments in development [24, 25]. While this may be attributed to the manifestations of the disease or repeated operations, it may also be explained by a reduction in appetite. One of the main features of this disease is weight loss and malnutrition [26]. These problems are not well understood [27]. Loss of appetite and symptoms such as abdominal pain, early satiety and diarrhoea are common causes for weight loss in these patients [28].

## Diagnosis

There is no singular known cause of IBD in general and CD in particular. A comprehensive review in 2012 noted that “CD seems to result from an impaired interaction of the intestinal commensal microbiota that is normally in a state symbiotic mutualism with the human host.” Such impairments may include altered gut microbiota, a compromised intestinal barrier allowing entry to more antigens, and an exaggerated immune response to gut microbes. Genetics plays a central role in these disease mechanisms. Genome-wide association studies have found at least 71 susceptibility loci across 17 chromosomes related to CD [29]. Twenty-nine percent of paediatric population with early-onset IBD inherited the gene from the family and 44% of children diagnosed are younger than three years of age [30]. One important putative manifestation of CD is a breakdown in the function of the intestinal epithelium. The gut is lined with a single layer of cells to carry out the appropriate absorption of nutrients from food while keeping unwelcome agents out. These cells are externally covered by a thin layer of mucus biofilm secreted by the goblet cells. The mucin gene MUC1 is down-regulated in the inflamed terminal ileum of patients with CD, creating a thinner layer of mucus biofilm. It suggests that such dysregulation has a role to play in the pathogenesis of CD [29].

There has been a longstanding interest on the effect of CD on gastric emptying and gastrointestinal motility [31-36]. In 1997, A study described a high prevalence rate of motility disorders in patients with inactive disease [32]. The study found that relative to healthy volunteers, fasting patients showed significantly fewer duodenojejunal contractions and substantially more propagated single and clustered contractions. Similarly, postprandial antral

hypomotility was overrepresented in CD patients. This has been repeatedly verified in the ensuing literature. In another study, the disease activity of IBD in general, and CD more specifically, is correlated with longer gastric emptying [37]. Reduced terminal ileal motility has been recently shown in CD patients with active terminal ileal disease [38]. A recent study showed a strong link between impaired ileal motility and the histological [38] and biochemical hallmarks of CD [39]. It also demonstrated that radiology [38] holds the potential role in the assessment of disease activity [38]. Intestinal hypomotility may be observed in proximal unaffected segments of small bowel as well [39].

Gastric emptying and gastrointestinal motility are intimately linked to the enteroendocrine (EC) hormonal responses. It has been shown that EC peptides are increased with exaggerated postprandial responses in CD patients with small bowel inflammation [28, 40, 41]. This is associated with symptoms of nausea and anorexia, and EC peptide expression can return towards normal in remission [28]. CD patients also experienced stronger increases in aversive symptoms after a meal challenge [42]. An increase in EC activity could potentially lead to altered appetite and symptoms of nausea through delayed gastric emptying and most importantly delayed small bowel transit. IBD patients, in general, have shown elevated concentrations of PYY, GLP-1, motilin, CCK, somatostatin, ghrelin, and gastrin. However, there are conflicting reports. Post-prandial CCK, for example, was initially seen to spike in CD patients, though this effect could not be replicated in a follow-up study [37, 40]. Also, plasma GLP-1 and CCK have been shown to alter gastric emptying in CD [37, 40], while PYY is thought to impair appetite and lead to nausea in small-bowel CD [28].

The phenomenon of impaired motility in CD has also been documented in paediatric patients. As early as 1985, researchers have known that gastric motility is dysregulated in children with CD, leading to delayed gastric emptying. This has important ramifications, and it may help explain malnutrition and growth deficits in paediatric patients. Importantly, poor gastric emptying occurs with or without duodenal involvement. Liquid caloric supplementation has been forwarded as a solution to the nutritional deficits while prokinetic agents may help ease symptoms [33].

## **Management**

A standard treatment now well established as an agent of induction is polymeric exclusive enteral feeds (e.g. Modulen®). Following their referral to hospital, some of the young patients are treated with pharmacological therapy (e.g. steroids) and some are put on Exclusive Enteral Nutrition (EEN). EEN is a recommended first-line therapy to treat active Crohn's disease. The formula-based (no solid foods) diet is designed to induce remission in patients. It is a short-term program and may extend six to 12 weeks. EEN provides a constant flow of formula, which prompts better absorption of nutrients and less distention, stomach pain and vomiting [43]. Exclusive enteral nutrition was proven its role and effectiveness in the treatment of CD patients over the last two decades. It is considered first line therapy in the induction of remission of active CD [44]. Moreover, EEN treatment had equivalent response to corticosteroids in children with active CD [45]. In Nottingham children's hospital, some CD patients are following the 8-week Liquid Diet (Modulen®) and there is a great need to study the effect of this treatment on their gastric emptying using MRI. This will open a new door to future studies.

### **1.2.2 Functional constipation**

Functional gastrointestinal disorders (FGID) are some of the most diagnosed conditions in gastroenterology and account for about 40% of all patient visits. Consensus definitions and diagnostic criteria have been developed over the years. Currently the accepted consensus is called the Rome IV process. It classifies the FGIDs according to the segment of gastrointestinal tract where the condition is believed to originate from [46, 47]. Further Rome IV classifications were developed for FGIDs observed in children and adolescents [48, 49].

#### **Factors behind development and progression of FGIDs:**

Environmental factors are believed to play a major role in the development and progression of many FGIDs. These factors include diets with insufficient amounts of fibre (mostly processed and refined food), high quantities of dairy products, insufficient water intake, low level of physical activity and sedentary lifestyle, as well as the use of certain medications. FGIDs are common in pregnancy. Stress and psychological problems can further worsen the symptoms of many FGIDs.

Although environmental factors appear to dominate in the majority of cases of FGIDs, in many disorders there are genetic components that predispose individuals to developing these conditions. For instance, the heritability of irritable bowel syndrome reaches as high as 57% by some estimates [50, 51]. It is believed that there is a genetic component to functional constipation in children, although specific mutations have not been identified so far [52]. In those disorders where a genetic element is present, the observed set of

symptoms can usually be linked to multiple genetic variants, each contributing only to a small risk increase.

### **Functional constipation (FC) or chronic idiopathic constipation**

Constipation features prominently as one of very common symptoms and a diagnostic criterion in multiple FGIDs. According to Rome IV, bowel disorders are categorised into three groups, (irritable bowel syndrome with predominant constipation (IBS-C), functional constipation and opioid-induced constipation) are associated with constipation [46]. Constipation can occur as a result of slow movement of stool within a colon that reduce gastrointestinal motility [53]. However, in addition to various FGIDs, the presence of constipation may be an indicator of other underlying disorders including diabetes, celiac disease, colon cancer [54].

The latest reviews puts the average prevalence of constipation at 16% worldwide [55]. In the US, The number of constipation-related emergency hospital visits increased by 41.5% between 2006 and 2011 [56]. Constipation is more common among women, as much as twice as common by some estimates [55]. Up to 25% of women at several weeks after pregnancy (postpartum stage) are often have constipation [57]. Most of constipation cases can be caused by one of three general mechanisms: disordered and obstructed defecation caused primarily by impaired rectal evacuation, irritable bowel syndrome with constipation, and slow transit constipation [58]. These mechanisms differ substantially, yet the major presentation of constipation is similar. Patient-specific diagnostic measures are therefore important for adequate treatment. Diagnosis of constipation is still a challenging task and

about 50% of patients that present with constipation do not leave satisfied with their treatment [58]. About 15-30% of all constipations are as a result of slow transit constipation. This reduced motility in the intestine caused by abnormalities of the enteric nerves also accounts for about 37% of women with constipation [59]. Constipation tends to occur more in elderly populations and reach up to 50-75%. In children, the rate of constipations is estimated at 14% [60]. Constipation is not linked to specific clinical disorders in the majority of paediatric cases, but it rather caused by changes in lifestyle or diet [61]. About 10% of pediatric constipation cases are caused by slow transit constipation [62].

### **Diagnosis of FC**

As the aetiology of constipation is multifactorial, the treatment of constipation requires a clear understanding and a differential diagnosis of its clinical causes [63]. Apart from physical examination (abdominal and rectal examinations), imaging and other tests may help to measure a number of parameters that can be very helpful in diagnostics. Traditional approaches to the investigation of constipation causes include abdominal X-ray and colonoscopy which are described in more detail below.

### **Treatments of FC**

Although the general symptoms of constipation appear very similar, the underlying mechanisms are very different and call for very different therapeutic approaches. The success of treatment depends on the proper diagnostics, which remains rather challenging. Almost half of all patients with constipation

remain unsatisfied with the received treatment [58]. Diagnostics must be performed before any non-symptomatic treatment is attempted.

### **1.3 Diagnostic approaches for imaging the GI tract and its function**

Different techniques are used to evaluate and visualise the anatomy and the pathology of the gastrointestinal tract to obtain the correct diagnosis and also to study transit time and motility in the gut. Some of these modalities will be explained briefly.

#### **1.3.1 X-ray**

Abdominal non-contrast X-ray radiography can be used to differentiate the accumulation of any usual or unusual gas and also the levels of air fluids and calcifications. With reference to FC, abdominal X-ray provides only limited information but can detect bowel obstruction and point to its potential causes. In addition, inflammation, emptying and pathological changes like stenosis, peristalsis and neoplastic changes can be imaged using positive and negative oral luminal contrast agents [64]. Some centres use X-ray radiopaque markers to assess transit times though this provides an ionising radiation exposure to the patient, which is particularly not desirable in paediatrics.

### **1.3.2 Gamma scintigraphy**

Gamma scintigraphy is an imaging modality used in visualising the upper GI. It is considered to be the gold standard examination for assessing gastric emptying with the use of radiolabelled test [65]. It is a simple, easy test that produce quantitative data on gastric emptying and GI transit with the use of small amount of ionising radiation [64]. Both scintigraphy and single photon emission computed tomography (SPECT) are used for transit and gastric emptying studies of the GI tract. Radiolabelled white cell scanning using  $^{111}\text{-indium}$  or  $^{99\text{m}}\text{Tc}$  has been used in evaluating inflammation and assessing disease extension in IBD [66-68].

### **1.3.3 Ultrasonography**

Abdominal ultrasonography (US) is an imaging technique that uses transmission and reflection of ultrasound waves to visualise disturbed wall morphology, wall thickening, lymphadenopathy, and oedema in inflammatory bowel diseases. US provides good soft tissue capabilities. US is considered to be a safe technique as no radiation exposure is used. Moreover, Doppler imaging techniques can be used to examine the perfusion of the intestinal wall and the surrounding tissues [64]. In addition, transabdominal US and colour Doppler US have been used to acquire qualitative and quantitative information regarding intestinal motility and gastric emptying [69]. Although US is a non-invasive technique and does not disturb the normal motility of the GI tract, it is an operator-dependant technique, and the views are limited by intestinal gas.

#### **1.3.4 Endoscopy**

Gastrointestinal endoscopy is a diagnostic and therapeutic modality that allows visualisation of the gut mucosa in combination with histology from biopsies. Colonoscopy is a version of endoscopy and involves insertion of flexible tube with a camera into colon through the anus. Colonoscopy allows visual examination of colon wall and can detect mucosal diseases and conditions such as polyps, haemorrhoids, ulceration, cancerous growth, anal fissures, and other physical barriers that prevent the normal movement of faecal mass. The removal of polyps (polypectomy) can also be performed during colonoscopy. The procedure allows visual examination of the whole length of colon. It is an initial tool to diagnose and classify different types of inflammatory bowel disease (IBD) [70]. It is also an important modality in assessing treatment responses and therapeutic outcomes. Colonoscopy does not allow quantitative measurement of many important diagnostic parameters such as colonic volume, colon wall thickness (that may indicate the presence of transmural inflammation), GI motility, and transit time. The procedure is performed when the colon is empty, which means that the functioning of colon in normal conditions cannot be assessed with this method. Besides, being an invasive procedure, colonoscopy is not entirely risk-free and can cause perforation of colon wall and bleeding [71]. A number of more recently developed methods can address these shortcomings [72]. Therefore, cross sectional imaging is required like computed tomography (CT) and MRI.

### **1.3.5 Computed tomography**

Abdominal computed tomography examination is used to assess the abdominal viscera [73]. Non-contrast enhanced CT allows the detection of free intra-abdominal air and intestinal obstruction [64]. Additionally, contrast materials in CT are used to enhance imaging of the bowel wall which is very useful in inflammatory bowel diseases [74]. Non-contrast and contrast enhanced CT scanning has been used to provide cross sectional images of the intestinal loops. The main advantage of modern CT scanners is that they permit fast acquisition of thin slices which reduces the motion artefacts; the images can then undergo multi-planar reconstruction in any direction. This has led to the development of dedicated protocols and two and three-dimensional reconstructions that are commonly termed 'virtual colonoscopy' yielding a minimally invasive examination of the lower intestine. Bowel preparation is required and this can be achieved with preparations delivered orally (enterography) or via rectal catheter (enteroclysis). The disadvantage of this modality is the use of ionising radiation [75].

### **1.3.6 Magnetic resonance imaging**

MRI is a medical imaging modality that provides excellent soft tissue contrast and spatial resolution compared to other modalities. Newly developed fast sequences and modified techniques have overcome the long acquisition time in GI MRI [76]. High resolution and better image quality using modified protocols and new sequences can enhance patient diagnosis [77]. This makes it the technique of choice in repeated scanning, also because of the lack of

radiation exposure. The three-dimensional capability of MRI also provide MRI ‘virtual colonoscopy’ methods, either as enterography or enteroclysis, using liquid or gaseous luminal contrast to distend the bowel lumen. Due to the relevance to this work, the MRI technique will be described in more detail below in Chapter 2.

### **1.3.7 Tracking systems**

Magnetic tracking system is the approach of using an external matrix of magnetic sensors to track the movement of an ingested small magnet through GI system [78]. Without exposure to any radiation, this technique allows reliable measurements of gastric, colonic, and whole gut transit time with good temporal resolution [79]. Magnetic tracking has also been used to investigate the specific details and the dynamics of the colonic propulsion [80]. Magnetic marker monitoring systems can be used not only for studying the transit through the GI tract but also for establishing the fate of oral dosage forms inside it. This is important for targeting delivery of drugs to specific parts of the gastrointestinal tract, including the colon. This method is limited as it provides no structural or diagnostic information about the gut, it provides only transit times. The use of X-ray with radiopaque markers provides some anatomical information but, as mentioned above, it also delivers a dose of ionizing radiation to the patients.

## **1.4 Aims and hypothesis**

Analysis of the literature showed that imaging of GI function in paediatric diseases of the GI tract is often neglected due to limitation of current modalities, particularly the radiation dose associated with nuclear medicine, X-ray and computed tomography. Knowledge of healthy gastrointestinal function in children is also limited for the same reasons. MRI offers a non-invasive, non-ionising alternative to such methods and its use has been increasing in studies of GI function in adults. To date its use in the paediatric population is however very limited and there is a clear unmet need.

Therefore, the overarching hypothesis underpinning this work was that new MRI methods and endpoints can be developed and deployed to study gastrointestinal function in paediatric health and disease.

Specifically, this work aims to:

1. Translate adult MRI measurement protocols of gastric emptying and small bowel motility to paediatric patients.
2. Evaluate safety and feasibility of new MRI methods to measure whole gut transit time in paediatric constipation.
3. Develop quantitative measurements of colon volume and colon length in paediatric health and constipation.
4. Develop and validate a protocol for quantitative MRI measurements of gastric emptying in the upright position.

## 1.5 Thesis outline

All the work described in this thesis was conducted by the author, except where specifically credited to collaborators, at the Sir Peter Mansfield Imaging Centre, the School of Medicine, the Nottingham Digestive Diseases Centre (NDDC) at the University of Nottingham, the Queen's Medical Centre (Nottingham University Hospitals NHS Trust) and the NIHR Nottingham Biomedical Research Centre (BRC) from 2018 to 2022. The research described in this thesis is original, unless otherwise stated.

The layout and content of the thesis chapters are as follows:

- Chapter one:** Introduction to the GI tract, its functional responses to feeding and imaging methods for its investigation.
- Chapter two:** MRI methods for measuring the GI tract's anatomy and physiology.
- Chapter three:** Quantitative measurements of gastric emptying and small bowel motility in paediatric patients with Crohn's disease.
- Chapter four:** Quantitative measurements of whole gut transit time in paediatric constipation.
- Chapter five:** Quantitative measurements of colon volumes and length in paediatric constipation.
- Chapter six:** Quantitative measurements of gastric emptying in the upright position.
- Chapter seven:** Overall discussion, impact of this work and possible future directions.

## **2 MRI FOR MEASURING GI TRACT ANATOMY AND PHYSIOLOGY**

This section provides a description of the basic principles behind MRI, and how to obtain an MR image. A summary of the literature on use and advantages of MRI in studying GI tract anatomy, physiology and disease is also presented.

### **2.1 Magnetic resonance imaging**

In 1945 two physicists, Felix Bloch and Edward Mills Purcell, discovered Nuclear Magnetic Resonance (NMR) at the same time. This research paved the way for the subsequent discovery of how to create images. In 1973, Sir Peter Mansfield in Nottingham and American chemist Paul Lauterbur, who was based in Illinois, independently made the discovery of MRI. The technique was initially known as NMR imaging, the later changed to MRI to differentiate it more from the nuclear medicine field. They shared the 2003 Nobel Prize in Physiology or Medicine in recognition of their work on magnetic resonance imaging. Mansfield reported the first cross-sectional MRI image of a live human abdomen in 1978 in Nottingham while using an entirely home-made scanner. In the 1980s, the first MRI systems for commercial use started to become available.

#### **2.1.1 Basic principles about MRI**

The hydrogen nucleus is the primary MR active nucleus used in for imaging because of its abundance in the body and its strong magnetic moment. The proton has an innate magnetism known as spin. The Larmor frequency, which is inversely proportional to the strength of the magnetic field, is the frequency

at which the spin magnetization rotates around a magnetic field in which the spin is placed.

The protons in hydrogen atoms begin to align parallel to the magnetic field  $B_0$  when a patient is positioned in the MR scanner's magnet. The precession of the proton spins exhibits phase coherence when a radiofrequency (RF) signal tuned to the hydrogen atoms' Larmor frequency is transmitted from an aerial (or coil) within the scanner. When the pulse transmission is completed, the excitation then yields a NMR signal which is picked up by the receiver coil as the magnetisation changes and cause an electric current to flow through the coil via Faraday induction.

The signal is attenuated by two relaxation processes identified by a respective time constant: T1 the spin-lattice relaxation time and T2 the transverse relaxation time. The T1 longitudinal (spin-lattice) relaxation time is a measure of the time taken for the net magnetic vector to recover to its ground state in the direction of  $B_0$ . At the same time, spins are dephasing from their aligned precession in the transverse plane, and the time constant of this process is termed the T2 transverse (spin-spin) relaxation time. Different types of tissues in the body have different T1 and T2 relaxation times. Differences in the T1 and T2 characteristics of a sample are captured by allowing a specific T1 or T2 weighting in the MRI imaging sequence, which yields the image contrast. Excitations and signal detection can be organised in complex sequences, which have some fundamental descriptive parameters. The repetition time (TR) is the time between the applications of the RF excitation pulse to the application of the next pulse. The echo time (TE) is the time between the application of the RF excitation pulse and the peak of the induced signal in the

receiver coil (Figure 2.1). If the TE in a T2 weighted sequence is very long, only signal tissues with very long T2 relation times such as fluid will be measured [81].

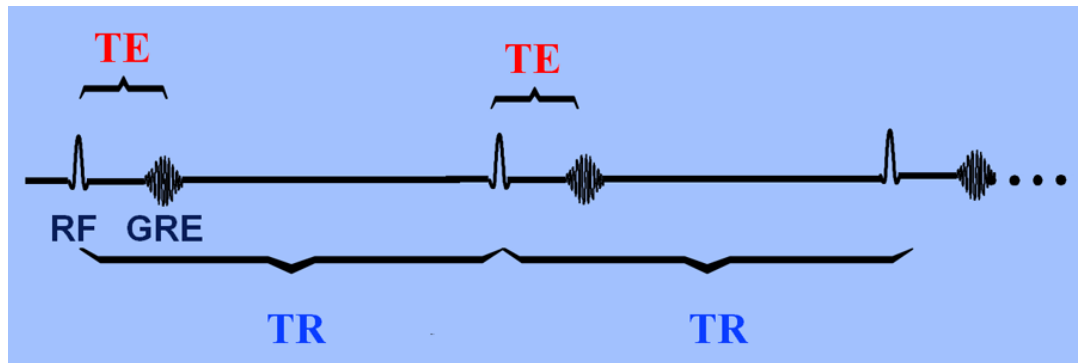


**Figure 2.1:** Diagram of a basic MRI pulse sequence showing repetition time (TR) and echo time (TE) [82].

The principal components of an MRI scanner are the magnet, RF coils, gradient coils, waveform controller and control computer. The quality of an MR image depends also on having a uniform strong static magnetic field.

The RF amplifier transmits radiofrequency energy into the patient and then detect and process the resulting MR signal from the patient's body using transmit and receive RF coils. The main body RF coil is used as the transmitter coil and is commonly located around the bore of the scanner. The receive coil is positioned as closely as possible to the body part being imaged.

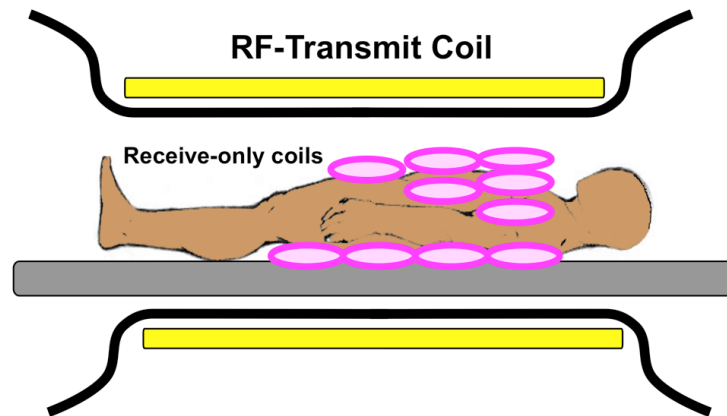


**Figure 2.2:** The location of RF coil in the MR scanner showing that the RF transmit in the scanner bore and a receive-only body array coil around the patient. cited from <http://mrquestions.com/receive-only-coils.html>

For the purpose of encoding into the MR signal a spatial information, magnetic field gradients are essential. They alter the primary magnetic field so that it changes linearly with spatial position inside the bore. Gradient coils are wire coils that are subjected to high currents (hundreds of Amperes) flowing at frequency of kilohertz, which alter the magnetic field strength in a precise manner. The isocentre is at the centre of all three gradients, aligned with the z, y, and x axes. The three orthogonal directions give rise to the names  $G_x$ ,  $G_y$ , and  $G_z$  for these. These gradients cause the nuclei to experience a slightly different total magnetic field at various points along the gradient direction, which in turn causes them to precess at a slightly different frequency this then is exploited to allow spatial localization for image reconstruction.

In slice selection, an RF pulse with a specific range of frequencies is applied at the same time with a slice select gradient ( $G_z$ ). The strength of the  $G_z$  gradient and the RF pulse will affect the slice thickness and thus only spins within this slice are tipped through 90 degrees into the x-y plane. The slice

thickness can be decreased by decreasing the bandwidth of the RF pulse or increasing slice selection ( $G_z$ ) gradient. Changing the carrier frequency of the RF pulse will result in a different slice position. Slice selection is shown in Figure 2.3.

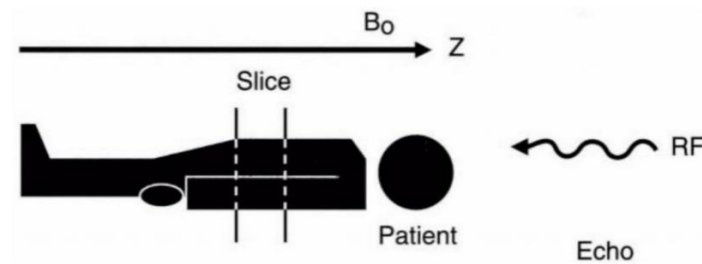


**Figure 2.3:** Selecting a slice of a certain thickness [83].

After selecting the slice, a phase encoding gradient ( $G_y$ ) is applied before the readout gradient and signal acquisition. When the  $G_y$  gradient is switched on the protons are spatially encoded based on higher and lower resonant frequencies in the y-direction. When the  $G_y$  gradient is switched off the protons return to the original Larmor frequency keeping their phase.

