University of Nottingham

# The use of magnetic resonance imaging to elucidate the mechanisms of gastrointestinal symptoms and complications in cystic fibrosis

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## Candidate statement

I confirm that all the work presented in this thesis are my own with the following exceptions:

Dr Caroline Hoad developed and prepared the MRI sequences and analysis program used to measure the MR outcomes.

Prof. Luca Marciani, Dr Caroline Hoad and Dr Neele Dellschaft assisted with a proportion of the MR analysis within the GIFT-CF 1 and GIFT-CF 2 studies.

Ms Melanie Lingaya performed the laboratory analysis for faecal elastase and faecal calprotectin for the GIFT-CF 1 and GIFT-CF 2 studies.

## Abstract

Over 72,000 people worldwide have cystic fibrosis (CF), an inherited life-limiting condition[1]. The underlying genetic defect causes secretions in the body to become thick and sticky, which can lead to chest infections and difficulties in digesting food[2].

Gut problems affect 65% of people with CF and can be an issue from birth[3]. Later in life, about 5% of people with CF per year experience serious bowel blockages, known as distal intestinal obstruction syndrome (DIOS)[4]. The introduction of pancreatic enzyme replacement therapy (PERT) improved overall nutritional outcomes; however gut symptoms still persist, which suggest a need to optimise PERT further.

Current treatments for DIOS have not been tested to current research standards[5], and there is limited understanding on the mechanisms of gut disease in CF. Factors that currently limit the evaluation of gut function in CF are the invasive and radiation nature of investigations[6]. Magnetic resonance imaging (MRI) avoids the above issues and over the last 20 years MRI has been used to evaluate other gastrointestinal disease.

This thesis will review the current understanding of CF gastrointestinal pathophysiology, complications and their treatment strategies. A systematic review of the literature will then be conducted to identify if there is any evidence on whether the administration of PERT, in relation to meal timings, has any effect on fat absorption or clinical gastrointestinal symptoms experienced. There will be an overview of

current tools used to investigate lower gastrointestinal problems before presenting a series of studies to evaluate the use of MRI to elucidate mechanisms of gut disease in CF.

The first study (GIFT-CF 1) will evaluate the feasibility for using MRI to identify changes in the gastrointestinal tract in CF compared to health.

The second study (GIFT-CF 2), a double-blind placebo-controlled randomised cross-over trial of tezacaftor-ivacaftor (TEZ/IVA), will use MRI to evaluate the gastrointestinal effects of TEZ/IVA.

This work has highlighted that there are gaps in the literature surrounding the timing of PERT administration and provided the research field with novel MRI findings on gastrointestinal physiology in CF. The use of MRI in CF gut disease could be useful in identifying sub-clinical complications and could be used in the understanding and development of new and existing CF therapies.

# Contents

Acknowledgements ..... i
Candidate statement ..... ii
Abstract ..... iii
Abbreviations ..... vii
List of figures ..... xii
List of tables ..... xvi
Chapter 1 Cystic fibrosis ..... 1
1.1. Epidemiology ..... 1
1.2. Diagnosis ..... 2
1.3. Genetics and classification ..... 3
1.4. CFTR pathophysiology ..... 5
1.5. Prognosis ..... 7
1.6. Gastrointestinal (GI) tract ..... 8
1.7. GI symptoms in CF ..... 26
1.8. Diagnosis and treatment of complications arising in the lower GI tract ..... 28
1.9. Treatment strategies ..... 33
1.10. Summary ..... 38
1.11. Aims of the thesis ..... 39
Chapter 2 A systematic review of timing of pancreatic enzyme replacement therapy doses in CF ..... 41
2.1. Background ..... 41
2.2. Aims ..... 44
2.3. Methods ..... 44
2.4. Results ..... 55
2.5. Discussion ..... 58
2.6. Conclusion ..... 62
Chapter 3 An overview of gastrointestinal investigations used in CF 65
3.1. Measures of transit ..... 65
3.2. Measures of pancreatic function ..... 70
3.3. Measures of absorption ..... 72
3.4. Measures of inflammation ..... 72
3.5. Other measures of physiological factors influenced by CFTR dysfunction ..... 73
3.6. Analysis of anatomical structures ..... 76
3.7. Summary of gastrointestinal investigations ..... 78

3.8. A simplified approach to MRI ..... 79
3.9. Summary ..... 105
Chapter 4 A study of the post-prandial changes in MRI parameters of gastrointestinal function in people with CF compared to non-CF controls ..... 107
4.1. Background ..... 107
4.2. Study aim and hypothesis ..... 108
4.3. Methods ..... 109
4.4. Results ..... 125
4.5. Discussion ..... 140
4.6. Summary ..... 152
Chapter 5 A double-blind, placebo-controlled, randomised crossover trial of the effects of tezacaftor-ivacaftor on gastrointestinal function, using MRI parameters in people with CF ..... 153
5.1. Background ..... 153
5.2. Study aims and hypothesis ..... 154
5.3. Methods ..... 155
5.4. Results ..... 170
5.5. Discussion ..... 190
5.6. Summary ..... 197
Chapter 6 Discussion ..... 198
6.1. Summary of original research ..... 198
6.2. Future directions ..... 203
6.3. Final remarks ..... 207
References ..... 208
List of appendices ..... 252
Appendix A: GIFT-CF 1 raw MRI and questionnaire data ..... 253
Appendix B: GIFT-CF 2 raw MRI and questionnaire data ..... 255
Appendix C: Example GI symptom questionnaires used in the GIFT-CF 1 and GIFT-CF 2 studies ..... 257
Appendix D: Rights and permissions ..... 260
Appendix E: Presentations arising from work done during this PhD ..... 261
Appendix F: Publications arising from work done during this PhD ..... 264

# Abbreviations

| | |
|---|---|
| ABC | ATP-binding cassette |
| AC | Ascending colon |
| AS | Alan Smyth |
| ATP | Adenosine triphosphate |
| au | Arbitrary units |
| AUC | Area under the curve |
| $B_0$ | Magnetic field |
| BMI | Body mass index |
| CENTRAL | Cochrane cystic fibrosis central registrar of controlled trials |
| CONSORT | Consolidated standards of reporting trials |
| CF | Cystic fibrosis |
| CF-QoL | Cystic fibrosis quality of life questionnaire |
| CFQ-R | Cystic fibrosis questionnaire revised |
| CFTR | Cystic fibrosis transmembrane conductance regulator |
| CH | Caroline Hoad |
| cm | Centimetre |
| CN | Christabella Ng |
| $CO_2$ | Carbon dioxide |

| | |
|---|---|
| COVID-19 | Coronavirus |
| CT | Computed tomography |
| DC | Descending colon |
| Delta TI | Post-prandial change in corrected small bowel water content before and after the second meal (at time points 240 and 300 minutes) |
| df | Degree of freedom |
| DIOS | Distal intestinal obstruction syndrome |
| ELISA | Enzyme-linked immunoassay |
| ENaC | Epithelial sodium channel |
| ESPGHAN | European society for paediatric gastroenterology, hepatology and nutrition |
| $FEV_1$ | Forced expiratory volume in 1 second |
| g | Gram |
| GE | Gastric half-emptying |
| GI | Gastrointestinal |
| GIFT-CF | Gut imaging for function and transit in cystic fibrosis |
| GM | Giles Major |
| GRADE | Grading of recommendations assessment, development and evaluation |
| GSRS | Gastrointestinal symptom rating score |

| | |
|---|---|
| HTA | Health technology assessment |
| $I^2$ | Heterogeneity |
| ICTRP | International clinical trials registry platform |
| ICC | Intra-class correlation coefficient |
| ISRCTN | International standard randomised controlled trial number |
| IQR | Inter-quartile range |
| k-space | Frequency space |
| kg | Kilogram |
| L | Litres |
| LM | Luca Marciani |
| $M_0$ | Net magnetisation |
| MHRA | Medicines and healthcare products regulatory agency |
| MHz | Megahertz |
| mins | Minutes |
| MIPAV | Medical image processing, analysis, and visualisation |
| MMC | Migrating motor complex |
| MR | Magnetic resonance |
| MRI | Magnetic resonance imaging |
| ms | Milliseconds |
| mSv | Millisievert |

| | |
|---|---|
| NBC | Sodium-bicabonate co-transporter |
| ND | Neele Dellschaft |
| NHE | Sodium-hydrogen co-transporter |
| NHS | National Health Service |
| NICE | National Institute for Health and Care Excellence |
| NIHR | National institute for health research |
| $O_2$ | Oxygen |
| OCTT | Oro-caecal transit time |
| PAC-QOL | Patient assessment of constipation quality of life |
| PAC-SYM | Patient assessment of constipation – symptoms |
| PERT | Pancreatic enzyme replacement therapy |
| Q25 | Lower quartile |
| Q75 | Upper quartile |
| RARE | Rapid acquisition with relaxation enhancement |
| RF | Radiofrequency |
| RS | Recto-sigmoid |
| SD | Standard deviation |
| SIBO | Small intestinal bacterial overgrowth |
| SBWC | Small bowel water content |
| SLC | Solute carrier |

| | |
|---|---|
| SPMIC | Sir Peter Mansfield Imaging Centre |
| T | Tesla |
| $T_1$AC | $T_1$ relaxation time of the ascending colon |
| TC | Transverse colon |
| TEZ/IVA | Tezacaftor-ivacaftor |
| $T_E$ | Echo time |
| $T_I$ | Inversion time |
| $T_R$ | Repetition time |
| UK | United Kingdom |
| US | United States of America |
| $V_0$ | Initial volume |
| WHO | World health organization |

# List of figures

Figure 1.1. Structure of CFTR ..... 3

Figure 1.2. Classes of CFTR mutations..... 4

Figure 1.3. GI tract highlighting sphincters and valve ..... 8

Figure 1.4. Large intestine anatomy ..... 13

Figure 2.1. CONSORT diagram to show the flow of studies in the systematic review ..... 56

Figure 3.1. Ussing chamber system to measure potential difference of rectal epithelial tissue CFTR ion channels..... 75

Figure 3.2. Diagram to show the magnetic field direction ..... 80

Figure 3.3. Diagram to show hydrogen atoms within a magnetic field. 81

Figure 3.4. Diagram to demonstrate net magnetisation..... 83

Figure 3.5. Image of person in the MRI scanner ..... 85

Figure 3.6. Graph to show the relationship of signal intensity and $T_R$ between fat, solid and water ..... 87

Figure 3.7. MR images of the same person to show difference between $T_1$ and $T_2$ weighted sequences..... 88

Figure 3.8. Diagram to show the proton (•) decaying back to its original direction (↑) in the z plane after a 90° radiofrequency pulse is applied 89

Figure 3.9. Coronal view of abdomen out of phase ..... 92

Figure 3.10. Fat and water spin in and out of phase at specific $T_E$..... 93

Figure 3.11. MRI images to show the head of the first meal arriving in the caecum..... 95

Figure 3.12. Single transverse slice of participant ..... 96

Figure 3.13. Graph generated to calculate gastric half-emptying time. 97

Figure 3.14. Colonic volumes ..... 99
Figure 3.15. Small bowel water content..... 101
Figure 3.16. Screenshot of $T_1$AC analysis using in-house software .. 103
Figure 4.1. Set meals provided for all participants..... 115
Figure 4.2. GIFT-CF 1 MRI study day schedule ..... 118
Figure 4.3. Participant positioned on scanner table..... 119
Figure 4.4. CONSORT diagram to show flow of study participants ... 126
Figure 4.5. Graph to show the time taken for a meal to reach the caecum..... 129
Figure 4.6. Graph to show the corrected SBWC (median, inter-quartile range [IQR]) over the scanning day. ..... 131
Figure 4.7. Boxplot of corrected delta TI for the CF and control group ..... 131
Figure 4.8. Bland-Altman plot to show the inter-observer variability in SBWC measurements ..... 132
Figure 4.9. Boxplot of corrected total colonic volume AUC for the CF and control group..... 132
Figure 4.10. Bland-Altman plot to show the inter-observer variability in ascending, transverse and descending colonic volumes..... 133
Figure 4.11. Bland-Altman plot to show the inter-observer variability in total colonic volumes (ascending, transverse, descending and recto-sigmoid colon) ..... 134
Figure 4.12. Chart to show the median domain scores of the CFAbd-Score between the control and CF group. ..... 135

Figure 4.13. Graphs to show reported GI symptoms for A. flatulence, B. bloating and C. abdominal pain during the study day in response to a test meal ..... 137
Figure 4.14. Boxplot to show the median and IQR of faecal elastase between control and CF groups ..... 139
Figure 4.15. Boxplot to show median and IQR of faecal calprotectin between control and CF groups ..... 140
Figure 5.1. Flowchart to show the two-period crossover trial design..159
Figure 5.2. GIFT-CF 2 MRI study day schedule ..... 160
Figure 5.3. CONSORT diagram to show participant flow. ..... 171
Figure 5.4. Graph to show the time taken for a meal to reach the caecum in the primary analysis ..... 173
Figure 5.5. Graph to show the time taken for a meal to reach the caecum in the secondary analysis ..... 173
Figure 5.6. Graphs for primary analysis of corrected SBWC and corrected delta TI ..... 176
Figure 5.7. Graphs for secondary analysis of corrected SBWC and corrected delta TI ..... 177
Figure 5.8. Graphs for primary analysis of corrected colon volumes. 179
Figure 5.9. Graphs for secondary analysis of corrected colon volumes ..... 180
Figure 5.10. Diagram to show median scores for each domain of the CFAbd-Score in the primary analysis ..... 181
Figure 5.11. Test-retest comparison for OCTT ..... 186

Figure 5.12. Test-retest comparison for corrected colon volumes AUC ..... 187
Figure 5.13. Test-retest comparison for corrected SBWC AUC ..... 188
Figure 5.14. Test-retest comparison for corrected delta TI ..... 189

# List of tables

Table 1.1. Summary of GI hormone secretion and actions..... 12

Table 2.1. Database search terms for systematic review. ..... 53

Table 2.2. Inclusion criteria for systematic review ..... 54

Table 2.3. Summary of reasons for exclusion of references for systematic review ..... 57

Table 4.1. Inclusion and exclusion criteria for GIFT-CF 1..... 112

Table 4.2. Summary of nutritional contents of GIFT-CF 1 set meals. 116

Table 4.3. Summary of analysts for MRI outcomes ..... 122

Table 4.4. Participant demographics ..... 127

Table 4.5. Post-prandial small bowel motility in arbitrary units ..... 138

Table 4.6. Pre and post-prandial $T_1$AC in seconds..... 138

Table 5.1. Inclusion and exclusion criteria for GIFT-CF 2..... 157

Table 5.2. Summary of nutritional contents of GIFT-CF 2 set meals. 162

Table 5.3. Participant demographics at baseline and baseline study visit measurements..... 172

Table 5.4. Summary of secondary outcomes. ..... 182

Table 5.5. Summary of exploratory outcomes ..... 184

# Chapter 1 Cystic fibrosis

Cystic fibrosis (CF) was first described by Dorothy Andersen in 1938 in a case series of young children where post-mortem revealed cyst formation and fibrosis of the pancreas[7]. Approximately 50 years later, Lap Chee Tsui and colleagues discovered that mutations in the CF transmembrane conductance regulator (CFTR) gene on chromosome 7 were responsible for the condition[8].

## 1.1. Epidemiology

CF is the most common life-limiting autosomal recessive condition in populations with northern European ancestry[2]. There are over 72,000 affected individuals worldwide[1], including just over 10,000 in the United Kingdom (UK); each year, around 200 people in the UK are newly diagnosed with CF[4]. In the UK an estimated 1 in 25 people are carriers of the faulty CF gene[9]. The carrier frequency varies between different ethnicities. In North America, the overall carrier frequency is 1 in 36, where Caucasians have the highest carrier frequency of 1 in 27 compared to Asians who have the lowest carrier frequency of 1 in 313[10]. In Australia, Massie *et al*[11] showed there to be approximately 1 in 25 carriers in women and couples who were planning or in the early stages of pregnancy.

## 1.2. Diagnosis

The diagnosis of CF has previously been by clinical presentation and family history. This meant some people were not diagnosed as a neonate and received a delayed CF diagnosis; especially those with milder phenotypes[12].

Since the introduction of newborn blood spot screening (2007 in the UK[4] and state by state gradually from 1997 in the United States of America, US[13]), the majority of people with CF are diagnosed as a neonate where newborn screening is available. In these areas, the number of new diagnoses in asymptomatic or minimally symptomatic infants have increased[14,15]. A heel-prick blood sample is taken from a neonate between days 2 and 5 of life to test for immunoreactive trypsinogen. A high immunoreactive trypsinogen is suggestive of CF, but further investigations such as sweat chloride testing and genetic testing are required to confirm a diagnosis[16].

Although most cases of CF are now diagnosed through newborn blood spot screening, approximately 10 to 20% of babies with CF will present with meconium ileus and some infants will already have faltering growth before screening results are received[4,15]. In 2020, the median age of all newly diagnosed individuals with CF in the UK was 2 months; with 74% of the diagnoses made by newborn screening and approximately 6% made in people over the age of 16 years. The median age of CF diagnoses in the UK, made before 16 years of age, was 22 days[4].

## 1.3. Genetics and classification

The role of the *CFTR* gene is to encode the CFTR protein, which is a chloride channel that functions at the epithelial cell surface to regulate chloride ions[17]. The CFTR protein (Figure 1.1) is part of the adenosine triphosphate (ATP) - binding cassette (ABC) gene family as it contains two ATP-hydrolysis nucleotide domains and two membrane-spanning domains (each with six membrane-spanning alpha helixes)[17,18]. The nucleotide and membrane-spanning domains send signals to each other to allow for ATP binding and hydrolysis, and subsequently the opening and closing of ion transport channels[18].

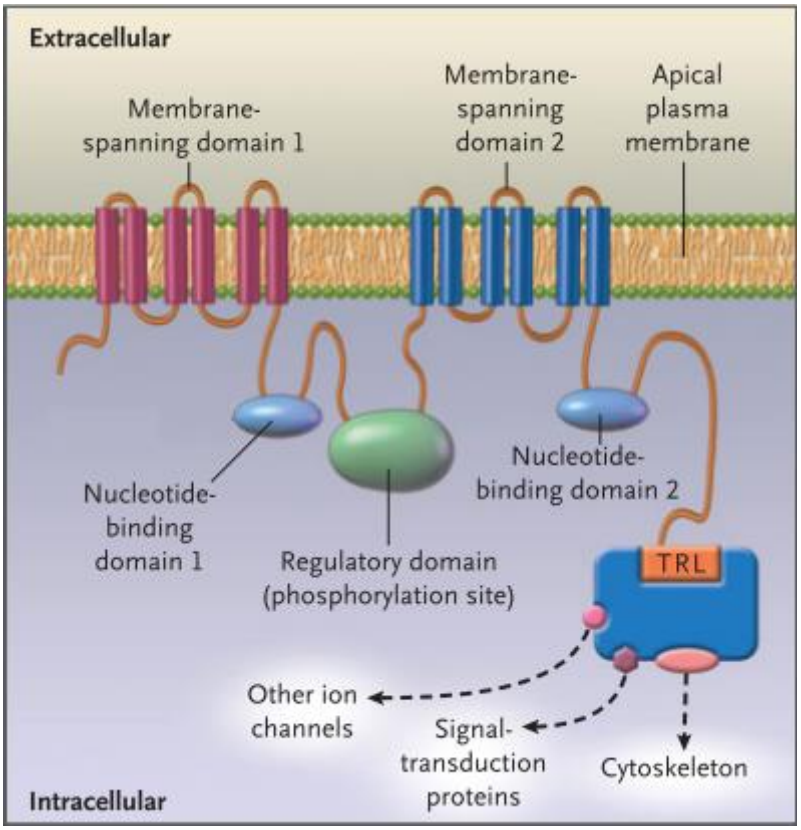



*Figure 1.1. Structure of CFTR. The TRL are amino acids (threonine, arginine and leucine) which hold the CFTR in position so it is close to other proteins. Reproduced, with adaptations and with permission from Rowe 2005[17], Copyright Massachusetts Medical Society.*

Traditionally, *CFTR* gene mutations are classified into six classes (Figure 1.2): I to VI based upon how the CFTR protein is affected. Mutations can range from those producing a protein with residual function to those producing a non-functioning CFTR protein. The CF phenotype varies accordingly[2]. Those with non-functioning CFTR proteins are more likely to have a severe phenotype. People who have residual CFTR function may present later in life.

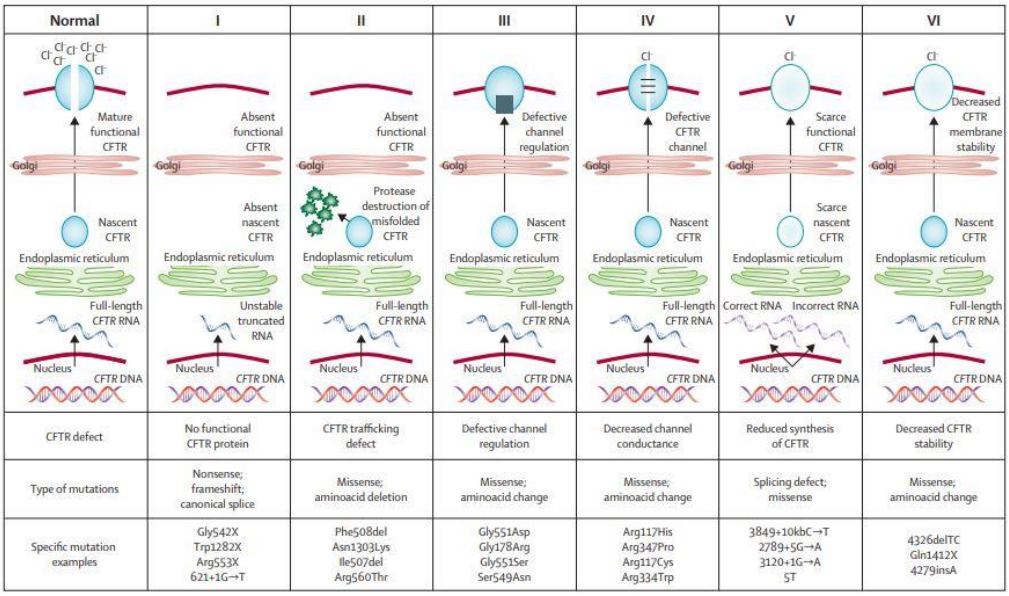



*Figure 1.2. Classes of CFTR mutations. Reproduced with permission from Elborn 2016[2].*

There are over 2000 *CFTR* gene mutations but only 382 mutations are believed to be CF-causing[19]. The most common mutation is p.Phe508del[2]. In the UK, approximately 89% of people with CF have at least one p.Phe508del gene mutation, with about 48% being p.Phe508del homozygous and 41% being p.Phe508del heterozygous[4].

## 1.4. CFTR pathophysiology

The CFTR protein is mainly responsible for chloride and bicarbonate transport at the epithelial surface but is also involved with other ion channels, such as the epithelial sodium channel (ENaC), to ensure surface epithelium lubrication. A dysfunctional CFTR protein causes thick viscous secretions which lead to ductal blockages and can lead to end-organ failure in multiple systems. The pathophysiology of the CFTR protein is best described in the airway and pancreas, and parallels can be made with the gastrointestinal (GI) tract[2].

Other manifestations of CF disease, such as bile acid stasis within the liver leading to CF related liver disease[20] and fertility problems[2], are out of the scope of this thesis and will therefore not be discussed.

Within normal airways, a balance in sodium absorption (via ENaC) and chloride secretion (via CFTR channels) is required to optimise mucus clearance from distal to proximal airways[21]. The CFTR negatively regulates ENaC function[21] and so, in CF, an absent or non-functional CFTR protein causes increased sodium absorption into the cell via ENaC and reduced CFTR-mediated chloride transport out of the cell[22]. As a result, more water is absorbed into the cell, leaving the airway surface liquid depleted. The airway surface liquid consists of a layer of periciliary liquid and a mucus layer, both of which are required to achieve muco-ciliary clearance[23]. When the airway surface liquid is depleted, both periciliary liquid and mucus layers are reduced so the cilia are unable to beat, leading to poor muco-ciliary clearance. There is a build-up of mucus with an impaired muco-ciliary escalator, which can

cause small airway obstruction. Mucus stasis is a favourable environment for bacterial growth and neutrophilic inflammation[17]. Recurrent chest infections can lead to the development of bronchiectasis[2].