During the signal acquisition, the frequency encoding gradient ( $G_x$ ) also known as the readout gradient is applied making the protons with the higher parts of the field gradient to resonate at a higher frequency than the protons in the lower parts. Therefore, the range of frequencies will be only acquired from the known spatial location in that case it will be from the x-direction.

The spatial frequency information is stored in a k-space matrix, acquired using the frequency and phase encoding gradients. The information from the phase encoding with the frequency encoding gradients will fill a single row of k-space.

Afterwards, the image is created by the inverse Fourier transform of the k-space matrix.

### **2.1.2 MRI and GI diseases**

The non-invasive and non-ionising nature of MRI coupled with the very flexible contrast ability rapidly revolutionised diagnostic radiology. However, applications of MRI to the abdominal region lagged behind other areas, particularly when looking at organ function. This is particularly due to the complexity of the GI tract anatomy and motion artifacts coming from both respiration and GI motility. However, the past decade advances in MRI have allowed many of these limitations to be overcome and for MRI to become the modality of choice for many GI diseases. The paragraphs below summarise briefly some of the MRI applications and benefits in selected GI diseases relevant to this work.

#### **Crohn's disease:**

Magnetic resonance imaging provides a complete view of the bowel and allow comprehensive examinations of the bowel wall and nearby extramural spaces [84, 85]. In adult studies, MRI has shown to be able to measure both disease activity, intestinal motility [38, 86] and whole gut transit [87]. The 'classic' MRI assessment of small bowel motility involves however ingestion of a large drink containing osmotic laxative to draw fluid in the small bowel and distend it before MRI imaging. This can be unpleasant and is likely to disturb normal physiology.

So far, established protocols for using MRI in CD diagnosis follow similar patterns. Generally, adult patients are made to ingest a biphasic contrast

medium about an hour before the MRI scan. Spasmolytics are usually used to decrease bowel motility and improve resolution. T2-weighted imaging with balanced steady-state free precession is typically preferred as it helps with fat suppression and allows for better visibility of the bowel wall inflammatory changes [18].

Many newer MRI techniques are increasingly being incorporated into clinical practice. Diffusion-Weighted Imaging, for example, is routinely used in the fields of oncology and neurology but has recently seen application in more fields. It has been shown in various studies to be useful for detecting bowel inflammation in adult CD patients, including paediatric cases [88, 89] .

Children present unique diagnostic challenges for CD in that the risks of radiation exposure and procedural complications are greater than in adults. Complications of colonoscopy in children include bleeding, cardiopulmonary complications due to sedatives and perforation.

MRI has become an established tool in monitoring CD especially in children [84, 85, 90]. MRI allows physicians to side-step most of these problems while still delivering excellent diagnostic value. MR enterography is also used to characterise bowel findings in children with IBDs and it was concluded that the technique “provides excellent bowel detail without the need for ionising radiation [91]”. The technique enables the examination of acute or chronic inflammation of the bowel wall, as well as its complications [91].

MR enterography allows specialists to view a child’s small bowel with good tissue contrast and, in the past decade, has become the standard imaging technique used to evaluate disease distribution, disease activity, and

progression of extraintestinal manifestations. Several features that appear on MR enterography are highly indicative of CD in children. These include bowel wall thickening to  $>3$  mm, oedemas, hyperenhancements, and ulcerations. Active CD will also show perienteric oedemas, lymphadenopathy, penetrating disease complications, and engorgement of the vasa recta. It is rare that the presence of only one or two of these factors have been tested for diagnostic accuracy. Typically, a constellation of multiple, co-occurring features will lead to a CD diagnosis [92].

The subsequent literature has confirmed these findings and improved on the technology. In a study MR enterography was compared to the gold standard histopathology in paediatric IBD patients. The study found that when compared to ileocolonoscopy, the radiographic technique delivered good sensitivity and specificity for detecting active bowel inflammation [93]. Another study showed that bowel distension was predominantly adequate in the participants, and that MR enterography is a feasible procedure to perform in a paediatric population, with performance estimates similar to that in adults [94]. In addition, the value of MR enterography in detecting lesions in the small and large bowels in children with known CD was also investigated. In that study, the performance of MR enterography was compared against reference techniques, such as ileocolonoscopy, barium studies, intestinal ultrasounds, and capsule endoscopies, which were performed within 15 days of MR enterography. The sensitivity and specificity values of MR enterography were 94.5% and 97%, respectively [95].

MRI is increasingly being implemented to measure CD activity through intestinal motility as noted in previous studies in adults. Small-bowel motility

and gut peptide responses to a standard test meal in CD by using MRI were investigated. There was a reduction in fasting small-bowel motility noted in adult CD patients which can be attributed to the increased fasting gut peptides [96].

MRI is also a versatile tool for many physiological parameters that can be monitored in a single scanning session. An optimised analysis technique using registered cine MRI datasets was deployed to assess adult small bowel motility in the physiological fasting and postprandial states. The method showed good concordance between measurements of intra- and inter-observers. The technique was also sensitive to changes in motility elicited by ingestion of a meal. Thus, this technique can also be used to study contractile activity and regional patterns throughout the gastrointestinal tract under physiological situations [22]. A study of eighty-two adult patients with CD who underwent colonoscopy and MR enterography was done to evaluate the accuracy of MRI-quantified small bowel motility for CD activity against endoscopic and histopathologic reference standards. The terminal ileal motility score in that study showed concurrence with endoscopic and histopathologic activity in CD [97]. Thus, the MRI technique to measure motility in CD patients is acceptable.

Moreover, fast T2-weighted MR imaging allows for multiple images within a single breath-hold, providing clinicians with high-resolution views of bowel motility. MRI can detect the changes in motility correlated well with biopsy-documented changes in the terminal ileum in adult CD patients [98].

To this date there are limited MRI studies investigating gastrointestinal function in children with CD although they often present with lack of appetite and early satiety. Ideally one would study the function of the undisturbed, unprepared gastrointestinal tract, not after the ingestion of a large volume of oral contrast media. Initial studies in adults in Nottingham developed a protocol to measure small bowel motility after a physiological meal stimulus. The protocol involved simply eating a soup meal and measuring the motility of the small bowel using MRI, without using any laxative drink [42, 96, 99]. Those experiments were successfully carried out in adults, both healthy and suffering from CD but to date there is no such experiment in children. Here this unmet need will be addressed and that type of protocol will be translated to the paediatric arena.

#### **Functional constipation:**

Functional constipation is common, and MRI would be particularly suited to study the function of the constipated bowel. However until recently only a small amount of such research was done with MRI. MRI has studied adults with constipation particularly looking at gut transit and colon volumes, and this will be described briefly below.

GI transit can be measured with MRI using capsule markers filled with water and/or a contrast agent, such as gadolinium (Figure 2.4). Measurements taken using these markers showed good correlation with the results obtained by other methods. MRI of the whole gut transit time was measured using marker capsules dimensions of 20 mm x 7 mm filled with gadolinium-based contrast agents was compared against the radiopaque markers technique [87]. The correlation coefficient  $r$  between two sets of measurements was 0.85, showing good correlation between the two techniques. A different group measured the

whole gut transit time in two groups of participants, one with normal gut function and one with slow transit constipation [100]. Both radiopaque markers and gadolinium-based marker capsules were used. The correlation coefficients between these two methods were 0.88-0.89 for both groups of participants, suggesting again that MRI can provide a good estimate of GI transit time in both healthy participants and patients with constipation.

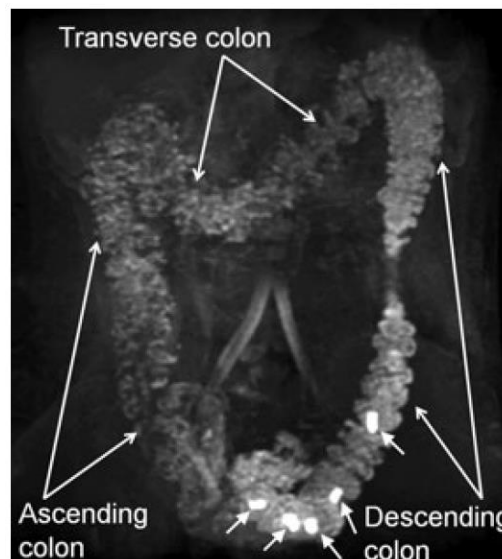


**Figure 2.4:** Example of magnetic resonance imaging (MRI) image showing five MRI marker capsules in the colon (indicated by close arrows [87].

An interesting alternative to standard water proton MRI imaging of intestinal transit, is the tracking of  $^{19}\text{F}$ -labelled capsules. The length, diameter, inner volume, and density of the capsules were 11.5 mm, 7.2 mm, 40mL, and 2.15 g/mL, respectively (Figure 2.5). Fluorine-based imaging methods provide relatively good sensitivity compared with methods based on the detection of  $^1\text{H}$ . Taking into account that natural occurrence of fluorine in human body is close to zero, the detection of fluorine signal allows for positive detection and contrast. In one study [101], the movement of fluorine-containing capsules ingested with a meal was monitored in participants over extended time periods

(up to 1–1.5 hour) while they remained in a supine position inside the scanner. This approach, in combination with high resolution anatomical reference scans, allowed accurate visualisation of the capsules journey inside the lumen.  $^{19}\text{F}$  MRI however requires dedicated receiver coils and multinuclear transmit/receive hardware which are not common and mostly based in a few research laboratories.

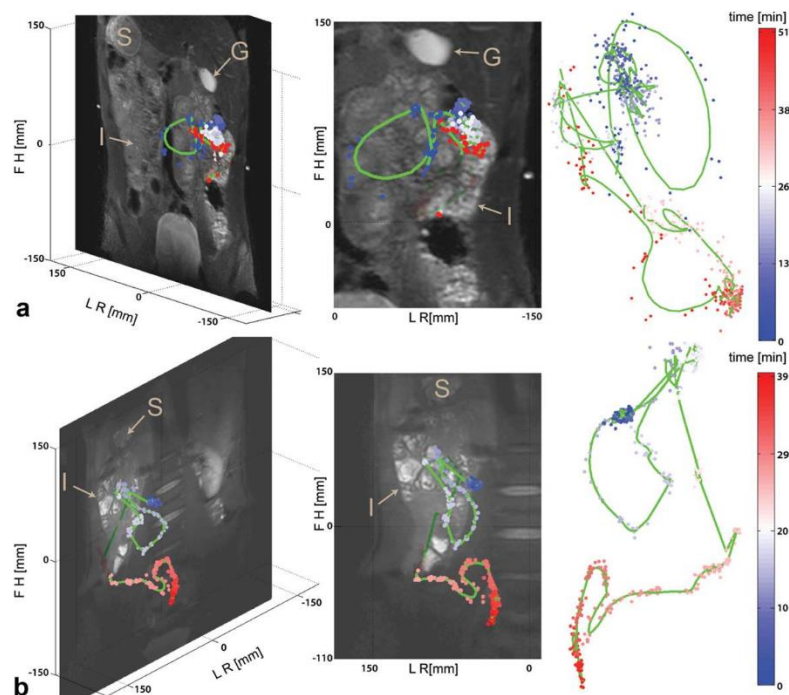


**Figure 2.5:** Example of Anatomical reference images of  $^{19}\text{F}$  capsule positions and the fitted intestinal course for (a) subject A and (b) subject B. Stomach (S), gall bladder (G) and small intestine (I) are denoted in the figure. The colour code reflects the time course of the two capsules [101].

In addition to GI transit time, one can visualise anatomy, measure colonic volume, colonic wall movement (motility), freely mobile water content, chyme relaxometry, gas content and abnormalities of colonic wall thickness [102-105]. Recently, a MRI tagging technique helped to visualise the movement of colonic chyme in subjects with and without constipation [58].

These new MRI methods have been developed in recent years. There are however still limitations associated with them and barriers to adoption in the clinical arena. One limitation is the need to process a large volume of data collected during a single scanning session. Manual segmentation of data is still commonly used, thus making the interpretation of findings technically challenging, time consuming and dependent on skilled operators [102].

## **2.2 Unmet needs in paediatric GI MRI**

Based on the brief review of the field made above there are still many unmet needs to bring more MRI to the field of study of paediatric GI disease, for example using food stimuli for bowel motility, new methods to look at transit and colon morphology, which the work described in the following chapters will address.

### **3 QUANTITATIVE MEASUREMENTS OF GASTRIC EMPTYING AND SMALL BOWEL MOTILITY IN PAEDIATRIC PATIENTS WITH CROHN'S DISEASE (MICP STUDY)**

Gastrointestinal motility in children is a largely unexplored area, particularly in gastrointestinal diseases where motility disturbances can be hypothesised, such as in CD. Building on previous work carried out in adult CD, this feasibility study aimed to translate MRI measurements of gastrointestinal function, and in particular of gastric emptying and small bowel motility, to the paediatric population. The first phase of the work developed the protocols, obtained ethics approvals, and run a study in healthy children. This assessed tolerance and acquired and analysed initial data in health. The second phase started to investigate children with CD.

#### **3.1 Introduction**

As previously mentioned in Chapter one, CD is a recurring, often chronic disorder of the gastrointestinal system, characterised by pockets of inflammation across the length of GI tract. It was estimated that CD affects 115,000 individuals in the UK alone, 33% of these cases being diagnosed in children and young persons below the age of 21 [19]. However a recent Nottingham study commissioned by the charity Crohn's and Colitis UK estimates that the prevalence is much higher, with over half a million patients living with CD or ulcerative colitis in the UK, or 1 in 123 people [106]. Features of the disease in children include also perianal infections, abdominal pain, diarrhoea, fatigue, loss of appetite, weight loss and malnutrition. CD is thought to affect also the overall function of the gastrointestinal system with high

prevalence of gastrointestinal motility disorders. Whilst gastrointestinal function has been studied in adult patients, little is known in children with CD, also due to the invasiveness or use of ionising radiation of current techniques.

The Nottingham GI MRI research group has developed non-invasive MRI techniques to assess post-prandial gastric emptying and small bowel motility in adult patients with Crohn's disease using a test meal challenge.

The purpose of this study was to translate for the first time this new MRI technique to a paediatric population, with a view to study paediatric CD. These unprecedented data will help to link gastrointestinal responses with symptoms, increasing knowledge of mechanisms of CD and providing objective biomarkers for future clinical trials of novel therapies.

The primary objective of the study was to develop the protocols and collect for the first time MRI data on the gastrointestinal response to the meal intervention in healthy children. The second objective was to collect data in paediatric patients with CD with these MRI techniques and to gain initial experience of using the same protocol with paediatric patients receiving enteral feeding (Modulen) for their CD.

### **3.2 Methods**

This study was sponsored by the University of Nottingham (Protocol number 20070) approved by the Health Research Authority (HRA), IRAS project ID: 284952, London - Queen Square Research Ethics Committee reference 20/PR/0971. All participants gave informed written assent/consent.

### **3.2.1 Study Participants**

Thirteen healthy volunteers (age  $12.4 \pm 3.2$  years, BMI  $21 \pm 5$  kg/m<sup>2</sup>) were recruited from the local campus population. At the time of writing, the first two patients with CD (age  $14.5 \pm 1$  years, BMI  $26 \pm 1$  kg/m<sup>2</sup>) were also recruited through specialist clinics at Nottingham University Hospitals NHS Trust. Eligibility criteria are shown below:

#### **Inclusion criteria (Healthy volunteer participants)**

- Aged 6-18 years, healthy with no previous known bowel disease.
- Able to provide informed consent or assent and parental/guardian consent if under 16yrs old.

#### **Inclusion criteria (Crohn's disease participants)**

- Aged 6-18 years with diagnosis of CD.
- Able to provide informed consent or assent and parental/guardian consent if under 16yrs old.

#### **Exclusion criteria**

Potential participants with any of the following criteria were to be excluded:

- Surgical Complications of CD (e.g. structuring disease)
- Any History of gastric surgery or bowel resections
- Respiratory disease or significant cardiovascular
- Thyroid disease
- Current Infection
- Neurological or cognitive impairment
- Renal failure or significant hepatic disease

- Use of any medication that may affects gastric emptying or small bowel transit (e.g. proton-pump inhibitor, hyoscine butylbromide or mebeverine) in the previous two weeks
- Contraindications for MRI scanning i.e. metallic implants, pacemakers, history of metallic foreign body in eye(s) and penetrating eye injury.
- Female participants who are pregnant during the course of the clinical investigation.
- Inability to lie flat and relatively still for less than 5 minutes
- Participants who have participated in another research clinical investigation involving an investigational product in the past 12 weeks.
- Dislike of the test meal.

### **3.2.2 Test meal**

- Cream of chicken soup (400g) (or mushroom for vegetarians) (Heinz, Wigan, UK) (Figure 3.1). The nutrient content of these meals/100g are: energy (kcal) 51, protein (g) 1.5, carbohydrate (g) 4.7, fat (g) 2.9 [28]. This test meal has been previously validated in experimental work in *Giardia enteritis* [107], CD [28] and in previous work in healthy adult participants [99].



**Figure 3.1:** Image of the cream of chicken soup and cream of mushroom soup meal used in the study (400g, Heinz, Wigan, UK).

- Modulen® (Nestlé, Vevey, Switzerland) (Figure 3.2) 6 scoops (50g) in 210ml of water, total of 250ml volume of water. The nutrient content of 100g: energy (kcal) 99, protein (g) 3.5, carbohydrate (g) 11, fat (g) 4.6.



**Figure 3.2:** Image of the Modulen, used in the study (Nestlé, Vevey, Switzerland).

### 3.2.3 Study design

This study had an open-label design. Participants were asked to be fasted since their dinner the night before but were allowed to drink a small glass of water on waking. On the day of the scan, the participants underwent a baseline fasting scan at approximately 0900 hours (defined at T = -30 min time point). Twenty-five minutes later they were asked to eat their test meal either Cream

of chicken soup (400g) (or mushroom for vegetarians) (Heinz, Wigan, UK) or Modulen® (Nestlé, Vevey, Switzerland) 6 scoops (50g) was prepared and administered by the parents or the young participants as their usual feeding treatment routine within a maximum time of 20 min so that at 0945 hours the subjects then underwent a first immediate postprandial scan (defined as  $T = 0$  min). This was followed with data collection (MRI and Visual Analogue Scale (VAS) questionnaire for appetite, satiety and abdominal pain) time points every 30 min up to 120 min.

At each time point, the positioning of the subject, setup and data collection took approximately 15 min. In between scans, participants were kept sitting upright in a quiet lounge next to the scanner (Figure 3.3). At each time point, the participants were asked to fill a symptoms questionnaire with the help of their parents or the researcher. Participants were offered refreshment (e.g. a snack and a water drink) at the end of the study.



**Figure 3.3:** The paediatric participants' lounge at the Sir Peter Mansfield Imaging Centre at the University of Nottingham.

The study day flow diagram is illustrated below in Figure 3.4.

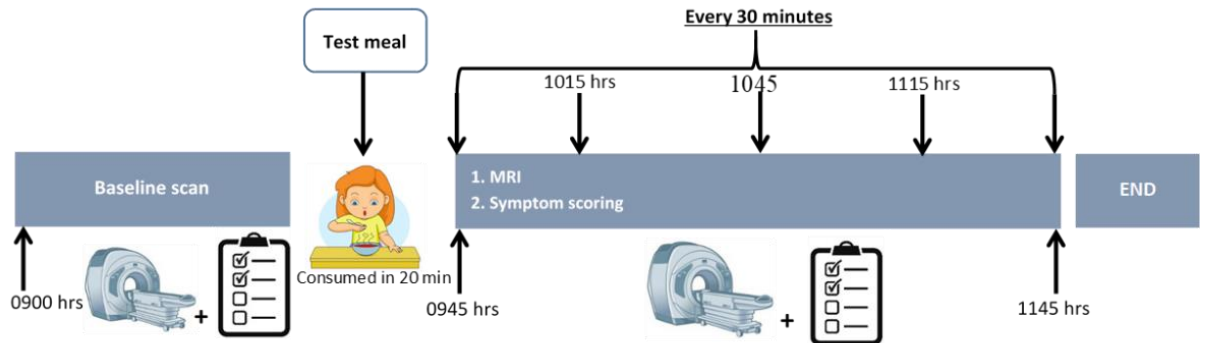


**Figure 3.4:** Flow diagram of study daytime points.

### 3.2.4 MRI protocol

MRI scanning was carried out by the Author as a scanner operator using a 1.5T GE HDxt MRI scanner (Figure 3.5). Participants were placed supine with 12-element abdominal receiver wrapped around the abdomen and scanned using a range of MRI sequences which are detailed in Table 3.1. The study consisted of 6 time points 30 minutes apart. At each time point the following assessments were performed:

- Gastric emptying [108] and gallbladder volumes were assessed on the same image stack using a FIESTA sequence acquiring 40 contiguous axial slices within one breath hold. This sequence provided good contrast between the stomach contents and other abdominal organs.
- Small Bowel Water Content was assessed as previously described and validated [109, 110] using a single-shot fast spin echo (FSE) sequence acquiring 32 contiguous coronal slices within four breath hold.

- Small bowel motility was assessed using a multi-slice Cine MRI acquisition comprising six contiguous parallel coronal planes through the small bowel (acquired in 3 stacks, to capture all the bowel loops). This data was acquired across the abdomen using a FIESTA sequence taking approximately 3 minutes to acquire.



**Figure 3.5:** 1.5T GE HDxt MRI scanner held at the Sir Peter Mansfield Imaging Centre at the University of Nottingham.

**Table 3.1:** Details of the 1.5T GE HDxt MRI sequence parameters used in this study.

| <i>MRI sequence</i>                    | <i>FIESTA</i>              | <i>FSE</i>                 | <i>FIESTA cine</i>                 |
|----------------------------------------|----------------------------|----------------------------|------------------------------------|
| <i>Image orientation</i>               | Axial                      | Coronal                    | Coronal                            |
| <i>Field of View</i>                   | 400 mm (RL) × 320 mm (AP)  | 400 mm (RL) × 400 mm (HF)  | 400 mm (RL) × 400 mm (HF)          |
| <i>Image stacks</i>                    | 1                          | 4                          | 1 with 60 dynamics                 |
| <i>Slices per stack</i>                | 40 - contiguous            | 8 - contiguous             | 2 - 20 mm spacing                  |
| <i>Length of breath hold per stack</i> | 16 s                       | 12 s                       | Gentle breathing, scan length 60 s |
| <i>ASSET acceleration factor</i>       | 2                          | N/A                        | 2                                  |
| <i>Signal averaging</i>                | 1                          | 1                          | 1                                  |
| <i>Flip angle</i>                      | 60°                        | N/K                        | 50°                                |
| <i>Repetition time TR</i>              | 3.2 ms                     | 1511.7 ms                  | 3.8 ms                             |
| <i>Echo times TE</i>                   | 1.1 ms                     | 326.0 ms                   | 1.9 ms                             |
| <i>Acquisition matrix</i>              | 256 × 256                  | 256 × 128                  | 256 × 256                          |
| <i>Acquired image resolution</i>       | 1.56 mm × 1.25 mm × 5.0 mm | 1.56 mm × 3.13 mm × 7.0 mm | 1.56 mm × 1.56 mm × 10.0 mm        |

### 3.2.5 Visual analogue scales (VAS)

Symptoms regarding appetite, satiety and abdominal pain were collected using a 100 mm VAS. The participants were asked to score their feeling of fullness, bloating, distension, abdominal pain/discomfort, and nausea at each time point using a previously validated questionnaire [28]. The VAS anchors for each symptom ranged from ‘not’ to ‘extremely’.

### 3.2.6 Data analysis

The assessment of gastric emptying, gallbladder volume, small bowel water content, and small bowel motility are described below.

#### Gastric emptying and gallbladder volumes

Stomach volumes were measured using Medical Image Processing, Analysis, and Visualisation software (MIPAV, NIH, Bethesda) from the axial FIESTA images. It was easy to identify the demarcation between stomach contents and air or surrounding tissues and then to manually draw ROIs around the contents of the stomach and then summing across all the volumes (Figure 3.6).

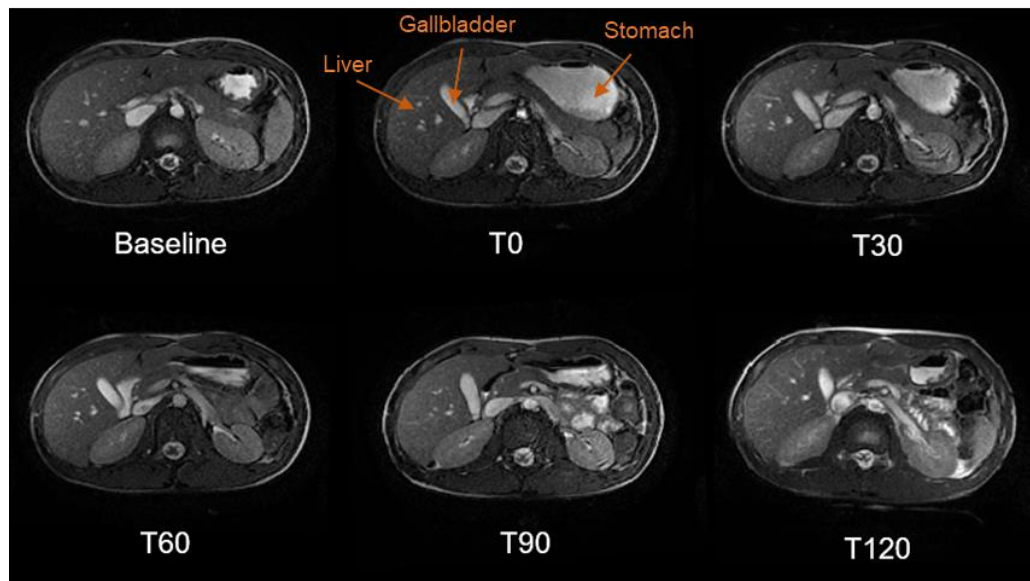


**Figure 3.6:** Illustrations of the gastric volume change before and after a meal and gastric emptying throughout study time.

Gallbladder volume was measured pre- and post-prandially, at each pre-determined time points, again from the FIESTA axial images. MIPAV was used again to draw ROIs around the GB in each visible slice (Figure 3.7).

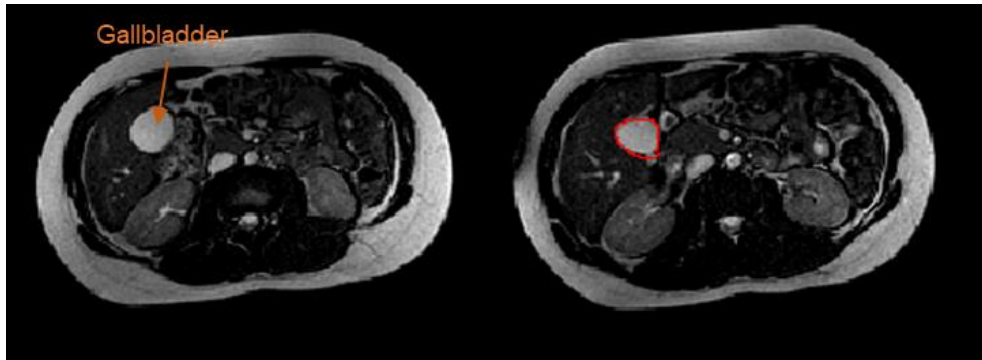


**Figure 3.7:** Illustrations of the processing analysis of gallbladder volume using MIPAV.

### **Small bowel water content**

Small bowel water content (SBWC) was assessed using an in-house software written in MATLAB (MathWorks, Natick, MA, USA) by Dr Caroline Hoad and as described previously [38] (Figure 3.8). The hypothesis behind SBWC assessment was that in the FSE images any pixel with a signal intensity above a given threshold was filled with "free" water. As this technique was based on MR signal intensity rather than measured fundamental parameters (e.g. relaxation times), the signal had to be normalised. Cerebral spinal fluid (CSF) was an ideal internal normalising marker because it is presented in most slices, and it was in the centre of the field of view.

The first step of this assessment was to select the centre of the CSF on each slice in which it was visible and then the ROIs were drawn manually around the small bowel loops and structures such as blood vessels, bladder, stomach, and gall bladder were excluded.

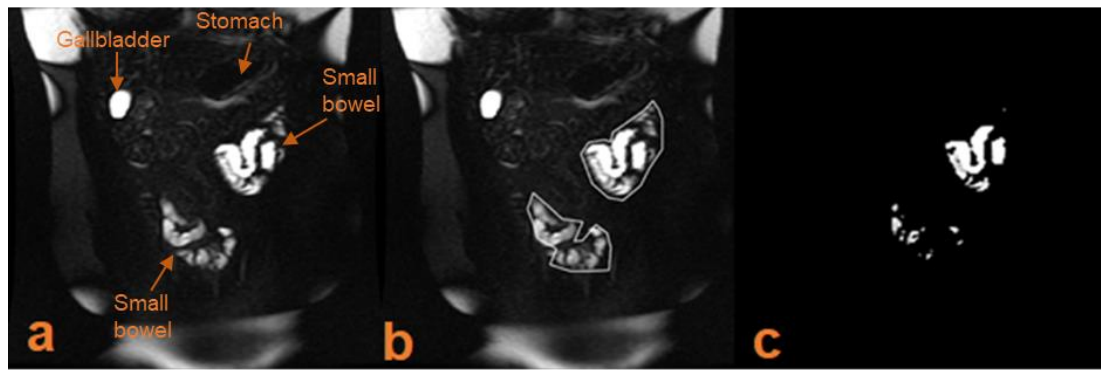


**Figure 3.8:** Example of SBWC analysis on the in-house written software. (a) The heavily T2 weighted images for a time point were loaded. Small bowel loops filled with water are visible in the abdomen as well as other structures. (b) Regions of interest are manually drawn around the small bowel loops only, excluding the other structures. (c) the software masks out all the rest of the body retaining only the desired regions of interest, before integrating all pixels above the intensity threshold calculated from the cerebro-spinal fluid.

### Motility assessment

Small bowel motility assessment was carried out in Motilent platform. Motilent Ltd. is a UK medical imaging technology company that offers imaging analysis services for the quantitative investigations of the GI tract. All anonymised dynamic data were sent to Motilent (in Digital Imaging and Communications in Medicine (DICOM) file format). The data were processed with Dual Registration of Abdominal Motion (DRAM) (Motilent, Ford, UK) techniques. DRAM first removes respiratory motion [111] before applying the optic flow registration [112] to capture local deformation caused by bowel wall motion and model intensity changes caused by bowel motion.

The Motilent platform then produces for the operator two images for each cine-MRI plane acquired: one was the reference registration anatomical MRI image (Figure 3.9 a) and one the corresponding local deformation motility map in colours (Figure 3.9 b). In the latter, for each pixel of the registered dataset a power spectrum of the intensity changes across the time series was calculated

and then summed across all frequencies. This metric was termed the 'total power' and is the MRI motility index used here. It reflects bowel motility, both in terms of segmental oscillations and bolus movement of contents which are typically seen postprandially. Each pixel value in the motility map (Figure 3.9 b) is therefore the motility index for that location. The map is then displayed to the operator using the same type of colour scheme used in conventional bowel manometric studies, whereby 'hot' red is high motility index and 'cold' blue is low motility index.

Once these maps were obtained, the main task was to segment out and calculate the overall small bowel motility index. For this purpose, the author first familiarised with the anatomy of that participant and then drew manually regions of interest (ROIs) over the small bowel loops visible on the MRI reference image for each of the 6 planes acquired at a given time point (Figure 3.9 a). The Motilent platform then copied automatically the same ROIs across onto the motility map, calculating a mean motility index and number of pixels for each ROI. All these values were then transcribed manually onto an Excel sheet to calculate the overall small bowel motility index for that time point as an average of all ROIs weighted by their size in pixels, which was termed the 'GI Quant weighted average score' in arbitrary units for that participant and time point. This process took approximately 20 minutes for each time point for each participant.

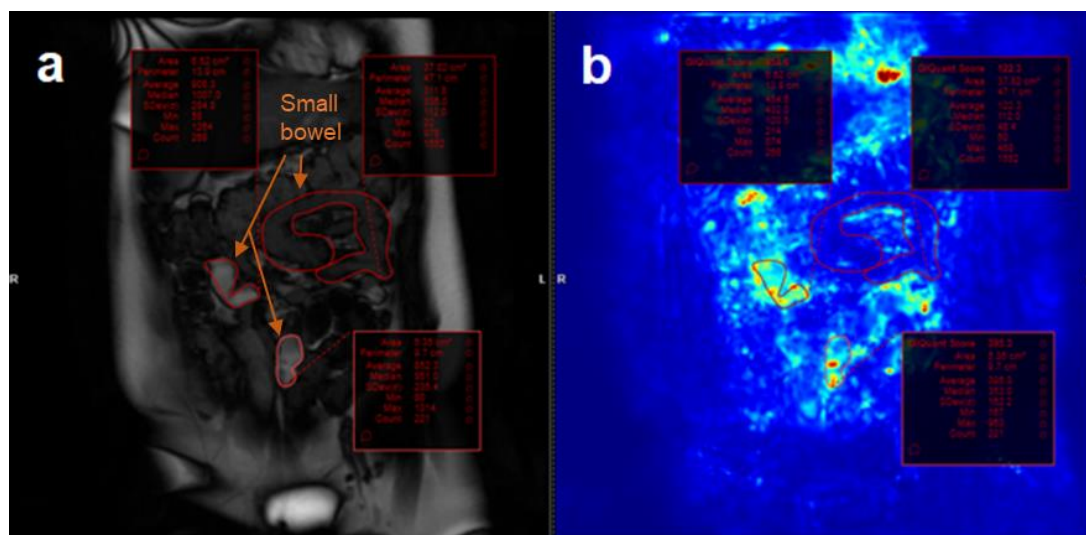


**Figure 3.9:** A screenshot of the motility assessment program (Motilent, Ford, UK). Example of a reference image (a) and a motility map (b) for a healthy volunteer. ROIs were placed on the reference image to include small bowel loops only.

### 3.2.7 Statistical analyses

The data are expressed as mean $\pm$ standard error of the mean (SEM). Normality of the data were assessed using Shapiro–Wilk’s test. Descriptive statistics and data plotting were carried out using Prism version 9 (Graph Pad Software Inc., La Jolla, CA, USA).

## 3.3 Results

All fifteen participants (4 female, 11 male, age  $13\pm 1$  years and BMI  $22\pm 1$  Kg/m<sup>2</sup>) completed the study and tolerated the experimental procedures well without any adverse event. Compliance with the study and breath holding was good and image data sets of good quality for processing were acquired throughout the study. Only on a couple of occasions a data set was repeated straight away by the scanner operator with the youngest participant (six year old) as it appeared blurred by poor breathing on the scanner console.

### 3.3.1 Gastric volumes

The baseline gastric volumes showed a small amount of resting gastric juices in all participants followed by the expected increase upon feeding. In HV the gastric volume increased from  $67 \pm 10$  mL to  $342 \pm 36$  mL. This was followed by a decline in volume as the stomach emptied, reaching baseline values at 120 min (Figure 3.10). The average time to empty half of the stomach contents ( $T_{1/2}$ ) in HV was  $53 \pm 4$  min. The time to empty half of the stomach contents ( $T_{1/2}$ ) for the patients appeared to be longer with 71 min for the patient with the soup meal intervention and 109 min for the patient with the Modulen meal.

The soup meal was accepted by the majority of the participants. One CD patient who received the soup felt that the whole amount was too much for them and managed only to consume about half of the soup volume.

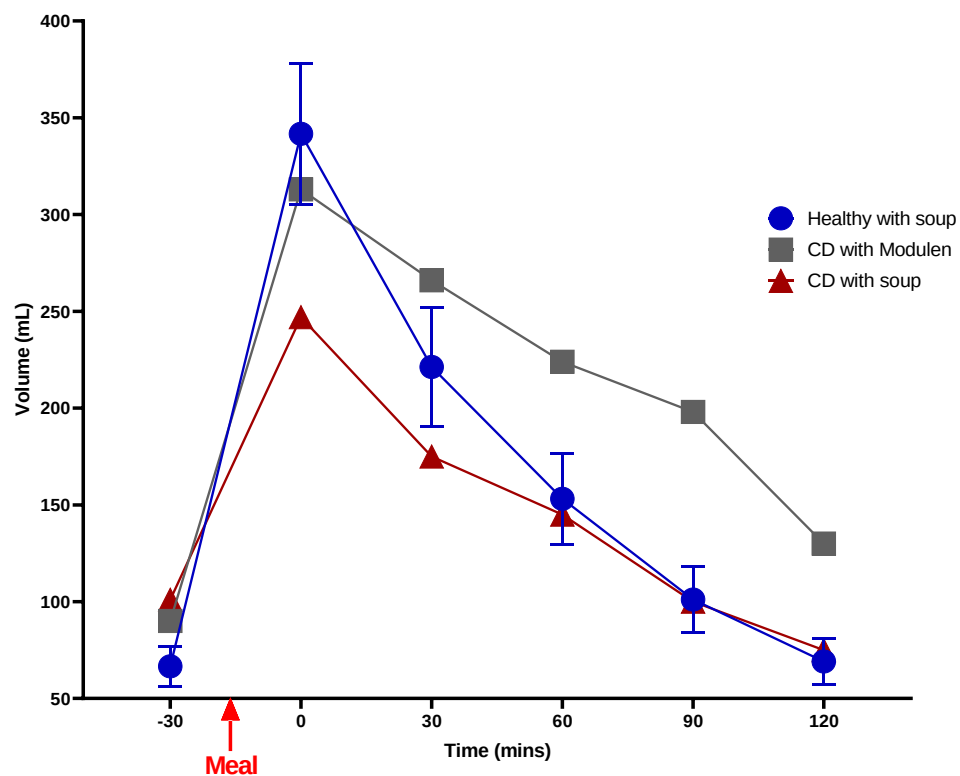


**Figure 3.10:** Time courses of gastric volumes in HV with soup, CD with soup, and CD with Modulen.

### 3.3.2 Gallbladder volumes

The gallbladder volume (Figure 3.11) decreased upon feeding in all participants and stayed stable throughout the postprandial period. The gallbladder contracted immediately upon feeding from fasting volumes of  $19\pm 2$  mL to  $10\pm 1$  mL in healthy volunteers. The gallbladder volumes after feeding were significantly lower than fasting baseline in HV with soup and in CD with Modulen. The gallbladder volume spiked high around 60 min postprandially in the CD patient who received the soup meal.

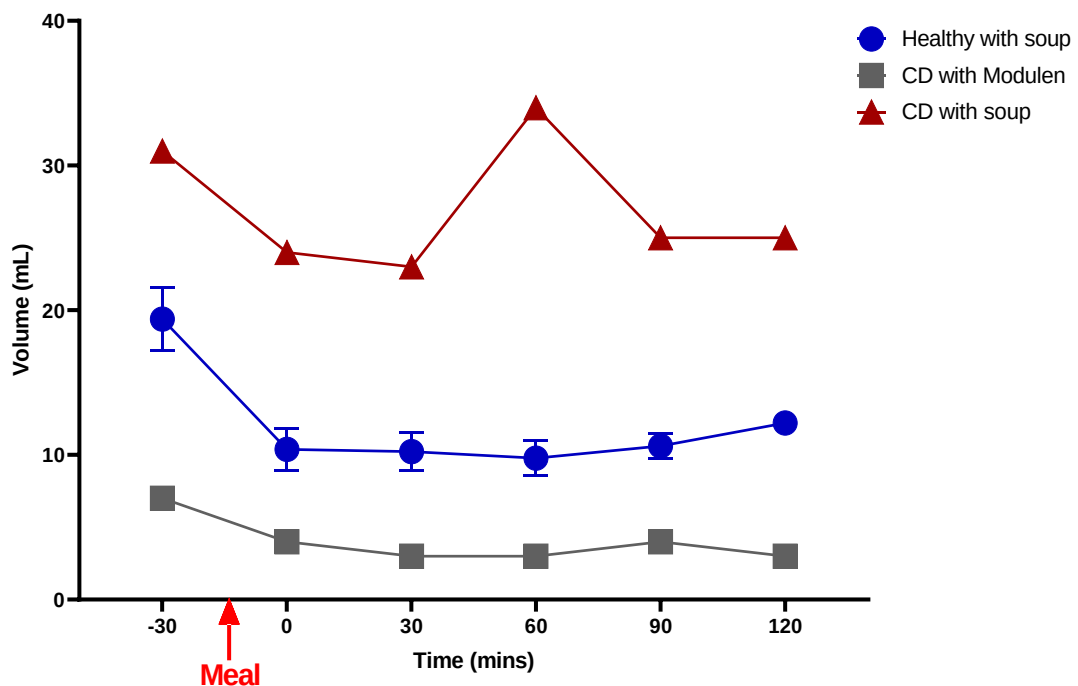


**Figure 3.11:** Time courses of gallbladder volumes in HV with soup, CD with soup, and CD with Modulen.

### 3.3.3 Small bowel water content

The data in Figure 3.12 showed a small amount of fasting small bowel water content in CD with soup HV with soup than CD with Modulen. Upon feeding, small bowel water volume in HV increased from  $17\pm 4$  mL to  $29\pm 7$  mL followed by a decrease of  $8\pm 1$  mL in 30 mins. SBWC in CD with Modulen doubled in volume from fasted stage at 30 mins then it decreased rapidly at 120 min.

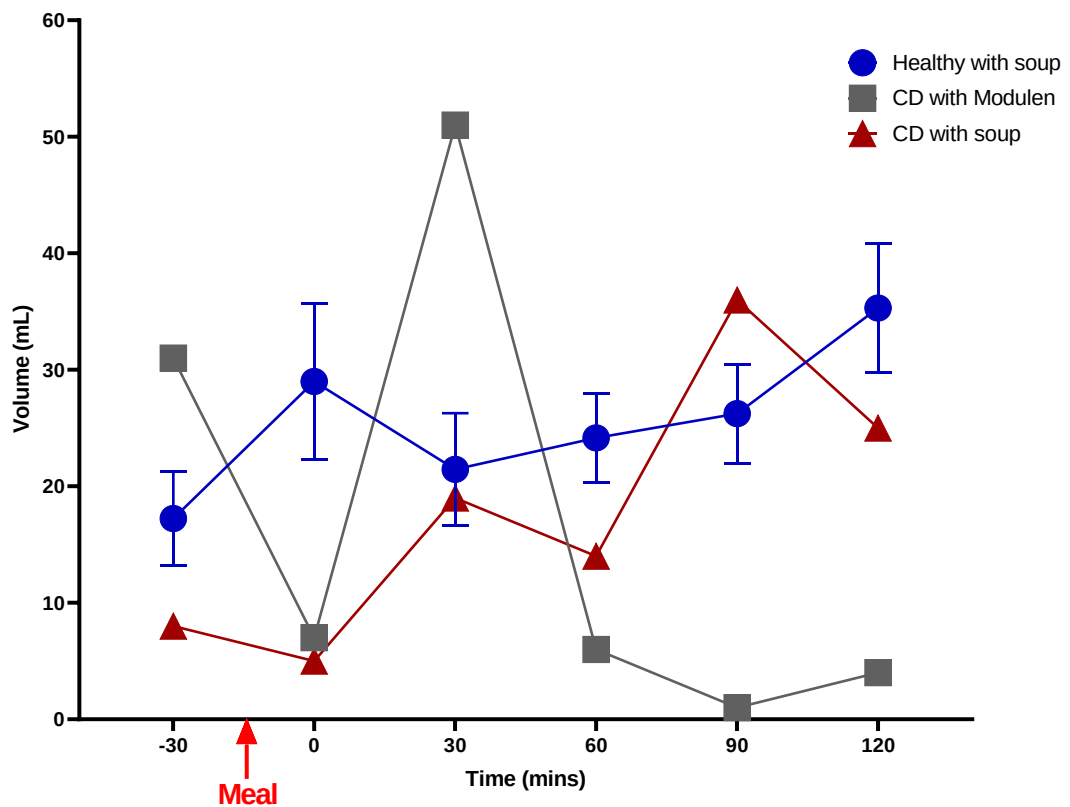


**Figure 3.12:** Time courses of small bowel water content in HV with soup, CD with soup, and CD with Modulen.

### 3.3.4 Small bowel motility

Looking at the data (Figure 3.13), small bowel motility increased from baseline for all participants at the first scan taken as soon as possible after feeding (T=0). This change was statistically significant ( $P = 0.0398$ , Wilcoxon's matched-pairs signed rank test, performed post-hoc and without correction for multiple comparisons). This change in motility was followed by a small decline in motility and a postprandial plateau. The small bowel motility data from the CD patients appeared hypomotile compared to healthy volunteers and also it appeared to decline more quickly postprandially.

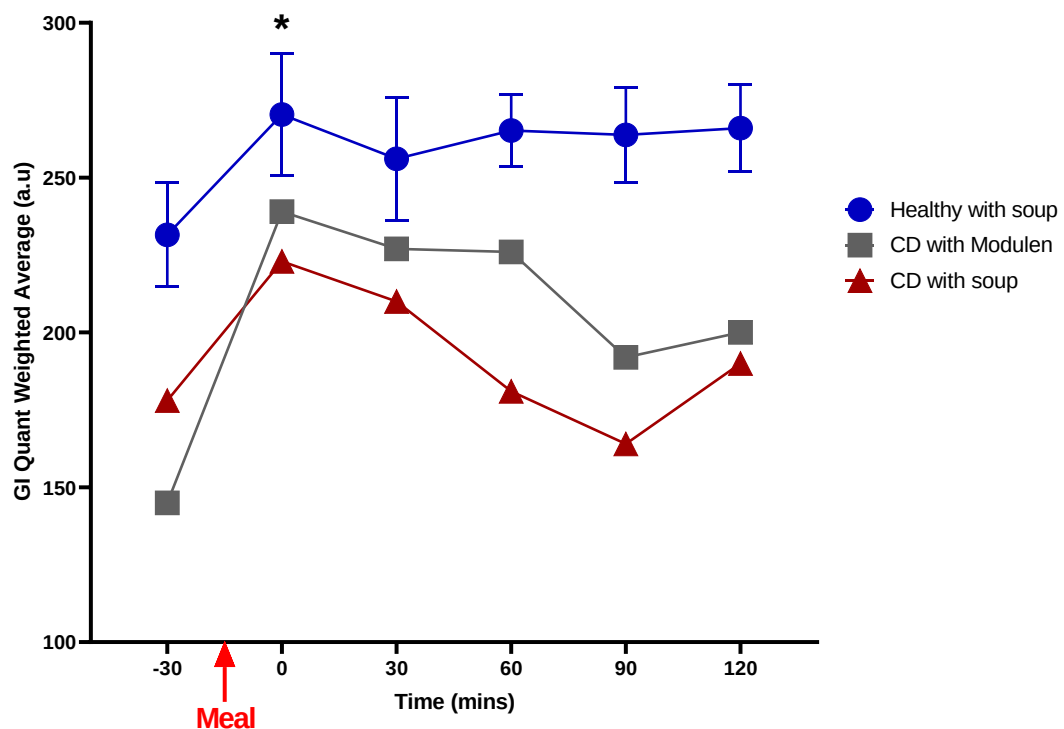


**Figure 3.13:** Time courses of the small bowel motility in n=13 HV with soup (\*  $P = 0.0398$ ), n=1 CD with soup, and n=1 CD with Modulen.

### 3.3.5 Symptom VAS data

The VAS score for fullness increased from  $9\pm 4$  mm to  $55\pm 5$  mm at 0 min and  $39\pm 5$  mm at 30 min postprandially ( $P < 0.0001$ ) for all participants. Bloating, distention, pain, and nausea scores were minimal and did not change significantly compared to fasting scores (Table 4.2)

**Table 3.2:** Visual analogue scale (100 mm VAS scores) \*\*\*\*  $P < 0.0001$  and \*\*\*  $P = 0.0002$  versus fasting value.

| Time point<br>(min) | Fullness       | Bloating | Distention | Pain/<br>discomfort | Nausea/<br>sickness |
|---------------------|----------------|----------|------------|---------------------|---------------------|
| -30                 | $9\pm 4$       | $0\pm 0$ | $0\pm 0$   | $0\pm 0$            | $0\pm 0$            |
| 0                   | $55\pm 5$ **** | $1\pm 1$ | $5\pm 2$   | $0\pm 0$            | $0\pm 0$            |
| 30                  | $39\pm 5$ ***  | $0\pm 0$ | $0\pm 0$   | $0\pm 0$            | $0\pm 0$            |
| 60                  | $30\pm 5$ **   | $0\pm 0$ | $1\pm 1$   | $0\pm 0$            | $0\pm 0$            |
| 90                  | $19\pm 4$      | $0\pm 0$ | $2\pm 2$   | $0\pm 0$            | $0\pm 0$            |
| 120                 | $10\pm 4$      | $0\pm 0$ | $2\pm 2$   | $0\pm 0$            | $8\pm 5$            |

### 3.4 Discussion

In this study we have established a novel platform for measuring undisturbed postprandial gastrointestinal function, including small bowel motility, in the paediatric population using MRI. This work shows that using such methodology is feasible.