A dysfunctional CFTR protein also causes a lack of bicarbonate secretion through the chloride-bicarbonate exchanger[24,25], which reduces the pH of the airway surface liquid and results in an impaired host defence. A more acidic pH in the CF airways impairs the action of antimicrobial enzymes (e.g. lysozyme) and proteins (e.g. lactoferrin) in the airway surface liquid against bacterial infection[26]. The lack of bicarbonate is also thought to contribute to the formation of viscous mucus, by preventing the mechanism which mucins unfold and expand to create a surface liquid. Viscous mucus subsequently contributes to mucus stasis[27].

In the pancreas, a dysfunctional CFTR protein results in reduced bicarbonate secretion in 85 to 90% of people with CF[28] and thus dehydrated pancreatic secretions[29]. The combination of reduced flow and volume of pancreatic secretions and high protein concentration (which affects pro-enzyme secretions) ultimately leads to obstruction, chronic inflammation, pancreatic exocrine insufficiency and end-organ damage[20,29]. Lipase has an important role in fat absorption, therefore a deficiency can lead to malabsorption and have adverse effects on nutrition and growth[30]. Low systemic concentrations of fat-soluble vitamins (vitamin A, D, E and K) have also been associated with pancreatic exocrine insufficiency[29].

## 1.5. Prognosis

CF was previously perceived as a paediatric condition due to the early mortality; for a person born between 1971 and 1973, the median survival was 20 years[31].

Chest disease remains the leading cause of death in CF to date[4,15]. However, over the last couple of decades due to improvements in nutrition and advances in treating chest disease, the survival of people with CF has improved and more than half of the people with CF in the UK are adults[4].

GI disease is an area of CF disease where there is little understanding despite the majority of people with CF experiencing GI symptoms[3]. In addition, around two thirds of people with CF miss school or work due to GI symptoms[32].

The focus in CF previously was to prolong life but in the era of CFTR modulators, there is a change in priorities for the CF community, which includes enhancing quality of life and addressing extra-pulmonary complications[32,33].

The principal focus of this thesis will be the dysfunction of the GI tract and impaired transit in CF, which lead to GI complications. First, section 1.5 will give an overview of GI physiology in health, followed by an overview of how the GI tract is affected in CF.

## 1.6. Gastrointestinal (GI) tract

### 1.6.1. A brief overview of GI physiology

#### *1.6.1.1. Movement through the GI tract*

To be able to identify abnormalities in disease, we must first understand GI physiology in health. The GI tract functions to digest complex molecules, absorb essential nutrients for metabolism and excrete waste products. Multiple areas of narrowing along the GI tract (sphincters and valves, see Figure 1.3) allow luminal contents to be held within different sections of the tract in order to optimise digestion and absorption before excretion. Other factors, including GI hormones and neural pathways, influence the rate of GI contents propulsion along the tract and through the sphincters.

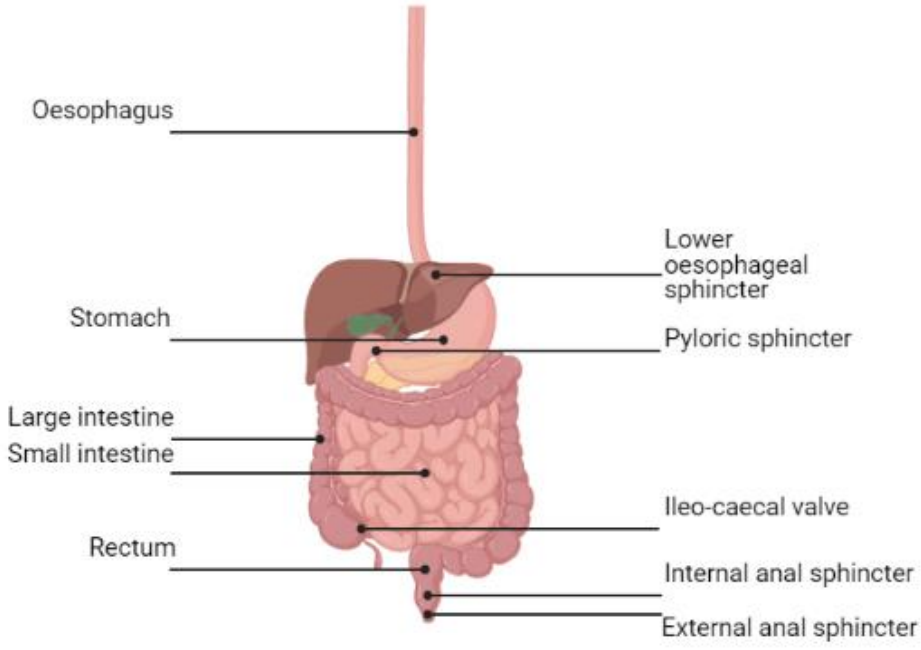



*Figure 1.3. GI tract highlighting sphincters and valve. Created with BioRender.com*

The GI tract wall consists of 5 layers: serosa, longitudinal smooth muscle layer, circular smooth muscle layer, submucosa and mucosa, and is innervated by the enteric nervous system. The smooth muscle contractions are controlled by the interstitial cells of Cajal and the myenteric plexus, which has both sympathetic and parasympathetic fibres[34].

Foods are usually complex molecules that are partially broken down in the oral cavity by salivary enzymes. Within 8 to 10 seconds of swallowing, the food bolus is quickly passed down the oesophagus into the stomach by peristalsis[35].

***Stomach***

On arrival to the stomach, the food bolus is exposed to gastric secretions in the fundus and body of the stomach. The lower oesophageal sphincter helps prevent reflux of gastric contents back to the oesophagus[35].

Neurotransmitters and gut hormones are released in response to food. Acetylcholine (a neurotransmitter) and the gut hormones gastrin and ghrelin are secreted in three phases: cephalic, gastric and intestinal, respectively, to control gastric acid secretion in the stomach. The vagus nerve releases acetylcholine whilst other cells within the stomach secrete gastrin and ghrelin. Gastric acid (hydrochloric acid) is secreted by the parietal cells in the upper two thirds of the stomach in response to gastrin. The hormones encourage motility and the acidic pH environment activates gastric enzymes for digestion[36].

The stomach is made up of five sections (cardia, fundus, body, antrum and pylorus). Peristaltic movements throughout the stomach allow for mixing of the gastric contents. The most vigorous peristaltic action is within the antrum. As the gastric pressure increases following a meal, the pyloric sphincter closes to encourage the gastric contents to mix into a suitable consistency for passing through the pylorus. The antrum secretes mucus and bicarbonate to aid this passage.

There is also an element of gastric sieving which occurs at the pyloric sphincter at the point of gastric emptying. Meals that enter the stomach are separated into their liquid and solid components[37]. Liquids and smaller particles in the stomach are emptied into the duodenum more quickly than larger particles. Particles which are too large may be prevented from emptying the stomach until they are a passable size[38]. Gastric contents are emptied into the duodenum through pyloric contractions, known as the antro-pyloro-duodenal coordination. These contractions are controlled by several factors, including the presence of glucose[39], triglycerides and fatty acids[40].

***Small intestine***

The small intestine comprises the duodenum, jejunum and ileum. Cyclic motor activity propels liquid chyme along the small intestine and cycles over four phases[41]:

- Quiescent phase 1: little or no contractions;
- Phase 2: Intermittent and irregular contractions;

- Phase 3: Maximum amplitude of irregular contractions from phase 2;
- Phase 4: regular contractions.

Together, these contractions are known as the migrating motor complex (MMC) and are initiated by the interstitial cells of Cajal. The MMC cycle can last up to 2 hours, but is interrupted by feeding[42]. Following a meal and in the presence of gastrin and cholecystokinin, the irregular contractions (phase 2) of the MMC aid digestion.

GI hormones secreted in the small intestine such as cholecystokinin, peptide YY, secretin, gastric inhibitory peptide and motilin are released in response to consumption of a meal. These hormones contribute to the production of gastric juices, motility and digestive enzyme secretions (Table 1.1)[34].

Other factors that can influence small bowel motility include the size of a meal and fat content in the ileum. Eating a meal stimulates events at the ileo-caecal valve where an increase in ileal peristaltic activity empties chyme from the ileum into the caecum, the first part of the large intestine[35]. This is known as the gastro-ileal reflex[37]. The ileo-caecal valve prevents reverse flow of luminal contents from the colon to the ileum and so enhances absorption in the caecum.

Gastric emptying, duodenal and jejunal motility are inhibited when fat is present in the ileum, the effect of which is to increase the amount of absorption and digestion in the small intestine prior to expulsion into the large intestine. This mechanism is known as the ileal brake[43].

*Table 1.1. Summary of GI hormone secretion and actions described in section 1.5.1.1.*

| **Hormone** | **Location of secretion** | **Response to** | **Action** |
|---|---|---|---|
| Gastrin | Stomach | Stomach distension | Stimulates gastric secretions and motility |
| Ghrelin | Stomach | Fasting | Stimulates gastric secretions, insulin secretion and motility |
| Motilin | Stomach, duodenum | Acid, fat | Stimulates motility |
| Secretin | Duodenum (S cells in the mucosa) | Acid, fat | Stimulates insulin, pepsin and pancreatic secretions and motility; inhibits gastric acid secretions |
| Cholecysto-kinin | Duodenum (I cells in the mucosa) | Fat, protein | Stimulates pancreatic secretions and gall bladder contraction; inhibits gastric emptying |
| Peptide YY | Small intestine, large intestine | Food consumption | Stimulates stomach distension and satiety Inhibits gastric emptying |

*Table 1.1. continued*

| Hormone | Location of secretion | Response to | Action |
|---|---|---|---|
| Gastric inhibitory peptide | Small intestine | Protein, fat, carbohydrate | Stimulates insulin secretion; Inhibits gastric secretions |

### *Large intestine*

The colon can be split into four segments: ascending, transverse, descending and sigmoid colon (Figure 1.4). Three longitudinally arranged bands of muscle form the taenia coli running from the caecum to the anal canal. The muscle layer within the colon also contains circularly arranged muscle fibres, which cause the formation of folds within the colon known as haustra[44].

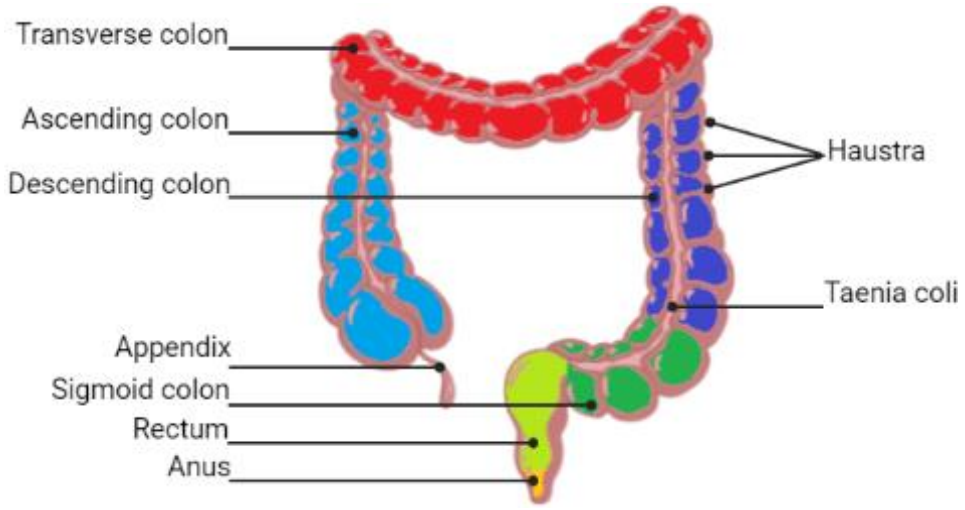



*Figure 1.4. Large intestine anatomy. Created with BioRender.com*

Peristalsis in the colon is triggered by the local nervous system, Auerbach's or myenteric plexus, in response to mechanical stimulation of the small bowel. Neurotransmitters such as serotonin, noradrenaline, adrenaline and dopamine are produced by the enterochromaffin cells within the GI tract, enteric nerves, post-ganglionic sympathetic nerve fibres or in the brain and are thought to also regulate peristalsis via the gut-brain axis[45].

Peristaltic contractions are formed of 2 phases[46]:

- Low amplitude segmental contractions for mixing luminal contents; and
- High amplitude mass movements for propelling luminal contents forward.

Peristaltic activity in the colon is thought to move distally but luminal content movement is not restricted to this forward motion. Segmental contractions occur more frequently than mass movement contractions to allow for mixing of luminal contents and absorption of water and electrolytes.

As the contents enter the rectum, the subsequent rectal distension causes the sensation of the urge to defecate. The external anal sphincter prevents defaecation until environmental conditions are optimised i.e. relaxation and correct positioning[44].

#### *1.6.1.2. Digestion and absorption*

Carbohydrates, fats and proteins from food and drink are broken down and absorbed in the body for growth and repair of cells.

The first part of carbohydrate digestion involves an enzyme called amylase, in the mouth, to hydrolyse starches into the disaccharide maltose. In the small intestine, the disaccharides (maltose, lactose and sucrose) are further digested by enzymes (maltase, lactase and sucrase) and split into monosaccharides (e.g. glucose, galactose and fructose), which can be absorbed from the gut directly into the bloodstream.

Protein is broken down into amino acids by a two-step process. Pepsin, an enzyme in the stomach, partially digests the protein to produce proteoses, peptones and polypeptides. In the small intestine, the proteolytic pancreatic enzymes (e.g. trypsin) further digest the polypeptides into amino acids that can be absorbed into the bloodstream[47].

Digestion of fat is more complex than carbohydrate and protein digestion and can be split into four sections: emulsification; pancreatic enzyme digestion; hydrolysis; and micelle formation[47].

Emulsification of fat by lipase and bile acids occur in the stomach and duodenum; large fat globules are broken down to smaller fat globules. Bile acids aid fat emulsification so fat globules are more dispersed, increasing the surface area for lipase to bind and digest the fat. Pancreatic lipases and co-lipases break down the emulsified fat into free fatty acids and monoglycerides.

Bile salts also form micelles with the free fatty acids and monoglycerides for transportation to, and absorption at, the intestinal

epithelial cells[47]. Chylomicrons are then formed within the smooth endoplasmic reticulum and are subsequently absorbed into the lymphatic system and eventually peripheral blood[47,48]. Some fatty acids are absorbed into portal blood.

Fat soluble vitamins (A, D, E and K) are absorbed through the intestinal lymphatic system. Vitamin A is the only vitamin that requires further metabolism prior to absorption after ingestion. Vitamin A is commonly consumed in the diet as pro-vitamin A and releases retinol esters when ingested, which need to be re-esterified to allow chylomicron formation[48].

#### *1.6.1.3. Intestinal absorption of water and electrolytes*

In an average adult, the small intestine is approximately 6 metres long[49] (and 3 centimetres, cm, in diameter[50]) and the large intestine is approximately 1.5 metres long[51] (and between 4 and 5 cm in diameter[51,52]). The luminal wall of the small intestine is made up of villi and microvilli to maximise the surface area for absorption of water, electrolytes and nutrients[47].

Approximately 7 to 10 litres (L) of water enters the small bowel over a 24-hour period. Around 70% results from GI secretions and the remainder is ingested[53]. Water is passively and actively absorbed through the intestinal wall by osmosis via transcellular and paracellular pathways. It can also be actively transported back into the intestinal lumen. The movement of ions within the intestine influences the amount of water movement[47]. Up to 80% of water and electrolytes are absorbed

in the small intestine and 2 L per day flow into the colon[54]. Up to 1.9 L per day of fluid is absorbed in the colon, leaving approximately 100 mL per day of fluid to be lost through stool[48].

Within the alimentary tract, *CFTR* gene expression is mostly in the small and large intestine, with highest expression in the duodenum and moderate expression in the large intestine[55,56]. In the intestinal wall, *CFTR* gene expression is highest in the crypts of the small intestine and at the crypt bases in the large intestine[55]. In these areas the CFTR protein is synthesised more quickly than other areas of the intestinal wall. The role of the CFTR protein in the intestinal wall is to regulate chloride, bicarbonate and fluid secretions[56].

The interactions between the chloride, sodium and bicarbonate ions are important in maintaining the electro-chemical balance in the small and large intestine. The transport of these ions is closely linked with the movement of water.  Sodium is actively absorbed in the intestine through the sodium-potassium ATPase pumps and specific sodium carriers (sodium-glucose co-transporter, sodium-amino acid co-transporter, sodium-hydrogen co-transporter (NHE2, NHE3, NHE8))[48]. More sodium is absorbed in the large intestine compared to the small intestine due to closer tight junctions which prevent diffusion of sodium ions back into the intestinal lumen[47]. In the descending colon, sigmoid and rectum, the main sodium transporter is the epithelial sodium channel (ENaC) and absorption is stimulated by aldosterone[48]. Since sodium moves down the electrochemical gradient, chloride ions are also absorbed across the basolateral membranes of epithelial cells via

CFTR-assisted chloride-bicarbonate exchangers allowing an electroneutral pathway[47,48,56]. Other anion exchangers involved in chloride absorption in the intestine belong to the solute carrier 26 and 4 (SLC26, SLC4) transporter families[48].

Bicarbonate is essential for two reasons. The first is to neutralise the acidic gastric secretions in the duodenum in order to protect the mucosa[47] and provide an optimum pH condition for pancreatic enzyme activity[36]. The second is to neutralise the products of bacterial activity, which are acidic[47]. Bicarbonate neutralises hydrogen ions that are secreted into the lumen by sodium-bicarbonate co-transporter 1 (NBC1) and NHE1[54], or formed as a by-product of bacteria in the large intestine. Bicarbonate and hydrogen combine to form carbonic acid before dissociating to water and carbon dioxide[47].

A study[57] using rat duodenal mucosa proposed that the CFTR protein is also essential for hydrogen ion absorption. For example, in environments containing high level of carbon dioxide caused by by-products of bacteria, it is possible that carbon dioxide is absorbed by epithelial cells and dissociates to bicarbonate and hydrogen ions. It is possible that bicarbonate is then released back into the lumen through CFTR and apical anion exchangers, whilst hydrogen ions are absorbed into portal venous blood[57].

Other bicarbonate transport mechanisms exist in the brush border of the duodenum, such as the SLC26A6 transporter which is thought to be

involved with the majority of chloride dependent bicarbonate movement within the epithelial cells[54].

### 1.6.2. GI pathophysiology in CF

#### *1.6.2.1. Movement through the GI tract*

Motility and transit of the GI tract in health are influenced by several factors including GI tract structure, GI hormones and food stimuli, as described in section 1.6.1.1. In CF, the underlying physiological abnormalities caused by CFTR dysfunction, alongside an imbalance of gut hormones[58,59] and a high fat diet could have an impact on GI motility and transit[59].

##### *1.6.2.1.1. Transit times*

Several studies have tried to evaluate whether there is delayed gastric emptying in CF using a variety of methods, but much debate persists. Evidence using scintigraphy methods are inconclusive as different studies have demonstrated slower[60], similar[61] and faster gastric emptying times in CF compared to controls[62] with statistical significance. Other studies, using magnetic pills[63] and motility capsules[64], support the proposition that there is no difference in gastric emptying times. A study[65] using ultrasound to evaluate gastric emptying times demonstrated a delay in CF of more than 5% with a liquid meal and more than 10% with a mixed meal.

In the small intestine, however, there is more of a consensus that there are delays in transit based on studies using wireless capsules[64], scintigraphy[61] and hydrogen breath testing[66]. Gelfond *et al*[64] describe

small bowel transit delays of 2 hours in people with CF whilst Rovner *et al*[61] demonstrated a delay in small bowel transit in CF by only 37.2% of radioactive tracer reaching the terminal ileum 6 hours after ingestion by study participants compared to 68.6% in the reference group. Bali *et al*[66] initially described a delay in small intestinal transit in CF using a hydrogen breath test, with a higher concentration of breath hydrogen over a prolonged fasting period. They estimated small bowel transit to be between 160 minutes and 390 minutes in CF compared with a small bowel transit time of between 50 minutes and 150 minutes in healthy controls[66]. The results from Bali's study also indicate there was small intestinal bacterial overgrowth[66]; a condition which is more prevalent in the CF population compared to a non-CF population[67] and is associated with prolonged small bowel motility[68]. Consistent with these measurements, Dalzell *et al*[69] also demonstrated delays in mean small bowel transit using the hydrogen breath test (CF 168 (9.8) minutes vs. controls 113 (7) minutes).

Only two studies[63,64] report colonic and whole gut transit times in CF and both agree that there was no difference in colonic or whole gut transit in CF when compared to controls. The whole gut transit time reported in CF using radio-opaque markers ranged from 0.5 days to 3.3 days[63], compared to between 1 day and 1.25 days when using wireless motility capsules[64].

In summary, there have been multiple studies evaluating the effects of GI transit in CF. Evidence is inconclusive regarding gastric emptying times in CF and there is limited evidence for colonic and whole gut

transit times. However, the delays in small bowel transit times shown by the hydrogen breath test studies are consistent with more recent gut transit studies using wireless capsules[64] and scintigraphy[61].

#### *1.6.2.1.2. Motility*

GI motility assesses the peristaltic actions and its consequences. Several studies describe that gut dysmotility exists in CF but few describe the exact underlying mechanism[70].

Few studies[71,72] use oesophageal manometry to report high pressures and link with increased gastro-oesophageal reflux and gastroparesis. However, as described in the previous section, there is a debate to whether delayed gastric emptying exists in people with CF.

Hydrogen breath tests have frequently described the presence of SIBO which is linked with small bowel dysmotility. However, there is a gap in the literature for intestinal motility in CF using intestinal manometry[70].

### *1.6.2.2. Digestion*

Between 80%[73] and 90%[74] of people with CF have pancreatic exocrine insufficiency caused by destruction of pancreatic tissue in early life. Andersen first described CF, on post-mortem examination in children who had a clinical history of fatty stools, as a disease where the pancreas had extensive fibrosis and cyst formation[7]. This was in keeping with the loss of pancreatic function. The CFTR protein is responsible for reduced water secretion in the pancreas and results in inspissated secretions, which leads to ductal blockages and abnormal pancreatic development[75]. As a consequence, there is inadequate

production of pancreatic enzymes[76], and reduced chloride and bicarbonate secretion[77]. Dilatation and atrophy of the pancreatic acini causes epithelial damage, which ultimately leads to inflammation, fibrosis and fatty deposition: “cystic fibrosis of the pancreas”[29,78].

Many studies use mouse models to understand the pathophysiology of CF but there are limitations[79]. Mice have a more diffuse and lobular pancreas whilst the human pancreas is more solitary and firm[80]. Although the pancreatic ducts in a CF mouse may become blocked, the overall lesions are not as severe as those seen in the human pancreas[79]. The difference in pancreatic function between humans and mice models may also be due to lower levels of CFTR protein in mice[80]. These pancreatic differences mean that the CF pathological changes in the mice models are less severe and have a milder phenotype than in humans[79,80].

The combination of inadequate pancreatic enzyme production and a relatively acidic environment in the duodenum in CF results in impaired fat digestion. Prior to pancreatic enzyme replacement therapy, children commonly presented with steatorrhoea, vitamin A deficiency and impaired weight gain[29]. The introduction of pancreatic enzyme replacement therapy was pivotal for people with CF[81]; allowing for improvements in fat maldigestion and malabsorption[82]. Better nutritional status in CF is associated with better pulmonary function[83,84], which is essential for extending survival because chest disease is a leading cause of mortality in CF[4].

Biomarkers of pancreatic dysfunction are detected by blood or stool sample, depending on the age. Within the first few days of life, the newborn bloodspot screening tests for serum immunoreactive trypsinogen: high levels indicate the presence of pancreatic destruction, causing trypsinogen from pancreatic cells to be released into the bloodstream. The coefficient of fat absorption, which requires a 72-hour stool collection, is the gold standard for assessment of fat absorption by using a calculation involving the amount of fat ingested and excreted[85,86]. A 72-hour faecal fat estimation of more than 7 g fat excreted per 24-hour period would indicate pancreatic insuffiency[85]. The amount of fat measured is the total fat content (a combination of neutral fats and fatty acids) not digested or absorbed by the GI tract[87]. This 72-hour test relies on participant compliance and comes with practical difficulties, so it is less commonly used. Stool elastase is also a marker of pancreatic function[88]: low concentrations indicate pancreatic destruction and less than 200 μg/g is considered a sign of pancreatic insufficiency[88,89]. Further discussion of these tests are described in Chapter 3.

#### *1.6.2.3. Absorption*

The CFTR protein is expressed throughout the GI tract, where it plays a role in the absorption and secretion of fluid[90], together with multiple other factors (such as gut hormones and motility)[91].

There are two hypotheses regarding the hydration status of gut luminal contents. The first is that a dysfunctional CFTR causes reduced chloride secretion into the lumen meaning that there is a reduction in

sodium and water secretion[92]. In addition, if the CFTR is inhibited, it could lead to activation of sodium-hydrogen exchange to secrete hydrogen into the lumen, decrease bicarbonate secretion or activate anion exchanges to absorb bicarbonate[57]. This in turn could lead to dry luminal contents. There are similarities between this hypothesis and the pathophysiology of the CF lung[76].

The second hypothesis is based upon a proposed passive chloride pathway causing water to remain in the jejenum in CF[93]. In the non-CF jejenum, sodium and chloride ions are actively and passively absorbed and water moves by osmosis through paracellular pathways. A reduction in active chloride secretion through the CFTR channels alongside reduced passive absorption of chloride via paracellular tight junctions could lead to an overall reduction in sodium and water absorption. This second hypothesis means that the ileum would subsequently receive a higher fluid volume.

Paracellular absorption is further support by Dalzell *et al*[69] who described a fourfold increase in disaccharide absorption, but normal level of monosaccharide absorption in CF compared to controls. In health, monosaccharides are absorbed by transcellular diffusion and disaccharides are absorbed paracellularly, and thus, the findings of Dalzell and colleagues[69] suggest the mechanism underlying the pathophysiological effects of CF in the gut could relate to transport mechanisms across cell membranes, as well as thickened intestinal secretions.

Animal studies have shown that CFTR is important in bicarbonate secretion in the intestine for mucus formation[94]. Without a functioning CFTR, there is reduced bicarbonate secretion and a failure of mucin polymers to unfold[95,96]. Inspissated intestinal mucus may therefore become trapped within the crypts[97] leading to a thicker mucus layer on the epithelial surface in people with CF[98]. However, a reduced secretion of bicarbonate could result in a higher bicarbonate concentration within the epithelial cells, which has been suggested to act as a protective mechanism for mucosa against gastric acid[54].

In addition to the abnormal ion channel hypotheses described, Werlin *et al*[99] describe the presence of intestinal inflammation via wireless capsule endoscopy and higher concentration of the faecal marker of inflammation (calprotectin) in people with CF. Intestinal inflammation can disrupt sodium absorption by inhibiting NHE3 co-transporters. Furthermore, inhibition of NHE3 could lead to dysbiosis (abnormal microbiota) and barrier defects[48].

The net effect of CFTR dysfunction in the intestine is still an area of uncertainty. Several mechanisms could affect intestinal function, including a disruption of ions and water balance affecting luminal hydration. This is an area where further evaluation of the intestinal absorption on a macroscopic level would be beneficial. It is likely that the three aspects of GI physiology (motility, digestion and absorption) affected in CF could contribute to the symptoms experienced by people with CF.

## 1.7. GI symptoms in CF

It is important to identify and address the abnormalities of intestinal function in CF because between 65%[3] and 100%[100] of children and adults with CF report intrusive gut symptoms over a period of several weeks. Over half report symptoms described to the lower GI tract[3] such as bloating, flatulence and abdominal pain. Registry data from the UK indicate that approximately 5% of people with CF *per annum* have a severe gut complication called distal intestinal obstruction syndrome (DIOS)[4,101]. The burden of GI symptoms, including absence from school or work, has been highlighted by the James Lind Alliance Priority Setting Partnership and hence, resolving GI problems is a priority for the CF community[33].

There have been recent efforts to describe the burden of GI symptoms in CF, such as the GALAXY study[102,103] in North America and Hayee *et al*[3] in the UK. The GALAXY study used existing patient reported outcome measures for GI symptoms in constipation (Patient Assessment of Constipation Symptoms, PAC-SYM[104], and Patient Assessment of Constipation Quality of Life, PAC-QOL[105]) to assess the prevalence of GI symptoms within a CF population at baseline and after 4 weeks of study enrolment[103]. Whilst Hayee et al[3] used existing patient reported outcome measures for GI symptoms in irritable bowel syndrome (Gastrointestinal Symptom Rating Score, GSRS[106], and Irritable Bowel Syndrome Symptom Severity Score, IBS-SSS[107]) to assess the prevalence of GI symptoms from one timepoint. They concluded there to be a high prevalence and increased burden of GI

symptoms in the CF community. In addition to the prevalence of GI symptoms, the GALAXY study also highlighted that a high proportion of participants experienced GI symptoms despite taking laxatives[103], which suggests there is an underlying pathophysiology that is not fully understood.

Another CF-specific GI patient reported outcome measure (CFAbd-Score) has also been derived[100] and validated[108] and confirms the presence of GI symptoms in people with CF. Unlike the PAC-SYM, PAC-QOL and GSRS questionnaires, the CFAbd-Score has followed the US Food and Drug Administration guidelines to develop the patient reported outcome measure. This includes structured interviews with people with CF and health care professionals to develop questions that assess GI symptoms and quality of life in people with CF. The CFAbd-Score also underwent testing for reliability and reproducibility within a group of people with CF and healthy controls. However, these studies for the CFAbd-Score were limited to one centre in Germany. Whilst these studies have highlighted and described the epidemiology of GI symptoms in CF, they do not describe the mechanisms of disease.

At present, the only CF specific questionnaires used in clinical practice are the CF Questionnaire Revised (CFQ-R)[109] and CF Quality of Life questionnaire (CF-QoL). These questionnaires have a limited number of questions relating to gut symptoms and until recently, gut symptoms in CF have been overlooked. The CFQ-R questions relating to gut symptoms are a recognised domain within the questionnaire and if extracted, may draw attention to some patients experiencing GI

symptoms and encourage further questioning[110]. However, the CFQ-R questions lack detailed interrogation to enable differentiation between upper and lower GI problems, stool type and stool frequency. Validated GI symptom questionnaires not specific to CF and rarely used by CF clinicians include: PAC-SYM[104], PAC-QOL[105], and GSRS[106]. Such GI symptom questionnaires adopted from other diseases should be used with caution as they have not been validated for use in people with CF and could have limitations such as under or over reporting of symptoms.

It is important for people with CF and their clinicians to identify the GI problems, because gut dysfunction adversely affects nutrition, which is associated with reducing life expectancy[111]. In order to treat these GI problems, further knowledge is required to understand the interactions between viscous intestinal chyme, dysmotility, dysbiosis and inflammation of the CF gut[101].

## 1.8. Diagnosis and treatment of complications arising in the lower GI tract

Following patient report of GI symptoms, through questionnaires or consultation, there may be a clinical diagnosis of a CF GI complication. This next section will give a brief overview of the possible CF complications arising in the lower GI tract to highlight the burden of GI problems in CF.

### 1.8.1. Constipation

Approximately 30% of all people with CF experience constipation[112] and within the paediatric CF population, approximately 50% have constipation[113]. In comparison, around 10% of the general population have constipation according to the Rome IV criteria[114]. Constipation is characterised by the reduced frequency of bowel movements and increased hardness of stool occurring over weeks to months. The exact pathophysiology of constipation is unclear but the symptoms experienced by people with constipation suggest the underlying issues lie within the colon[115]. It is possible that there is a disruption of water movement, which causes stool to be drier in nature in constipation compared to no constipation[115], but this is not usually due to a physiological abnormality. Constipation is confirmed by a clinical diagnosis and commonly treated with oral laxatives (see section 1.9.1 for further details).

### 1.8.2. Distal intestinal obstruction syndrome

The most severe GI complication in CF is distal intestinal obstruction syndrome (DIOS). Approximately 5% of people with CF in the UK[4] are treated for DIOS every year, with a 77% lifetime risk of recurrence[116]. In comparison to constipation where there are drier colonic contents which lead to hard stools on defaecation, DIOS is thought to be a result of build-up of viscous material in the terminal ileum and caecum, which leads to partial or complete intestinal obstruction. On three operative reports, Snyder et al[117] describe the intestinal obstruction to be clay-like, inspissated, hard and putty-like within the terminal ileum, appendix

and caecum. Furthermore, in one patient, the description included difficulty in removing adherent faeces from the small and large bowel and they had recurrent episodes of DIOS caused by impacted faecal matter in the terminal ileum. Although it is likely that the underlying pathology exists within the small bowel, the exact pathophysiology is unknown.

DIOS presents acutely with varying degrees of abdominal pain, distension, nausea and vomiting. There are two categories of DIOS based on the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) definitions: partial and complete obstruction. The palpation of a faecal mass in the ileo-caecal region alongside abdominal pain or distension can provide a clinical diagnosis of a partial DIOS. Complete obstruction is diagnosed if signs of a partial obstruction are accompanied by bilious vomiting or small bowel fluid levels are seen on abdominal radiographs.

Hospital admissions for the treatment of DIOS is required in at least 85% of cases, with the average length of stay being 4 days[118]. Medical treatments of DIOS are primarily osmotic laxatives (described in Section 1.9.1) but stimulant laxatives may also be used. In severe cases, nasogastric decompression and intravenous hydration may be needed. Surgical relief to prevent bowel perforation may be required if medical treatment has failed[119].

Preventative treatments for DIOS include the osmotic and stimulant laxatives, given in regular lower doses as opposed to acute treatment

doses. Pancreatic enzyme replacement therapies play a role in improving fat absorption and thus reduce steatorrhoea in some cases[120], which could indirectly influence other gut hormones such as gastric inhibitory peptide due to less fat presence. However, pancreatic enzymes do not directly improve GI motility[121].

Although these treatments are commonly used for DIOS, recent Cochrane Systematic Reviews have concluded that little evidence exists for current preventative measures[122] and treatment[5] of DIOS.

### 1.8.3. Small intestinal bacterial overgrowth

Small intestinal bacterial overgrowth (SIBO) is the presence of excessive bacteria in the small intestine[123] and diagnosed in 30 to 40% of people with CF[101]. SIBO is not exclusive to people with CF. Others also at risk of SIBO include: people who have gut dysmotility or structural abnormalities such as irritable bowel syndrome and coeliac disease; people with metabolic disorders such as diabetes; and people with organ dysfunction such as cirrhosis and pancreatitis[123]. The mechanism of SIBO is poorly understood but altered pH levels, reduced intestinal motility and intestine structural pathology (e.g. dysfunctional ileo-caecal valve) are thought to contribute, and are not specific to CF[124].

SIBO causes non-specific symptoms such as abdominal distension, bloating, diarrhoea and fatigue, and complications of SIBO including fat malabsorption, bile acid diarrhoea and nutrient deficiencies can occur[101]. There is no gold standard test for SIBO diagnosis. A hydrogen

breath test can be used as a diagnostic tool[123], but can provide false negative results if there are non-hydrogen producing bacteria or delayed gastric emptying; or false positive results due to colonic fermentation[125]. Despite this, hydrogen breath tests have been used in CF research and demonstrated the high frequency of SIBO in the CF population[126].

The aim of SIBO treatment is to eliminate the bacterial overgrowth using antibiotics and to replace nutrient deficiencies with supplements[127].

The presence of SIBO as a CF gut complication suggest there may be an underlying predisposition to the CF population. Evidence suggests that the gut microbiome in people with CF is different compared to healthy controls and that these differences correlate with declining lung function[128]. However, this is out of the scope of this thesis and will not be discussed in more detail.

### 1.8.4. Small intestine and colorectal cancer

The incidence and prevalence of lower GI cancers in people with CF have increased but this is largely due to the longer life expectancy of people with CF and diagnosis of intestinal cancers in the larger age group[129]. The standardised incidence ratio (observed cancers to expected cancers) is 6 times higher in people with CF compared to the general population for colon cancers and 11 times higher for small intestine cancers[130]. In addition, the standardised incidence ratio for a person with CF post lung transplant to develop colorectal cancer is

increased by between 24 fold[131] and 30 fold[130] and possibly due to immunosuppressive therapy required to prevent organ rejection[131]. Other risk factors for intestinal cancers include CF-related diabetes and gastro-oesophageal reflux[129]. There is some evidence to suggest that factors influencing the CFTR protein could be a driver for intestinal cancers[132] and there is still debate to whether additional colorectal cancer screening for people with CF is necessary, but this is beyond the scope of this thesis.

## 1.9. Treatment strategies

Section 1.6.2 has discussed the three broad abnormalities in CF gut disease: maldigestion, malfunction (dysmotility) and malabsorption. By targeting these abnormalities, current treatment approaches aim to alleviate the complications (such as laxatives for constipation), the consequences of the physiological abnormality (such as pancreatic enzyme replacement therapy, PERT, for pancreatic insufficiency) or the underlying abnormality caused by the CFTR mutation (such as modulators for CFTR dysfunction). This section will provide a brief overview of the mechanisms of action of laxatives, PERT and CFTR modulators.

### 1.9.1. Laxatives

Laxatives are primarily used for treating functional constipation, where the mechanism of action is to rehydrate faecal matter and encourage peristalsis.

Constipation and DIOS in CF are also treated with laxatives based on the same principles as functional constipation and the need for rehydrating viscous faecal matter[133]. Osmotic laxatives such as lactulose, polyethylene glycol or the radiological contrast agent sodium meglumine diatrizoate (Gastrografin®), draw water into the large intestine lumen to encourage the movement of stool. Gastrografin® is used in radiology to detect bowel obstructions or strictures but has a secondary laxative effect. Stimulants such as senna and sodium picosulphate are another type of laxative, which primarily encourage peristalsis. Sodium docusate is a laxative with two mechanisms of action. It stimulates peristalsis and acts as a faecal softener.

There are no clinical trials which support the use of laxatives for the treatment of DIOS[5] in CF.

### 1.9.2. Mucolytics

Mucolytics, such as oral n-acetylcysteine, break down viscous mucus by opening disulphide bonds to reduce the viscosity[5]. It is used as an adjunct therapy to laxatives in DIOS, with variable effects[119]. No clinical trials evaluate the effect of mucolytics for DIOS treatment[5].

### 1.9.3. Pancreatic enzyme replacement therapy with high fat high calorie diets

Pancreatic enzyme replacement therapy (PERT) was first introduced as part of treatment for exocrine pancreatic insufficiency in the 1930s[134]. PERT is made up of the pancreatic enzymes including lipase, amylase and protease and is used to treat pancreatic exocrine insufficiency. It is

important for exogenous PERT to mimic the effect of endogenous pancreatic enzymes. Hence, PERT formulations have been further developed with an enteric coating so that the enzymes are not denatured by gastric acid and are active when they reach the duodenum[120]. Somayaji *et al*[134] have found that there is little evidence to guide appropriate PERT dosage in CF. The recommended PERT dosage (per gram (g) of fat or units per kilogram (kg) of body weight) is based on consensus guidelines[135]. A maximum PERT dose of 10,000 units of lipase per kg per day was implemented due to the increased prevalence of fibrosing colonopathy in the presence of high dose PERT[136,137]. Furthermore, these doses are also titrated according to clinical symptoms[135].

In addition to the introduction of PERT, there are several reports[138,139] that associate improved nutrition with better survival and indirectly improved lung function[111]. In the late 1980s, Corey et al[111] compared CF populations in Toronto (Canada) and Boston (US) who received different PERT dosages and types of diet. People with CF in Toronto, who received approximately 20 to 30 PERT capsules a day alongside a high fat high calorie diet, were seen to have improved growth and nutrition and subsequently better survival when compared to people with CF in Boston, who received few PERT capsules alongside a low fat high calorie diet[111]. A high fat and high calorie diet aims to compensate for malabsorption or maldigestion and the higher energy expenditure due the chronic inflammatory processes caused by CF[140]. Other small studies[141,142] have included behavioural therapies to

encourage infants to tolerate the diet described due to previous difficulties with encouraging children to have a higher than recommended daily intake. A high fat high calorie diet that is between 110% to 200%[141] is recommended in the existing UK[135] and US[81] CF guidelines.

The combination of PERT and a high fat high calorie diet has led to significant improvements in nutritional status, which subsequently correlates with improved lung function and ultimately improved life expectancy[143].

Although high fat high calorie diet has been part of routine CF clinical care for the past few decades[81,135], it is possible that with the new CFTR modulator therapies, there may be a change in recommendations. This is due to multiple cases where people with CF, who have initiated CFTR modulator treatment, have rapidly gained weight and more people with CF are in the overweight and obese weight category[144].

### 1.9.4. CFTR modulators

CFTR modulators are licensed for use in individuals with specific CF mutations. CFTR modulators aim to correct the underlying physiological defect caused by CF mutations, as described previously. There are two types of CFTR modulators: potentiators and correctors.

Ivacaftor was the first CFTR modulator to be developed and is a potentiator. A potentiator enables CFTR channels at the cell surface to remain open[145]. Ivacaftor is licensed for people with class III CF

mutations (Figure 1.2) only, when used as a single CFTR modulator therapy.

Correctors such as lumacaftor[146], tezacaftor[147] and elexacaftor[148], correct CFTR misprocessing which in turn allows more CFTR channels to reach the cell surface. Correctors are used in combination with potentiators to enhance CFTR function[146-148] and are licensed for a wider range of CF mutation types[149-151].

In the phase 3 studies for CFTR modulators, the primary focus was to assess the changes in lung function. Although the phase 3 studies[145-148,152] and others who pooled data[153] evaluated the nutritional effects of CFTR modulators, there has been little focus on GI function[99,154,155].

Compared to placebo, ivacaftor alone showed a 10.6% greater change from baseline in percentage predicted $FEV_1$ (forced expiratory volume in 1 second) by 24 weeks of treatment and weight increased by 3.1 kg at 48 weeks of treatment compared to 0.4 kg with placebo[145]. Discontinuation of PERT after commencing ivacaftor has been described in several case reports[156-158] and case series[159-161]. An increase in faecal elastase has been described in paediatric clinical trials[162,163].

The combinations of either lumacaftor[146] or tezacaftor[147] with ivacaftor have each shown modest improvements in lung function in people with CF who are homozygous for p.Phe508del. Lumacaftor-ivacaftor is currently licensed for people with CF homozygous for p.Phe508del aged 6 years and over[151]. Tezacaftor-ivacaftor (TEZ/IVA) is licensed for

people with CF aged 6 years and over who are homozygous for p.Phe508del or who have 1 copy of the p.Phe508del gene and 1 copy of a CF-causing gene specified as having residual function[149].

Elexacaftor-tezacaftor-ivacaftor is a triple combination therapy and significantly improves lung function. After 24 weeks, elexacaftor-tezacaftor-ivacaftor showed a mean treatment difference of 14.3% (relative to placebo) in change in $FEV_1$ percentage predicted from baseline. The mean treatment difference in body mass index (BMI) was +1.04 $kg/m^2$ (relative to placebo) in the same trial[148]. As of 11$^{th}$ January 2022 in the UK, elexacaftor-tezacaftor-ivacaftor is licensed for people with CF aged 6 years and over who have at least one copy of the p.Phe508del mutation[150].

## 1.10. Summary

CF is an inherited multi-organ disease that can present at birth with meconium ileus and in early life with malnutrition. After the introduction of PERT and high calorie diet as part of routine clinical care for pancreatic insufficient CF, the life expectancy of people with CF dramatically improved and the focus of CF care has been to prolong life by treating chest disease.

However, the treatment of CF is changing and the focus has shifted from primarily treating respiratory complications to now including management of extra-pulmonary disease such as gut complications. The CF community have requested that CF researchers should

prioritise how to relieve GI symptoms[33] and therefore, it is crucial that clinicians also have a better understanding of the underlying CF gut pathology so that research provides more evidence for the use of treatments in GI complications.

## 1.11. Aims of the thesis

This thesis will aim to answer the following key questions arising from the gaps in the literature:

i. Is there sufficient evidence to support the administration timings of PERT?
ii. Is there a tool that could be used to further understand the GI pathophysiology of CF without adding burden to people with CF?
iii. Do new CF therapies (CFTR modulators) improve GI function and symptoms?

In order to answer the questions, the next chapter in this thesis will be a systematic review evaluating whether current practices for administering PERT, in relation to meals, can be optimised.

Chapter 3 will summarise the current abdominal investigations used in CF and provide a simplified explanation to magnetic resonance imaging (MRI) as the later chapters describe the use of MRI to assess gut function and transit in CF.

Chapter 4 aims to evaluate the use of MRI in CF and compare GI function and transit in people with CF to healthy individuals, before assessing the GI effects of TEZ/IVA in people with CF using MRI in Chapter 5.

The final chapter will draw conclusions from the original research presented in this thesis and propose the future direction of GI research in CF.

# Chapter 2 A systematic review of timing of pancreatic enzyme replacement therapy doses in CF

This chapter will systematically review the literature to evaluate the efficacy and effectiveness of pancreatic enzyme replacement therapy (PERT) administration timings. The protocol and full review were published in the Cochrane Database of Systematic Reviews in December 2019[164] and August 2021 respectively[165]. A summary of this systematic review has also been published in the journal Paediatric Respiratory Reviews[166].

## 2.1. Background

As described in Chapter 1, up to 90% of people with CF have pancreatic insufficiency caused by a destruction of pancreatic tissue in early life. PERT has been used to treat pancreatic insufficiency since the 1930s[134]. Without pancreatic enzymes, people with exocrine pancreatic insufficiency can experience gut symptoms such as bloating, abdominal pain and fatty foul-smelling stools (steatorrhoea) due to maldigestion and malabsorption[120]. In people with pancreatic insufficient CF, a combination of PERT alongside a high calorie diet has improved nutritional status, which correlates with improved lung function and ultimately increased life expectancy[111]. In the early 1990s, a series of reports suggested that the use of high dose formulation of

PERT was linked with colonic strictures: a condition termed fibrosing colonopathy[167-169]. Between 1990 and 1994 there were 35 cases of fibrosing colonopathy reported to the US CF Foundation registry which prompted the development of PERT consensus guidelines for maximum daily dosing[170]. At present, the prevalence of fibrosing colonopathy is less than 0.1% of people with CF in the UK[4] and US[15].

PERT may be formulated as capsules or granules (for younger children) which contain enzymes that are of porcine origin, in most cases[120,171]. PERT contains mostly pancrelipase but may also contain amylase and protease. Doses of PERT are calculated based on their lipase content[120]. The aim of the therapy is to mimic the normal physiological digestive response by replacing the inadequate pancreatic enzyme levels, in the small bowel lumen, caused by CF. Pancreatic enzymes work best at pH 7[171] and therefore PERT may be denatured when exposed to gastric acid on their passage through the stomach[120]. This has led to pharmaceutical companies developing their PERT further to include an enteric coating, with the aim for the coating to dissolve at pH above 5.5. The ultimate aim of this mechanism was to enable enzymes to be released into the duodenum without being deactivated by acidic conditions[120,171]. PERT activity is thought to peak within the first hour of ingestion before beginning to diminish after two hours post ingestion[172]. In spite of this, a systematic review by Somaraju *et al*[134] highlights there is controversy in the effectiveness of enteric coated PERT. A similar argument is proposed in the systematic review[173] that evaluates the use of adjunctive therapies (e.g. proton

pump inhibitors) which increase the stomach pH environment to optimise PERT mechanism of action.

In CF, the luminal environment in the duodenum is of a lower pH than normal[64] due to a lack of duodenal bicarbonate secretion, which may impair the action of PERT. In addition, PERT is only effective in the presence of fat within the partially digested food. Therefore, PERT delivery to the duodenum, before the enzymes are activated, may optimise the action of PERT[120].

Current PERT doses are based upon consensus guidelines, which recommend calculating the amount of PERT dependent on meal fat content in g or per kg of body weight. UK consensus guidelines suggest doses of 500-4000 lipase units per g of fat with a mean requirement of 1800 lipase units per g of fat per day to achieve optimal absorption of fat and other nutrients[135].The maximum recommended daily dose is 10,000 lipase units per kg of body weight per day[135-137]. One of the aims of PERT is to improve the symptoms of malabsorption such as steatorrhoea, with the UK CF Trust nutritional guidelines suggesting the possibility to titrate PERT doses in response to clinical signs and symptoms[135] (for example weight gain, stool type and frequency). Current advice is for PERT to be taken with meals but there is no set recommendation on whether PERT should be taken before, during or after the meal is eaten[136].