This study was tolerated well by all participants. One out of the fifteen participants didn't quite like the soup, but they tolerated to consume more than half of the soup tin. It is difficult in study design to choose between giving to all

participants the same test meal for consistency, despite the very different body sizes, or to adjust the meal dose to body size, after which however the energy and volumes given would vary considerably. The soup was used as it had been used successfully in a previous adult participants study in a similar setting [96, 99]. The soup meal was reasonably well accepted but not ideal with this study population. Potentially a different meal might have been more suitable for such a young age groups particularly as this soup meal was given as a breakfast.

All participants completed the entire study, taking 20 min breaks in between the MRI scans playing video games at the participants' lounge. All MRI scans were successful with collecting all the needed data. Informal feedback from the participants' parents and carers was positive.

Motility assessment was carried out using the Motilent platform. The platform had few initial glitches while registering the data due to the particular scanner data format and the file naming for multiple timepoints. The platform has a user-friendly interface with easy access to the tools.

Fasting and postprandial small bowel motility was quantified in both the healthy paediatric participants and the CD patients, without disturbing the bowel. The study findings were consistent with previous studies in adults [96, 99]. The soup meal challenge was effective in eliciting physiological responses. Moreover, this study has imaged Modulen inside the bowel for the first time.

The mean of the small bowel motility peaked immediately postprandially. This early increase may be due to the fact that meal bolus moves rapidly through

the duodenum and upper jejunum. Additionally, a rapid gastro-ileal reflex would facilitate emptying of the distal small bowel content into the ascending colon. At approximately 60 minutes and when the emptying of the stomach has almost completed, the small bowel motility decreased and for the healthy participants reached a plateau which was still higher than baseline. Small bowel water content rose likely due to an increase in intestinal and pancreatico-biliary secretions. This pronounced bowel distention could be a stimulus itself for smooth muscle contraction [113] and might cause the observed increase in the measured small bowel motility. There was an initial rapid gastric emptying in the first 0 to 30 min, followed by slower phase of emptying. There was an immediate postprandial increase in mean reported fullness VAS fasting state with a subsequent decline to baseline.

## **4 QUANTITATIVE MEASUREMENTS OF WHOLE GUT TRANSIT TIME IN PAEDIATRIC CONSTIPATION (MAGIC STUDY)**

Our team has recently developed a new mini-capsule medical device to measure whole gut transit in paediatric constipation using MRI. The team was setting up the first clinical investigation and this thesis work comprised participants' recruitment, participants' MRI study days, data collection, data analysis, and final study report. The study was successful. and the data from 35 young participants showed that the device had the intended purpose and performance. A version of this work has been published in the Journal of Pediatric Gastroenterology and Nutrition (JPGN) [114]. This Chapter describes briefly the background context of paediatric constipation before detailing the actual study and results with their discussion.

### **4.1 Introduction**

Functional constipation in childhood is common, with estimated prevalence of 14% [60, 115-118]. Paediatric constipation forms 3% of all hospital referrals to paediatric practice and up to 25% to paediatric gastroenterologists [117], but less than 5% of these children have an identifiable underlying cause of their constipation [119].

Whole gut transit time imaging studies have long been used [120] and such objective measure can assist to stratify the patients and direct early selection of therapy. Gut transit studies have been reported to help characterise normal transit [121], anorectal retention (outlet obstruction) [122], non-retentive faecal incontinence [123] and slow transit constipation [122, 124] and can be

particularly useful when medical history and/or physical examination is unreliable [124]. Current methods include gamma scintigraphy and X-ray radiopaque markers (ROMs). Both techniques involve exposure to ionising radiation, which is undesirable in young persons [125, 126]. Scintigraphy uses a radiolabelled tracer meal to determine transit time. Standardisation [127] and availability are limitations. In the conventional Metcalf ROMs method [128], the patients ingest a number of small, inert plastic pellets on 3 consecutive days after which an abdominal X-Ray is taken to assess the location of the markers inside the gastrointestinal tract, thereby determining WGTT. The ROMs method is more widely available. However, it is often difficult to determine accurately the location of the radiopaque markers on an X-ray film due to the tortuous structure of the large bowel and the limitations of two-dimensional X-ray projection imaging (Figure. 4.1). MRI has already revolutionised diagnostics in many fields and is recently coming of age for diseases of gastrointestinal function [102]. WGTT has been recently measured using adult-sized (20 mm×7 mm), MRI visible capsules in health and constipation [87, 100, 104, 105].

The present adult capsules are too big for children to swallow and are also too big to represent true gut physiological conditions. Additionally, the predominantly water-based nature of the filling could increase the difficulty of detecting them in the bowel among residual meals.

We aimed to miniaturise the adult prototype MRI marker capsules and fill them with a liquid that would provide a unique MRI signal signature.

The primary objective of this study was to develop the new device and technique and to assess its feasibility in paediatric constipation population. The secondary objectives were: (i) to describe WGTT of controls and patients. (ii) to describe the safety of the technique. (iii) to describe the effect of the technique on Quality of Life (QoL).

The primary endpoint for feasibility was the number of successful measurements. The secondary endpoints were WGTT of controls and patients, the number of Adverse Events (AE) for all participants and changes in quality of life as assessed by questionnaires.

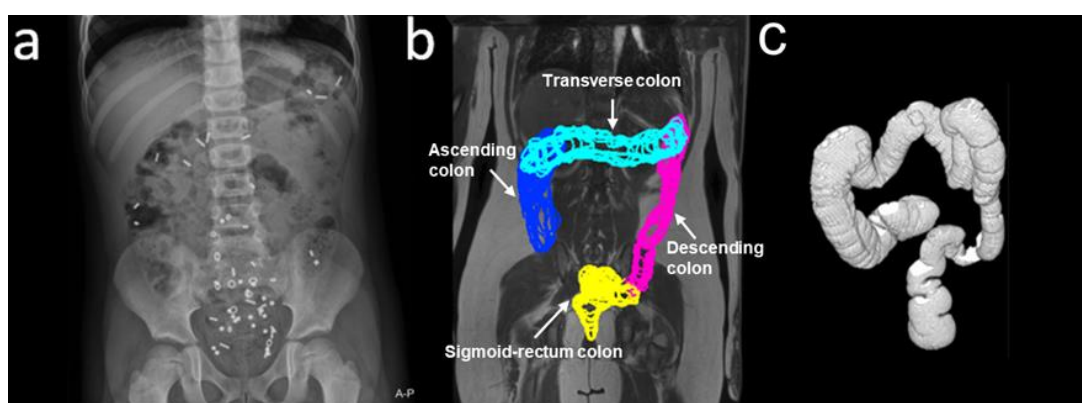


**Figure 4.1:** Difficulty in interpreting an X-ray ROM image (a). The colon is poorly depicted in the X-ray [121]. Also, the ‘theoretical’ appearance of the large bowel represented in (b) is not what is encountered in practice, whereby the colon is convoluted with often overlapping segments as shown in the two-dimensional projections (c) taken from [129]. It can therefore be difficult to assign accurately the location of the radiopaque markers on an X-ray film.

## 4.2 Methods

This study was approved by the UK National Research Ethics Committee (17/WM/0049), by the Medicines and Healthcare products Regulatory Agency (MHRA) (CI/2017/0054) and registered on Clinicaltrials.gov (NCT03564249).

#### **4.2.1 Study Participants**

Recruitments took place between April 2018 and June 2019. We contacted 50 potential participants. The CONSORT Diagram for the study [130] is shown in Figure 4.2.

Patients with childhood functional constipation were identified using The Rome IV criteria following a referral either from primary or secondary care into a specialist clinic at Nottingham University Hospitals NHS Trust. The 16 patients studied were 7 male and 9 female, age  $11\pm3$  years old, weight  $50\pm20$  kg, height  $1.4\pm0.2$  m and body mass index BMI  $25\pm9$  kg/m<sup>2</sup>. The patients were at various stages of treatment when recruited and there was no expectation that they would change treatment plan whilst participating as this was a feasibility study of the new methodology.

During the same period healthy participants, with normal bowel habit and not suffering from constipation or diarrhoea, were recruited by advertisement from the local community. The 19 healthy controls studied were 8 male and 11 female, age  $16\pm2$  years old, weight  $69\pm17$  kg, height  $1.7\pm0.1$  m and body mass index BMI  $25\pm5$  kg/m<sup>2</sup>.

**Inclusion criteria**

- Young person aged 7 – 18 years old
- Male or female
- Able to give assent or have a parent able to give informed consent
- Willing to allow his or her General Practitioner and consultant, if appropriate, to be notified of participation in the clinical investigation.

**Inclusion criteria specific for functional constipation**

- Young persons presenting with intractable constipation at secondary or tertiary care and considered for possible treatment. Possible treatment may include strong laxatives, secretagogue drugs, prokinetic drugs, peristeen irrigation, Antegrade colonic enema (ACE) surgery.

**Inclusion criteria specific for Healthy volunteers**

- Young persons who have normal bowel habit and do not suffer from constipation or diarrhoea.

**Exclusion criteria**

The participant may not enter the clinical investigation if ANY of the following apply:

- Female participants who are pregnant, lactating or planning pregnancy during the course of the clinical investigation.
- Any history of gastrointestinal surgery that could affect gastrointestinal function, such as colectomy or small bowel resection.

- Existing ACE procedure before the first MRI scan for Group 1 participants
- Significant renal or hepatic impairment.
- Contraindications for MRI scanning i.e. metallic implants, pacemakers, history of metallic foreign body in eye(s) and penetrating eye injury.
- Inability to lie flat and relatively still for less than 5 minutes
- Poor understanding of English language
- Any other significant disease or disorder (other than intractable constipation for Group 1) which, in the opinion of the Investigator, may either put the participants at risk because of participation in the clinical investigation, or may influence the result of the clinical investigation, or the participant's ability to participate in the clinical investigation.
- Participants who have participated in another research clinical investigation involving an investigational product in the past 12 weeks.

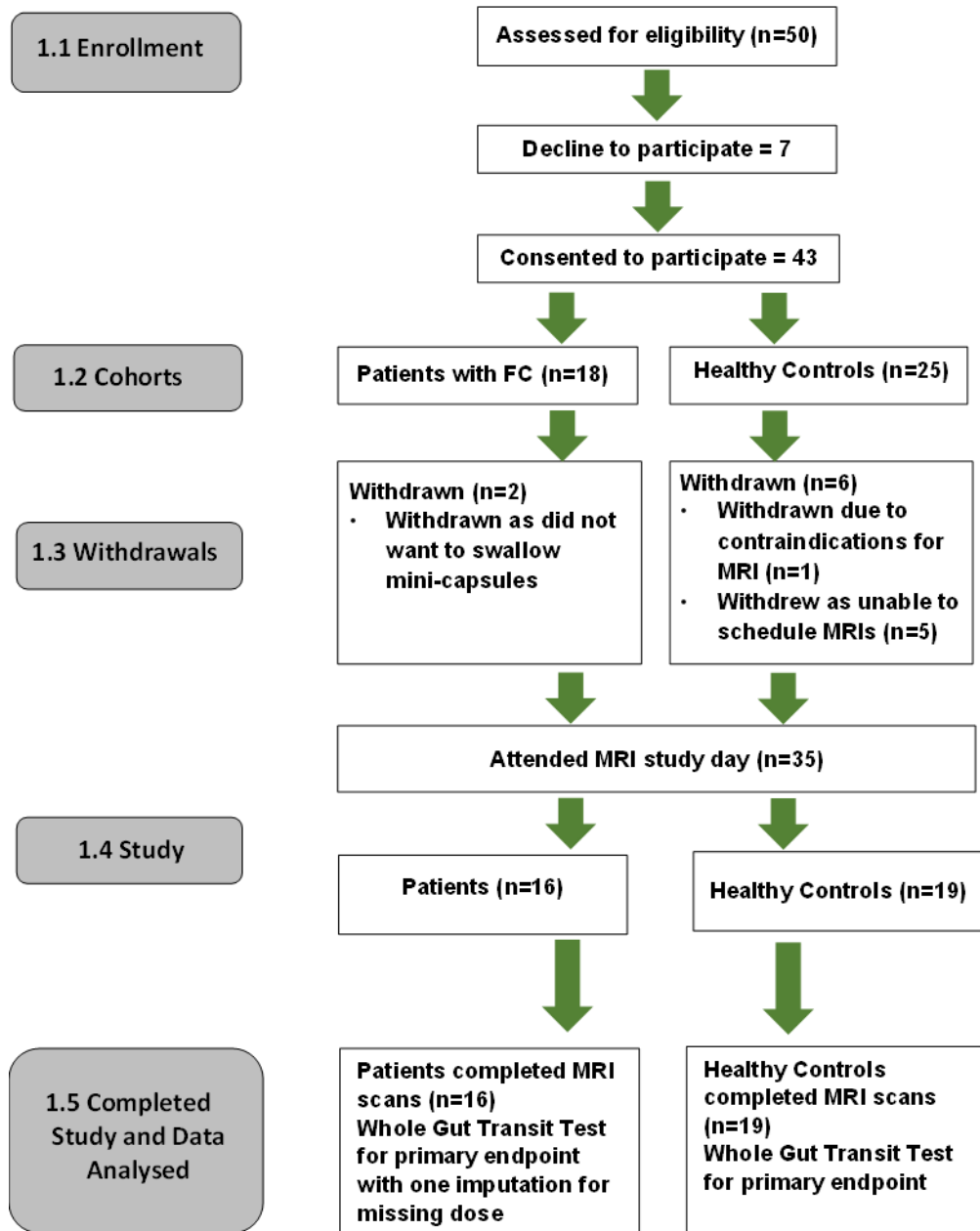


**Figure 4.2:** Magic study CONSORT diagram [114].

#### 4.2.2 Mini-capsules

The mini-capsules were an inert marker device (European Union Class IIa) that enters the body via the oral cavity and travels inside the GI tract where it can be located using MRI, (in good analogy with X-ray ROMs). The mini-

capsules (Figure 4.3) were made from medical grade polypropylene polymer (8 mm×4 mm). The polypropylene shell is invisible to MRI thus the mini-capsules were filled with an MRI-visible solution, (oil and water, with trace amounts of (1µM) of Gadolinium (Gd) contrast agent for increased visibility) and the shell is designed to ensure that the contents are not released in the GI tract. The combination of fat and water allowed us to exploit standard fat and water MRI sequences providing a unique, positive signal, MRI signature for the mini-capsules.

Renfrew Group International (Abbey Meadows, Leicester, UK) made the initial design working with patient and public involvement (PPI) in partnership with our Young Person Advisory Group, our Parent Advisory Group and technology consultants from the Enteric Health Technology Cooperative. The children and the parents helped with the design by providing direct feedback to the designers and consultants on shape, feel and colour of the mini-capsules and also on the type of packaging. The development and manufacture of the mini-capsules were made by JEB Engineering Design Limited (Hampstead Avenue, Mildenhall, UK). At the time of the study, the mini-capsules were non-CE marked and not FDA approved. They have subsequently been patented in 7 countries and trademarked as TransiCap™.

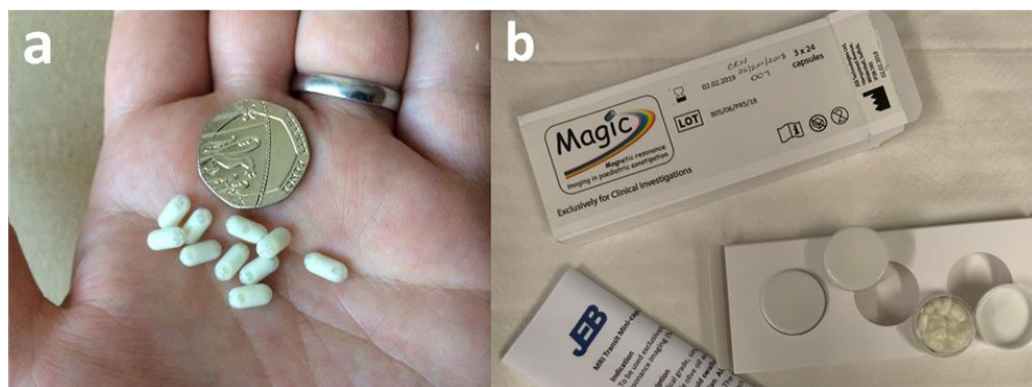


**Figure 4.3:** The mini-capsules are classed IIa an inert marker ingestible medical device (a). mini-capsule paediatric MRI transit markers (8 mm×4 mm) (b). The study pack given to the study participants, containing 3 pots of 24 mini-capsules each.

### 4.2.3 Study Design

This was an open label, feasibility, case-control study in a university setting. The intervention in this study mirrored a common X-ray ROM protocol [128]. The rationale behind it is to load the gastrointestinal tract with repeated daily doses of a plurality of markers to achieve a steady state and then image the location of the markers in the gastrointestinal tract at predetermined time points [120]. Many variants of this type of protocol have been proposed and the one chosen here is the one involving 3 daily doses of 24 markers each, which was also used for example for the common Sitzmarks® ROMs product [120, 131].

More specifically: Each participant was given an information sheet (related to different age group) with both information for the participant and the carer. After fully understanding the study protocol the participants were asked few questions to check against the inclusion and exclusion criteria. Upon accepting to take part of the study, both participants and parents were asked to sign the Participant Assent Form, MRI Safety Screening Questionnaire were also

signed by the participants. The participants were asked to swallow 24 mini-capsules each morning at home for 3 consecutive days (72 mini-capsules in total), by either mixing the mini-capsules in yoghurt, or swallowing with water or fruit juice before MRI scan day. The participants underwent a short MRI scan on day 4. If mini-capsules were still seen in the bowel, a second short MRI scan was performed on Day 7. If mini-capsules were still seen in the bowel, they underwent a third MRI scan around day 26-28. This last scan was not part of the WGTT test but performed to collect data on possible retention of mini-capsules. EQ-VAS (visual analogue scale) questionnaire data (young people health on the day) [132] was collected at baseline and each day of the study (day 0, before they started taking the mini-capsules to day 8). EQ-5D-Y data [132] was also collected at baseline. The questionnaire evaluates health-related life domains such as mobility, self-care and pain. The lowest scores set possible for the EQ-5D-Y is 1,1,1,1,1 indicating no issues with any of the 5 domains. Scores higher than 1 in one or more domains indicate loss of QoL. All questionnaires were used with permission. The study flow chart is shown in Figure 4.4.

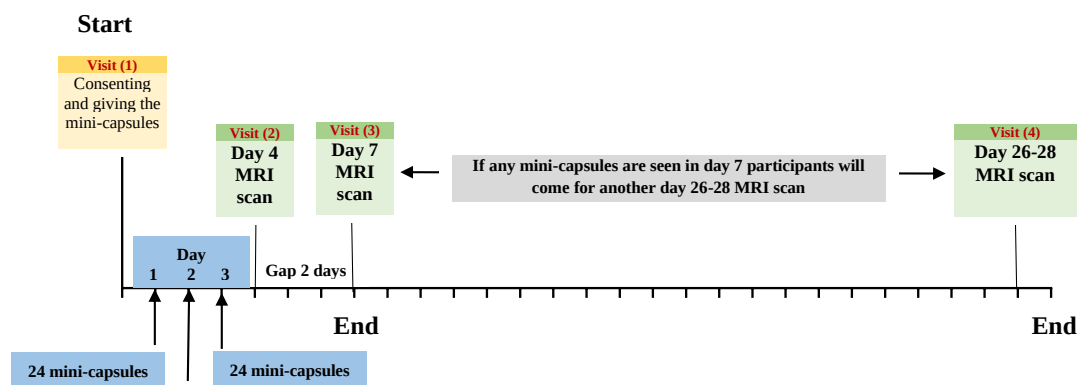


**Figure 4.4:** Flow diagram of study daytime points.

#### 4.2.4 MRI protocol

The MRI procedure involved placing the participants in a 3T, wide-bore Ingenia scanner (Philips, Best, The Netherlands) (Figure 4.6). Participants were not sedated and lay feet first on the scanner bed, in the supine position with standard abdominal receiver wrapped around the abdomen.



**Figure 4.6:** Ingenia MRI scanner at the Sir Peter Mansfield Imaging Centre at the University of Nottingham.

A 3-dimensional 2-echo, mDIXON [133] scan was then acquired to locate the mini-capsules. This provided 4 image types for each slice acquired: water only, fat only, fat and water in-phase and out-of-phase images [133]. The liquid filling of the mini-capsules had been previously optimised in conjunction with the imaging sequence parameters so that on a derived subtraction of the out-of-phase image from the in-phase image the signal of the mini-capsules would be maximised compared to gastrointestinal chyme, intestinal water and surrounding organs, facilitating their detection. The sequence was acquired

both in coronal and axial planes, breaking down the 3D volume into stacks of short breath-holds to minimise respiratory motion. The whole MRI procedure took approximately 15-20 minutes. The MRI sequence parameters are shown in Table 4.1.

**Table 4.1.** Details of the 3T Ingenia MRI sequence parameters used in this study.

| <i>MRI sequence</i>                               | <i>3D T1 weighted TFE (mDIXON)</i> |                            |
|---------------------------------------------------|------------------------------------|----------------------------|
| <i>Image orientation</i>                          | Axial                              | Coronal                    |
| <i>Field of View</i>                              | 350 mm (RL) × 280 mm (AP)          | 348 mm (RL) × 250 mm (HF)  |
| <i>Image stacks</i>                               | 5                                  | 6                          |
| <i>Slices per stack</i>                           | 33                                 | 27                         |
| <i>Length of breath hold per stack</i>            | 12.3 s                             | 13.5 s                     |
| <i>SENSE acceleration factor</i>                  | 2                                  | 2                          |
| <i>Signal averaging</i>                           | 1                                  | 1                          |
| <i>Flip angle</i>                                 | 20°                                | 20°                        |
| <i>Repetition time TR</i>                         | 10 ms                              | 10 ms                      |
| <i>Echo times TE<sub>1</sub> / TE<sub>2</sub></i> | 1.32 ms / 2.2 ms                   | 1.32 ms / 2.2 ms           |
| <i>Reconstruction matrix</i>                      | 400 × 400                          | 400 × 400                  |
| <i>Acquired image resolution</i>                  | 1.8 mm × 1.8 mm × 4.4 mm           | 1.8 mm × 1.8 mm × 4.4 mm   |
| <i>Reconstructed image resolution</i>             | 0.88 mm × 0.88 mm × 2.2 mm         | 0.87 mm × 0.87 mm × 2.2 mm |

#### 4.2.5 Data Analysis

Counting of the mini-capsules was performed preferentially on the coronal plane views because the anatomy of the colon is simpler to follow in that orientation. If the coronal images were blurred by motion or partly obscured by

a very full bladder, then the axial images were of additional value for the counting. These were observations made at post-processing stage. Four participants had some data sets that were more difficult to read because of blurring induced by respiratory motion caused by poor breath-holding during some of the imaging image acquisitions but possibly also by gastrointestinal motility due to a recent meal. When blurring was noted by the radiographer at the time of scanning some image sets could be repeated immediately, which only required a few additional breath-holds.

The mini-capsules remaining in the gut were located and counted on the derived in-phase minus out-of-phase images using an IDL Development Environment program (program to count pills in colon) designed by Dr Caroline Hoad, June 2018 (Figure 4.7) and the WGTT subsequently calculated following the Sitzmarks ROM method [128]. The calculation of WGTT assumes that that by loading the gut with repeated ingestion of the mini-capsules over three days, a steady state is reached so that:  $\text{WGTT in hours} = (72 \text{ hours} / \text{the number of mini-capsules ingested over three days}) \times \text{the total number of mini-capsules remaining in the colon}$ . The total number of mini-capsules ingested in this study over 3 days, 72, simplifies the multiplying factor so that  $\text{WGTT} = \text{the total number of mini-capsules remaining in the colon at Day 4}$ . When some mini-capsules are remaining at Day 7, their contribution to the total transit time is simply added to the count at day 4 using the same method. The principle of the WGTT marker method relies on loading of the bowel with a plurality of markers not necessarily on their specific total number [120] hence the formula above can be adapted in case some of the mini-capsules were mistakenly not ingested, by simply changing the denominator for 'the number of mini-capsules

ingested over three days'. This happened in one instance in this study leading to one data imputation. The feasibility study was open label, and the person analysing was not blind to which group the patients or controls belonged to, but they did not have knowledge of the individual patient histories.