PERT has been shown to improve fat breakdown of triglycerides to free fatty acids *in vitro*. PERT also improves fat absorption and thus reduces

steatorrhoea[171,174,175], especially if an individual has less variation between PERT doses (lipase unit per g of fat) at each meal or snack[176]. However, some people continue to experience symptoms such as abdominal pain and bloating despite taking PERT, which may relate to maldigestion and malabsorption[3]. The interactions between fat absorption and proposed CF pathophysiology are complex, for example any variation in gastric emptying could have effects on PERT delivery to the duodenum[177]. Inevitably, there are variations in clinical advice on PERT use despite the consensus guidelines and attempts to standardise care[178,179]. The low grade of evidence for PERT administration timings in consensus guidelines highlights the need to systematically review whether there is sufficient robust evidence evaluating the efficacy and effectiveness of PERT administration timings to make a standard recommendation.

## 2.2. Aims

The aim of this systematic review was to evaluate the literature for the efficacy and effectiveness of different PERT administration timings in people with CF.

## 2.3. Methods

A systematic review of the literature was conducted to evaluate the timings of PERT in people of all ages with a confirmed diagnosis of CF (by genetic testing or sweat chloride testing). The protocol described in

this section was peer-reviewed and published prior to conducting the literature search[164].

A literature search of the Cochrane Cystic Fibrosis and Genetic Disorders Trials Register (compiled of the Cochrane Cystic Fibrosis Central Registrar of Controlled Trials (CENTRAL), weekly MEDLINE searches, Embase and Pediatric Pulmonology and Journal of Cystic Fibrosis) for trials of the timing of pancreatic enzymes was conducted by a member of the Cochrane Cystic Fibrosis and Genetic Disorders team and myself (CN). A search of the International Standard Randomised Controlled Trial Number (ISRCTN) registry, the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP), and ClinicalTrials.gov was also conducted using the search terms in Table 2.1. Multiple synonyms were used for pancreatic enzymes to ensure all relevant studies were included in the search.

After conducting the searches, the references were imported to Covidence (covidence.org; Melbourne, Australia: Veritas Health Innovation) and duplicates removed. Covidence is an online resource that allows reviewers to screen references independently and upload references as required, to reduce the risk of bias. It also directly extracts the references required for full text review and streamlines discussions, where the reviewers do not agree on including or excluding a reference from the systematic review.

Two researchers (CN, and Dr Giles Major, GM) independently reviewed the eligibility of references based on the inclusion criteria summarised

in Table 2.2. References were screened based on their title and abstract before the full text was screened for inclusion or exclusion.

For references where the two researchers (CN, GM) were unable to agree on inclusion or exclusion, a third researcher (Prof. Alan Smyth, AS) was asked to review the reference and make the final decision.

### 2.3.1. Justification of inclusion criteria

#### *2.3.1.1. Study type*

All randomised controlled trials were included because they are the gold standard in evaluating the efficacy of treatments[180] and have the highest GRADE (Grading of Recommendations Assessment, Development and Evaluation) rating[181].

Randomised crossover trials were included only if there was a minimum two-week duration for each of the treatment periods and washout period. The washout period was defined as a period without PERT or run-in period with the second treatment arm. It was essential to specify a washout period to reduce the treatment carry-over effect. PERT is eliminated via stool and in health this could take up to 72 hours[182]. Transit times are slower in CF and a two-week washout period was therefore a sufficient timeframe to buffer for any treatment carry-over effect. If a randomised crossover trial had a sufficient treatment period but insufficient washout period, only data from the first treatment period were to be used to reduce risk of carry-over effect and risk of reporting bias.

Quasi-randomised controlled trials (where randomisation is not truly random) were also included providing the baseline characteristics of the groups were similar. A quasi-randomised controlled trial can estimate a relationship between the exposure and outcome, but the exposure is not entirely controlled. For example, a quasi-randomised controlled trial may withhold a treatment until a specified outcome is measured, and therefore does not randomly allocate the treatment length[183].

Non-randomised controlled trials and studies using historical data were excluded.

#### *2.3.1.2. Study participants*

To ensure all types of PERT timings were assessed for use in CF, the criteria for study participants were restricted to a confirmed diagnosis of CF by genotype or sweat chloride testing.

#### *2.3.1.3. Interventions*

Studies that pre-specified PERT administration timings in relation to meals as an intervention were considered for the review. The type of preparation or strength of PERT used was not restricted. Studies were also only considered if the comparator used was a specified timing of PERT.

#### *2.3.1.4. Outcomes*

The outcomes for this review were chosen to evaluate the efficacy of PERT in a controlled clinical trial settings (fat absorption) and effectiveness of PERT to reflect the real-world conditions (nutritional status, lung function, quality of life) of PERT timings at 2 and 4 weeks,

then monthly. Shorter term outcome measures such as coefficient of fat absorption are complimented by measures that assess trends over longer time period such as weight, BMI and patient reported symptoms. The overlap in the evaluation of efficacy and effectiveness is helpful in assessing the external validity of the clinical trials.

For the first primary outcome, the gold standard to measure fat malabsorption[85], coefficient of fat absorption based on a 72-hour stool collection, was chosen. Although a 72-hour stool collection is much more challenging than collecting a single spot sample, it is more accurate and reflects the overall action of fat digestion and therefore PERT action rather than sampling the level of pancreatic enzymes, which may not be excreted in stool.

Weight, height and BMI were also chosen as primary outcomes as they are a measurable indicator of nutritional status. Changes from baseline can be easily identified and used for comparing data.

The third primary outcome was adverse events relating to the GI system.

The secondary outcomes chosen were primarily to assess the effectiveness of PERT timings. Lung function correlates positively with nutritional status and measurements of forced expiratory volume, forced vital capacity and lung clearance index are routinely used as outcome measures in CF clinical trials.

Finally, participant reported outcome measures were planned for collection to provide information on how people perceived their

symptoms and how intervention affected the individuals' body and quality of life.

### 2.3.2. Plan for data management

#### *2.3.2.1. Data collection*

For all included studies, collection of the following data were planned: number of participants; characteristics of participants; study design; PERT type; PERT timing; and treatment effect (primary and secondary outcomes) at 2 weeks, 4 weeks and monthly.

The treatment effects for continuous outcomes (coefficient of fat absorption, weight, height, BMI, lung function and participant reported quality of life and symptom scores) were planned to be calculated by the mean difference and 95% confidence intervals between groups. The treatment effects for dichotomous outcomes (adverse effects) were planned to be calculated using risk ratios for each event occurring.

By ensuring the treatment effects were described in the same way, the summary statistics would have a balance of data consistency between studies and would be easily interpretable to enable readers to apply the conclusions to clinical practice[184].

#### *2.3.2.2. Study designs*

When reviewing a study design, it is important to identify when and how randomisation occurs to ensure the number of observations are the same as number of participants randomised. This is to avoid inappropriate reporting and summary of results. For example, for crossover trials, analysis would be completed as described in section

2.3.1.1. For cluster randomised trials, we planned to use the intra-cluster correlation coefficient and the design effect to determine the treatment effect using the effective sample size. This would allow for a more appropriate comparison with other randomised controlled trials.

For studies with multiple treatment arms, it is important to avoid double counting and multiple comparisons. To mitigate this risk, we planned to include only the intervention and comparator groups relevant to the systematic review to use a fixed-effect meta-analysis across the comparisons within the same study. Using a fixed-effect meta-analysis assumes that all effect estimates have the same underlying treatment effect.

#### *2.3.2.3. Missing data*

We planned to make every effort to contact the original study authors for missing data in included studies. If such data were retrieved, we planned to analyse the data so treatment effect could be compared to other studies. This meant that potential publication bias would be reduced for studies where data were not randomly missing, but rather the reasons for missing data were not described in the publication (e.g. poor outcomes or insignificant results).

A sensitivity analysis was planned to impute the missing data and repeat the analysis, to ensure the study data collection was robust.

#### *2.3.2.4. Heterogeneity and bias*

The heterogeneity within a systematic review is the variability in study participants, study design and evaluation of outcomes. An assessment of heterogeneity ($I^2$) was planned using the following equation:[184]

$$I^2 = \left(\frac{Q - df}{Q}\right) \times 100\%$$

Where *Q* is chi-square and *df* is the degree of freedom. This analysis was planned only if a minimum of 10 studies could be included in a random-effects meta-analysis.

Whilst the percentage of heterogeneity may be influenced by factors such as the GRADE of evidence and the magnitude and direction of effects[185], the following categories guide the amount of heterogeneity in the meta-analysis[184]:

0-40%: may not be important;

30-60%: may have moderate heterogeneity;

50-90%: may have substantial heterogeneity; and

75-100%: considerable heterogeneity.

Bias can occur at any point throughout a trial: from inviting participants to publishing data. For this reason, bias was planned to be assessed using the risk of bias tool (www.riskofbias.info[186]) in the following domains: randomisation process, study conduct and measured and reported results[180]. Elements of non-reporting biases was planned to be further assessed in the following subgroups: publication, time lag,

multiple publication, location, citation, language and outcome reporting[187,188].

#### *2.3.2.5. Subgroup analysis*

Had there been a sufficient number of studies included in the meta-analysis, a subgroup analysis would aid clinical application. The ages of participants were planned, a priori, to be divided into infants under 3 years old; pre-school age children aged 3 to 5 years old; school age children aged 5 to 16 years old; and adults.

PERT type was planned, a priori, to be categorised into the following groups: granules; capsules; and tablets of different strengths.

#### *2.3.2.6. Summary of data*

We planned to summarise all of the included studies into one summary of findings table, which included the primary and secondary outcomes outlined in Table 2.2. Subgroup analyses were planned to be summarised in the summary of findings table if there were sufficient data. The quality of evidence was planned to be assessed using the GRADE approach[185].

*Table 2.1. Database search terms for systematic review.*

| **Database** | **Search terms** | **Date of search** |
|---|---|---|
| ISRCTN Registry (www.isrctn.com) | (Cystic fibrosis OR mucoviscidosis) AND ("pancreatic enzyme" OR “pancreatic exocrine” OR pert OR creon OR pancrex OR pancrease OR Nutrizym OR forte OR ultrase OR pancrelipase OR pancrecarb OR liprotamase OR Sollpura) | 19 May 2021 |
| WHO ICTRP (https://ictrptest.azurewebsites.net/Default.aspx) | **Condition**: cystic fibrosis OR mucoviscidosis. **Recruitment status**: All **Intervention**: pancreatic enzyme OR pancreatic exocrine OR pert OR creon OR pancrex OR pancrease OR Nutrizym OR forte OR ultrase OR pancrelipase OR pancrecarb OR liprotamase OR Sollpura. | 19 May 2021 |
| ClinicalTrials.gov (www.clinicaltrials.gov) | **Condition/disease**: cystic fibrosis OR mucoviscidosis. **Study type**: Interventional Studies **Intervention/treatment**: “pancreatic enzyme” OR “pancreatic exocrine” OR pert OR creon OR pancrex OR pancrease OR Nutrizym OR forte OR ultrase OR pancrelipase OR pancrecarb OR liprotamase OR Sollpura | 19 May 2021 |

*Table 2.2. Inclusion criteria for systematic review.*

| | |
|---|---|
| **Study type** | Randomised controlled trials, including cross-over trials with a minimum treatment and washout period of 2 weeks each;<br>Quasi-randomised controlled trials considered if baseline characteristics of each group were similar. |
| **Study participants** | People with CF of all ages with a confirmed diagnosis of CF by genotype or sweat chloride test.<br>Pancreatic function status was not required. |
| **Interventions** | PERT regimens with pre-specified administration timings in any dosage or formulation. |
| **Outcome measures** | At 2 and 4 weeks of each treatment period, then monthly if the study period was longer.<br>Primary: 1. Fat malabsorption (absolute coefficient of fat absorption by 72-hour stool collection);<br>2. Nutritional status based on weight in kg, height in cm and body mass index in $kg/m^2$;<br>3. Adverse events relating to the gastrointestinal system or other systems.<br>Secondary: 1. Lung function change from baseline ($FEV_1$, forced vital capacity, lung clearance index);<br>2. Change in participant-reported quality of life score (CF-QOL, CFQ-R, or equivalent);<br>3. Change in participant-reported symptom score (CFQ-R, CFAbd-Score, or equivalent). |

## 2.4. Results

The searches from the Cochrane Cystic Fibrosis and Genetic Disorders Trials Register identified 190 individual references, ClinicalTrials.gov and WHO ICTRP identified 72 studies. No studies were identified from the ISRCTN.

After importing the references to Covidence, 48 duplicates were identified. Following screening of titles and abstracts, a total of 10 studies (29 references) were retrieved for full text review.

No studies met the inclusion criteria in the review (Figure 2.1). Therefore, no data were extracted and no meta-analysis was performed. There was no requirement to complete the risk of bias assessment tool, measures of treatment effect, mitigate unit of analysis issues or missing data, assess heterogeneity or report any biases.

The studies excluded after full text review were due to cross-over studies with insufficient treatment periods, PERT timings not assessed or insufficient data available (see summary in Table 2.3).

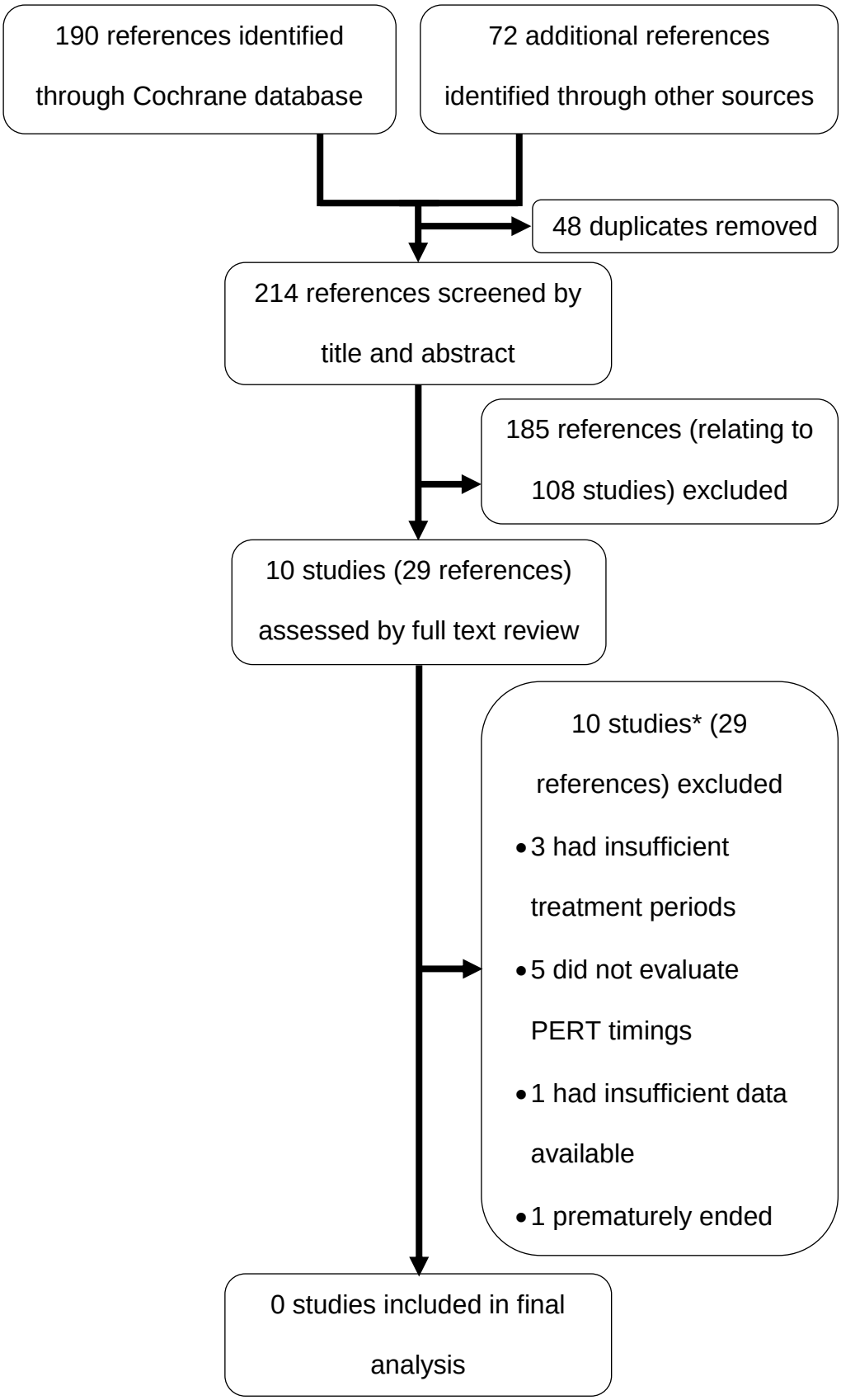



*Figure 2.1. CONSORT diagram to show the flow of studies in the systematic review. *Further detail on excluded studies are presented in Table 2.3.*

*Table 2.3. Summary of reasons for exclusion of references for systematic review.*

| Reference | Reason for exclusion |
|---|---|
| Williams 1990[189] | Did not compare PERT timings |
| Brady 1992[190] | Cross-over study: insufficient treatment period |
| Lancellotti 1996[191] | Cross-over study: insufficient treatment period |
| Stapleton 2001[192] | Study evaluated a new educational program for PERT and did not compare PERT timings |
| Borowitz 2005[193] | Did not compare PERT timings |
| Kalnins 2005[194] | Did not compare PERT timings |
| Heubi 2007[195] | Did not compare PERT timings |
| Van der Haak 2016[196] | Cross-over study: insufficient treatment period |
| Brekke 2019[197] | Insufficient data in the published abstract as data analysis was still ongoing. No full text available at the time of searches. |
| Katzenstein 2020[198] | Prematurely ended. No data available. |

A study by Katzenstein et al[198] was identified through the WHO ICTRP search and the study protocol suggested it may have met the inclusion criteria of the systematic review. This study aimed to evaluate the PERT timing at meals and assess fat malabsorption. However, the registered protocol suggested the study had prematurely ended and despite multiple attempts to contact the study investigators to clarify if the study would re-open, no information was obtained.

## 2.5. Discussion

The aim of this systematic review was to evaluate in more detail PERT administration timings in relation to meals. This systematic review has shown that there are a limited number of studies that assess whether the timing of PERT administration in relation to meals has an effect on fat malabsorption. Whilst randomised controlled trials are the gold standard for providing robust clinical evidence, this systematic review is limited by not reviewing any non-randomised controlled data. None of the studies reviewed met the methodological criteria for inclusion in this review. Furthermore, where cross-over studies[190,191,196,197] have assessed PERT timings, they did not meet eligibility criteria for a two-week minimum treatment period or minimum washout period of two weeks.

This review has shown that there is still a need for research studies to answer the question when is best to take PERT in relation to meals. Other medications such as rapid-acting insulin is an example of a medication that has undergone multiple clinical trials to ascertain the optimal timing of administration in relation to a meal for people with insulin-dependent diabetes. A review[199] demonstrated the variation that can occur in glucose control in relation to insulin administration up to 20 minutes before a meal, immediately before the meal or 20 minutes after a meal. Such review highlights the importance of evaluating the time-dependent effects of a replacement therapy to optimise treatments[199].

Previous studies and reviews[134,173] have assessed the overall efficacy and effectiveness of PERT but they have never reviewed the finer

details such as specific timings. The risk of fibrosing colonopathy associated with high doses of PERT[167] has highlighted the need to ensure the overall safety as well as efficacy and effectiveness of PERT.

An example of two studies that evaluated the efficacy and effectiveness, but not administration timings are by Graham *et al*[171] and Stead *et al*[200]. Graham *et al*[171] compared enteric coated PERT with non-enteric coated PERT and found no improvement in coefficient of fat absorption in the presence of enteric coated PERT compared to non-enteric coated PERT. Although not a directly comparable study, Stead *et al*[200] showed that enteric coated tablets were preferred by participants and most commonly reasons were due to its reduction in number of bowel motions and changing stool consistency from loose to formed stool, compared to non-enteric coated PERT with the adjunct cimetidine (a drug used to inhibit gastric acid production). However, the difference in faecal fat and coefficient of fat absorption were not statistically significant between the enteric and non-enteric coated PERT, and could be due to the small sample size. These studies comparing different PERT coatings confirm that despite attempting to mitigate acidic conditions, other factors may be contributing to the efficacy of PERT such as timings with meals.

The inclusion criteria for length of treatment and washout periods if in a crossover trial were pre-specified to be a minimum of two weeks each. A two-week period for treatment would allow the body to adjust to PERT and a two-week washout period would ensure the effects of a change in PERT timing (such as altered fat absorption) were no longer

evident. Although Williams[189], Borowitz[193] and Kalnins[194] did not compare PERT timings in their studies, they each described the effects of different preparation and combination of PERT on fat malabsorption using the coefficient of fat absorption as a pre-specified outcome after treatment periods between 2 weeks and 1 month. In each study, the length of treatment period was justified by the need to allow participants to adjust to different PERT. PERT is not absorbed in the GI tract and is excreted through defaecation. This suggests that the time taken for PERT to be eliminated from the body is the total gut transit time. Other studies evaluating whole gut transit time in health suggest average transit times varies from approximately 30 hours[201,202] to 53 hours[203]. Whole gut transit in CF has only ever been reported in two studies: one using radio-opaque markers[63] and one using wireless motility capsules[64]. The average whole gut transit of 10 adults with CF were reported to be between 0.5 and 3.3 days[63], using radio-opaque markers, and between 1 day and 1.25 days[64], using the wireless motility capsules. However, about 30%[112] of people with CF have constipation, including up to 50%[113] of children with CF, and whole gut transit times have been reported to be significantly longer in constipation[115], with transit times of between 60 hours[204] and 80 hours[201]. By pre-specifying the length of treatment and washout periods to allow for participant adjustment to PERT and PERT to be excreted from the body, a two-week treatment period would ensure the outcomes would be clinically meaningful. A washout period aims to reduce the risk

of carry-over effect; however, it should ideally be longer than two weeks.

The primary outcomes of the review were coefficient of fat absorption, nutritional status (weight, height and BMI) and adverse events. These outcomes are important in assessing PERT efficacy by evaluating fat absorption and wider clinical benefits and harms.

In spite of the gold standard for measuring fat malabsorption, several studies opted to use other techniques. Techniques such as breath tests and faecal spot tests to determine pancreatic enzyme activity only measure activity at one time point and therefore are less reliable than a 72-hour stool collection to measure the coefficient of fat absorption. Measures of nutritional status can be influenced by PERT and trends over time will inform on the efficacy of PERT but these markers can also be subject to confounders such as intercurrent illness. Van der Haak *et al*[196] did evaluate the effects of PERT timings by assessing lipase activity however the treatment was only given on the study days rather than for consecutive days. The study was excluded from the review since it did not follow the pre-specified methodological requirements.

Brekke *et al*[197] also studied the effects of PERT timings in a crossover trial with a treatment period of 4 weeks but no washout period in between treatment arms. The authors did not assess the coefficient of fat malabsorption and only reported abdominal symptoms and quality of life measures. The study methods suggested anthropometric

measurements were obtained and analysis was still ongoing. These measurements were not reported in the published abstract and there was insufficient information in the published abstract to extract data. At the time of the review, only the abstract was available and therefore, the study was excluded from this systematic review. However, since the completion of the systematic review, the study has been published and should be considered for inclusion in future updates of this systematic review.

## 2.6. Conclusion

The literature for randomised controlled trials regarding the timing of PERT doses in CF have been systematically reviewed, however the review has not examined non-randomised controlled trials. Although this review has not concluded whether PERT is more effective when taken before, during or after a meal, it has confirmed that there is a need to re-evaluate study design for future PERT clinical trials. To answer the clinical question, it would be unethical to ask participants to stop taking PERT for any length of time and therefore the use of crossover design may not be suitable. It may be possible to design a crossover trial where the washout period is the first two weeks of the second treatment arm before outcomes are measured after a further two weeks. If crossover designs are used in future trials, an alternative approach to a systematic review should be to include the first treatment period only in order to avoid the risk for carry-over effect[205]. Double-blinded parallel group randomised controlled trials that compare specific

timings with placebo or “standard care” without the need to stop PERT are required to determine whether alternative timing of PERT dose in relation to meals or snacks is superior to current practice.

The outcomes in clinical trials of PERT should continue to assess fat absorption using coefficient of fat absorption but evaluating fat digestion, such as enteric losses of triacylglycerol and free fatty acids, could also assess the efficacy of PERT. Outcome measures over a longer timeframe will provide a better indication of the efficacy of PERT compared to outcome measures that are limited to one time point.

This systematic review has highlighted that there are over 100 clinical trials evaluating the use of PERT in pancreatic insufficient CF and fat absorption but there is little evidence of how this could be refined by timings of PERT delivery. The fact remains that people with CF still experience gut symptoms despite PERT and there is little understanding of the gut function in CF.

The 2018 James Lind Alliance priority setting partnership by Rowbotham *et al*[33] has emphasised the importance of listening to the CF community (people with CF, carers and health care professionals) and finding a solution to gut problems was ranked as the second most important research topic. In order to find a solution to the problem, we need to understand the mechanisms underlying gut symptoms; the extent to which existing therapies normalise these mechanisms; and, where physiology remains abnormal in spite of existing treatment, ensure that new treatments are developed. In particular, it will be

important to evaluate whether CFTR modulators help normalise gut physiology in CF because this class of drug is relatively new and now widely used in CF centres. Therefore, the next chapter will aim to evaluate the current tools used to investigate gut disease in CF.

# Chapter 3 An overview of gastrointestinal investigations used in CF

This chapter will provide an overview of the different GI investigations used clinically and in research to assess motility, digestion and absorption; the mechanisms thought to be affected in the CF gut.

## 3.1. Measures of transit

Transit times are a measure of the physiological GI tract motility and can be measured by different modalities such as scintigraphy, radiograph, wireless motility capsule, and hydrogen breath test. A dysregulation of peristalsis in the GI tract causes abnormal GI motility and can affect GI transit times. Measures of lower GI motility such as manometry have been used in other GI diseases but have not been reported in CF disease.

### 3.1.1. Scintigraphy

Scintigraphy is the gold standard for assessing whole gut transit times. The procedure involves ingesting a set volume of radioactive substance, which emits gamma rays. At set intervals, a gamma receiver measures the amount of emitted gamma rays[206]. Calculations based upon the amount of radioactive material remaining in the stomach, small bowel and ascending colon determines the rate of gastric emptying, small bowel transit and colonic transit respectively[207].

The percentage of radioactive material left in the stomach 4 hours post ingestion provides an assessment of gastric emptying, although this method may assume a constant emptying rate if no other interval measurements are recorded.

Small bowel transit times can be calculated using different methods to obtain the percentage of radioactive material that arrives at the terminal ileum or caecum. There is no standardised method used, but it most commonly involves calculating the time taken for 10% or 50% of radioactive material to arrive at the terminal ileum or caecum[207]. Other methods include using formulas to account for gastric emptying and colonic filling; or calculating the difference in transit time between the proximal and distal small bowel[208].

There are two methods for calculating colonic transit: the first by measurement of radioactive material in specified regions of the colon, and the second by calculating the time for half of the ascending colon to empty[207].

Scintigraphy does have other limitations in addition to its use of radioactive material. As described in Chapter 1, the GI tract produces secretions in response to ingestion of food or liquid. These secretions are unlabelled in scintigraphy. Since such secretions are unlabelled and therefore unaccounted for in scintigraphy measurements. Whilst unlabelled secretions would not affect the transit time reading, it could provide potential reasons for differences in transit times. Furthermore, it is important to acknowledge that if no interval measurements are taken,

there is an assumption that transit velocity is linear and is not consistent with what is known about the migrating motor complex (MMC).

### 3.1.2. Abdominal radiographs

The different materials of bone and soft tissue mean different fractions of the x-ray beams are absorbed[209]. Plain x-rays of the bone and lower GI tract deliver one of the highest radiation doses using plain x-rays and are 0.7 millisievert (mSv) and 7.2 mSv, respectively[210]. One mSv is the average accumulated background radiation dose to an individual for one year. Abdominal x-rays produce the highest amount of radiation to the gonads at 5 mSv in adults. Abdominal x-rays expose children to a moderate dose of radiation (0.5 mSv for females and 1.5 mSv for males)[209].

Abdominal radiographs use x-rays to provide a snapshot picture of the bowel in the acute setting and may aid the diagnosis of DIOS in CF. Signs of bowel obstruction on abdominal radiographs include dilated loops of bowel and air-fluid levels (on upright or decubitus films)[118]. The diagnosis of constipation should be a clinical diagnosis and does not require an abdominal radiograph.

Radiopaque markers, in combination with abdominal radiographs, are used determine transit time. Patients ingest approximately 20 radiopaque markers and serial abdominal radiographs taken daily for 3 days. Based upon the location of the markers, mathematic formulae described by Metcalfe et al[203] can be used to calculate the mean mouth to anus transit time, mean colonic transit time or segmental colonic

transit times. Variations on this method have also been documented[211,212].

It is worth noting that, despite chest disease being the leading cause of death in people with CF, O'Connell et al[213] have previously described in a retrospective study 11.9% and 25.6% of patients in a single centre underwent abdominal radiography and fluoroscopy respectively. Furthermore, nearly 50% of the cumulative radiation exposure were attributed from abdominal imaging and have the potential to increase the risk of developing radiation-induced malignancies[213].

### 3.1.3. Wireless motility capsules

More recently, the use of wireless motility capsules (such as the SmartPill®) have become popular, as they do not require radioactive exposure[206]. Instead, the wireless motility capsules measure transit times by taking continuous pH measurements throughout the GI tract; the change in pH signals where the capsule is, in the GI tract [64]. An increase by more than 2 units indicates the capsule has entered the duodenum and drop by more than 1 unit afterwards demonstrates that the capsule has entered the colon through the ileo-caecal valve[64,214]. The capsule pH readings are timestamped. Video capsule endoscopy (further described in Section 3.4.2) are timestamped and can also determine transit times by visual detection of the capsule passing through sphincters within the GI tract.

### 3.1.4. Hydrogen breath test

Hydrogen breath tests are used clinically to diagnose SIBO and carbohydrate malabsorption. Previous CF clinical trials have used hydrogen breath tests to assess GI transit times[206].

For this test, the patient drinks a substrate that can be metabolised by bacteria to hydrogen, such as glucose and lactulose. Glucose may be used for the hydrogen breath test because it is not absorbed as readily in the presence of SIBO. Lactulose may be used because it is not absorbed in the small bowel[125].

Human cells do not produce hydrogen. Therefore, an assumption is that all the hydrogen produced is due to bacterial metabolism in the bowel. The amount of hydrogen expired is measured at set time intervals. If lactulose is used as the substrate for the hydrogen breath test, the oro-caecal transit time can be determined when there is a continued rise in breath hydrogen levels of at least 10 parts per minute (ppm) over at least three consecutive time points[215]. The lactulose breath test may produce two peaks in breath hydrogen, with the first peak indicating SIBO and the second peak indicating the remaining lactulose passing into the colon. High levels of breath hydrogen or early hydrogen peaks (i.e. more than 20 ppm at 15 minutes before the second peak) can reflect SIBO. It is worth noting that if a patient has had a high fibre intake prior to the test, it can lead to a false positive result[125]. Whilst the hydrogen breath test does not directly measure gut transit times, an early rise may also reflect a faster small bowel

transit[215]. The absence of hydrogen producing bacteria can lead to false negatives[125].

## 3.2. Measures of pancreatic function

Insufficient pancreatic function in CF, in particular lipase deficiency, is the primary cause of maldigestion. Markers of pancreatic function can be measured by concentration of enzymes in the blood or stool; or by measuring the products of fat digestion.

### 3.2.1. Faecal elastase

Faecal elastase is a marker of pancreatic function, because elastase-1 is produced by the pancreas and not degraded within the GI tract[88]. This test is used to diagnose pancreatic insufficiency in CF so patients who need PERT can be identified. Values are reported as a concentration so can be falsely low when stool water content is high (i.e. in diarrhoea).

### 3.2.2. Serum immunoreactive trypsinogen

Serum immunoreactive trypsinogen is a precursor to the enzyme trypsin and thus also a marker of pancreatic function. Trypsinogen is secreted into both the small intestine and the blood[206]. However, when the pancreatic ducts are blocked by viscous secretions, there is more secretion into the blood stream[216]. Newborn blood spot screening includes the measurement of serum immunoreactive trypsinogen because high trypsinogen levels are present in newborns with CF[206,216].

A high serum immunoreactive trypsinogen level indicates there is likely to be an element of pancreatic insufficiency[217].

### 3.2.3. Secretin stimulation test

The secretin-cholecystokinin or secretin-pancreozymin stimulation test is also a direct marker of pancreatic function[206]. Following intravenous secretin and cholecystokinin or pancreozymin, at set intervals, duodenal secretions are collected via a nasojejunal tube[206,218]. Measurements for pH, bicarbonate, lipase, trypsin, chymotrypsin and amylase are taken from the duodenal secretions[218]. These stimulation tests are not used in clinical practice but have previously been used in clinical research studies to distinguish between pancreatic insufficient and pancreatic sufficient CF[218,219].

### 3.2.4. Carbon-13 isotope label

Aside from measuring the enzyme concentration, it is possible to use stable carbon-13 isotope label, $^{13}C$, to determine the adequacy of fat digestion[220]. By drinking a test meal containing $^{13}C$ labelled fat, it is possible to track the fatty acid pathway[220] and measure enteric triacylglyceride and fatty acid losses[206]. These help to differentiate whether there is a problem in digesting (high stool triacylglyceride concentrations labelled with $^{13}C$) or absorbing (high stool fatty acid concentrations labelled with $^{13}C$) fats[220]. Radioactive labels to assess fat digestion are not used in the clinical setting[206].

## 3.3. Measures of absorption

### 3.3.1. Coefficient of fat absorption

The coefficient of fat absorption is the gold standard for measuring how well fat is absorbed in the GI tract after ingestion. The percent of coefficient of fat absorption is calculated using the following formula:

$$\frac{dietary\ fat\ intake\ (g) - stool\ fat\ excretion\ (g)}{dietary\ fat\ intake\ (g)} \times 100$$

where the dietary fat and stool fat are calculated over the same 72-hour period[221]. The coefficient of fat absorption does not take into account different mechanisms of fat absorption and does not differentiate between triacylglyerides and fatty acids.

Other markers of fat absorption, such as free fatty acid absorption tests and malabsorption blood tests[222], are not currently used in clinical practice[206] and will not be discussed further in this thesis.

## 3.4. Measures of inflammation

### 3.4.1. Faecal calprotectin

As described in Chapter 1, intestinal inflammation could affect intestinal absorption.[99] Faecal calprotectin is a non-specific marker of inflammation and used to aid diagnosis and acute exacerbations of inflammatory bowel disease. A faecal calprotectin concentration of over 100 µg/g is suggestive of intestinal inflammation and a faecal calprotectin concentration of less than 50 µg/g is considered normal[223]. Studies evaluating faecal calprotectin in people with CF suggest that

over 60% have a degree of intestinal inflammation using these thresholds[99,224] or seen on wireless capsule endoscopy[99].

### 3.4.2. Endoscopy

Fibreoptic endoscopy and video capsule endoscopy (such as Pillcam™) are useful in assessing the intestinal luminal wall macroscopically to identify areas of inflammation or abnormal anatomical structures such as polyps and cancer. Fibreoptic endoscopy allows microscopic assessment of the bowel wall by taking a biopsy; however, this is more invasive than video capsule endoscopy. For both endoscopy methods, the patient is required to take oral bowel preparation to cleanse the bowel in order to be able to evaluate the bowel wall effectively[119,225]. The amount of bowel preparation required in CF is more than in other diseases due to the viscid nature of intestinal secretions and luminal contents[226,227].

## 3.5. Other measures of physiological factors influenced by CFTR dysfunction

### 3.5.1. pH study

Multichannel oesophageal intraluminal impedance and pH monitoring is used both clinically and in research to diagnose gastro-oesophageal reflux in CF[228]. The pH catheter is passed through the nasal cavity and positioned into the lower part of the oesophagus, where it records the oesophageal pH every 4 to 8 seconds for 24 hours[229]. Gastro-oesophageal acid reflux episodes are confirmed if the pH is less than 4

for at least 15 to 30 seconds[229]. It is normal for individuals older than 1 year to have a reflux index (percentage of total monitored time with pH <4) of up to 6%[228,229].

### 3.5.2. Intestinal current measurements

The use of intestinal current measurements are becoming more popular both clinically and for research. The electrical response (intestinal current) of rectal epithelial tissues from biopsies reflects CFTR function. This is an *ex vivo* technique where the biopsies are submersed in a fluid reservoir within an Ussing chamber[230] (Figure 3.1) and the transepithelial short-circuit currents across the rectal epithelial tissues is calculated[231]. The fluid reservoir contains a buffer solution for ions to pass across the epithelial tissue and also contains glucose as an energy source to the tissue[230]. A carbogen gas (95% oxygen, $O_2$; 5% carbon dioxide, $CO_2$) is continuously passed through the reservoir to oxygenate the liquid and ensure the liquid is mixed[231,232].

In CF, the aim of the short circuit current measurements is to determine the amount of ion transport across the epithelium after stimulation by chloride secretagogues (for example carbachol and forskolin)[232,233]. A transepithelial short circuit current response of more than 10 μA/cm$^2$ is suggestive of a normal functioning CFTR whereas a short circuit current of less than 10 μA/cm$^2$ is suggestive of CFTR dysfunction[232]. Intestinal current measurements can clinically assist in the diagnosis of CF in individuals with atypical presentations[233,234].

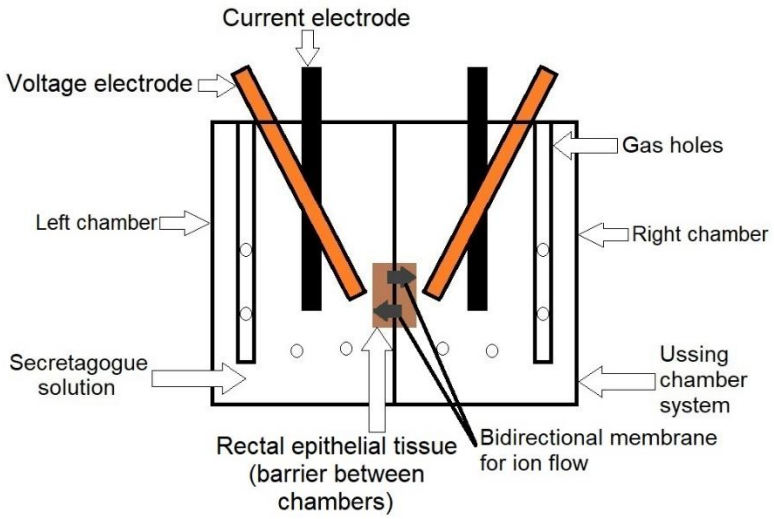



*Figure 3.1. Ussing chamber system to measure potential difference of rectal epithelial tissue CFTR ion channels. The rectal epithelial tissue is submersed in a buffer solution, which provides the tissue with a glucose energy source. The current electrodes send a current across the tissue at set time intervals set by a technician. The voltage electrodes measure the potential difference across the tissue. The gas holes pump carbogen gas (95% $O_2$ and 5% $CO_2$) into the chemical solution to mimic the oxygenation by the systemic arterial blood supply. Figure drawn by Christabella Ng.*

### 3.5.3. Intestinal organoids

The formation of intestinal organoids, a three-dimensional model which can be derived from rectal biopsies, is such that they resemble intestinal mucosa and used for assessing CFTR function in different treatments[206]. One method of assessing CFTR function in intestinal organoids is to measure the amount of forskolin-induced swelling, where functioning CFTR proteins allow chloride secretion into the centre of the spheroid organoid and so function correlates with increased swelling[235]. The use of intestinal organoids is a new and emerging area of CF research. Although it is an invasive and could be seen as unpleasant, in the future could be a tool used to personalise CF treatments by predicting a patient's response to therapy[236].

## 3.6. Analysis of anatomical structures

Anatomical structures are best analysed by imaging the area of interest. Medical imaging provides the clinician with structural (and sometimes functional) information to aid diagnosis and potential treatment options. There are benefits and drawbacks to each different imaging modality used.

### 3.6.1. Ultrasound

Ultrasound uses no radiation. Piezoelectric crystals transmit pulses from, and receive echoes to, the ultrasound transducer. The crystals form an image when echoes are received by vibrating and then converting vibrations to electrical signals. The echoes reflected vary based on the material imaged. The difference in impedance between air, tissue and bone means organ structure can be evaluated[237].

In CF, abdominal ultrasound is used in clinical practice to assess solid organs. Whilst ultrasound has been used to assess other gut diseases such as inflammatory bowel disease[238], at present it is not routinely used in the assessment of CF gut disease. It has, however, previously been used clinically to diagnose fibrosing colonopathy[239,240] and in clinical research to determine the relationship between fibrosing colonopathy and high-dose pancreatic enzymes[241-245].

### 3.6.2. Computed tomography

Computed tomography (CT) transmits x-rays in an arc around the body and a computer detects the x-rays from the opposite side of the body. The amount of transmitted x-rays and the location of x-rays is computed

to produce an image, where air appears black, water appears grey and bone appears white. The images provided by CT show slices of the body, with the ability to vary slice thickness[246].

The radiation dose delivered for abdominal CT scans is typically between 8 mSv to 15 mSv[247], but can be higher when using contrast agents[248] or multiphase scans[249]. This is a greater dose than plain x-rays (radiation doses between 0.7 mSV and 7.2 mSv,) and carries a higher risk of developing radiation-associated cancer in later life. A routine head CT scan could have an adjusted lifetime attributable risk of 0.23 cancers per 1,000 patients (range 0.03-0.70 cancers per 1,000 patients). In comparison, a multiphase abdomen and pelvis CT scan that could have an adjusted lifetime attributable risk of up to 4 cancers per 1,000 patients (range 0.83-11.1 cancers per 1,000 patients) for a 20 year old female. The risk of developing a radiation-associated cancer is reduced in patients who are exposed to radiation when they are older or if they are male[249].

Abdominal CT scans can also aid diagnosis of bowel manifestations in CF such as DIOS and intussusception[250].

### 3.6.3. Magnetic resonance imaging

Magnetic resonance imaging (MRI) uses no radiation and instead produces images by applying a magnetic field around the subject. The human body is made up of many hydrogen ions and when a magnetic field is applied, these ions spin and can align with each other[251]. Dependent on the tissue and scan sequence, different signals are sent

to the receiving coil to produce an image. Water produces strong signals, whilst air produces weak signals.[252]

MRI is used to clinically assess the small bowel in other gut diseases but often requires bowel preparation or hyoscine butylbromide injection to obtain an image of small bowel anatomy without artefacts secondary to peristalsis. Intravenous gadolinium can also be used to aid the assessment of the bowel wall. Both MRI techniques using hyoscine butylbromide and gadolinium have previously been reported in CF by Almberger *et al*[253]*,* who demonstrated a reduction in bowel wall thickening after an adjustment in PERT. There were no reports for the use of MRI without the use of bowel preparation or contrast agents in assessing the function or structure of the luminal GI tract in CF[206], at the start of the thesis studies presented in the following chapters.

## 3.7. Summary of gastrointestinal investigations

There are a variety of tools that may be used to investigate the GI abnormalities in CF. However, the majority of these investigations can be invasive and unpleasant (e.g. endoscopy, rectal biopsy) or expose individuals to radiation (e.g. x-rays, CT). Furthermore, endoscopy and enterography requires individuals to drink bowel preparation to clear luminal contents or have a hyoscine butylbromide injection, respectively, in order to obtain adequate images. These techniques inevitably disturb the intestinal environment and limit the physiological assessment of the bowel.

MRI is a technique used to assess gut function and transit in gut diseases, such as inflammatory bowel disease[238] in the clinical setting and research studies have previously assessed gut physiology in irritable bowel syndrome[254]. However, MRI has not been used previously to assess gut function and transit in CF. In Chapters 4 and 5, I will describe clinical studies where I explore the potential of using MRI to evaluate the gut in CF compared to healthy controls and in response to a CFTR modulator therapy, respectively.

The next section in this chapter will summarise the basics of MR physics and the techniques used to assess the GI tract to provide a basic understanding for its application to studying the gut in CF, as presented in Chapters 4 and 5. Detailed discussion of the intricacies of MRI physics are beyond the scope of this thesis.

## 3.8. A simplified approach to MRI

MRI was first described in 1973 by Paul Lauterbur who demonstrated that it was possible to use nuclear MR to create a 2-dimensional image[255]. Four years later, Sir Peter Mansfield obtained the first MR images of live human structures and subsequently, he developed the rapid acquisition of images in less than 100 milliseconds[256].

### 3.8.1. The magnetic field

#### *3.8.1.1. Generating a magnetic field*

The Earth has a natural magnetic field, which has a strength of 0.5 Gauss[251]. MRI scanners are classed by their magnetic field strength

(Tesla, T) and range from 0.5 to 10 T, where 1 T is equivalent to 10,000 Gauss[252].

Scanner magnetic fields are generated using cryogenic superconducting magnets. The magnet is immersed in liquid helium to reduce its temperature close to absolute zero to create a huge current[252,257]. The magnetic fields generated have both magnitude and direction and therefore a vector can represent the overall magnetic field. The magnitude is the strength of the magnetic force for which the direction of the external magnetic field is parallel. The spin of the proton is perpendicular to the direction of magnetic field (Figure 3.2)[251,252].

Creating an MR image is based upon a hydrogen nucleus (single proton) spin, due to its nuclear spin quantum number ½ (the spin energy level) and high abundance[252]. Since the human body is largely composed of water and fat, which contain hydrogen, strong MR signals can be produced and create an image[251,252,257].

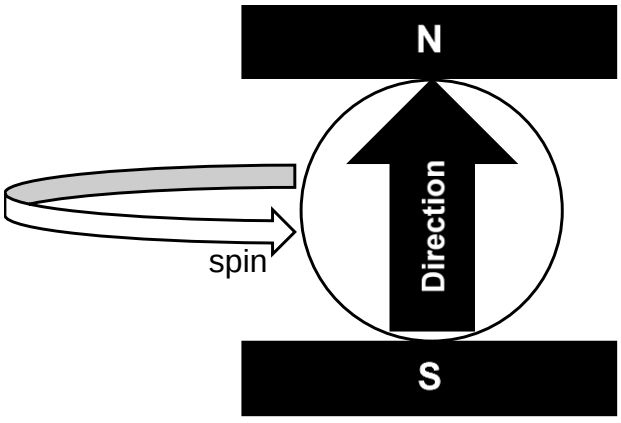



*Figure 3.2. Diagram to show the magnetic field direction. Magnetic field (↑) applied to proton (circle) to generate spin. N = north, S = south. The higher the magnetic field strength, the higher the energy spin. Figure drawn by Christabella Ng.*

Hypothetically, if a set number of hydrogen atoms are in an area with no external magnetic field, each atom has equal magnitude but spins in different directions. This results in the overall vector to be zero. Once placed inside a magnetic field, the hydrogen atoms line up and spin around the axis in the direction of the magnetic field (Figure 3.3)[251,252].