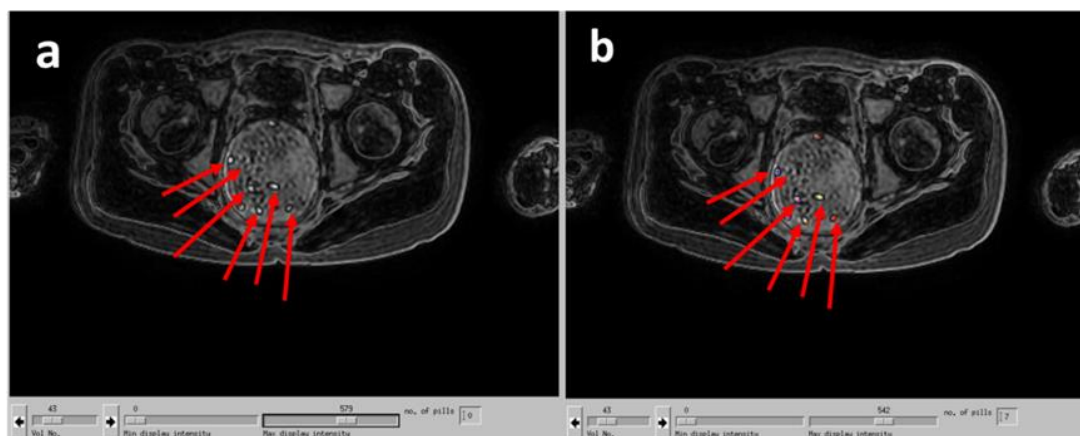


**Figure 4.7:** IDL pill counting program: (a) MRI axial slice shows the rectum with mini-capsules. (b) Shows the pill counting tool that highlights each indicated pill location.

#### 4.2.6 Statistical analyses

There was no prior data on the new mini-capsules to estimate sample size and N=25 participants are considered as an acceptable sample size for a feasibility study [134, 135] and allowing for dropouts.

All statistical analyses were carried out using Prism version 6.07 (Graph Pad Software Inc., La Jolla, CA, USA). Normality of the data was assessed using D'Agostino and Pearson test. The comparison of WGTT and EQ-VAS between patients and controls was carried out *post-hoc*. The WGTT data were normally distributed and differences in WGTT between the patients and controls were compared using two-tailed, unpaired t test. The EQ-VAS were compared between the baseline time point before the start of the study procedures and

the day after the study procedures were completed (Day 8). Data were considered significantly different at  $P < 0.05$ .

### **4.3 Results**

Thirty-five young persons were studied (Figure 5.8). These comprised 16 patients (7 male; 9 female;  $11 \pm 3$  years old; BMI  $25 \pm 9$  kg/m<sup>2</sup>) and 19 healthy young controls (8 male; 11 female;  $16 \pm 2$  years old; BMI  $25 \pm 5$  kg/m<sup>2</sup>). The age between the two groups had a modest but significant difference (Mann Whitney  $P < 0.001$ ).

#### **4.3.1 Number of successful measurements and feasibility**

All the 35 participants that ingested the mini-capsules completed the study. Only one of the subjects forgot to ingest one of the daily doses and the WGTT calculation was corrected for this. This showed very good acceptability of the mini-capsules and MRI procedures. Feedback from the participants and the parents, albeit not formally recorded, was positive.

The mini-capsules were imaged successfully in the colon of all participants using MRI (Figure 4.8).

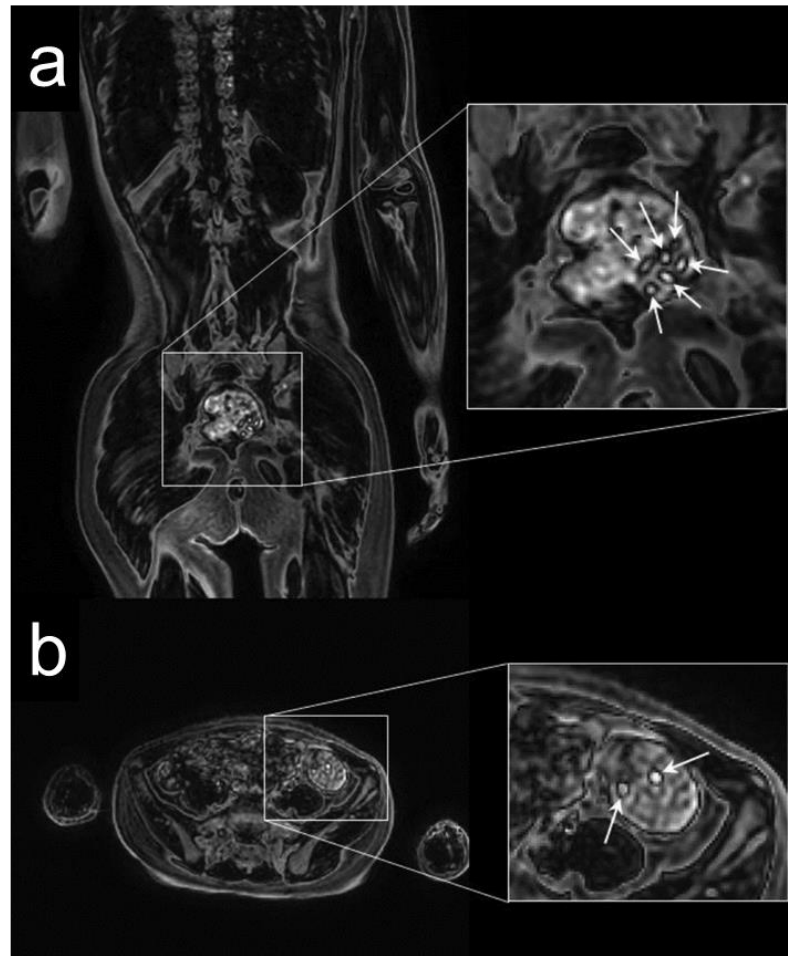


**Figure 4.8:** (a) coronal image slice clearly showing the mini-capsules with bright, positive signal against the darker colon contents of a participant. The image shown in Fig. 4.8 (b) is a projection reconstructed from a multi-slice three-dimensional set of the whole abdomen of another participant. The appearance of the markers in the segmented colon here is more similar to X-ray imaging with radiopaque markers, an example of which from a different study is shown in Fig. 4 (c) for comparison (taken from [114]).

### 4.3.2 Whole Gut Transit Time

From the MRI images, it was possible to count the mini-capsules in the gastrointestinal tract of all the participants. The WGTT (Figures 4.9 and 4.10) calculated from the mini-capsules count, was  $78 \pm 35$  hours for the young patients with constipation, significantly longer than that for the healthy controls  $36 \pm 16$  hours,  $P < 0.0001$ .

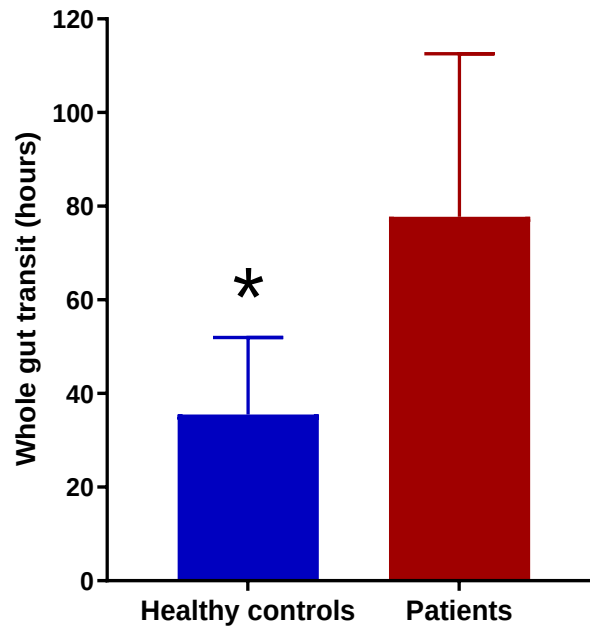


**Figure 4.9:** Whole gut transit time (mean and SD) for patients (n=16) and healthy controls (n=19). \*  $P < 0.0001$ .

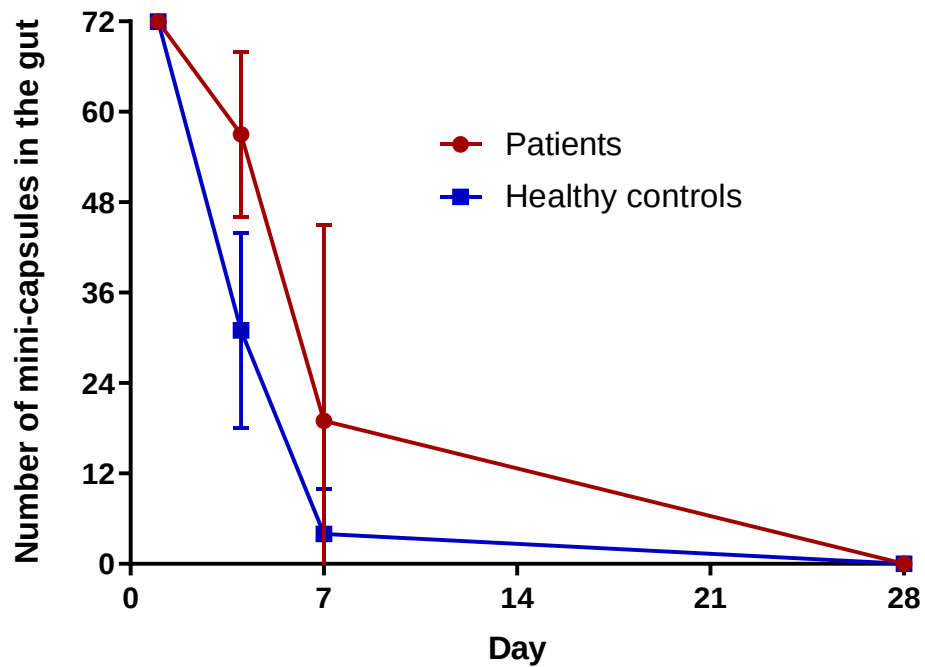


**Figure 4.10:** Number of mini-capsules (mean±SD) detected in the colon of young patients (n=16) and healthy controls (n=19) at day 4, day 7 and day 28 after ingestion.

### **4.3.3 Number of Adverse events and safety**

There were no Serious Adverse Events (SAEs) in the study. There was only one Adverse Event (AE) in the study. This was a single episode of vomiting. It was categorised as mild and quickly resolved, requiring no follow up and possibly related to the study procedures.

The number of mini-capsules detected in the gut decreased with time for all participants. In the patient group, the number significantly decreased from  $57 \pm 11$  mini-capsules at day 4 to  $19 \pm 26$  mini-capsules at day 7,  $P = 0.02$ . For the healthy controls the count significantly decreased from  $31 \pm 13$  at day 4 to  $4 \pm 6$  at day 7,  $P < 0.0001$ .

Some mini-capsules were present at day 7 in  $n=11$  patients and  $n=9$  healthy controls. These participants were recalled for a final scan at day 26-28 post ingestion. No mini-capsules were detected in any of the participants at this final time point, thus in part confirming the European Union Class IIa classification of the device.

### **4.3.4 EQ-VAS scores**

There was no significant difference in EQ-VAS visual analogue scores provided by all participants pooled together at baseline before any procedure ( $87 \pm 13$ ) and after completing the mini-capsules and MRI intervention at Day 8 ( $85 \pm 18$ ), Wilcoxon's  $P = 0.79$ .

The young patients' EQ-VAS total mean was about 10% lower than for healthy controls with no significant differences between groups ( $P = 0.78$ ). There are also no significant differences in EQ-VAS scores (Figure 4.11) before and after

the mini-capsules and MRI test for patients (Wilcoxon's  $P = 0.54$ ) and for healthy controls (Wilcoxon's  $P = 0.55$ ).

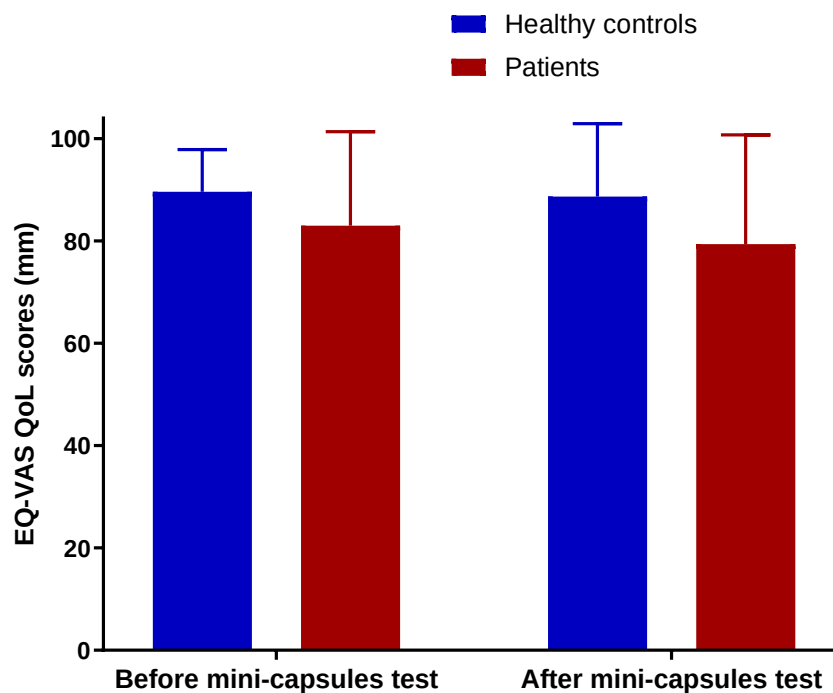


**Figure 4.11:** Young participants' EQ-VAS scores before and after the mini-capsules test.

#### 4.3.5 EQ-5D-Y

All healthy controls scored their QoL at the lowest score 1 for all dimensions on the EQ-5D-Y questionnaires. Four patients indicated a loss of QoL in the questionnaires. A clerical error prevented the administration of the second batch of questionnaires after the MRI study procedures therefore those data were not available for analysis.

#### **4.4 Discussion**

The study was a ‘first-in-child’ clinical investigation of a novel medical device. As such we set out to assess feasibility, safety, and performance of using the new device in the intended population, for the intended purpose. The study was successful on various points. We have been able to image the new mini-capsule device in the colon of 35 young people with and without constipation successfully, using MRI. The intended method to image the mini-capsules, using a derived in-phase minus out-of-phase fat and water MRI imaging worked well. The TransiCap™ mini-capsules appeared distinctively, with a marked bright positive contrast against the colonic chyme. Having imaged the TransiCap™ in the colon of these participants it has been possible to count them, which provided a successful measure of whole gut transit time using the simple algorithm described in the methods. Therefore, the primary outcome measure from the study demonstrated that our new device met the intended purpose of being imaged in the gut, monitoring gut transit time using MRI and of measuring the intended primary end point of the study in paediatric constipated participants and healthy controls.

A reference mean and a range of whole gut transit time was also collected for the healthy controls, which was a secondary objective of the study.

Another strength of the whole project was the active participation of children and young persons in the co-design of the mini-capsules and of the feasibility study. This work was extensive and went on for years with also the Author’s involvement to the point of publication in Research Involvement and Engagement journal [136].

When comparing our transit time results with other paediatric data in the literature, Gutierrez et al. [137] found transit time in a constipated group of 49.6h versus 29.1 hours in a control non-constipated group. They used a radiopaque marker method involving ingestion of 10 markers per day for 6 days followed by an X-ray on day 7. Zaslavsky et al. [138] similarly found a transit time of 54.3 hours in an adolescent constipated group versus 30.2 hours in a control non-constipated group, a delay in transit time of 40% compared to controls. They used 20 radiopaque markers ingested each day for 3 days followed by an X-ray on day 4. The data collected here with our new mini-capsules showed a highly significant difference of 54% in whole gut transit time between our young patients with constipation and healthy controls. This further corroborates evidence that the new device was able to detect a clinically significant difference between paediatric constipation and healthy controls.

In this study we measured WGT by counting the number of capsules remaining in the bowel at predetermined time points. Measuring segmental transit times in different tracts of the colon was not an outcome for this study. Segmental transit times were once more popular particularly to inform segmental surgical resection but recently controversy has been reported about this treatment and its uncertain benefits [54, 139]. The cross-sectional quality of MRI can provide an advantage over X-ray to assign the location of markers as bowel loops can overlap in the two-dimensional X-ray abdominal film, thought this needs to be assessed formally.

The study had a very good safety record. There was only one episode of vomiting categorised as an adverse event.

The EQ-VAS scores showed no difference before and after the study procedures indicating that undergoing the study and the procedures did not alter the score of the patients and of the healthy participants.

The study had some limitations. The baseline EQ-5D-Y for the participants were not repeated after the study due to a clerical error thus preventing an appropriate assessment of changes in QoL.

The patient participants reached the specialist clinics in the hospital as they required expertise more than that available within primary and secondary care. Our pool included also those referred from other community paediatric clinics and general paediatric clinics within the region. As such, they were at various stages of treatment, when they participated in the study. There was no expectation that patients would change treatment plan whilst being investigated. It is interesting that despite this and their heterogeneity we demonstrated a longer WGTT in the patient group. At the same time this did not allow us to collect meaningful data on changes in gut transit upon treatment. There was a significant difference in age between the two groups though this was modest and did not seem to affect this feasibility study.

Constipation is a very common problem and whilst the vast majority of children and young persons will respond to simple treatment with stool softeners and bowel stimulant, there is a significant proportion of patients who do not show signs of improvement despite follow up. It is important to in these cases to evaluate the WGTT in order then to individualise therapy.

A non-contrast paediatric MRI scan to a single area of the body cost more than an X-ray (in the UK health system about 4 times more) so MRI cost and availability are issues to be taken into account. In the paediatric population,

avoiding ionising radiation exposure is a definite advantage of the new technique that should counterbalance the economic argument. The additional cross sectional imaging additional information on the bowel anatomy that can be gathered within the same scan is also an advantage of MRI. In terms of availability, it is true that MRI units are particularly busy with clinical routine. However, when new bowel imaging scans prove their worth they enter that clinical routine as has happened for MRI cholangiopancreatography and MRI enterography.

The X-ray ROM protocol that was copied for this initial study (3 daily doses of 24 markers) is a common one but it is not necessarily the only one used in past literature. The new MRI mini-capsules could be used in the future with other imaging protocols such as a single dose, single imaging time point [120] and also in adults, though further investigation and validation will be needed. A multi-centre study of the mini-capsules in paediatric constipation was funded (Trial ID: ISRCTN42273449) but later failed to run due to the COVID-19 pandemic.

## **5 QUANTITATIVE MEASUREMENTS OF COLON VOLUMES AND LENGTH IN PAEDIATRIC CONSTIPATION (MAGIC STUDY)**

The MAGIC study described in the previous Chapter was successfully completed and published. That work focused on the primary outcome which was whole gut transit time. The data however contained additional information beyond the transit time hence the Author carried out further post-hoc analyses.

The colonic volume work has already been published in the journal *Diagnostics* [140].

This Chapter describes post-hoc analysis for colon volume and length measurement in paediatric constipation and healthy controls. For these measurements of different colon segments were firstly segmented manually. Secondly the colon volumes were calculated by integrating the regions of interest. Thirdly a method to calculate colon segment lengths using a skeletisation method in 3D was developed and used. This study yielded the first data on the undisturbed colonic volume and colon length in paediatric patients with FC and healthy paediatric controls using MRI.

### **5.1 Introduction**

The importance of understanding functional constipation in childhood was explained in the previous chapter. Despite its prevalence, the aetiology of paediatric constipation is still poorly understood [122, 141] and diagnosis principally based on symptom reports alone [46, 142, 143]. Increased knowledge of colon physiology and morphology could provide healthcare professionals with additional, objective parameters to evaluate. It could also

aid to develop hydrodynamic modelling of the colon mass flow and drug dissolution modelling.

In previous reports, the volume of the adult human colon, either as a whole or divided in anatomical segments, has been estimated using different measurement techniques such as cadaver dissection studies [144, 145], perioperative assessment [146], abdominal computed tomography or barium contrast studies [147]. However, these techniques are invasive, often require preparation of the colon and use contrast media which can disturb the physiology or use ionising radiation which is particularly undesirable in children [120].

Studies of the volume of the adult colon have been carried out using MRI. The advantages of MRI are the excellent soft tissue contrast, cross sectional image, speed and also the lack of use of ionising radiation which can provide comprehensive 3D views of each colonic segment (ascending, transverse, descending colon and sigmoid-rectum) without preparation or use of contrast agents for the measurement of colonic volume [103, 148, 149]. Several MRI studies in adults have demonstrated the responsiveness of the total colonic volume to intervention. Randomised placebo controlled trials, showed an increase in response to fibre-based bulking agents like psyllium and kiwifruit [150, 151]. Intraluminal volumes have been measured before and after feeding interventions [149, 151] and treatment with bulking agents and laxatives [150, 152], to assess the colon volume functional responses. MRI studies have reported increased volume of the un-prepared, un-disturbed colon in adults with FC and irritable bowel syndrome compared to healthy controls [104, 105]. They also showed that colonic volume in healthy subjects decreases by

around 1/3 after defecation indicating that delayed defecation would be predicted to significantly increase total colonic volume [153]. Delayed defecation with chronic faecal impaction is an important cause of FC in children but whether it would increase colonic volume has until now been unclear [154].

Whilst much data on adult colon volume is available as mentioned above, A limited amount of on information on colon length in infants, children, and adults is available, and this originates mostly from invasive techniques such as surgical intervention [155], barium enema [147], cadavers study [145] or computed tomography [156]. There is general agreement that colon length increases with children's age. However, the measures for total colon length found in the literature vary depending on the reports and technique used. Using the retrospective computed tomography study by Mirjalili [156] the colon length increases from 52 cm in children between 0 and 2 years of age to 95 cm in children aged 9-11 years, compared to adult values reported by others between 108 and 132 cm. There is an obvious benefit in using non-invasive imaging methods to capture the undisturbed physiological length of the colon. A recent study by Mark *et al* used MRI in conjunction with a new electromagnetic 3D-Transit capsule technique to measure the length of the colon in healthy adults [157]. Total colon length was measured from the MRI images using a 3D topological skeleton algorithm in Matlab [157] and the value had an average of 95 cm. To our knowledge this is the only study available using MRI and it was performed in adult healthy participants.

Therefore, the primary aim of this retrospective study was to measure colonic volume and colon length in children with functional constipation and also

provide initial normal range values for healthy controls. We hypothesised that the colon volume would be larger, and the colon length would be longer in patients with FC compared to healthy controls.

## **5.2 Methods**

As this was a post-hoc study for the data acquired from the study in chapter 4. Study participants, study design, and MRI protocol were mentioned in the previous chapter.

### **5.2.1 Data analysis**

This section will be divided into two parts. Firstly, describing colon volume data analysis followed by colon length data analysis.

#### **Colon volume**

Colonic volumes were measured from coronal mDIXON fat and water in-phase images in keeping with previous work in adults [105, 149, 158]. Individual regional colon volumes were manually segmented on each slice using Medical Image Processing, Analysis and Visualisation software (MIPAV, NIH, Bethesda). The data analysis was carried out not blinded. The colon of the participants was divided into 4 regions: ascending colon (AC), transverse colon (TC), descending colon (DC) and sigmoid colon and rectum region (SC-R). Regional boundaries commenced at the caecum (ascending) and were fixed in a coronal plane at the superior point of the hepatic flexure (ascending to transverse), at the splenic flexure (transverse to descending) and terminated at the sagittal plane of the commencement of sigmoid colon where the descending colon deviates posteriorly or medially. Each colon region was identified within each coronal image slice, and a region of interest (ROI) drawn

around it (Figure 5.1), building a 3D representation of the morphology from which the volume of each region was measured by summing up the areas of the corresponding ROIs and multiplying by slice thickness. If any difficulties were found in visualising the anatomy, available axial views were also used to aid navigation.

The colon volume data were corrected for body surface area (BSA) using the Mosteller formula as the square root of (height (expressed in cm)  $\times$  weight (expressed in kg)/3600) [140, 158, 159]. Linear regression was used to explore the relationship between total colon volume from this study and the individual participants' WGTT values from chapter five [114].