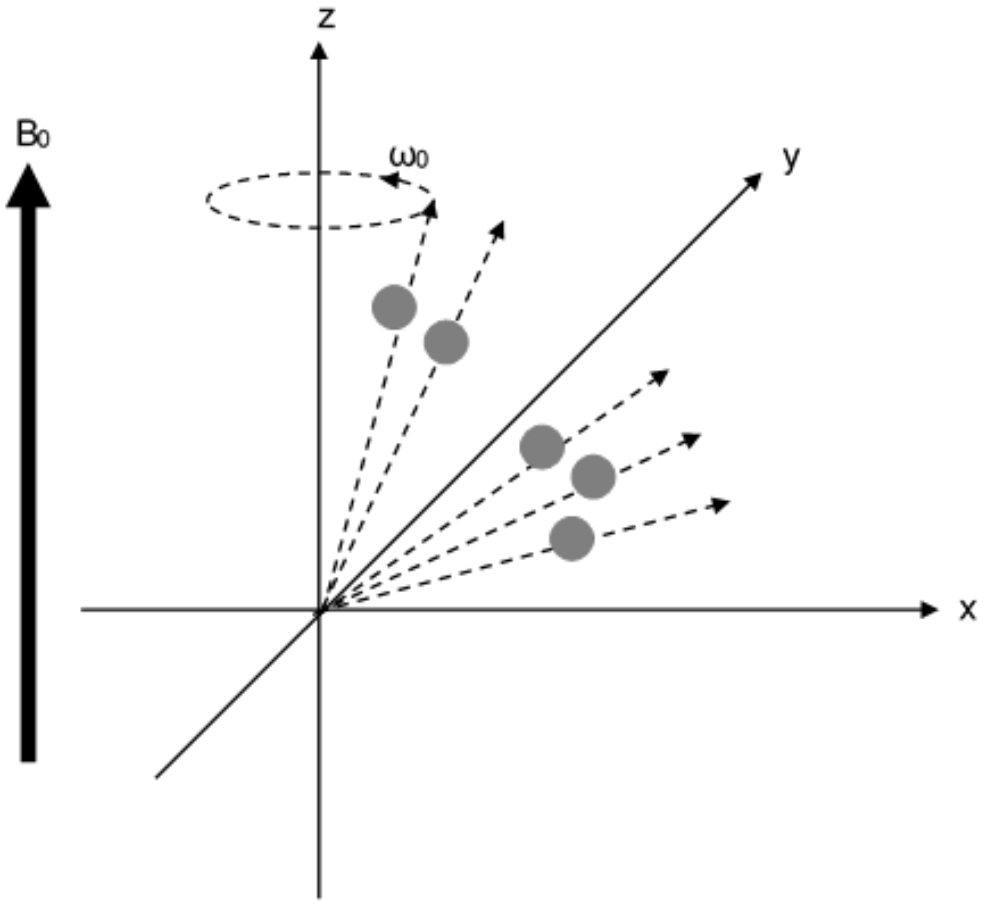



*Figure 3.3. Diagram to show hydrogen atoms within a magnetic field. The 3-dimensional space is described in the x, y, z coordinate system. Within a magnetic field ($B_0$), the hydrogen atoms line up and spin around the axis (z) parallel to the magnetic field. Reproduced, with adaptations and with permission from Dale et al 2015[252]. Figure drawn by Christabella Ng.*

The rate of precession (or rate of rotation about the axis of the magnetic field) is expressed by the Larmor equation:

$$\omega_0 = \gamma B_0$$

Where $\omega_0$ is the Larmor frequency in megahertz (MHz);

$B_0$ is the magnetic field strength in tesla (T);

$\gamma$ is a constant for each nucleus (gyromagnetic ratio).

Protons can exist in two energy states depending on how they align to the magnetic field. They can align parallel to the magnetic field in a natural state with a low energy or can align in the opposite direction (anti-parallel) with a high energy state, similar to when compass needles line up to the Earth's magnetic field[251]. So, when the magnetic field ($B_0$) is applied, some protons spin anti-parallel (spin down) to the direction of $B_0$ with a higher energy. The overall difference in energy is the net magnetisation ($M_0$). Without any other interactions, the direction of $M_0$ is the same as the $B_0$ (Figure 3.4)[251,252,257].

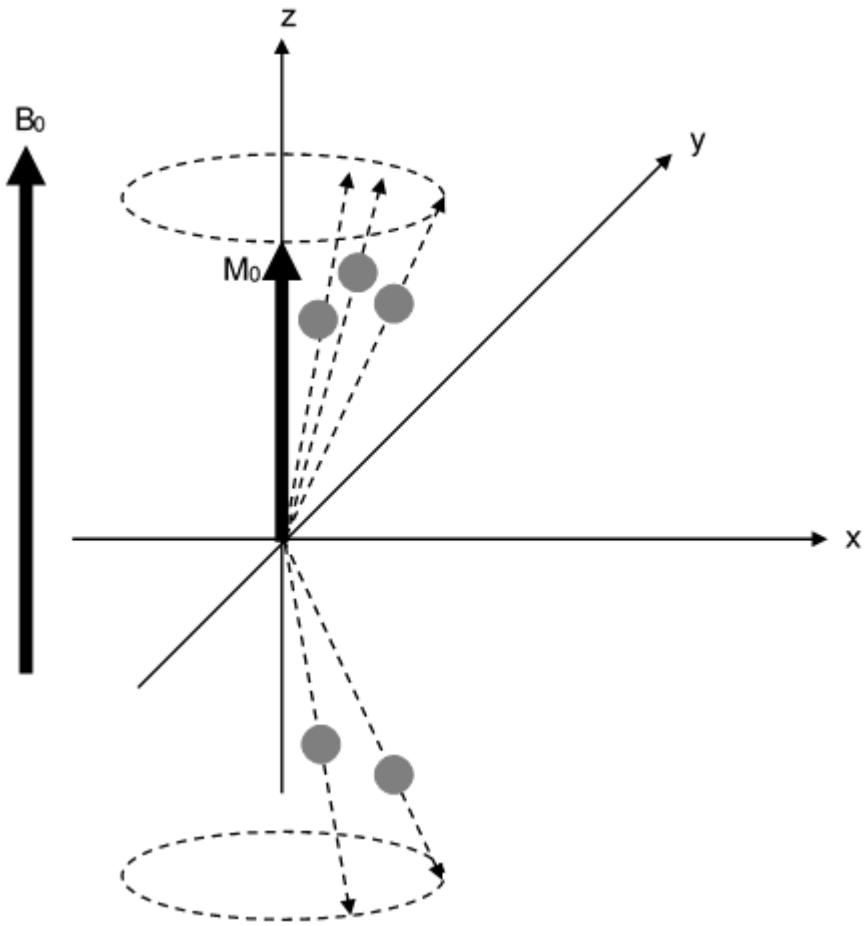



*Figure 3.4. Diagram to demonstrate net magnetisation. The overall energy of the z component is positive so the net magnetisation ($M_0$) goes in the direction of the magnetic field ($B_0$). In the diagram, there are three protons spinning parallel to $B_0$ and two protons spinning anti-parallel. Reproduced, with adaptations and with permission from Dale et al 2015[252]. Figure drawn by Christabella Ng.*

#### *3.8.1.2. Using the magnetic field to create an image*

The magnetic susceptibility of a substance is "*a measure of how magnetised they get"*[251] and reflect how much signal will be produced for an MR image. There are three categories of magnetic susceptibility: diamagnetic, paramagnetic and ferromagnetic. Diamagnetic substances have paired orbital electrons (two electrons each spin in opposite directions to cancel out their moment) and are non-magnetic; when placed in a magnetic field, they produce a weak negative signal.

Paramagnetic substances have unpaired orbital electrons so when placed in a magnetic field, they produce a weak positive signal[252]. Ferromagnetic substances are magnetic and do not require an external magnetic field to be magnetised; they produce a large positive signal. The majority of tissues within the human body are diamagnetic and therefore when placed into a magnetic field, the protons within the body can be realigned. The different types of tissue within the body will produce different signals to create the MR image[251].

To create an image using MRI, the application of an electromagnetic wave (or pulse) generates a signal. A receiver measures this signal. The electromagnetic wave used is directly proportional to its frequency and is called the radiofrequency (RF) pulse. The signal generated is a free induction decay, which means that over time, the signal becomes weaker and uses a sinusoidal waveform. It is the change in alignment of the protons within the object that generates the signal.

Coils are required to generate (gradient coil) or detect (RF coil) the magnetic field and are made of multiple electrical loops of wire. The RF coil can transmit and/or receive a RF pulse. Only the signal transmitted from the region of interest (where the receiver RF coil is positioned, Figure 3.5) creates an image.

In the MRI scanner (Figure 3.5), a magnetic field is generated by a transmitter coil and 3 gradient coils (slice-selection, phase-encoding, frequency-encoding) perpendicular to each other[252]. A process called spatial encoding uses gradient coils to determine the signal position in

relation to the person. The direction of the gradients determines the plane of imaging (axial, sagittal or coronal).

An image is then created when the RF coil transmits a RF pulse to the person in the scanner and receives the RF pulse signal generated from the person[251].

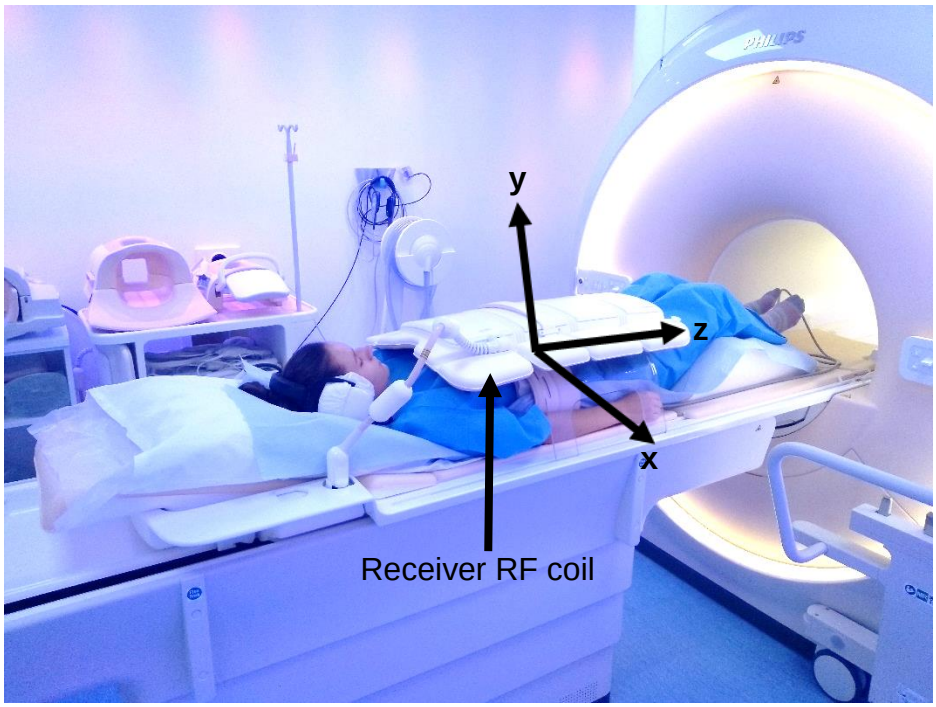



*Figure 3.5. Image of person in the MRI scanner. Axes of gradient coils (x, y, z) required for imaging. Permission given for photography and publications.*

### 3.8.2. MR terminology

#### *3.8.2.1. Repetition time*

During a scan sequence, in addition to the background magnetic field, gradient and RF pulses are generated. The time between each RF pulse sequence is called the repetition time ($T_R$). The shortest a $T_R$ can be is the shortest amount of time required for the pulse sequence to be completed. However, this may not produce the best image. A longer $T_R$

will allow the MR signal to fully recover and enhance the tissue contrast[251,252].

#### *3.8.2.2. Echo time*

The echo time ($T_E$) is the time period between the initial excitation pulse and receiving the signal measurement[251].

#### *3.8.2.3. Longitudinal ($T_1$) relaxation time*

When a 90° RF pulse is applied, magnetisation is flipped 90° from the z plane into the x-y (transverse) plane and the protons in fat and water are in phase (see section 3.8.3.2 for further description). When a 180° RF pulse is applied, magnetisation is inverted but remains in the z plane.

The longitudinal $T_1$ relaxation time is the time constant that characterises the time taken for the protons to recover to 63% of their baseline equilibrium state (the lowest energy state after the RF pulse has stopped) and realign in the longitudinal axis (z-axis) so magnetisation in the z-plane recovers to produce a signal. An exponential growth curve represents the longitudinal relaxation. At a value five times the $T_1$, the majority of protons have recovered to their baseline equilibrium state[251].

The rate at which energy transfers from the proton to its surrounding lattice (the structure of the proton arrangement) is most efficient when the proton's natural motional frequency in a magnetic field is at the Larmor frequency. Protons that have an inefficient frequency, such as water, have a longer $T_1$ and lower signal intensity (darker image). Fat or

tissues with high protein content have a natural motional frequency similar to the Larmor frequency of a proton in a magnetic field and therefore have shorter $T_1$ relaxation times with higher signal intensity (Figure 3.6)[252].

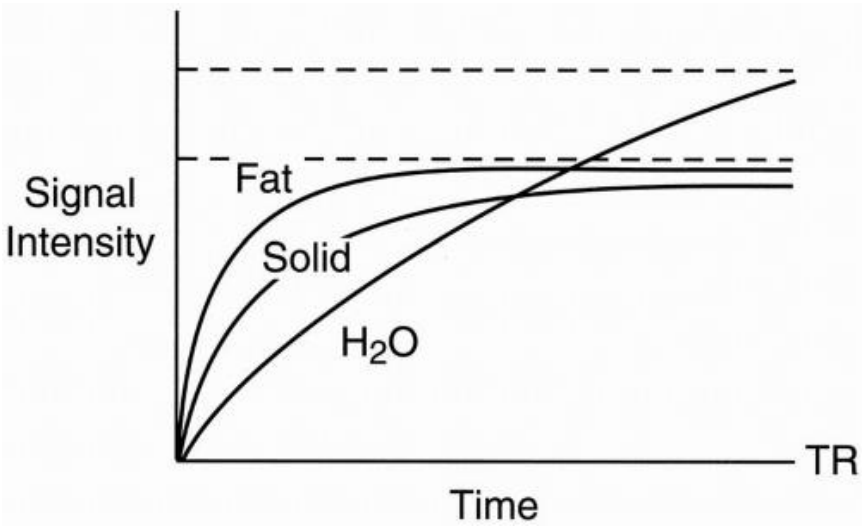
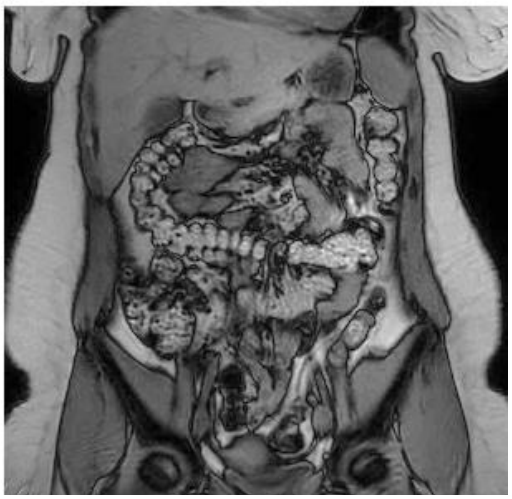


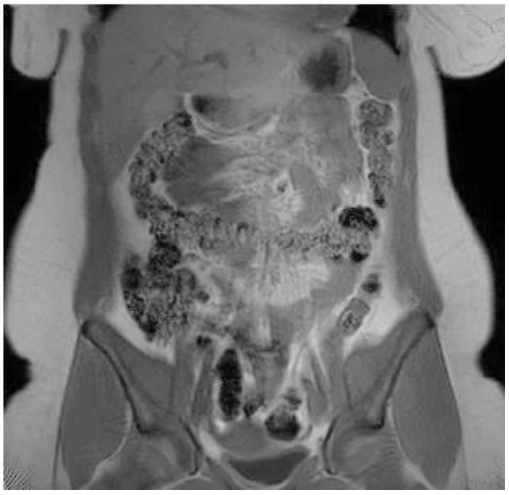

*Figure 3.6. Graph to show the relationship of signal intensity and $T_R$ between fat, solid and water. Reproduced, with adaptations and with permission from Hashemi et al.[251]*

A $T_1$-weighted image (Figure 3.7A, Figure 3.7B) is produced when the $T_R$ and $T_E$ are short[252]. This means when an image is taken, fat and solids will produce a higher signal intensity than water and are brighter on the MR image.

A.



B.



C.

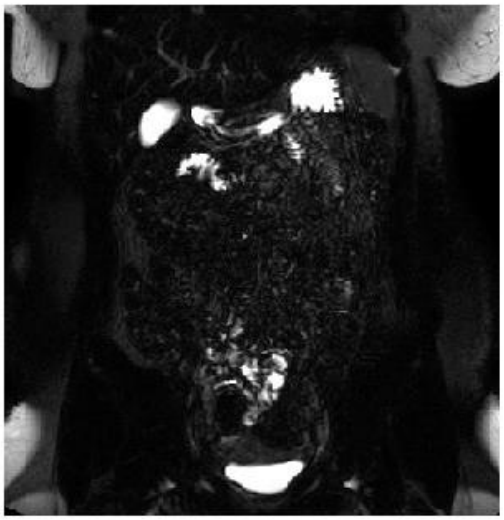


*Figure 3.7. MR images of the same person to show difference between $T_1$ and $T_2$ weighted sequences. A. $T_1$-weighted (out of phase); B. $T_1$-weighted (in phase); C. $T_2$-weighted. Images from the research presented in Chapter 4.*

#### *3.8.2.4. Transverse ($T_2$) relaxation time*

As described in section 3.8.2.3, a 90° RF pulse flips the magnetisation from the z plane into the x-y plane. The $T_2$ relaxation time is the time constant that characterises the time taken for the magnetisation in the transverse plane (x-y plane) to decay by 63% (Figure 3.8) and the protons to become out of phase after the RF pulse is turned off[251].

The $T_2$ relaxation occurs more quickly than the $T_1$ relaxation time due to spin dephasing, which causes spins to get out of phase more quickly[251]. There are two reasons for dephasing: spin-spin interaction and external magnetic fields. Once the RF pulse stops, protons spinning next to each other can have an effect on each other's magnetic field, known as spin-spin interaction. Variations in the external magnetic field can affect the spin decay but it is beyond the scope of this thesis[251].

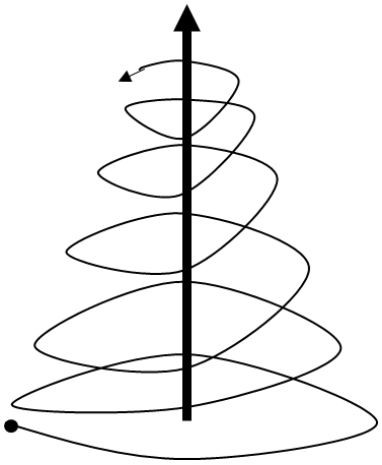


*Figure 3.8. Diagram to show the proton (•) decaying back to its original direction (↑) in the z plane after a 90° radiofrequency pulse is applied. Figure drawn by Christabella Ng.*

A longer $T_2$ relaxation time means the tissue signal has taken a longer time to decay. For example, water has a long $T_2$ relaxation time

because the protons are far apart so there is less interaction between the protons (also known as spin-spin interaction). Solids' protons are more closely packed so there is more spin-spin interaction, leaving a shorter $T_2$ relaxation time.[252]

A $T_2$-weighted image (Figure 3.7C) is produced when the $T_R$ and $T_E$ are long. In contrast to a $T_1$-weighted image, in a $T_2$-weighted image water will have a higher signal intensity (brighter) whilst solid tissue will have a lower signal intensity (darker).[251]

### 3.8.3. MR echo type

An echo results after the application of one or a series of RF pulses to a subject. The type of RF pulse sequence will influence the type of echo received and image generated[251].

#### *3.8.3.1. Spin echo*

Spin echoes generate images after two RF pulses, one flipping the spins by 90° and one flipping the spins by 180°, are applied. An echo follows the RF pulses and each echo generated is stored in a frequency space (k-space), an analog file of the data[251,258].

Historically, generating the images using the conventional spin echo were time consuming. Each $T_R$ was processed in a different k-space and produced a different image for each echo. Now using the fast spin echo method, multiple echoes within a $T_R$ can efficiently fill the same k-space after the single 90° RF pulse and multiple 180° refocussing pulses are applied. For example if the number of echoes in a fast spin echo was eight (i.e. the echo train length is eight), one k-space would

be filled with eight echoes instead of eight k-spaces in the conventional spin echo method. This means a reduction in scan time by eight-fold. The fast spin echo technique, or rapid acquisition with relaxation enhancement (RARE), means it is quicker to obtain images compared to the conventional spin echo. Spin echoes can be $T_1$-weighted with a short $T_E$ and $T_R$ or $T_2$-weighted where the $T_E$ and $T_R$ are longer[251].

#### *3.8.3.2. Gradient echo*

The aims of a gradient echo are to shorten scan times by reducing the $T_R$. Gradient echoes may also be used to generate 3D images[251].

Instead of using a pair of RF pulses, as seen in the spin echo technique, the application of a smaller flip angle (the rotation of the magnetisation) for gradient echoes reduces the $T_R$. Therefore, the second RF pulse (180° refocussing pulse) is not used.

When the RF pulse stops, a negative lobe gradient in the x-axis dephases the protons followed by a positive lobe gradient in the x-axis that rephases the protons to produce an echo. To produce a dual echo, a second gradient process dephases and rephases the decay again. The decay has already lost some signal at the point of the second gradient process and therefore, the $T_E$ is different compared to the first $T_E$. This is particularly useful to acquire fat and water data from an image slice[251,252].

The structure of hydrogen protons in fat and water are slightly different and therefore their ability to spin in comparison to the Larmor frequency for hydrogen is different. Fat and water protons go in and out of phase

with each other at different $T_E$. Taking echoes in and out of phase means there is some separation between the signal intensity of fat and water (Figure 3.7A, Figure 3.7B). When the protons are out of phase (Figure 3.7A), the fat and water protons spin 180° out of phase (Figure 3.10). This means the spins will cancel each other out. If an $T_E$ at this point is selected, the image will produce a boundary effect where a dark outline is seen around fat surrounded organs, i.e. where both fat and water are present within a voxel (Figure 3.9).

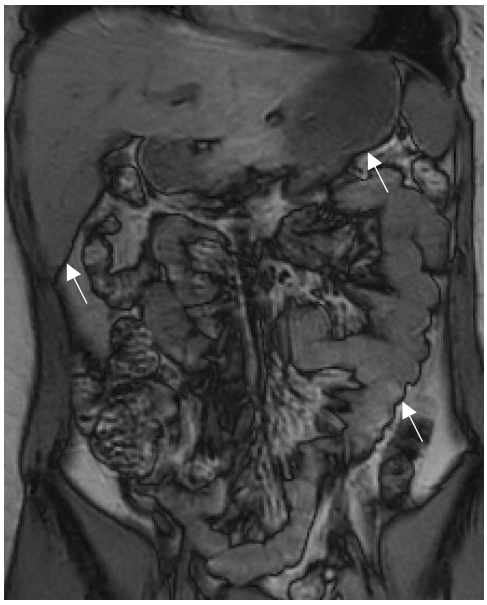


*Figure 3.9. Coronal view of abdomen out of phase. White arrows to show the boundary effect outline around organs. Image from the research presented in Chapter 4.*

When protons are in phase (Figure 3.7B), the fat and water signals are aligned in the same direction (Figure 3.10) and therefore will provide a brighter signal on the image[251].

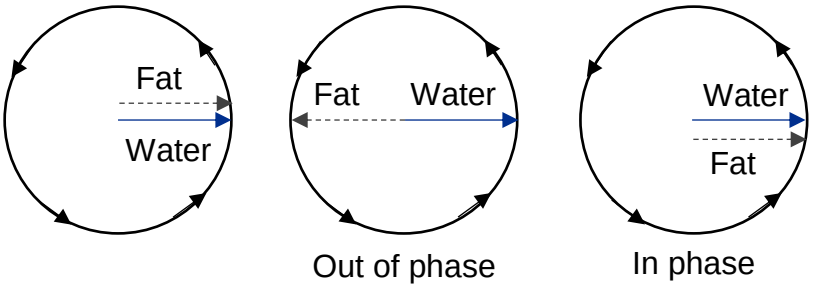



*Figure 3.10. Fat and water spin in and out of phase at specific $T_E$. Fat and water spin at 180° out of phase and spin together when in phase. The circle with arrows indicates the spinning movement. Figure drawn by Christabella Ng.*

3D images are generated using multiple thin slices and extra parameters (phase encoding in the *z* direction and slab-selection) to the original gradient coils described in Section 3.8.1.2. The extra phase-encoding steps allow for volume imaging but can cause artefacts in some slices at the end of each pulse sequence. As a result, these slices are discarded and a slab of slices remains[251].

There are various gradient echo methodologies used to generate different images[251]. In the abdomen, Pritchard *et al*[254] describe a MRI technique using a coronal dual echo gradient echo sequence to evaluate the undisturbed colonic volumes. Menys *et al*[259] have previously demonstrated that GI motility can be captured using balanced gradient echo sequences. Details colonic volume and motility analysis are described in the next section.

### 3.8.4. MR analysis

Over the last two decades, the GI MRI group at the University of Nottingham, alongside other institutions, have developed the use of different MRI parameters to assess GI function and transit for research.

This next section will discuss the methods used to analyse the following parameters: oro-caecal transit time, gastric volumes, colonic volumes, small bowel water content, $T_1$ relaxation time of the ascending colon and small bowel motility. These parameters do not require participants to ingest bowel preparation, oral contrast, or receive intravenous hyoscine butylbromide.

#### *3.8.4.1. Oro-caecal transit time*

Transit time measurements alongside imaging techniques have previously been limited to using scintigraphy (as described in section 3.1.1) or using radio-opaque marker pills to track the time taken for food to pass from the mouth and through the GI tract to the large bowel by identification on serial abdominal radiographs[204]. Both techniques expose individuals to radiation. More recently, Chaddock *et al*[202] describe the use of capsules containing gadolinium, a MRI compatible contrast agent, to be used as transit markers with MRI. Furthermore, Sharif *et al*[201] describe the use of gadolinium-filled mini-capsules in the paediatric population. However, these gadolinium-filled capsules add to the investigational burden on individuals, as they need to remember to take the capsules in the days leading up to the MRI scan.

Oro-caecal transit time (OCTT) is defined as the time taken for the head of a meal to arrive in the caecum. Chaddock *et al*[202] have shown that by performing serial MRI scans, it is possible to identify when the head of the meal arrives in the caecum without the use of gadolinium-filled capsules.

Participants undergo a MRI scan immediately following ingestion of a set rice pudding meal and then sequential MRI scans at set intervals. The head of the meal is identified in the caecum by the first change in signal intensity in the caecum (Figure 3.11). The time at which the scan is performed is the OCTT.

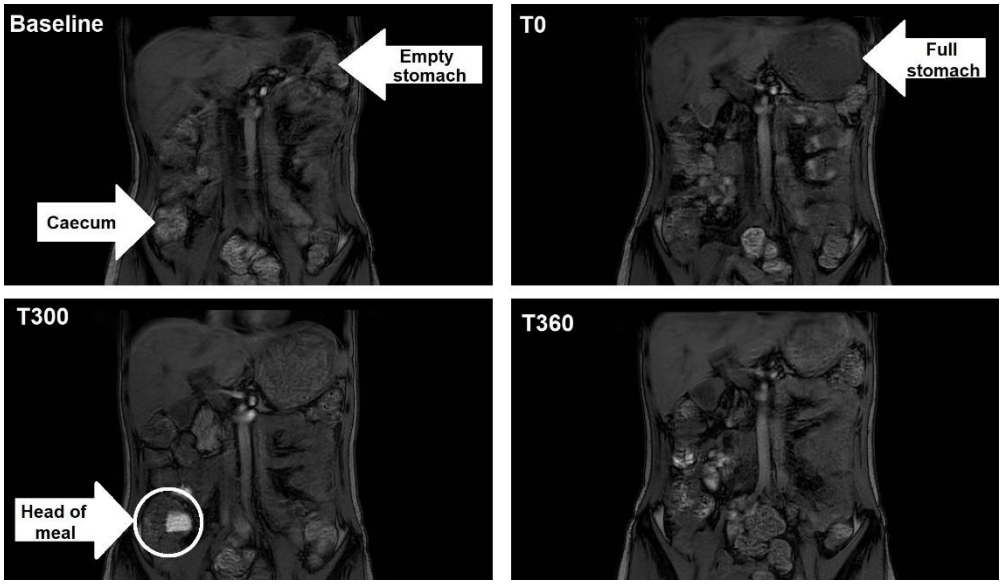



*Figure 3.11. MRI images to show the head of the first meal arriving in the caecum in one participant, identified by high intensity material at T300. Baseline: fasting scan. T0: scan taken immediately after first meal. T300: scan taken at 300 minutes following the meal. T360: scan taken at 360 minutes following the meal. Images from the research presented in Chapter 4.*

#### *3.8.4.2. Gastric half-emptying times*

Parker *et al*[260] developed a MRI technique to analyse gastric content volume and calculate gastric half-emptying times, using serial MRI scans, after a test meal is given. The gastric half-emptying (GE) time is the time taken for the initial gastric content volume, immediately following ingestion, to halve. This in turn aids the interpretation of the OCTT and the researcher can differentiate whether delays, if any, occur in the stomach or small bowel.

The MR sequences used to generate the transverse images are such that the researcher is able to differentiate between gastric content that is solid, liquid or gas, and the stomach wall.

Using image analysis software (Medical Image Processing, Analysis, and Visualisation [MIPAV], NIH, Bethesda[261]), the researcher drew around visible gastric contents on each transverse slice (Figure 3.12). A constant interval of 10 mm between the slices allows the measured areas to be combined to determine the volume of gastric contents.

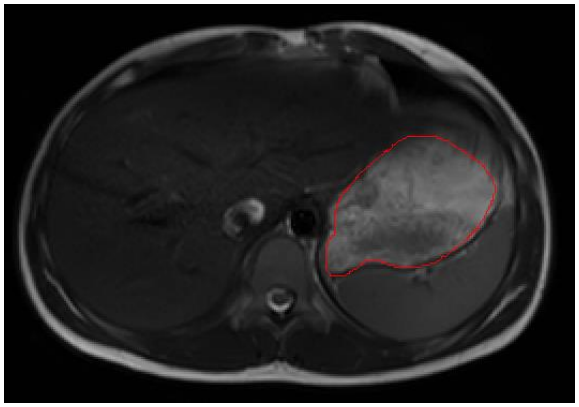


*Figure 3.12. Single transverse slice of participant. Region of interest drawn around gastric contents (red). Images from the research presented in Chapter 4.*

The raw gastric volume data at each time point were modelled to a gastric emptying curve generated by a 5-parameter model described by Parker *et al*[260,262]. The model fitted the data until the gastric content volume was less than 100 mL, as volumes of less than 100 mL are likely to consist mainly of gastric secretions. Using an in-house software written in MATLAB (Mathworks) using this model, the gastric half-emptying times were calculated (Figure 3.13).

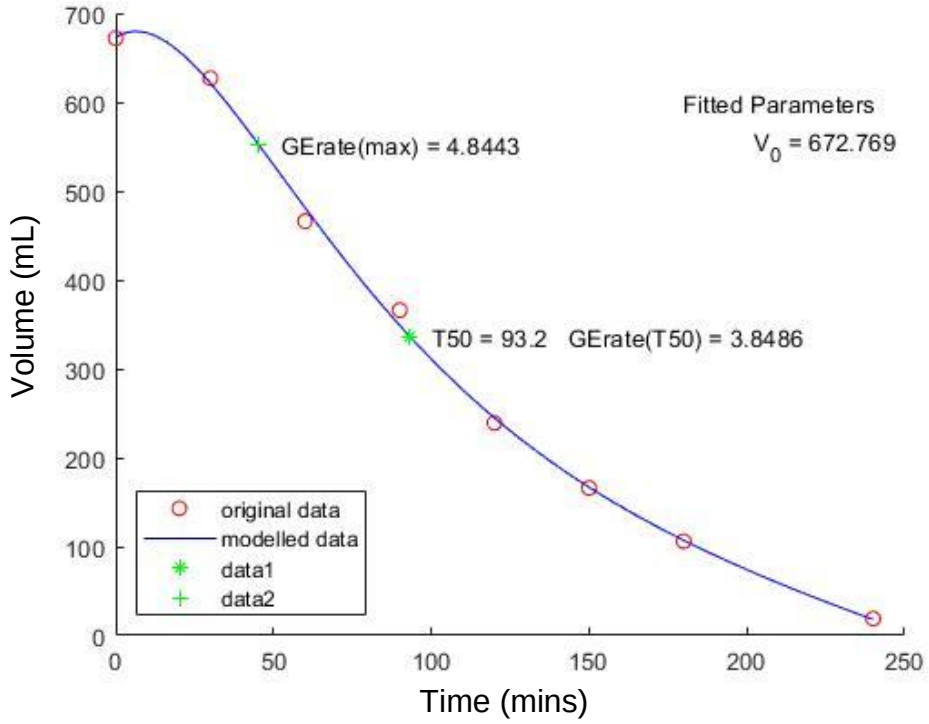



*Figure 3.13. Graph generated to calculate gastric half-emptying time. $V_0$: fitted volume at the first scan immediately after a meal. T50: time in minutes for the stomach contents to decrease to half of the initial volume ($V_0$), in this example 93.2 minutes. GErate_(T50): emptying rate at T50 in mL/min. GErate(max): the fastest rate in mL/min of gastric emptying over the given time period. Blue line modelled data is the line of best fit. Images from the research presented in Chapter 4.*

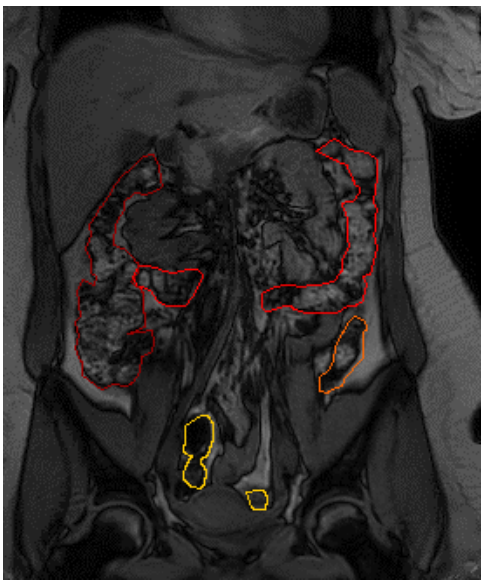
#### *3.8.4.3. Colonic volumes*

Pritchard *et al*[254] have previously described the use of MRI in measuring colonic volumes. By scanning individuals in the fasted state and without oral bowel preparation, the colon is undisturbed and in its usual physiological state. There are limited alternative methods of measuring colonic volumes through radiological methods, which expose patients to radiation,[52] and historical colonic measurements are derived from cadavers[263].

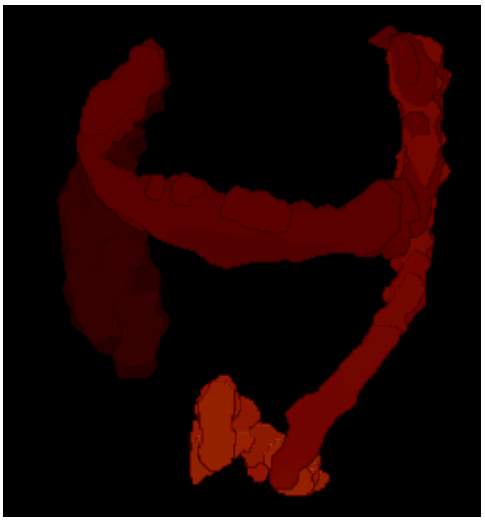
The MR sequences are $T_1$-weighted and allow the researcher to differentiate the colon wall from its surrounding tissue. Using the same

MIPAV software as for the gastric volumes, the researcher drew around colonic segments in each coronal slice (Figure 3.14A). Colonic segments can be divided into ascending, transverse, descending and recto-sigmoid colons. A constant interval of 7 mm between the slices allows the measured areas to be combined to determine the colonic volume. Through the 3D volume renderer tool in MIPAV (Figure 3.14B, Figure 3.14C), the researcher could confirm the colonic segment locations and calculate the colonic volume.

**A.**



**B.**



**C.**

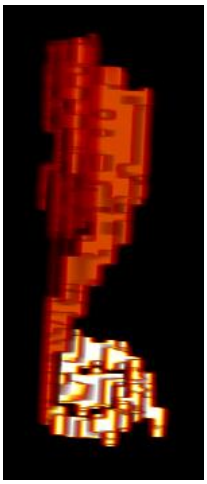


*Figure 3.14. Colonic volumes. A. Coronal slice of participant with colonic segments drawn (dark red – ascending colon; bright red – transverse colon; orange – descending colon; yellow – recto-sigmoid colon). B. 3D coronal view of colonic segments. C. 3D sagittal view of colonic segments. Images from the research presented in Chapter 4.*

#### *3.8.4.4. Small bowel water content*

Measuring small bowel water content (SBWC) using MRI has been validated against intubation studies. Hoad *et al*[264] showed that by instilling a measured volume of mannitol saline solution into the first part of the duodenum via a nasojejunal tube at set intervals, there was a strong positive correlation with the amount of small bowel water measured by MRI. They reported that the mean difference between the instilled volume and measured SBWC was 2% with a standard deviation of 10%. Their intra-observer variation was between -15% to +17%[264].

Using in-house software written in IDL® 6.4 (Research Systems Inc. Boulder, Colorado, USA) or MATLAB, the researcher selected areas of small bowel to calculate the SBWC using $T_2$-weighted images (Figure 3.15A). To ensure only free water was measured, the participant's cerebrospinal fluid was used to calculate the water threshold. Areas that do not contain small bowel were removed (Figure 3.15B). The final small bowel water volume was calculated by combining the content of each coronal slice (Figure 3.15C).

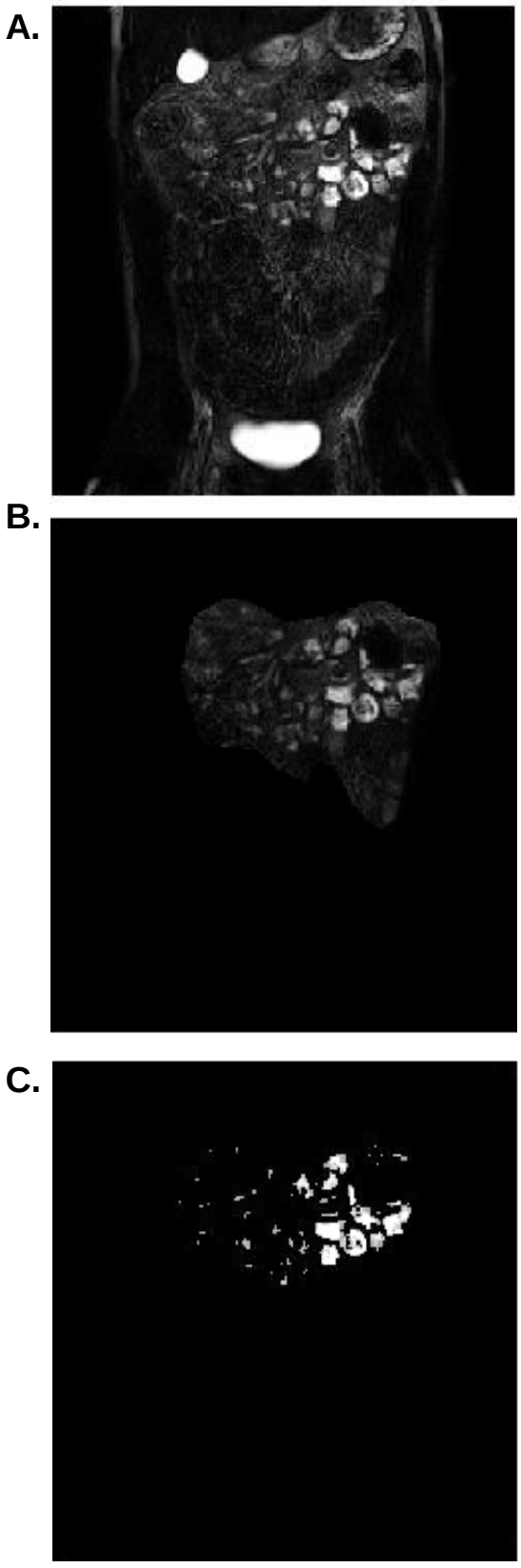



*Figure 3.15. Small bowel water content. A. Strongly T2-weighted coronal slice; B. Small bowel region of interest selected; C. Voxels and areas under the water/cerebrospinal fluid threshold excluded to allow the small bowel water volume to be calculated. Images from the research presented in Chapter 4.*

#### *3.8.4.5. $T_1$ relaxation time of the ascending colon*

As discussed in Section 3.8.2.3, the time constant that characterises the time taken for the restoration of protons alignment after a RF pulse is applied, is the $T_1$ relaxation time.

To calculate the $T_1$ relaxation time of the ascending colon ($T_1$AC), eight sagittal images of the ascending colon were obtained, using eight inversion times ranging from 25 milliseconds (ms) to 5000 ms. The inversion time ($T_I$) is the time taken between a 180° RF pulse and the first 90° RF pulse. The average $T_1$, using a 3 T MR scanner, for tissue such as liver is approximately 800 ms[265] and for cerebrospinal fluid is approximately 3800 ms[266].

Therefore, by selecting an area of chyme within the ascending colon for analysis, the water mobility in the ascending colon ($T_1$AC) can be determined. Major *et al*[115] have previously validated this outcome measure using a 1.5 T MRI scanner and made comparisons with percentage of water content within faecal matter determined by freeze-drying stool. They reported that in a group of 16 adults with no bowel disorders, the $T_1$AC (median, interquartile range) was 720 milliseconds (572 – 904). It is worth noting that the higher the magnetic field strength, the longer the $T_1$ value.

Using in-house software written in IDL® 6.4 or MATLAB, the researcher selected the same area of ascending colonic chyme on each slice to determine the mean signal intensity on each sagittal $T_1$AC image (Figure 3.16). The estimated $T_1$ value of the selected colon contents

was calculated using a code written in IDL® 6.4 or MATLAB. The code used multiple complex equations to model the evolution of magnetisation and then using other model parameters ($T_1$, $T_2$ and $M_0$) the acquired data was best fit to the model data to determine the ascending colon chyme $T_1$. The average $T_1$AC of the ascending colonic chyme for each scan was calculated by taking an average $T_1$AC of three regions of interest selected at the bottom, middle and top of the ascending colon.

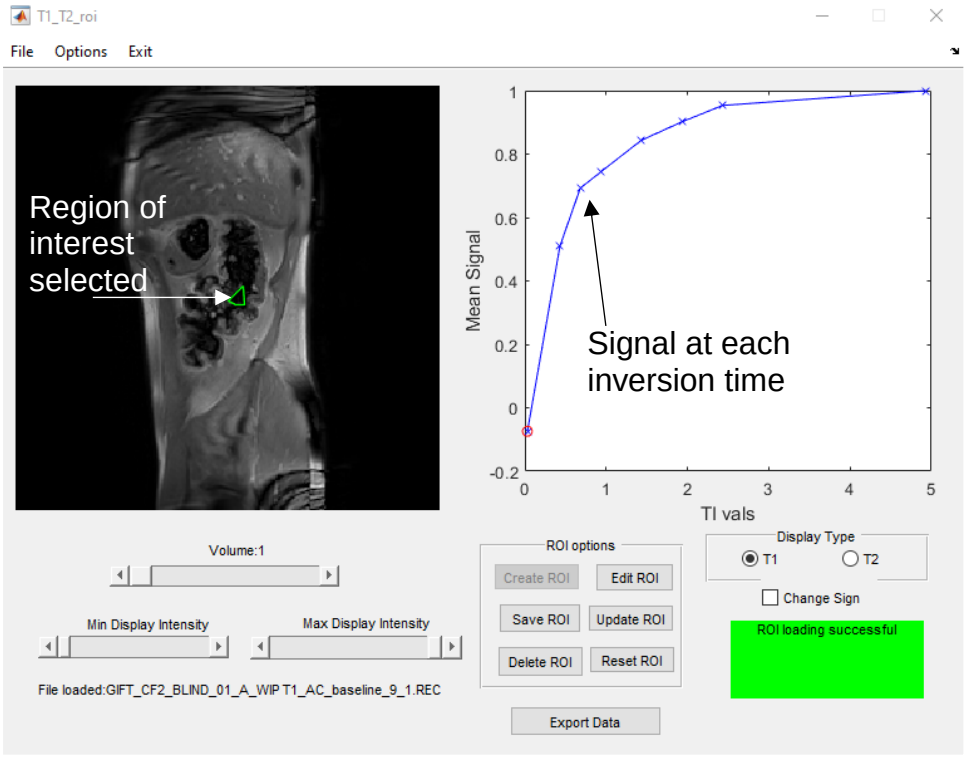



*Figure 3.16. Screenshot of $T_1$AC analysis using in-house software to show an example of when the region of interest is selected, the mean signal is plotted on a graph for each inversion time. Images from the research presented in Chapter 4.*

#### *3.8.4.6. Small bowel motility*

A marker of motility is small bowel contractility. The small bowel is difficult to access with endoscopy and manometry. CT and MR enterography can aid diagnosis of inflammatory bowel disease, in which motility is reduced. These enterography techniques require the individual to drink at least 1 L of mannitol solution prior to scanning to prepare their bowel and then the patient drinks oral contrast at the time of scanning. CT and MR enterography are limited to images taken at set points to determine if the contrast has passed through the small bowel and it is from the transit time readings that the degree of small bowel motility is inferred. These techniques do not specifically assess the bowel wall motility.

Cine imaging is an alternative MR sequence to be used in assessing small bowel motility. Menys *et al*[267] have reported a MR cine image technique to measure small bowel motility without the need for individuals to take bowel preparation or hold their breath during MRI scans. They demonstrated by stimulating and inhibiting small bowel motility using neostigmine and butylscopolamine, respectively, that a quantitative measure of small bowel motility is achievable[268].

Cine imaging occurs by taking a series of echo images in the same position or slice to capture the small bowel movement. Each cycle of movement (e.g. a peristaltic wave) is called a phase. The number of phases and $T_R$ duration determines the image resolution. A greater number of phases leads to a better resolution[251].

If a person breathes freely during the cine imaging of the small bowel (without the use of neostigmine and butylscopolamine), the movements seen on the images obtained are affected by the breathing motion and cause artefacts in the motility analysis. This artefact can be removed by a program called GIQuant™ (Motilent, Ford, London, UK)[269]. GIQuant™ also proposes to take into account through-plane motion (motion caused by local displacement of the bowel wall) and luminal changes[270].

After the breathing motion artefact is removed, the region of interest (part or the entire small bowel) is selected for each slice and the changes in intensity over time are calculated in MATLAB to formulate the total power in arbitrary units (au). This metric reflects the small bowel wall motility including segmental oscillation and bolus movements[271]. A higher total power correlates with a higher small bowel motility.

## 3.9. Summary

GI investigations in CF have limited utility and can be invasive or expose individuals to radiation. There is little knowledge and understanding on how the CF gut differs from the healthy gut or how new and existing CF therapies affect gut function.

Multiple MRI parameters have been used previously to assess gut function and transit in health and other GI diseases. Thus, using the MRI techniques described, this thesis will first assess the feasibility of

MRI in CF and evaluate the differences in gut function and transit between CF and health in Chapter 4. Chapter 5 will then evaluate the GI effects of a CFTR modulator in CF, using MRI.

# Chapter 4 A study of the post-prandial changes in MRI parameters of gastrointestinal function in people with CF compared to non-CF controls

This chapter will describe the gut imaging for function and transit in CF 1 (GIFT-CF 1) study: a study designed to assess the feasibility of using abdominal MRI in CF.

The UK CF Trust, US CF Foundation and Nottingham University Hospitals Charity funded this work. The work was first published online in the Journal of Cystic Fibrosis in June 2020[272] and in December 2021[273].

## 4.1. Background

Relieving GI symptoms in CF is a priority for patients and healthcare professionals, as highlighted by Rowbotham *et al*[33]. However, an understanding of the mechanisms of CF gut disease is required to develop new or effectively repurpose existing treatments.

Chapter 1 and Chapter 3 discussed the poor understanding of the mechanisms of GI disease in CF and how the majority of investigations used to evaluate GI disease are invasive or expose individuals to radiation[6].

The evaluation of gut function and transit using MRI is a technique used in research involving healthy volunteers and other gut diseases such as irritable bowel syndrome and constipation. MRI is advantageous in a patient group who already have a high investigational burden as MRI is both non-invasive and has no exposure to radiation.