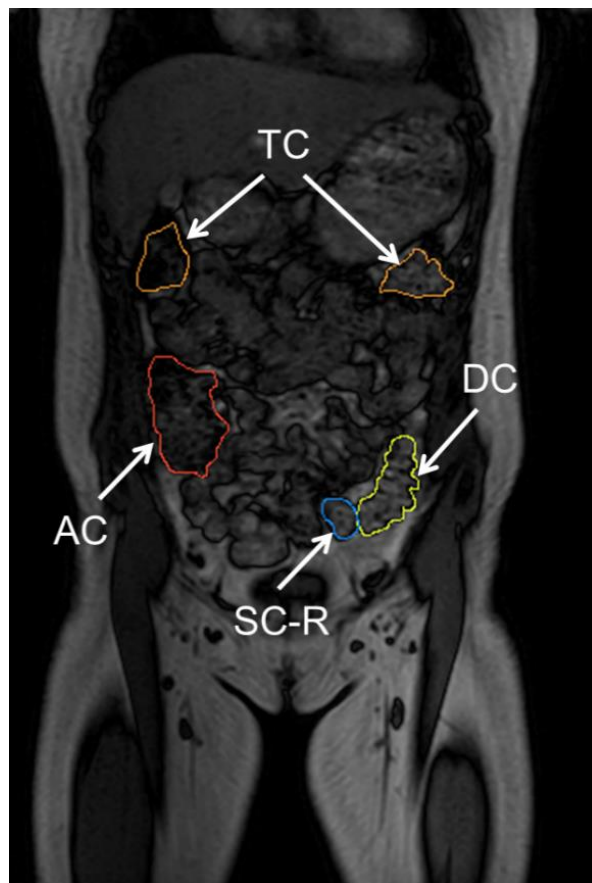


**Figure 5.1:** Region of interest (ROI) was drawn to divide the colon into 4 regions (AC, TC, DC, and SC-R).

## Colon length

Colon lengths were measured using the AnalyzeSkeleton plug-in [160] in ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). Skeletonization is an image analysis process that used binary thinning to reduce three-dimensional anatomical structures to a central line (skeleton) which preserves the length connectivity in the original object whilst erasing all other foreground pixels (Figure 5.2). The program then tagged all pixel/voxels in the skeleton image and counted all its junctions, triple and quadruple points and branches, and measured the average and maximum length of the individual segments. After the 3D skeletonization process the plug-in Analyze Skeleton2D/3D was applied to quantify the length of the colon regions. When required by comparisons the colon length data was also corrected for body surface area following the same formula mentioned above.

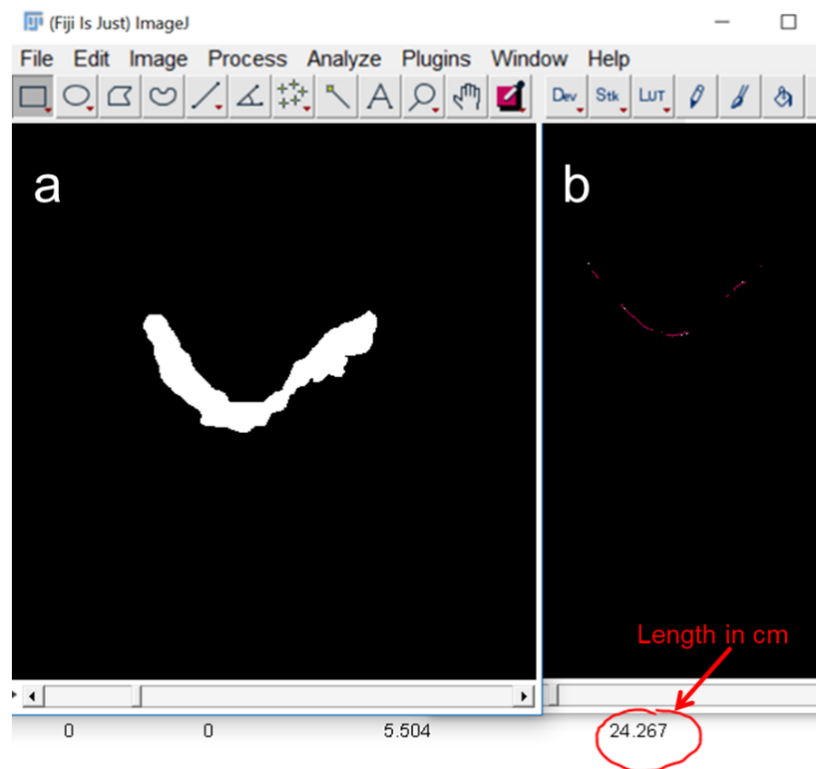


**Figure 5.2:** (a) binary image for a segmented transverse colon. (b) the skeleton3D for the segmented transverse colon in ImageJ software.

### 5.2.2 Statistical analysis

Statistical analysis was carried out using Prism 9 (GraphPad Software Inc., La Jolla, CA, USA). Normality of the data was checked using Shapiro-Wilk normality test. Depending on normality, comparisons between groups were performed using two-tailed t-test or Mann–Whitney rank sum test for unpaired data. Differences were considered significant at  $P < 0.05$ . Linear regression was used to assess the strength of the relationship between colon length and colon volume and WGTt respectively.

## 5.3 Results

Good quality images of the colon were obtained in the majority of the 35 participants. The images from 4 participants displayed some breathing artefacts. This, however, did not prevent identification and mapping of the colon. All data from the 35 participants were included in the image analysis.

### 5.3.1 Colon volume

There was a moderate positive linear correlation of colon volume with height ( $R = 0.49$  for patients  $P = 0.058$  and  $R = 0.60$  for controls  $P = 0.0068$ ) and weight ( $R = 0.45$  for patients  $P = 0.0802$  and  $R = 0.30$  for controls  $P = 0.2163$ ). Moreover, the height and weight of the young participants varied markedly, with some being nearly twice as tall (range 105 cm to 185 cm) and/or 4 times heavier (range 22 kg to 106 kg) than others, which underpinned the use of the colonic volume correction for body surface area.

Example colon reconstructions are shown in Figure 5.3. A 13 year old patient with FC with a BMI of  $17.7 \text{ kg/m}^2$  had the largest total colon volume corrected for BSA in the patient group ( $712 \text{ mL/m}^2$ ), with a WGTt of 140 hours. This

patient presented with a megarectum (Figure 5.3a), with a sigmoid colon and rectum volume of 243 mL/m<sup>2</sup>. By comparison Figure 5.3b shows the colon reconstruction for a 17 year old healthy control with a BMI of 23.4 kg/m<sup>2</sup> who had the largest colon volume corrected for BSA (360 mL/m<sup>2</sup>) among the control group and a WGTT of only 16 hours. This control participant was one of the tallest ones, with a height of 185 cm.

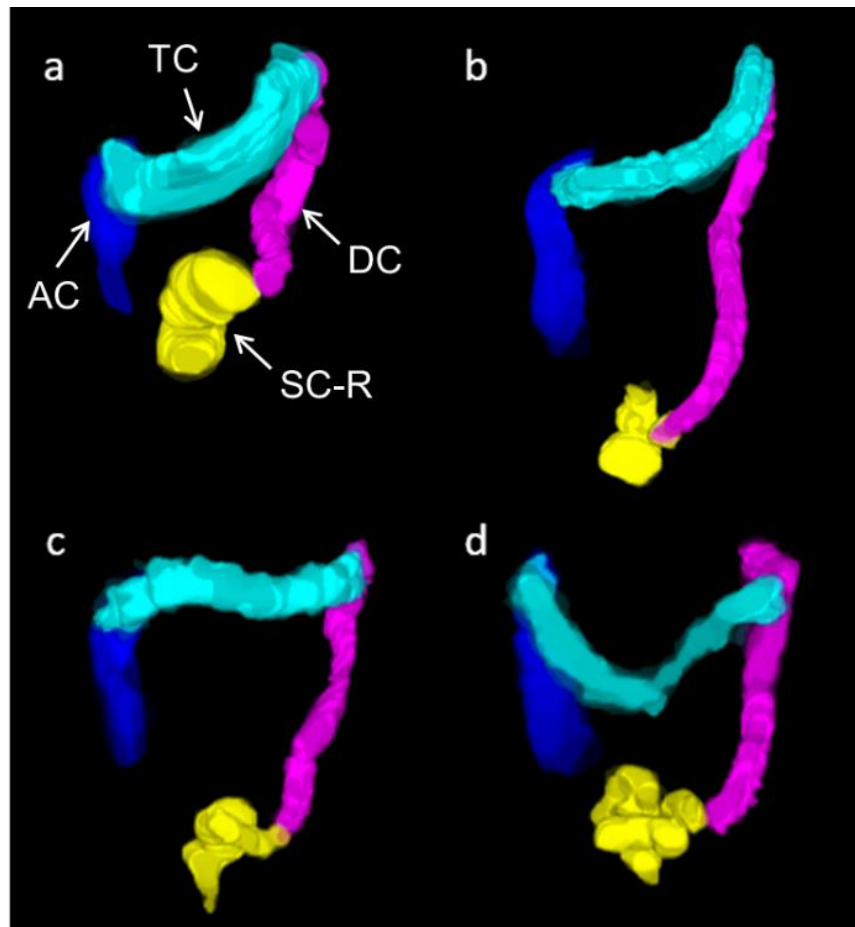


**Figure 5.3:** 3D representations of the colon morphology from four different participants whose characteristics were: (a) Patient, 13 years old, weight 42 Kg, height 154 cm, BMI 17.7 kg/m<sup>2</sup>. Total colon volume corrected for BSA TCV = 712 mL/m<sup>2</sup>, whole gut transit time WGTT = 140 Hours. (b) Healthy control, 17 years old, weight 80 Kg, height 185 cm, BMI = 23.4 kg/m<sup>2</sup>. TCV = 360 mL/m<sup>2</sup>, WGTT = 16 Hours. (c) Patient, 9 years old, weight 48 Kg, height 130 cm, BMI 21.4 kg/m<sup>2</sup>. TCV = 237 mL/m<sup>2</sup>, WGTT = 75 Hours. (d) Healthy control, 10 years old, weight 42 Kg, height 145 cm, BMI = 19.9 kg/m<sup>2</sup>. TCV = 227 mL/m<sup>2</sup>, WGTT = 26 hours.

The total colonic volume corrected for BSA (Figure 5.4) was 309mL/m<sup>2</sup> (243-384mL/m<sup>2</sup>) median (interquartile range) for the young patients with constipation, significantly larger than that for the healthy controls 227mL/m<sup>2</sup> (180-263mL/m<sup>2</sup>) ( $P=0.0081$  Mann-Whitney two-tailed test).

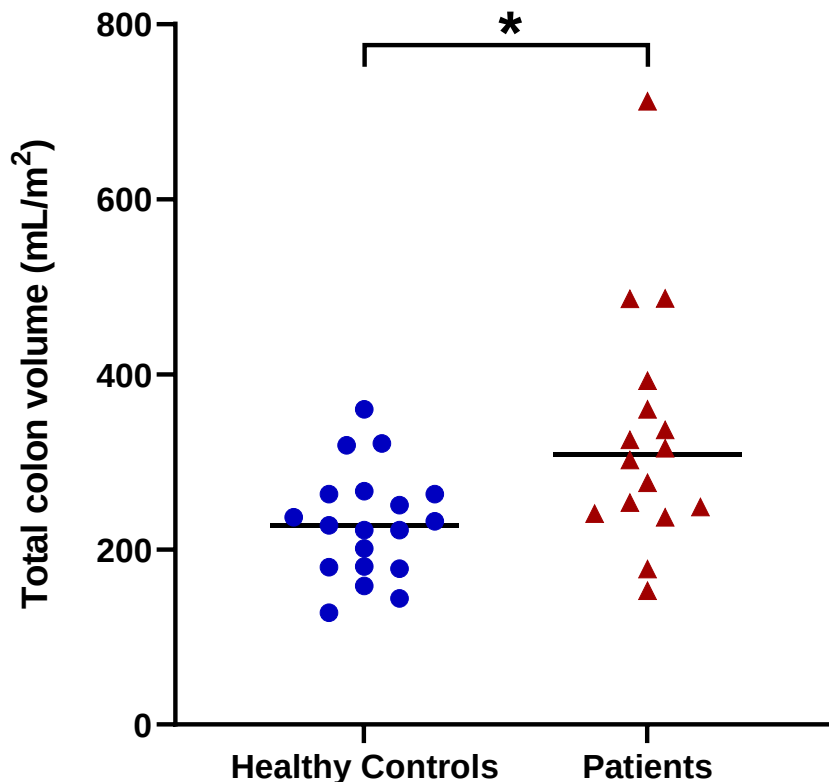


**Figure 5.4:** Total colon volume corrected for both BSA of n=19 control and n=16 patients with constipation. The horizontal lines indicate the median, \*  $P=0.0081$  two-tailed Mann-Whitney test.

Regional colon volumes are shown in Figure 5.5 and show that the larger increase between controls and patients was in the SC-R region ( $P=0.0410$  Mann-Whitney one-tailed test) with 9 constipated patients having SC-R volume above the 95% CI of the control values.

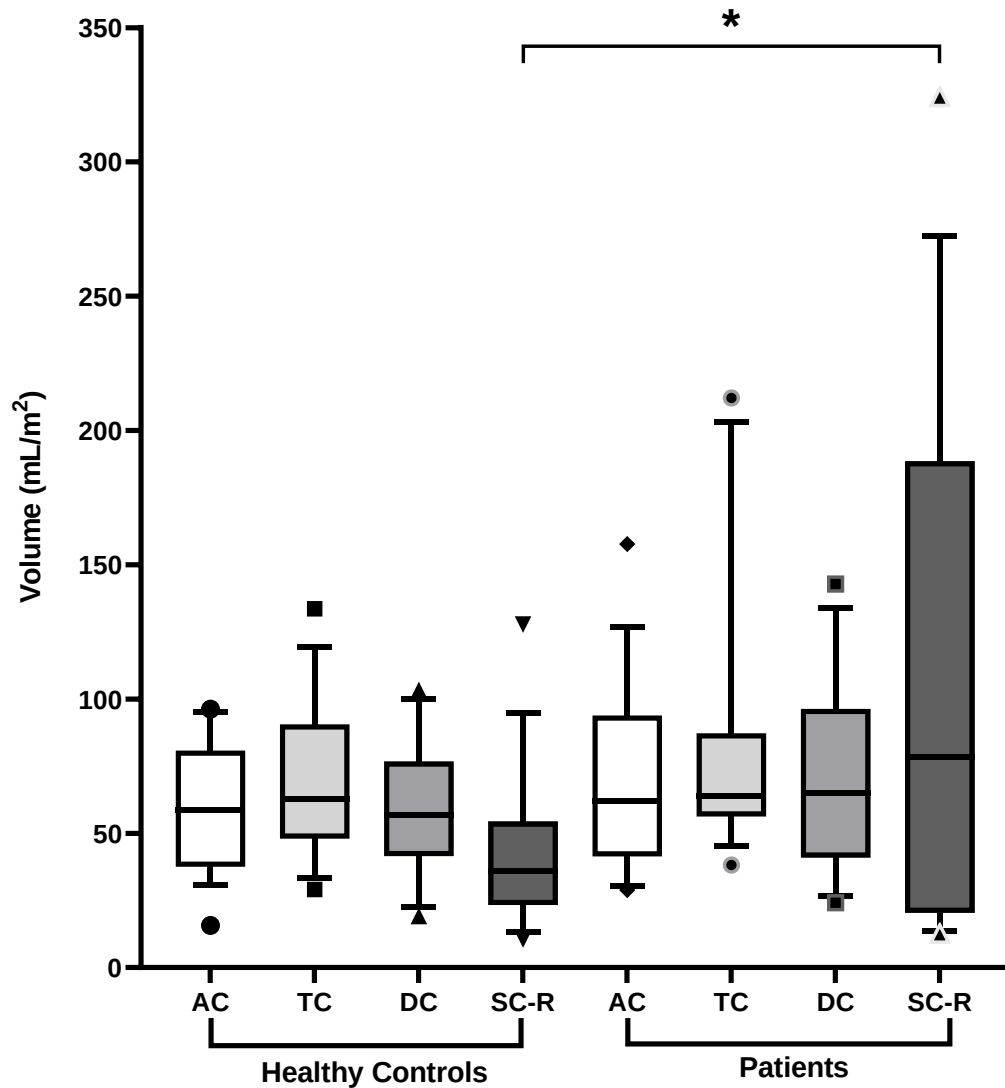


**Figure 5.5:** Regional colon volumes corrected for body surface area (ascending colon [AC], transverse colon [TC], descending colon [DC], sigmoid colon and rectum [SC-R]) for 19 controls and 16 patients with constipation. The box and whiskers plot shows the mean (+) and median (–) values of the regional ascending, transverse, descending, sigmoid colon and rectum volumes. The box represents the 25th–75th centile and the whiskers represent the 10th–90th centile ranges, respectively. \*  $P = 0.0410$  one-tailed Mann-Whitney test.

Median and IQR data of the colon of controls and patients are summarised numerically in Table 5.1.

**Table 5.1:** Volume of colon segments for 19 healthy controls and 16 patients with functional constipation. The data are shown both with actual values are also after correction for Body Surface Area. The data are presented in median (interquartile range) \*  $P < 0.05$  and \*\* $P < 0.001$  versus control.

| Colon segment        | Volume for healthy controls (mL) | Volume for patients with FC (mL) | Volume for healthy controls corrected for BSA (mL/m <sup>2</sup> ) | Volume for patients with FC corrected for BSA (mL/m <sup>2</sup> ) |
|----------------------|----------------------------------|----------------------------------|--------------------------------------------------------------------|--------------------------------------------------------------------|
| Ascending colon      | 82 (73-136)                      | 80 (40-132)                      | 59 (38-81)                                                         | 62 (41-94)                                                         |
| Transverse Colon     | 110 (81-152)                     | 88 (60-143)                      | 63 (48-91)                                                         | 64 (56-87)                                                         |
| Descending colon     | 100 (76-122)                     | 73 (42-163)                      | 57 (41-77)                                                         | 65 (41-96)                                                         |
| Sigmoid-rectum colon | 61 (36-106)                      | 92 (31-202)                      | 36 (23-54)                                                         | 78 (20-189)*                                                       |
| Total colon          | 383 (306-498)                    | 402 (285-549)                    | 227 (180-263)                                                      | 309 (243-384)**                                                    |

There was a positive correlation between the total colonic volume and WGTT (Figure 5.6), coefficient of correlation  $R = 0.56$ ,  $P = 0.0005$ .

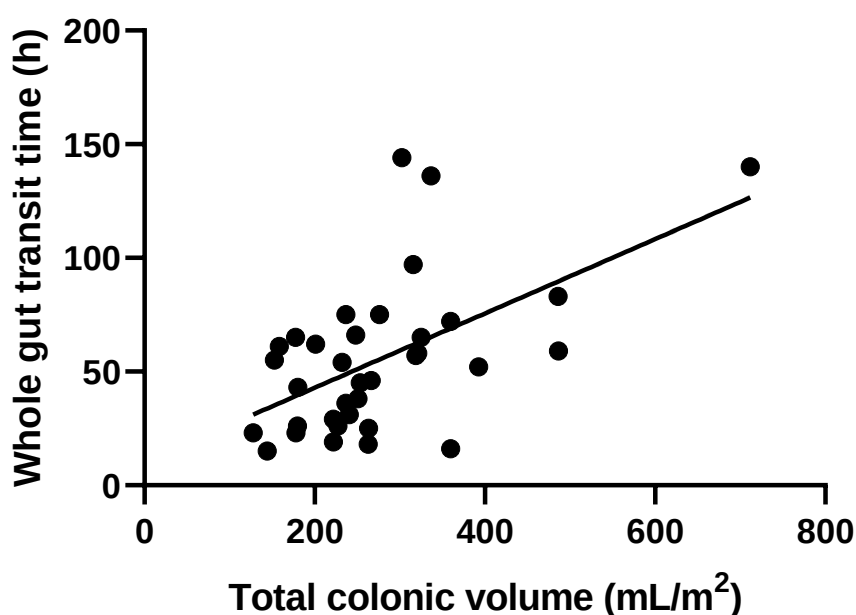


**Figure 5.6:** Correlation between whole gut transit time (WGTT) and total colonic volume, corrected for body surface area, for 19 controls and 16 patients with constipation coefficient of correlation  $R = 0.56$ ,  $P = 0.0005$ .

### 5.3.2 Colon length

It was possible to carry out the 3D skeletonization analysis for all participants and all colon segments. The process of determining the colon length of all segments for one participant, from existing colon region volume segmentations, took only a few minutes. These regions are shown for one patient participant in Figure 5.7. Next, binary masks of each segmented colon region were formed. Figure 5.7b shows a 2D projection of the overall 3D binary mask for the whole colon. Figure 5.7c shows a 2D projection of the overall 3D binary mask for the whole colon.

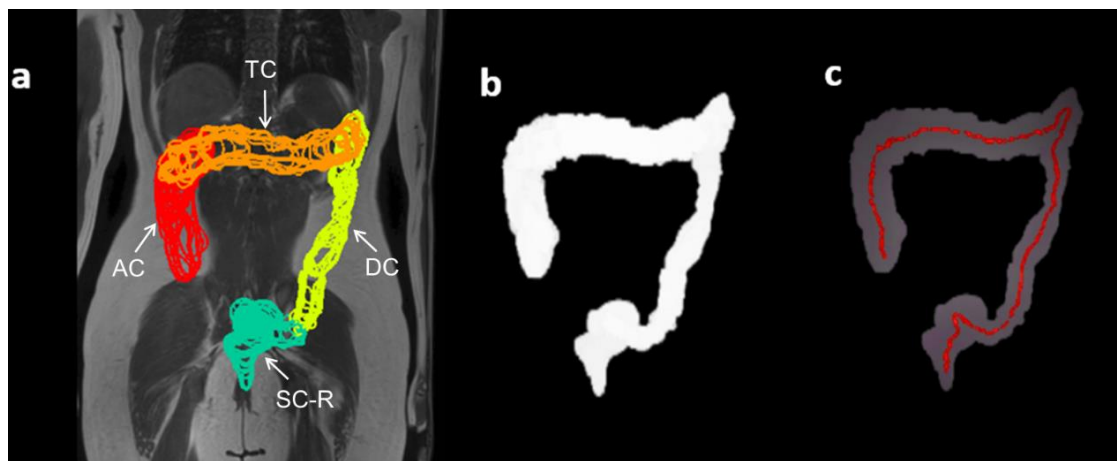


**Figure 5.7:** (a) 2D projection of the 3D segmentation of the 4 regions of the colon of a participant superimposed to a coronal MRI scan of their body. The regions are color coded as follows: ascending colon in red, transverse colon in orange, descending colon in yellow and sigmoid/rectum colon in green. (b) 2D projection of the overall 3D binary mask for the whole colon whereby white is colon and everything else is set to black. (c) 2D projection of the 3D longest shortest path line (shown in red) for the colon of this patient participant superimposed to a shaded colon projection outline.

In three participants the recto-sigmoid region was more challenging than the other regions due to its tortuosity and required re-segmentation of that colon region prior to application of the skeletonization process.

The data for colon lengths are reported in Table 5.2.

**Table 5.2:** Length of colon segments for 19 healthy controls and 16 patients with functional constipation. The data are shown both with actual values are also after correction for Body Surface Area. Values are mean $\pm$ SEM.

| Colon segment        | Length for healthy controls (cm) | Length for patients with FC (cm) | Length for healthy controls corrected for BSA (cm/m <sup>2</sup> ) | Length for patients with FC corrected for BSA (cm/m <sup>2</sup> ) |
|----------------------|----------------------------------|----------------------------------|--------------------------------------------------------------------|--------------------------------------------------------------------|
| Ascending colon      | 19 $\pm$ 1                       | 17 $\pm$ 1                       | 11 $\pm$ 1                                                         | 13 $\pm$ 1                                                         |
| Transverse Colon     | 27 $\pm$ 1                       | 24 $\pm$ 2                       | 16 $\pm$ 1                                                         | 18 $\pm$ 1                                                         |
| Descending colon     | 31 $\pm$ 2                       | 26 $\pm$ 1                       | 18 $\pm$ 1                                                         | 20 $\pm$ 1                                                         |
| Sigmoid-rectum colon | 20 $\pm$ 1                       | 22 $\pm$ 2                       | 11 $\pm$ 1                                                         | 17 $\pm$ 1                                                         |
| Total colon          | 96 $\pm$ 3                       | 90 $\pm$ 5                       | 56 $\pm$ 2                                                         | 69 $\pm$ 3                                                         |

Total colon length for all participants correlated significantly with their age ( $R=0.45$ ,  $P=0.0064$ ), height ( $R=0.49$ ,  $P=0.0031$ ) and weight ( $R=0.46$ ),  $P=0.0059$ ) but not with their BMI ( $R=0.17$ ,  $P=0.3265$ ).

The total colon length corrected for BSA (Figure 5.8) was 55 $\pm$ 2 cm/m<sup>2</sup> (mean $\pm$ SEM) for the healthy controls. Total colon length was significantly longer (69 $\pm$ 3 cm/m<sup>2</sup>) for the young patients with constipation compared to the controls ( $p=0.0037$  Mann-Whitney two-tailed test).

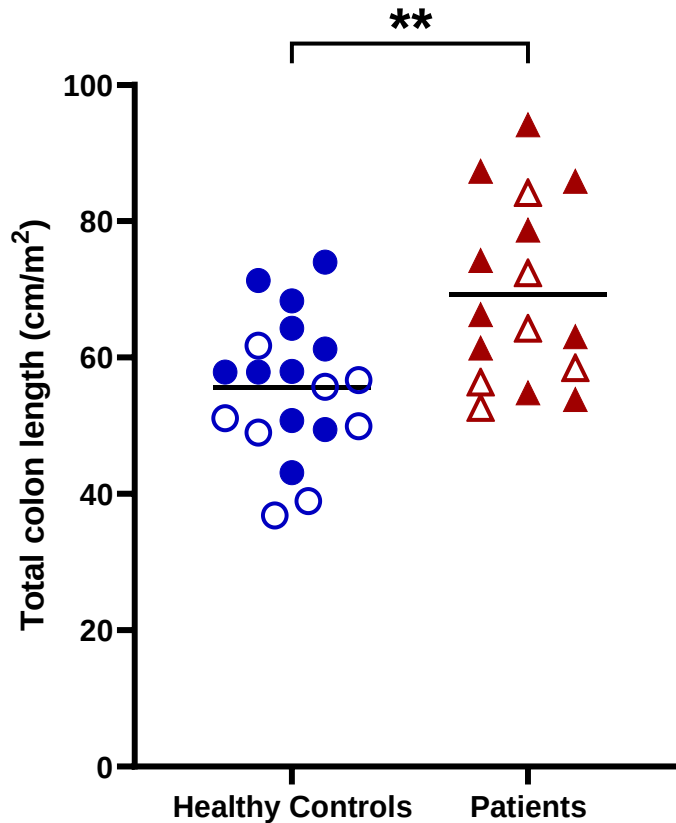


**Figure 5.8.** Total colon length corrected for both body surface area of  $n=19$  control and  $n=16$  patients with constipation. Open symbols indicate male participants and solid symbols indicate, respectively, female participants. The horizontal lines indicate the mean, \*\*  $P = 0.0037$  two-tailed Mann-Whitney test.

The total colon length corrected for BSA was significantly longer for female controls  $60 \pm 10$  cm/m<sup>2</sup> than for male controls  $50 \pm 9$  cm/m<sup>2</sup> ( $P = 0.0361$  unpaired two tailed t test). It was also longer for female patients  $74 \pm 5$  cm/m<sup>2</sup> than for male patients  $63 \pm 4$  cm/m<sup>2</sup>, but the difference was not significant ( $P = 0.1119$  unpaired two tailed t test). There was no correlation between age and actual (uncorrected for BSA) total colon length in the control group ( $P = 0.9759$ ), whilst these correlated strongly in the constipation group ( $R = 0.68$ ,  $P = 0.0039$ ).

Regional colon lengths, corrected for BSA, are shown in Figure 5.9. The AC and SC-R regions were shorter in the control group compared to the patients group,  $P = 0.0479$  and  $P = 0.0003$  respectively (Mann-Whitney one-tailed test).

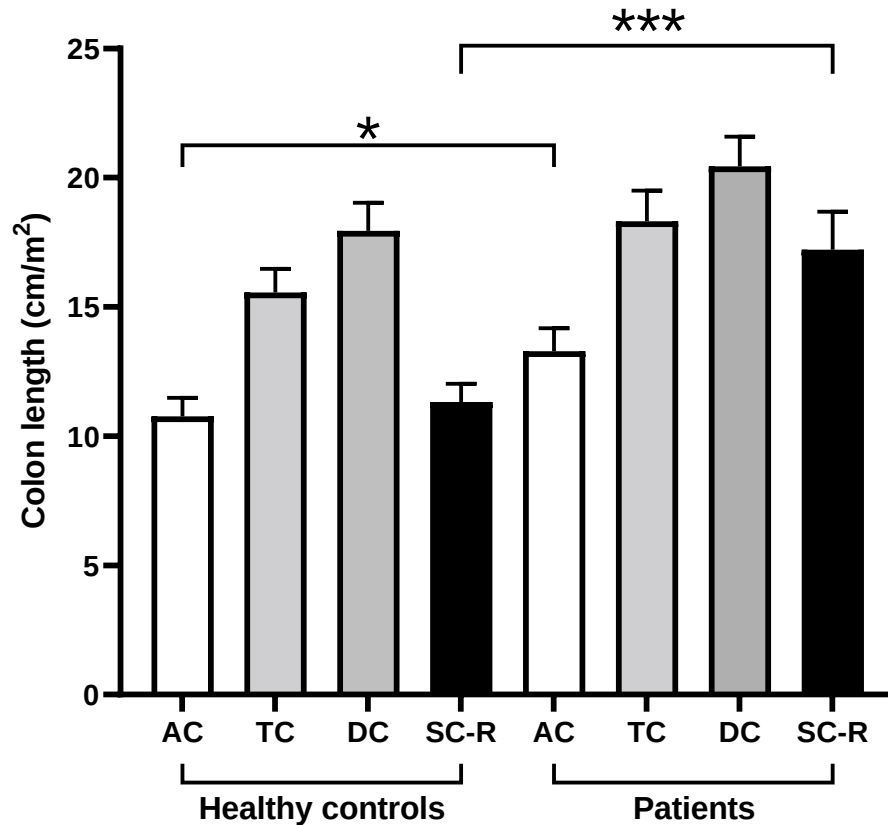


**Figure 5.9:** Regional colon length (Mean±SEM) corrected for body surface area (ascending colon [AC], transverse colon [TC], descending colon [DC], sigmoid colon and rectum [SC-R]) for 19 controls and 16 patients with constipation. \*  $P=0.0479$ , \*\*\*  $P=0.0003$  one-tailed Mann-Whitney test.

There was a positive, significant correlation between total colon length and total colon volume in the healthy controls, coefficient of correlation  $R = 0.56$ ,  $P = 0.0132$ . There was also a positive, significant correlation between total colon volume and total colon length in the patients with FC, coefficient of correlation  $R = 0.63$ ,  $P = 0.0093$ .

There was no correlation between total colon length and age in the healthy control group  $P=0.9759$ . There was however a positive, significant correlation between total colon length and age in the patients with FC, coefficient of correlation  $R = 0.68$ ,  $P = 0.0039$ .

No correlation between total colon length and whole gut transit time was found in the control group ( $P=0.5333$ ) nor in the patients' group ( $P=0.2582$ ).

## 5.4 Discussion

Colon volume has been shown to be increased in adult functional constipation [104, 105] but data on paediatric constipation was not available. This is the first comparative study on the undisturbed colonic volume in paediatric patients with FC and healthy paediatric controls using MRI. An early paper from 1973 investigated colonic volume in idiopathic constipation but the volume was derived from invasive constant infusion studies on unphysiologically lavaged colons [161]. In that report adult data were mixed with the data from the paediatric participants making it difficult to compare.

In our study the total colon volume was found to be larger in the paediatric patients with FC than in healthy controls, where 63% of patients with FC had a colonic volume above the 75% centile of normal control values and 25% of patients had the colon volume above the 95<sup>th</sup> centile (upper limit of normal) of the healthy control values. There are no similar studies to compare our data against. As expected, data suggested also some degree of correlation between colonic volume and height and weight in the different age groups. These considerations led to the use of a common Mosteller correction for body surface area [159] to allow for a more realistic comparison between organ volume as previously reported for MRI colon volume in children and adults with cystic fibrosis [158].

We also found a moderate but significant correlation of transit with colonic volume. Intervention studies in healthy adults and adults with constipation showed that feeding bulking agents such as ispaghula [150] and kiwifruit [151] increase colonic volume, most obviously in the proximal colon but these agents did not alter transit. Defecation has been shown to reduce colonic

volume, mostly after emptying the distal colon [153]. One can infer that defecation would influence the total colon volume of children with FC more since the distal colon represents a bigger proportion of their total colon volume than in adults. For most adult subjects' colonic volume is determined by many factors other than whole gut transit time. Constipation can also be associated with changes in microbiota [162] so metabolic changes and influence on fermentation cannot be excluded.

We were able to use 3D skeletonization methods to measure the length of colonic regions from MRI images in a group of healthy children and a group of children with FC. These data were collected in a physiological state, not disturbed by purges or oral/enteral contrast media (liquid or gas). We are not aware of similar paediatric FC data being available in the literature.

The large differences in size of the participants and the significant correlations of colon length with the height and weight of the participants justified the use of the Mosteller formula to correct for BSA to allow for more meaningful comparison between groups [140, 158, 159].

As hypothesised, colon length was longer in the patient group than in the controls, showing that the colon of these young patients not only has a larger volume [140] but also a greater length. This suggests that also the longitudinal muscles might be affected. Colons in females were longer than in males.

A limitation here is the lack of correlation with clinical data which the original feasibility study did not collect. This may increase the likelihood of chance findings, although the differences shown here have a high degree of statistical significance.

Attempts to calculate the whole colon length in one single step were impaired by the fact that sometimes the binary masks had a missing connection between segments in the representation of the entire colon due to image quality or lack of continuity between some image slices of the colon. Therefore, the colon length measurements were carried out in the binary masks for each individual segmented colon region and then the total colon length were measured by summing up the regional colon length values. The colon length analyses were relatively easy to apply in most of the colon regions, but the method was somehow more difficult to apply in the rectosigmoid region due to the complexity of the rectosigmoid colon shape. The regional segment lengths were exploratory outcomes and the p values here were not corrected for multiple comparisons. Correction for multiple comparison would still show that the SR-C differences were significant. Low numbers mean that some possible correlations with sex and were difficult to explore. It has been reported that the age of puberty can coincide with the time when decreased bowel frequency and other symptoms are first noticed [163] but in this study data on the age of puberty were not collected so it is not possible to comment on colon length differences before and after puberty.

Future studies should specifically stratify by pubertal status. Colonic length could also be assessed dynamically after prokinetic and antispasmodic drugs which may well act differently on longitudinal versus circular muscle and hence alter length / volume ratios.

This study provides novel data on colonic volume and colon length healthy children and children with functional constipation. These new data will help improve our understanding of colon pathophysiology in children. The data will

also be useful for the pharmaceutical industry whereby better knowledge of colon morphology parameters will improve pharmacokinetic modelling accuracy for colon-targeted drug systems. Further work is needed to increase the number of participants studied and continue to investigate the relationship between colonic volume, colon length and WGTT.

## **6 QUANTITATIVE MEASUREMENTS OF GASTRIC EMPTYING IN THE UPRIGHT POSITION (UPRIGE STUDY)**

The Sir Peter Mansfield Imaging Centre has recently installed a 0.5T, open design MRI scanner which could be used to study gastric emptying in the upright position, a more physiological and also patient-friendly set up. Unlike the conventional MRI scanners described in Chapter 2 and used for the work described in the previous Chapters, the upright MRI scanner has unique characteristics. This Chapter will firstly describe the upright scanner. It will then describe the development and then the demonstration of an upright gastric emptying protocol that could be suitable for children.

### **6.1 Introduction**

An upright, open-design MRI scanner has a dipolar superconductive magnet configuration different from that of the conventional, horizontal 'tunnel' clinical MRI scanners. The open design allows to position the participants both in a vertical (upright, weight bearing) or horizontal (recumbent) positioning. The open design allows and dynamic kinetic flexion and extension manoeuvres. The more open space around the patient can also be used for MRI-guided interventional procedures.

The upright MRI scanners are designed with a completely open gap between the magnetic poles that are placed on either side of the patient so that the magnetic field transverses the body axially as opposed to the conventional, horizontal bore magnets whereby the main magnetic field is aligned along the patient longitudinally (Figure 6.1).

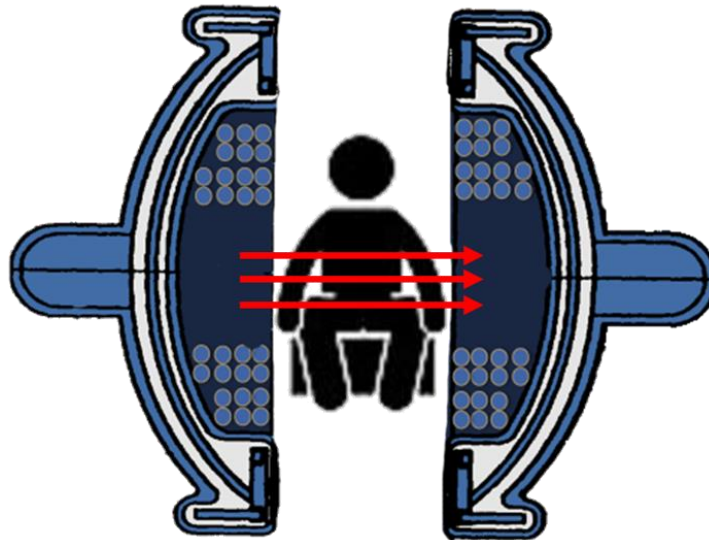


**Figure 6.1:** An illustration of the upright MRI scanner, the individual is placed inside the two vertical poles so that the magnetic field (red arrows) transverses the body in a horizontal direction.

The design limits the available field strength that can be achieved and current upright MRI scanners generally use low/medium field magnets of 0.5T. The open design impairs the static magnetic field homogeneity and increases distortions outside of a relatively small 'sweet spot'. The low field also yields less signal from the hydrogen protons and images are usually acquired with a lower resolution than on standard higher field horizontal bore scanners.

This configuration of open-bore scanners also places different constraints on the design of gradient, shim, and radio-frequency coils.

The open-design MRI scanners have attracted increasing interest from the community although in most radiology departments they are not yet available. In the UK they are rarely found on National Health Service (NHS) sites. Up to 10% of the population are claustrophobic at some point during their lives and specific research into claustrophobia in MRI has concluded that on average

1.2 to 2.3% of MRI examinations are abandoned due to patient anxiety and this can be higher in some centers [164, 165]. Based on NHS figures for MRI activity in 2013-14 [166] this would equate to over 30,000 patients failing to undergo their MRI scan. With an increase in MRI referrals of about 12% per year [166] this figure will grow, which suggests that it may be viable to offer an upright scanner service that could not only provide upright scanning but also help accommodating some of the more claustrophobic patients.

Researchers at the Sir Peter Mansfield Imaging Centre (SPMIC) have long developed MRI methods and image analysis protocols to determine gastric emptying non-invasively from changes in volume of stomach contents. These assessments were at first made on a home-built 0.5T horizontal bore scanner and are now made on conventional (mid-field 1.5T and high field 3T) MRI scanners. The participants have to lie supine within the machine, but this postural orientation may influence the rate that contents leave the stomach [185], particularly when different nutrients layer inside the stomach. To offset this potential impact, the time that the participants spend supine is usually minimised in the design of these studies, with participants sitting upright between different measurement time points. However, this involves repetitively setting up the participants for scanning and can restrict the frequency of assessments that can be made and could potentially also introduce a degree of mixing of the stomach contents.

The SPMIC has recently installed a low-field ASG Paramed MROpen 0.5T MRI scanner (ASG Medical Systems, Genoa, Italy, Figure 6.2) which will address the problems of measuring gastric emptying rate in a supine position.

The initial attempts to image the stomach for gastric emptying studies for this project were not encouraging. The scanner had been designed for high resolution static joint imaging and was now required to deliver single shot, T2-weighted, multi-slice images of good enough quality and within breath-hold range. This was due to limitations of both existing hardware and the software.

A recent upgrade of the scanner console, thanks to an Engineering and Physical Sciences Research Council (EPSRC) grant, made the development of a gastric emptying protocol possible. The section below consists of two parts. Part one is about the development of setting up of the gastric emptying protocol and part two is the demonstration of the gastric emptying protocol.



**Figure 6.2:** The 0.5T ASG Paramed MROpen MRI scanner at the Sir Peter Mansfield Imaging Centre at the University of Nottingham.

## **6.2 Optimising the upright MRI scanner**

Extensive in-vivo test MRI work was carried out to allow familiarisation with the scanner and then to optimise the protocols prior starting the actual developmental study.

### **In-vivo MRI test scans**

Pilot adult human work was carried out initially to understand the scanner capabilities and to gain information about RF coil positioning for seated participants, initial parameter optimisation for different sequences, and how to reduce scan time to breath hold range.

#### **Before the upgrade:**

The example images shown in Figure 6.3 were taken with a HASTE sequence which was tested to acquire both coronal and axial abdominal image stacks. As mentioned above, single-shot fast sequences were not available in the manufacturer's scanner library. The sequence had to be coded manually and all images needed to be reconstructed off-line, outside the scanner console and post processed to try to reduce noise and artifacts. This initial work was carried out with the expert help of Dr Joshua Macateer and Dr Olivier Mougin under guidance of Professor Penny Gowland. The laborious, off-line processing meant that the operator could not interact 'live' with slice positioning nor monitor quality. In addition, the MRI scanner didn't have an option for breath-hold triggering, and the coded sequences still took longer than desired.

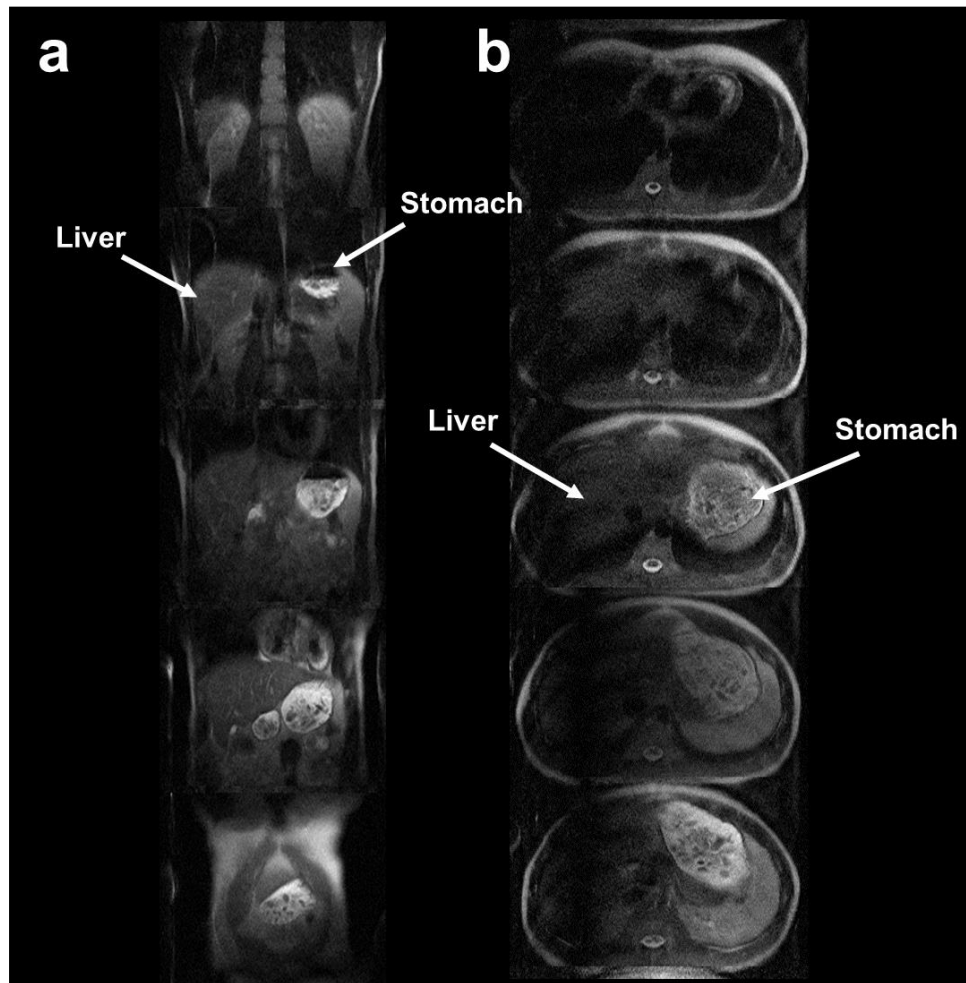


**Figure 6.3:** Example of a 'home-coded' HASTE (a) coronal and (b) axial abdominal scan, 5 slices 1 cm thick.

### **After the upgrade**

The above work presented great challenges for a viable gastric emptying protocol on the upright scanner. During that time a new Paramed scanner console became available, and Prof Penny Gowland was awarded an EPSRC grant that included the purchase and installation of the new hardware (waveform controller, reconstructor) and software.

The installation finally allowed good quality fast spin echo imaging, with immediate reconstruction and viewing on the scanner's graphical user interface. Strangely the upgrade still did not include the ability to stop the

sequence after preparations steps to instruct the participants to hold their breath. This problem was circumvented by Dr Olivier Mougin who coded in the scanner software the ability to stop the sequence after the preparation steps and restarting upon participant's instruction. This was done using an external trigger signal delivered by the scanner operator from the control room to the waveform controller using a waveform scope generator.

From this point onwards more pilot development work was carried out using healthy adult human volunteers. Figure 6.4 shows a complete multi-slice, coronal stack comprising 12 images taken using a 19 second breath-hold. A small (2 mm) slice gap was used to minimise cross-talk between slices and also to achieve better spatial coverage of the trunk within a single breath hold. The healthy participant had ingested 500 mL of water with 75 g table sugar. The scan has markedly better quality than the previous attempts and was repeated serially for 2 hours.



**Figure 6.4:** A multi-slice coronal abdominal series taking for 2 hours for a healthy volunteer with 500 mL of water with 75 g table sugar.

The optimised sequence scans parameters are detailed in Table 6.1.

**Table 6.1:** Details of the ASG Paramed MROpen 0.5T MRI upright MRI sequence parameters used in this study.

| <i>MRI sequence</i>                    | <i>Fast spin echo (FSE) gastric emptying</i> |
|----------------------------------------|----------------------------------------------|
| <i>Image orientation</i>               | Coronal – participant sitting at 60° angle   |
| <i>Field of View</i>                   | 350 mm (HF) × 301 mm (RL)                    |
| <i>Image stacks</i>                    | 1                                            |
| <i>Slices per stack</i>                | 12 – spacing 12 mm                           |
| <i>Length of breath hold per stack</i> | 10 s                                         |
| <i>Acceleration factor</i>             | N/A                                          |
| <i>Signal averaging</i>                | 1                                            |
| <i>Flip angle</i>                      | N/K                                          |
| <i>Repetition time TR</i>              | 3192 ms                                      |
| <i>Echo times TE</i>                   | 102 ms                                       |
| <i>Acquisition matrix</i>              | 116 × 116                                    |
| <i>Acquired image resolution</i>       | 3.02 mm × 2.60 mm × 10.0 mm                  |

### 6.3 Gastric emptying developmental study

Having optimised a good multi-slice scan the next task was to demonstrate the feasibility of a complete study protocol and, if possible, to validate it.

At that time Dr Liz Simpson, from the Nottingham's David Greenfield Human Physiology Unit, was running a separate gastric emptying study using a clinical <sup>13</sup>C-acetate breath test method, based on a <sup>13</sup>C-acetate labeled glucose tolerance test drink. Ingested acetate is rapidly absorbed from the small intestine after emptying from the stomach and broken down to carbon dioxide

which is then expelled in the breath. The stable isotope is then detected in breath samples by mass spectrometry and it provides a marker to estimate gastric emptying. This is a well-used, standard method to assess gastric emptying.

We joined forces and planned to run the MRI upright gastric emptying protocol using that same test drink and running concomitantly the MRI and the clinical <sup>13</sup>C breath test gastric emptying methods. By doing so we would firstly be able to demonstrate the feasibility of the MRI upright gastric emptying protocol. We would then secondly store the breath samples so that we could at a later stage compare MRI gastric emptying and <sup>13</sup>C breath test gastric emptying for validation of our MRI protocol. The glucose drink was particularly attractive as it would provide good MRI signal on moderately T2-weighted sequences, it would empty from the stomach slower than plain water, which empties too quickly from the stomach for the purpose of this study. Conversely, alternative solid meal, or a mixed macronutrient test meals, depending on composition, could take too long to empty from the stomach and/or not provide a good enough signal.

The aim of this study was therefore to establish and demonstrate feasibility of a gastric emptying protocol on the upright MRI scanner and to collect an initial data on healthy adult volunteers in an upright position.

#### **6.4 Methods**

This study was approved by Faculty of Medicine and Health Sciences Research Ethics Committee (FMHS REC, approval reference number FMHS 48-0822).

#### **6.4.1 Study participants**

At the time of writing, six healthy volunteers (5 female, 1 male, age  $25\pm 3$  years, BMI  $24\pm 1$  kg/m<sup>2</sup>) were recruited from the local campus population. Eligibility criteria are shown below:

##### **Inclusion criteria**

- Aged between 18 and 60 years.
- Healthy.
- Able to fully comprehend the informed consent process and give informed consent.
- BMI  $<30$  kg/m<sup>2</sup>.

##### **Exclusion criteria**

Potential participants with any of the following criteria were to be excluded:

- Inability to complete the informed consent process and/or MRI safety questionnaire.
- Known contraindication to MRI scanning (for example, pacemaker/implanted defibrillator, aneurysm clips, implanted programmable device, intra-ocular metallic fragment, claustrophobia).
- Major psychiatric, cognitive or mood disorder.
- Any other significant chronic medical conditions, including diabetes (type 1 and 2), asthma and epilepsy.
- Any acute major illness or surgery within one year prior to participation.
- Implanted metalwork in the torso (close to the stomach) which would degrade the MR image quality.

- Glucose intolerance, as assessed by a finger-prick test at screening.
- Having taken part in a research study in the last 3 months involving invasive procedures, ionising radiation or an inconvenience allowance.

#### **6.4.2 Test drink**

75 g glucose dissolved in 300mL of water, to which 150 mg of  $^{13}\text{C}$ -labelled sodium acetate have been added.

#### **6.4.3 Study Design**

This was a pilot study in a university setting. At the screening visit, the individual was asked to complete a general health screening questionnaire and an MRI Safety Screening questionnaire. The participant's height and weight were measured and a finger prick blood sample was taken to measure blood glucose using a portable glucose meter with disposable strips (both Accu-Chek Performa, Roche Diabetes Care, Inc). The participants were then asked to attend one MRI scanning session at the SPMIC MRI unit on A Floor Medical School, in the morning, after an overnight fast. Upon arriving the participants were asked to change into disposable scrubs and to provide 2 baseline breath samples by blowing in separate bags. They were then asked to sit on the scanner bed, configured as a 60° reclined chair position, the abdominal coil was placed around them, secured in position with straps. Padding was added between the chest and the coil and under the knees to make the participants more comfortable. The scanner bed was then sent to the isocentre of the magnet and fine adjustments in the height and depth of positioning were then made using MRI scout scans, until the empty stomach

appeared to be entirely inside the image field of view. This positioning was to be maintained until the end of the study unless the participants asked to come out, for example for a toilet break. The participants were then asked to ingest quickly the study test drink from a bottle while they were in their seated position inside the scanner (Figure 6.5). Third year undergraduate medical student Miss Aliya Ali helped with recruitment, study days and some of the data processing (meal volumes and interobserver variability).



**Figure 6.5:** Upright MRI scanner at the Sir Peter Mansfield Imaging Centre at the University of Nottingham. (Permission to use was obtained).

After this MRI of the stomach and breath samples were collected every 5 minutes in the first hour after the drink and every 10 min in the second hour. A flow diagram of the study is shown below (Figure 6.6).

After this time, the participants were taken out of the scanner and offered refreshments (drink and snack) before leaving the MRI imaging centre. The visit lasted no longer than three hours in total, with the total time in the scanner being the time needed to perform set up and baseline scans (10-15 minutes) plus the postprandial time of 120 min.

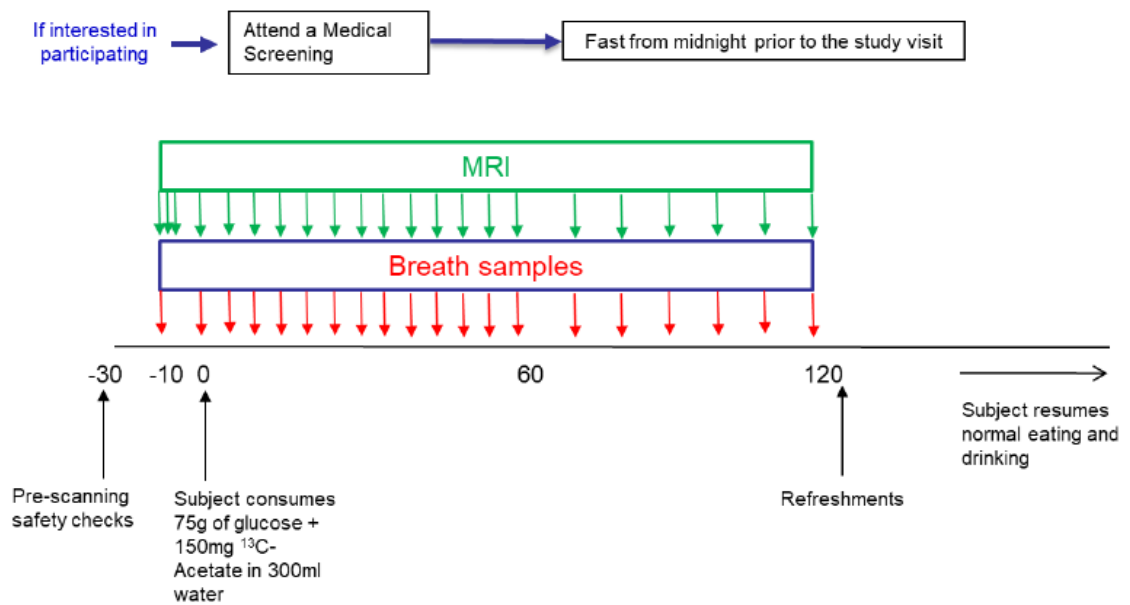


**Figure 6.6:** Flow diagram of study.

#### 6.4.4 Breath samples collection

At each time point the participants were handed one empty breath bag, whilst sitting in the scanner, and asked to blow breath inside the bag and pass it back to the researcher. The air in the bags was then decanted into two breath sample vacutainers by attaching the mouthpiece of the breath bag to a two-way tap with a line and a needle. Two vials were used so that the samples were stored in duplicate. They will be analysed at a later point using mass

spectrometry and the amount of  $^{13}\text{C}$  with time will be fitted to a common mathematical model yielding half gastric emptying time.

#### 6.4.5 Data analysis

Stomach volumes were measured using Medical Image Processing, Analysis, and Visualisation software (MIPAV, NIH, Bethesda) from the coronal FSE images. It was relatively easy to identify the stomach and its contents against the surrounding tissues (Figure 6.7) and then to manually draw ROIs around the stomach and its contents and then summing across all the volumes using the same MIPAV software and techniques described in previous Chapters. A second operator (Miss Aliya Ali) was trained in the MIPAV data analysis and analysed independently all-time points for one participant to assess interoperator variability.

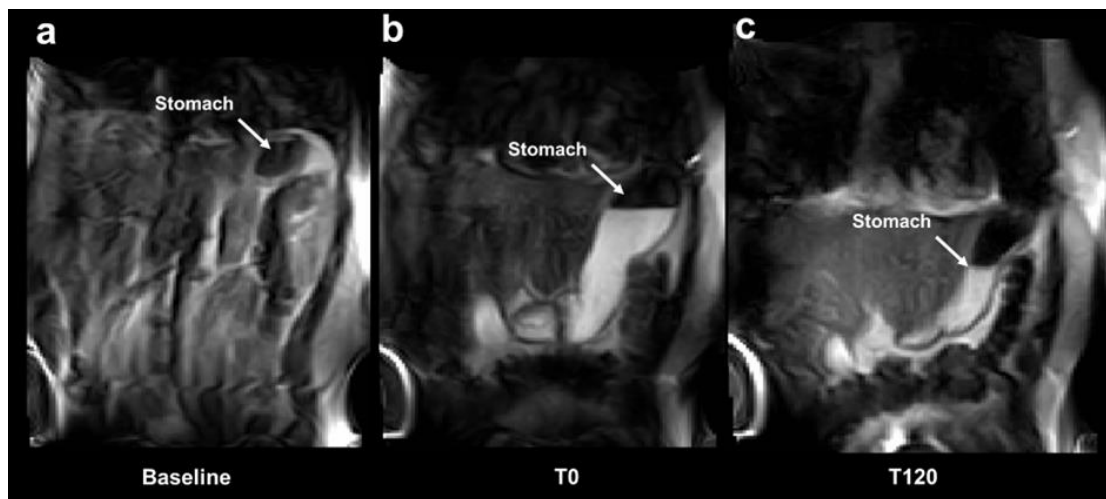


**Figure 6.8:** Illustrations of the gastric volume change in a healthy volunteer (a) before having the drink (89 mL), (b) after the test drink (478 mL) and (c) after two hours (214 mL).

#### **6.4.6 Statistical analyses**

The data are expressed as mean $\pm$ standard error of the mean (SEM). Descriptive statistics and data plotting were carried out using Prism version 9 (Graph Pad Software Inc., La Jolla, CA, USA). Half gastric emptying time was calculated from data fit as described in previous Chapters. Interoperator variability was assessed using linear regression and the coefficient of correlation between two operators.

### **6.5 Results**

All six participants completed the upright MRI study well without any adverse event. The test drink and study procedures were tolerated well and good quality data were collected throughout.

#### **6.5.1 Total gastric volumes**

The baseline average total gastric volume was 83 $\pm$ 11 mL, reflecting some resting gastric juices. The average total gastric volume increased after ingestion the test drink to 461 $\pm$ 25 mL (Figure 6.8). The average time to empty half of the stomach volume (T1/2) was 152 $\pm$ 20 min and to empty half of the meal volume was 115 $\pm$ 10 min.

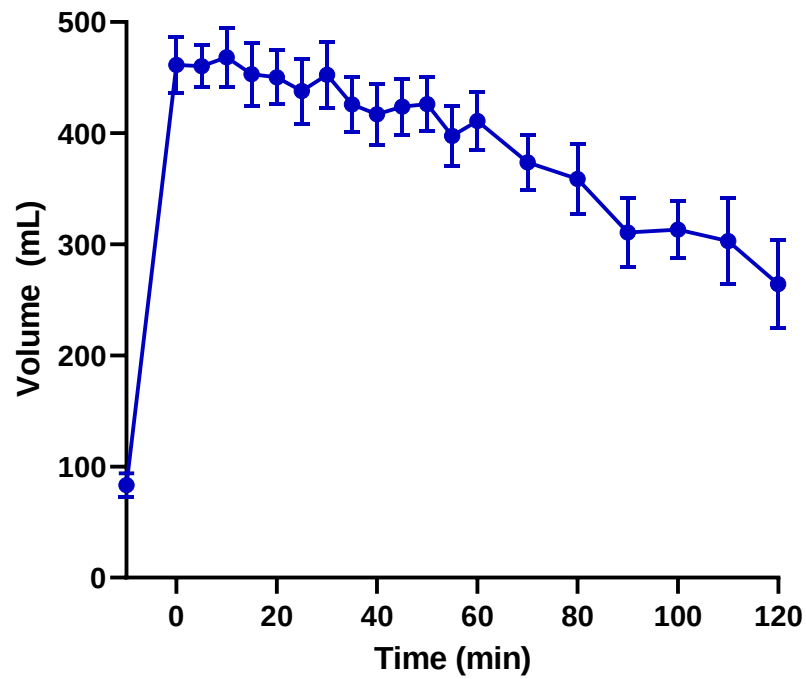


**Figure 6.8:** Time courses of total gastric volumes in 6 healthy participants who ingested 300 mL of glucose test drink. Data are shown as mean $\pm$ SEM.

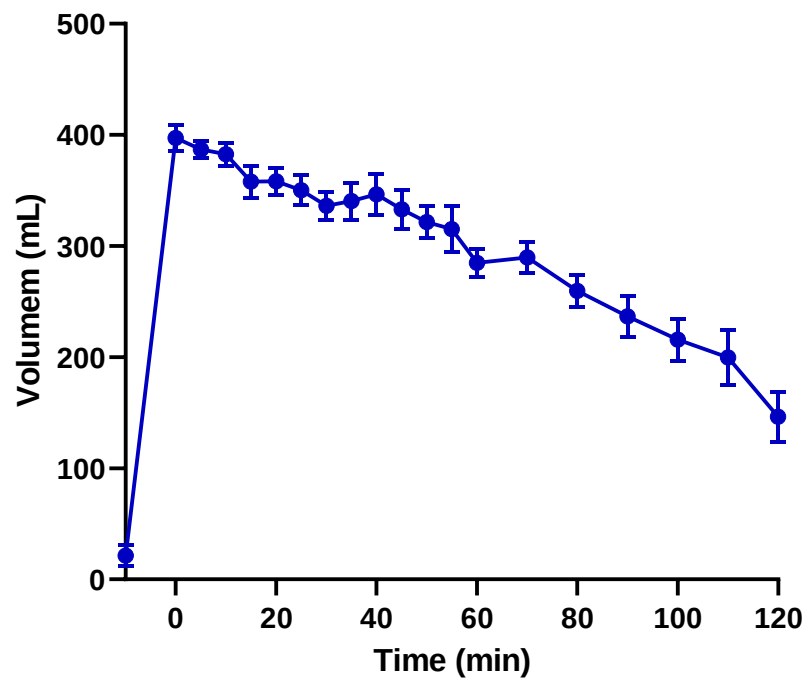


**Figure 6.9:** Time courses of test meal gastric volumes in 6 healthy participants who ingested 300 mL of glucose test drink. Data are shown as mean $\pm$ SEM.

### 6.5.2 Interobserver variability

The total gastric volumes analysed by the two operators lies along the line of identity over the whole range of volumes and correlated very well with correlation coefficient  $r = 0.95$  and the correlation was highly significant with  $P < 0.0001$ .

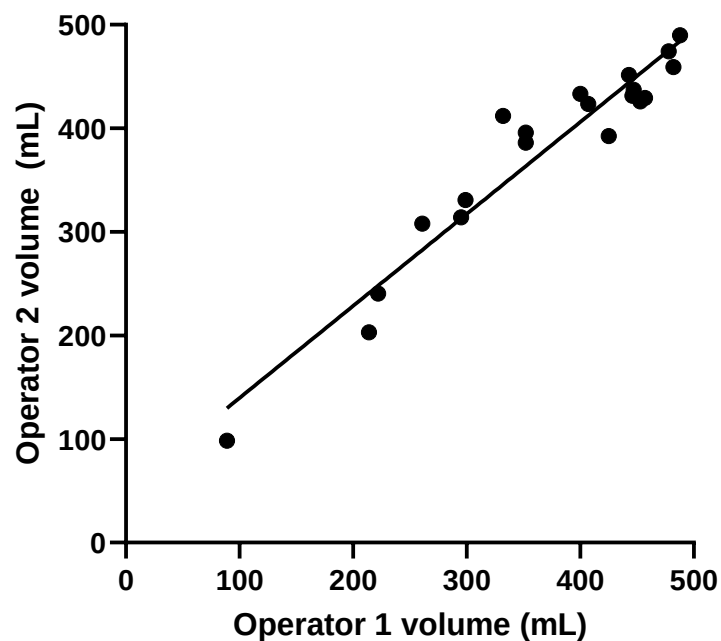


**Figure 6.10:** Comparison of total gastric volumes from one participant analysed independently by two operators. The solid line represents the line of identity. Correlation coefficient  $r = 0.95$  ( $P < 0.0001$ ).

## 6.6 Discussion

Traditionally, non-invasive methods to quantify gastric emptying rate in the upright position have required the ingestion of radioactive isotopes and gamma camera imaging. Ultrasound imaging has also been used. Alternatively, methods using surrogate markers of gastric emptying such as paracetamol appearance in the blood or, as discussed above, stable isotope appearance in the breath have been used. These methods have limitations. The nuclear medicine methods require participants to be exposed to ionising radiation and do not depict the anatomy. Ultrasound methods are more limited to views of the antrum with assumptions about total stomach volumes, and the beam is disrupted by air. The surrogate markers of gastric emptying could be influenced by other factors such as absorption rate from the gut, gut blood flow and metabolic rate, which can introduce error and increase the variability of the assessment. MRI provide a unique method to measure gastric emptying non-invasively and with good anatomical resolution. However the supine position can be unphysiological, particularly when layering of multicomponent meal drive emptying of nutrients depending on where the pylorus sits compared to the meal. This was shown previously in Nottingham using a fat and water meal, whereby the position of the participant on the right or left hand side changed gastric emptying dramatically depending on whether the water or the fat emptied first [167]. Because of this in some MRI studies the participants were positioned right side down so that the pylorus was lower than the fundus [168]. In the only example in the literature of a gastric emptying MRI study using an open design scanner similar to the one used here the

investigators showed that for a homogeneous liquid meal, gastric emptying of healthy participants positioned upside down is not markedly different from when they were standing up [169]. That study showed that gravity is not the main driver of gastric emptying but rather more a pressure-pump effect.

This study was successful. A new gastric emptying protocol was implemented for the first time on the Nottingham upright MRI scanner. The images obtained after the optimization phase are of good quality. In the study phase the feasibility of running a full gastric emptying protocol was demonstrated on 6 healthy adult participants. The setup is patient-friendly, particularly for paediatric patients, in that the upright position is more comfortable than lying down on a conventional scanner bed for a protracted period. The open design is less claustrophobic and allows better communication with the operators. Also, the 0.5T upright scanner is much less loud than a conventional clinical scanner. All these factors would provide a better experience to young patients. The upright scanner would suit well also early life studies where the baby/child could sit on a parent's lap. In this case the flexible belt receiver coil would probably be more suitable than the hard body coil used for this study. Initial experience made with that receiver was positive too.

A limitation of the study was that the stomach had not quite completed emptying within the two hours of data collection. After that period the participants started feeling uncomfortable in the sitting position, which suggests a less caloric meal could be used to shorten the time it takes to the stomach to empty. Alternatively, to study more caloric and/or mixed solid/liquid meals it should be possible to plan to take the participants in and out of the

scanner at given intervals so that they can stretch their back and legs and/or go to the toilet if needed.

As future work, in the period subsequent to the submission of this thesis additional scans and breath samples will be collected to carry out a meaningful comparison between the upright gastric emptying MRI method developed here the clinical  $^{13}\text{C}$  breath test gastric emptying test. Also, the author made an observation about stomach shape which appeared different in the upright scanner than laying down as seen in the data from gastric emptying in Chapter 3. The idea that the stomach shape may change depending on body position had been already mentioned in the 'upside down' gastric emptying study [169]. Further investigation is needed using quantitative mathematical methods to describe three dimensional shapes. This was however beyond the scope of this work.

## 7 DISCUSSION

Developing a better understanding of the mechanisms behind disorders of gastrointestinal function in children and young people is one of the top research priorities of the pediatric gastrointestinal community worldwide. This is for example stated in the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) Pediatric Gastroenterology Research Agenda [170]. Despite its importance, this area of interest is however under-researched. Barriers to paediatric research often include difficulty to recruit participants and problems with paediatric endpoints, difficulties commonly experienced also in the fields of paediatric pharmaceuticals [171] and paediatric medical devices [172]. In the case of gastrointestinal function, more specifically relating to gastric emptying, gut motility and transit, another problem is posed by the limitation of the current techniques, including catheters insertion or use of ionising radiation [120], which are particularly unsuitable for paediatric patients.

MRI has recently been taking a prominent role in investigating adult GI function non-invasively, however to date its applications to paediatric GI function in health and disease have been scarce. This work built on previous work from the Nottingham GI MRI research group and sought to address aspects of this unmet research priority.

The work presented here was successful in achieving its objectives and generated novel data and insights that add to the pre-existing body of knowledge and literature. The discussion below describes highlights of the work, putting them in context with current knowledge and literature.

## **7.1 Gastric emptying**

Monitoring of gastric emptying by MRI, using conventional horizontal bore scanners, is one of the more established techniques, going back to the early days of the groups in Nottingham [173, 174] and Switzerland [175]. The novel aspects here were to evaluate the feasibility of translating to a paediatric setting a previously developed adult MRI protocol, and to develop an upright gastric emptying protocol on a new open-design MRI scanner.

The first part of the task was more straightforward as the FIESTA sequence on the 1.5T GE scanner required only small adjustments and the soup meal had been used and characterised in previous adult studies [42, 96]. Gastric emptying data were collected successfully on 13 healthy children and will be used as reference range. The soup meal provided a good and reliable stimulus for GI responses but was not ideal for the age of the participants. Another breakfast, more child-friendly test meal with mixed macronutrient composition and enough caloric load could be considered in the future. Due to the pandemic effect on the local clinics, despite the effort, only two patients could be enrolled and this part of the work will be continued.

The second part of the task, the upright gastric emptying protocol, required additional development work. The original scanner console was ultimately unsuitable for breath-hold multislice, T2-weighted scanning but the new console allowed it, with some in-house coding help. The optimised imaging sequence performed very well and the complete study using the glucose drink test meal was very well accepted by the 6 participants scanned so far. The set up and protocol in an open design magnet will be ideal for children and for their parent/carers too.

It is expected that the MRI monitoring of upright gastric emptying will attract attention from the food industry, particularly the sector working with infant nutrition. There are many unanswered basic questions on infant physiology including gastric filling and emptying [176, 177] and the effect of formula feeds [178, 179]. There will be also interest from the developers of raft formulations for gastroesophageal reflux disease. Previous imaging studies on alginate raft formation and retention were carried out on conventional horizontal bore scanners [180, 181] whilst the more physiologically normal upright position would bring the rafts to float closer to the gastroesophageal junction.

## **7.2 Motility**

Cine-MRI to assess cardiac function developed quickly from the late 1980s [182] to become the 'gold-standard' for assessment of myocardial function [183, 184]. The development of MRI of bowel motility lagged behind. One reason being that bowel motility is not periodic. Another reason is that respiratory motion can affect the measurements substantially. In recent years the latter problem has been overcome using image coregistration methods such as the ones used in this work.

The principal area of application of these techniques has been CD [185]. Most studies exploit the use of oral contrast agent which is an unphysiological stimulus. Studies in Nottingham used a more physiological meal stimulus on an unprepared bowel, studying the time courses of small bowel motility in adult health and CD [42, 96, 99].

The translation of MRI motility methods to children and young people is a natural evolution of that work and the MICP study chapter presented here built on those protocols.

### **7.3 Whole gut transit time**

The MRI gut transit methods provide the non-ionising radiation equivalent of the radiopaque markers. This requires the use of MRI-visible markers and quick scans which are available on most clinical platforms. The attraction of using MRI for gut transit instead of ionising radiation methods lies particularly in the area of children and women in childbearing age for whom the gut transit test is indicated. The MRI measurement of WGTT presented here requires a short scan twice and could potentially be reduced in the future to one short scan following other X-ray ROM protocols, subject to a successful validation. The method is still limited to research settings, however the mini-capsules have been patented and trade-marked with the name TransiCap, and are approaching regulatory 'marking' approval procedures for sale.

### **7.4 Colon volumes and length**

This part of the work was successful too and provided unprecedented data on colon volumes and length in health and paediatric constipation.

These new data will help improve our understanding of colon pathophysiology in children. The data will also be useful for the pharmaceutical industry whereby better knowledge of colon morphology parameters will improve pharmacokinetic modelling accuracy for colon-targeted drug systems.

## **7.5 Limitations**

As mentioned above for WGTT, some of the techniques described here could translate to the clinical arena. Others require lengthy data processing, like the colon volume segmentation, or serial scans over a period of a couple of hours, which would be more difficult to fit in the routine busy schedule of an MRI unit. As such they are more likely to remain part of research clinical trials carried out to investigate aspects and phenotypes of disease or mode of action of drugs. An example of this is provided by the current Nottingham GIFT-CF studies in cystic fibrosis [158] and mode of action of novel transmembrane modulator drugs which are currently expanding to encompass children and young people with cystic fibrosis.

From the point of view of the studies presented here, the pandemic detrimental effect on the functioning of the paediatric gastroenterology clinics and, in turn, on recruitment affected the patient part of the MICP study.

## **7.6 Future directions**

In the short term, it is already planned to complete both the MICP series of patients and the comparisons against healthy controls, and the validation of upright gastric emptying by MRI against standard breath test.

In the medium to longer term, the applications of MRI to study GI function in children and young people are bound to develop further and to become more established. It is envisaged that applications of the upright scanner techniques will extend not just to children but also to early life whereby a neonate/infant could sit on the mother's lap with a belt coil around the abdomen to study milk ingestion and emptying both from breastfeeding and from bottle-feeding.

Automation as seen for the Motilent platform for small bowel motility has considerably speed up some aspects of data analysis. However, manual segmentation for stomach, gallbladder and colon volumes/length analysis is still a time-consuming burden for the operator. The use of machine learning and artificial intelligence in radiology is increasing exponentially and the paediatric work described here will certainly benefit from data analysis automation, which in turn will increase the appeal and chances of translation of these methods to the clinical arena.

## **7.7 Conclusions**

This thesis work has shown for the first time that a range of MRI techniques, both novel or previously used in adult studies, can be translated and applied to children and young people to study their gastrointestinal function. The techniques provided MRI endpoints to measure a range of parameters including gastric emptying, gallbladder contraction, small bowel motility, whole gut transit time, colon length and volume in an undisturbed bowel. The MRI data on WGTT and colon volumes and colon length is completely original and adds to the body of knowledge of gut morphology and function in paediatric health and constipation. The protocols were well received by the young participants, both the healthy controls and the patients, and the new upright gastric emptying protocol opens up a whole new space for work in the field.

These novel magnetic resonance imaging approaches and data can therefore increase our knowledge of paediatric gastrointestinal function and its dysfunction; this will help, in turn, diagnosis and treatment of paediatric patients.

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