Using MRI to assess gut function and transit in CF is novel. It is not clear whether people with CF can tolerate lying flat in a MRI scanner for long periods with multiple breath-holding instructions or whether MRI can detect the underlying GI pathophysiological changes in CF that could be used as a surrogate marker, similar to how $FEV_1$ is used as a surrogate marker for respiratory health. This study will provide information as to whether GI MRI is an appropriate tool for assessing GI physiology in CF and whether clinically meaningful outcomes can be derived from MRI. This study will provide the initial steps towards using GI MRI in future research and clinical care for CF.

## 4.2. Study aim and hypothesis

The first aim of the work presented in this chapter was to assess the feasibility of assessing gut function and transit in people with CF, using MRI. The hypothesis tested was that 12 people with CF are each able to complete a one day scanning protocol to obtain images to assess MRI parameters of gut function and transit.

The second aim was to compare gut physiology assessed by MRI in people with and without CF. For the second aim, the hypotheses tested were:

I. Oro-caecal transit times (OCTT) are longer and small bowel motility is reduced in CF due to viscous secretions;
II. Colonic volumes are increased in CF due to the increased prevalence of constipation in CF;
III. Ascending colon contents are drier and small bowel water content (SBWC) is reduced in CF due to abnormal CFTR function;
IV. GI symptoms correlate with physiological abnormalities.

The third aim was to compare stool markers relating to pancreatic function and GI inflammation in people with and without CF. The hypotheses tested were:

I. Faecal elastase concentrations are lower in CF due to pancreatic exocrine insufficiency;
II. Faecal calprotectin concentrations are higher in CF due to intestinal inflammation.

## 4.3. Methods

### 4.3.1. Study design

GIFT-CF 1 was a pilot study conducted at the University of Nottingham and Nottingham University Hospitals NHS Trust.

The study was prospectively registered on clinicaltrials.gov (NCT03566550) and was approved by the West Midlands – Coventry and Warwickshire Research Ethics Committee (18/WM/0242). The study was carried out according to the principles of Good Clinical Practice in accordance with the Declaration of Helsinki.

### 4.3.2. Study population

Potential participants with CF (who receive their clinical care from the paediatric or adult CF services at Nottingham University Hospitals NHS Trust) were pre-screened using the UK CF registry and digital health records. Members of the clinical team approached potential participants first (i.e. during a clinical interaction when patients attended hospital for an outpatient appointment or inpatient visit) before the research team provided further information. Control participants (who did not have CF) were recruited through open advertisement in the Nottingham area and matched for age and gender to participants with CF. A member of the research team (myself, Dr. H. Barr, Dr. N. Dellschaft or J. Longmate) obtained written informed consent from all participants. Participants aged under 16 years were given the option to provide written assent alongside written parental consent. All participants had a minimum of 48 hours to read the participant information sheets before giving consent.

Table 4.1 provides a summary of the inclusion and exclusion criteria followed. A lower age limit was imposed to ensure participants were able to understand and follow instructions while in the MRI scanner. An upper age limit was imposed as it is more likely for a person with CF to

have worsening lung function with increasing age. People with a worse lung function are also less likely to tolerate lying flat and hold their breath for 10 seconds. For this reason, there was a lower limit of inclusion for predicted forced expiratory volume in 1 second ($FEV_1$). Participants with CF were only included if they had a confirmed diagnosis of CF and were homozygous for p.Phe508del, to reduce the heterogeneity within the CF group. Since potential CF participants do not have routine faecal elastase measurements, their pancreatic function status was not included in the inclusion or exclusion criteria.

*Table 4.1. Inclusion and exclusion criteria for GIFT-CF 1*

| Inclusion criteria | Exclusion criteria |
|---|---|
| – Age 12 – 40 years<br>– Capacity to consent, or to understand the requirements of the study where parental consent is needed<br>– Patients: confirmed CF (homozygous p.Phe508del)<br>– Controls: no clinical evidence or suspicion of CF | – $FEV_1$ <40% predicted using Global Lung Initiative criteria[274]<br>– Contra-indication to MRI scanning, such as embedded metal or pacemaker<br>– Pregnancy<br>– Unable to stop medications directly prescribed to alter bowel habit, such as laxatives or anti-diarrhoea medicines, on the study day<br>– Previous resection of any part of the GI tract apart from appendicectomy or cholecystectomy. Surgical relief of DIOS or meconium ileus were permitted unless clinical records showed excision of intestine length >20cm<br>– Intestinal stoma<br>– Diagnosis of inflammatory bowel disease or coeliac disease confirmed by biopsy<br>– GI malignancy<br>– Unable to comply with dietary restrictions required to complete the study |

### 4.3.3. Study procedures

All participants attended the Sir Peter Mansfield Imaging Centre (SPMIC) at the University of Nottingham, for a day of serial MRI scans. Participants fasted from 20:00h the night before and were allowed to drink water until bedtime. They were able to have a small amount of water with essential medicines in the morning.

On arrival to the SPMIC at 08:00h, the participant completed a MRI safety questionnaire to ensure they had no contraindications to undergo the MRI scan. The participants also completed two GI questionnaires described in Chapter 1, section 1.7 (PAC-SYM and CF-AbdScore, Appendix C) to assess their GI symptoms over the preceding two weeks. A recall period of two weeks is often used in inflammatory bowel disease[275]. Short recall periods of less than a week may not capture quality of life measures accurately, whilst long recall periods of more than a month may introduce recall bias[276].

Data were collected for MRI scans and GI symptom report (a 3-point Likert-scale to assess flatulence, bloating and abdominal pain, Appendix C) at 11 time points over a 7-hour period (Figure 4.2) for each participant. A full description of the MRI protocol, including analysis, can be found in section 4.3.3.1.

MRI scans were taken at baseline (fasting state), immediately after the first meal then every 30 minutes for 4 hours and every 60 minutes thereafter. The first set test meal (Figure 4.1A, Table 4.2), consisting of 300 g creamed rice pudding mixed with 30 g double cream and 25 g

seedless raspberry jam and a drink of 100 mL orange juice with 240 mL water (all Sainsbury's®, UK), was provided immediately after the baseline scan. The rice pudding meal was chosen because it demonstrates physiological SBWC trends without absorbing the too much small bowel water, which is seen with a bread[37] or porridge[277] meal. Furthermore, the rice pudding meal empties quicker than other meals and the head of the meal can be identified in the caecum. The addition of fat to the rice pudding meal reflects the high fat high calorie meals routinely ingested by people with CF.

The second set test meal (Figure 4.1B, Table 4.2), consisting of 400 g macaroni cheese (Sainsbury's®), 100 g strawberry cheesecake (Rhokett®) and 240 mL water, was given immediately after the scan at 240 minutes. Participants with CF took their usual prescribed dose of PERT with their meals.

**A.**



**B.**

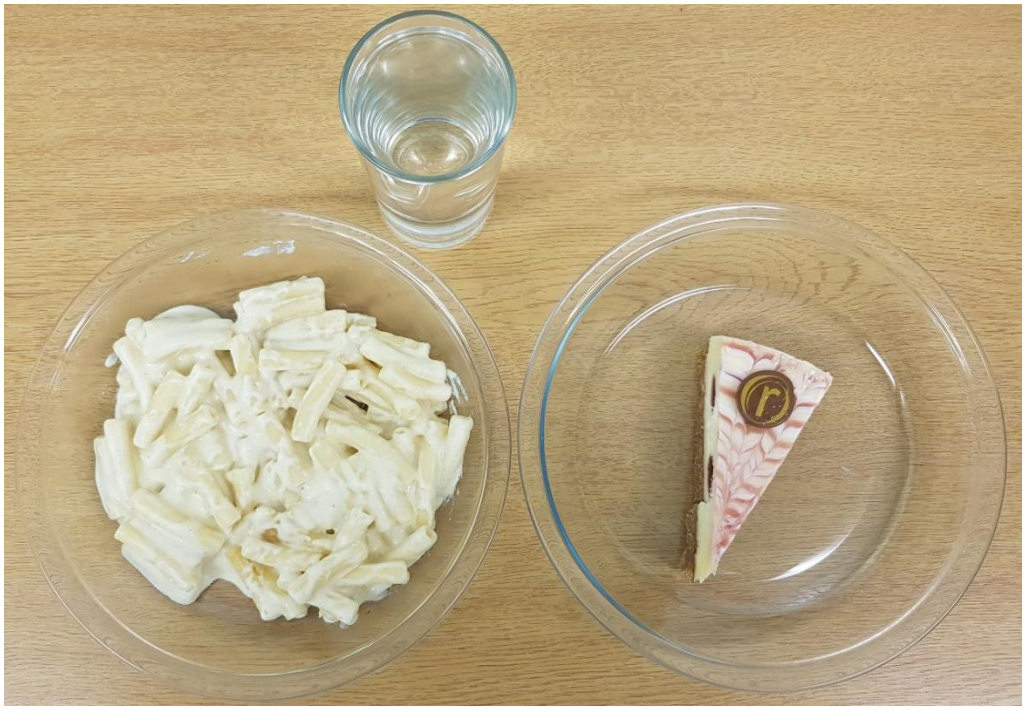


*Figure 4.1. Set meals provided for all participants. (A) Set meal 1: Rice pudding mixed with double cream and jam, glass of orange juice and water. Total energy 519 kcal. (B) Set meal 2: macaroni cheese, strawberry cheesecake and a glass of water. Total energy 1110 kcal. Photos taken by Christabella Ng.*

*Table 4.2. Summary of nutritional contents of GIFT-CF 1 set meals.*

| | **Energy (kcal)** | **Carbohydrate (g)** | **Protein (g)** | **Fat (g)** |
|---|---|---|---|---|
| 300 g Sainsbury's creamed rice pudding | 282 | 52.5 | 8.1 | 4.2 |
| 30 g Sainsbury's double cream | 132 | <0.5 | <0.5 | 14.3 |
| 25g Sainsbury's seedless raspberry jam | 62.75 | 15.4 | <0.5 | <0.5 |
| 100 mL Sainsbury's pure orange juice from concentrate | 42 | 8.6 | 0.6 | <0.5 |
| **Set meal 1** | **519** | **77** | **9** | **19** |
| 400 g Sainsbury's macaroni cheese | 755 | 78.4 | 31.9 | 33.1 |
| 100 g Rhokett® strawberry cheesecake | 355 | 37.8 | 3.7 | 21.2 |
| **Set meal 2** | **1110** | **116.2** | **35.6** | **54.3** |

At the end of the day, participants provided anonymous feedback to rate the ease and comfort of the study protocol.

In addition to the MRI scans and questionnaires, participants provided a stool sample within 24 hours of the study visit. Ms. M. Lingaya, at the Nottingham Digestive Diseases Centre, processed the stool samples

for faecal elastase and faecal calprotectin. Each stool sample had a sample of approximately 100 mg of stool extracted for analysis using the ScheBo® Master Quick-Prep™ extraction system. Each extracted stool sample then underwent an enzyme-linked immunoassay (ELISA) test using the ScheBo® Pancreatic Elastase 1™ stool test and an ELISA test using the BÜHLMANN fCAL® ELISA kit. Stool samples were also processed for microbiome analyses at the Manchester Metropolitan University but this forms part of another PhD[278], and therefore will not be further described in this thesis.

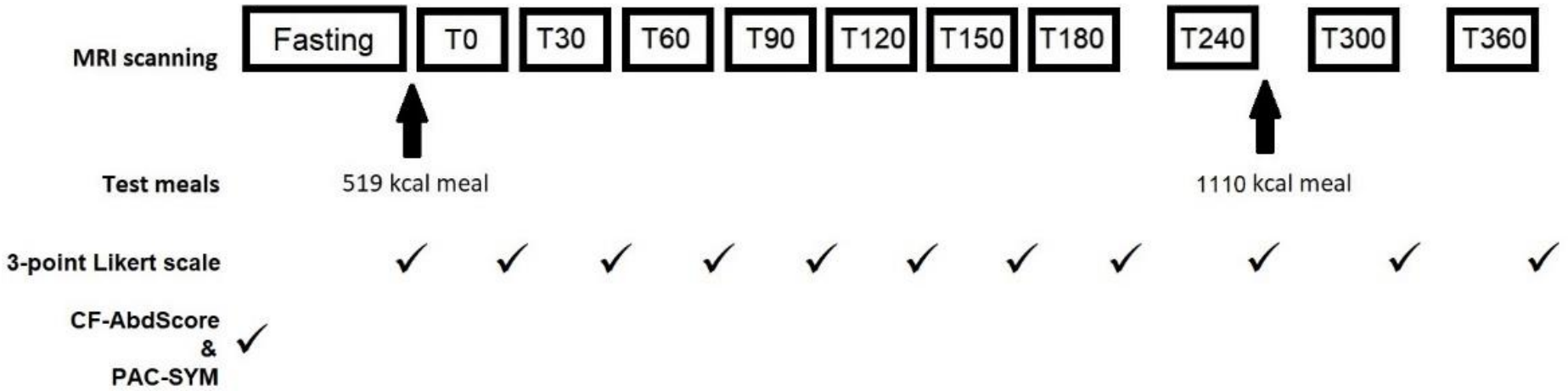



*Figure 4.2. GIFT-CF 1 MRI study day schedule. Test meals were the set meals given to each participant. "T" relates to the time of MRI scan in relation to when the first meal was eaten. For example, T0 was the MRI scan taken at 0 minutes after the first meal was eaten. Post-prandial MRI scans were performed immediately after the first test meal, then at half hourly intervals for 180 minutes with further scans at 240, 300 and 360 minutes. The second test meal was given immediately after the T240 scan. The CFAbd-Score and PAC-SYM questionnaires were completed prior to the fasting scan. The 3-point Likert scale questionnaires were completed after every scan.*

#### *4.3.3.1. MRI protocol*

At each time point, the participant was positioned supine on the 3 T Philips Ingenia (Philips Healthcare, Best, The Netherlands) MRI scanner table, as in Figure 4.3, and advanced into the magnet bore feet first.

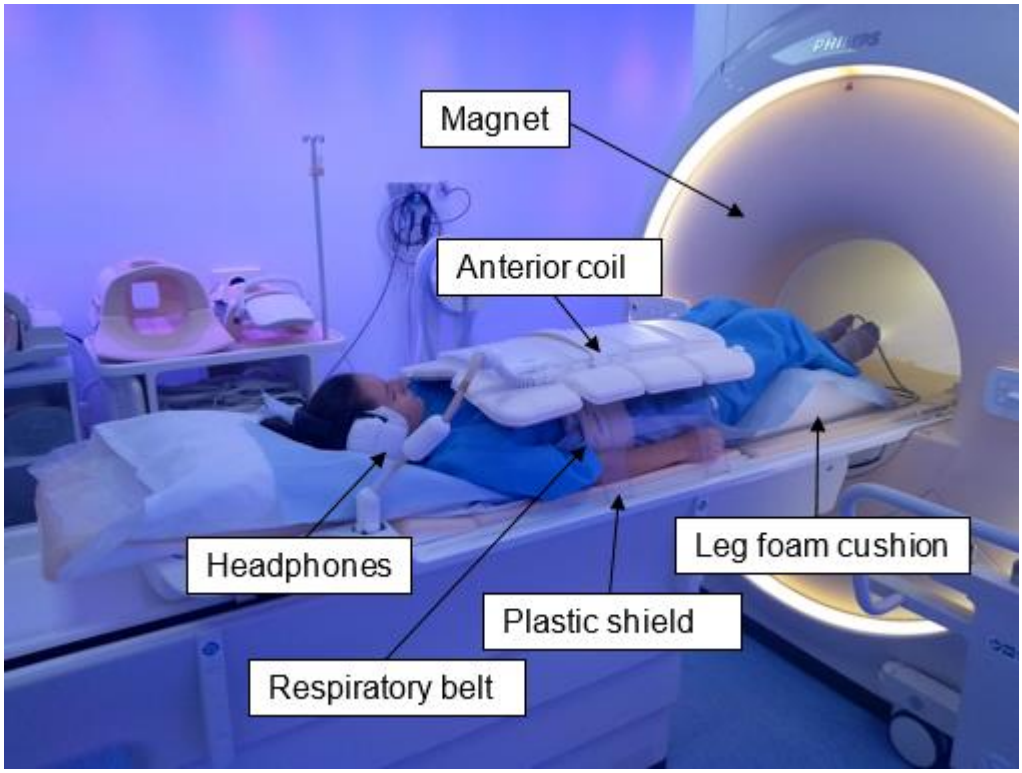



*Figure 4.3. Participant positioned on scanner table with headphones, respiratory belt, plastic shield and anterior coil. Permission given for photography and publication.*

For each time point, an initial survey scan was performed to determine positioning of abdominal organs, followed by a range of MR scan sequences to:

1. Identify OCTT and measure colonic volumes: a coronal dual echo gradient echo ($T_E$ = 1.15 ms and 2.3 ms, $T_R$ = 110 ms, 60° flip

angle) to acquire 22 coronal slices (each 7 mm thick with 1 mm gap) with voxel size 1.6 × 1.6 × 7 mm;

2. Aid identification of head of the meal for OCTT: high resolution balanced turbo field echo sequence ($T_E$ = 1.2 ms, $T_R$ = 2.4 ms, 42° flip angle) to acquire 8 sagittal slices (each 7 mm thick with 0.6 mm gap) with voxel size 1.5 × 1.5 × 7 mm;
3. Measure gastric volumes to calculate the gastric half-emptying times: half Fourier turbo spin echo sequence ($T_E$ = 60 ms, $T_R$ = 508 ms, 90° flip angle) to acquire 20 transverse slices (each 10 mm thick with no gap) with voxel size 1.59 × 1.99 × 10 mm;
4. Measure SBWC: strongly $T_2$-weighted turbo spin echo sequence or RARE ($T_E$ = 400 ms, $T_R$ = 1263 ms, 90° flip angle) to acquire 24 coronal slices (each 7 mm thick with no gap) with voxel size 1.4 × 1.76 × 7 mm;
5. Measure ascending colonic chyme composition: $T_1$- weighted balanced turbo field echo sequence ($T_I$ = 100 ms to 5000 ms after a 180° inversion pulse) to acquire 8 sagittal slices; and
6. Measure small bowel motility: free breathing cine balanced turbo field echo sequence to acquire 3 sets of 2 coronal slices from. Acquisition of each pair of coronal slices was over 1 minute with temporal resolution of 1 second, and spaced at 10mm antero-posteriorly. Using the dual echo gradient echo scan performed earlier in the MR sequence, the first 2 coronal slices were positioned immediately behind the abdominal wall muscles. The following 2

sets of coronal slices were then positioned 10mm and 20mm posterior from the first slice.

All sequences required the participant to hold their breath for up to 10 seconds at a time, except for the small bowel motility sequence. The time required for each breath hold was an adaptation of previous study protocols in healthy volunteers. Three variables were altered to allow for the reduction in breath holds: 1) multiple breath holds to create an image were used instead of a single breath hold; 2) the $T_R$ for the echo sequences were reduced; and 3) the number of slices acquired per time point were also reduced.

Unless otherwise stated, I undertook all study procedures, with the assistance of a qualified scanner operator (Mr. A. Cooper, Mr. C. Bradley, Mr. J. Paul or Dr. M. Kaviani) on the MRI study visits.

#### *4.3.3.2. MRI analysis*

An independent researcher re-labelled all the MRI data collected to ensure that the observer remained blind to the participant's group (CF or control) during image analysis. MR data were analysed as described in Chapter 3, Section 3.8.4. I completed a training set of MR data prior to analysing the study images to ensure my analysis reached an adequate level of performance compared to other analysts. This is to reduce inter-observer variation between a more and less experienced observer. Table 4.3 details the analysts for each MRI outcome.

For OCTT analysis, in order to ensure the technique was robust, two researchers (myself and Dr Dellschaft (ND)) independently analysed

the images. If there were disagreements that could not be resolved by discussion, a third researcher (Prof. L. Marciani (LM) or Dr C. Hoad (CH)) was asked to review and make a final decision.

For colonic volume and SBWC analyses, to ensure the technique was robust, ND randomly selected 10% of the data to analyse.

*Table 4.3. Summary of analysts for MRI outcomes. For outcomes where there was one analyst, a second analyst reviewed 10% of the data. For OCTT, a third researcher (CH or LM) resolved any disagreements. CN = Christabella Ng; ND = Neele Dellschaft; CH = Caroline Hoad; LM = Luca Marciani.*

| Outcome measure | Analysts |
|---|---|
| OCTT | CN, ND, CH, LM |
| Gastric half-emptying time | CN |
| Colonic volume | CN |
| SBWC | CN |
| $T_1$AC | CN |
| Small bowel motility | ND, CH |

### 4.3.4. Outcomes

#### *4.3.4.1. Feasibility*

Feasibility of the proposed study was described by the success of recruitment and retention of participants. Tolerability and acceptability of the study were described by the proportion of completed study visits and through feedback questionnaires relating to comfort and enjoyment of the study visit.

#### *4.3.4.2. Gut physiology*

The primary MRI endpoint was OCTT, defined as the time taken for the head of the meal to arrive in the caecum and detected by a change in signal intensity at the caecum. The secondary endpoints were gastric half-emptying times, SBWC corrected for body surface area (area under the curve, AUC), total colonic volumes corrected for body surface area (AUC) and GI symptoms using validated questionnaires as described in Chapter 1 (PAC-SYM, CFAbd-Score, 3-point Likert-scale from the GSRS questionnaire to assess flatulence, bloating and abdominal pain). For all the questionnaires a higher score reflects increased symptoms. Exploratory outcomes were small bowel motility where a lower score is indicative of less motility, $T_1$ of the ascending colon ($T_1$AC) where a shorter time is indicative of drier contents, faecal elastase and faecal calprotectin.

#### *4.3.4.3. Correction for body surface area*

In view of the study populations' age range, it was important to account for differences in height and weight. Therefore, to accommodate, the colonic volumes and SBWC were corrected for body surface area using Mosteller's formula[279]:

$$Corrected\ colonic\ volume\ or\ SBWC = \frac{Colonic\ volume\ or\ SBWC\ (AUC)}{\sqrt{\frac{height\ (cm) \times weight\ (kg)}{3600}}}$$

### 4.3.5. Sample size and statistical analysis

Prior to this study, there were no data using MRI outcomes for gut function in CF and the data generated were pilot data. A sample size of 12 for this pilot study was chosen and deemed acceptable as there are

few gains in precision about the mean and variance when the sample size is increased above 12[280].

The data were planned to be presented as mean (standard deviation, SD) if parametric and as median (lower quartile (Q25), upper quartile (Q75)) if non-parametric. Since there are multiple time points for colonic volume and SBWC, the AUC was used as the endpoint to reduce multiple comparison testing.

For the primary MRI endpoint OCTT, a survival analysis was used to evaluate the number of participants whose OCTT was complete within the 6 hour postprandial scanning period. A log rank test was used to test for statistical significance. Those participants who had a longer OCTT than the time of the last scan were censored on the survival analysis.

For the secondary and exploratory endpoints, the Wilcoxon matched-pairs signed-rank test was performed in view of the small sample size and paired study design. A descriptive analysis was used for the 3-point Likert-scale symptom questionnaire.

A p-value of less than 0.05 was considered statistically significant. This study was an exploratory study to evaluate the use of MRI for GI physiology in CF and therefore the risk of false discovery due to type 1 error was accepted. All statistical analysis was completed using RStudio, PBC (Boston, MA, USA)[281].

## 4.4. Results

### 4.4.1. Recruitment

Study recruitment took place between September 2018 and February 2019. I recruited the planned sample size of 12 participants with CF and 12 age and gender-matched controls (see Figure 4.4 for the CONSORT [consolidated standards of reporting trials] diagram). The recruitment rate was 4.2 participants per month and the retention rate was 86%. The first participant enrolled to the study was a control participant to ensure the protocol was acceptable to an individual with no respiratory problems. This control participant was excluded from the final analysis because I was unable to match a CF participant. Their data were not analysed but the feedback was taken into consideration. Reasons for excluding participants were if they were not able to eat the test meals, could not complete the MRI scans, or were unable to attend the study day. Participant demographics are summarised in Table 4.4. It was not possible to match the height and weight of participants in the two groups due the nutritional deficiencies experienced by people with CF leading to lower weight for age measurements[282].

All participants with CF took PERT with the set meals. Half of the participants with CF (6/12) took a proton pump inhibitor (omeprazole or lansoprazole) as part of their routine CF treatment.

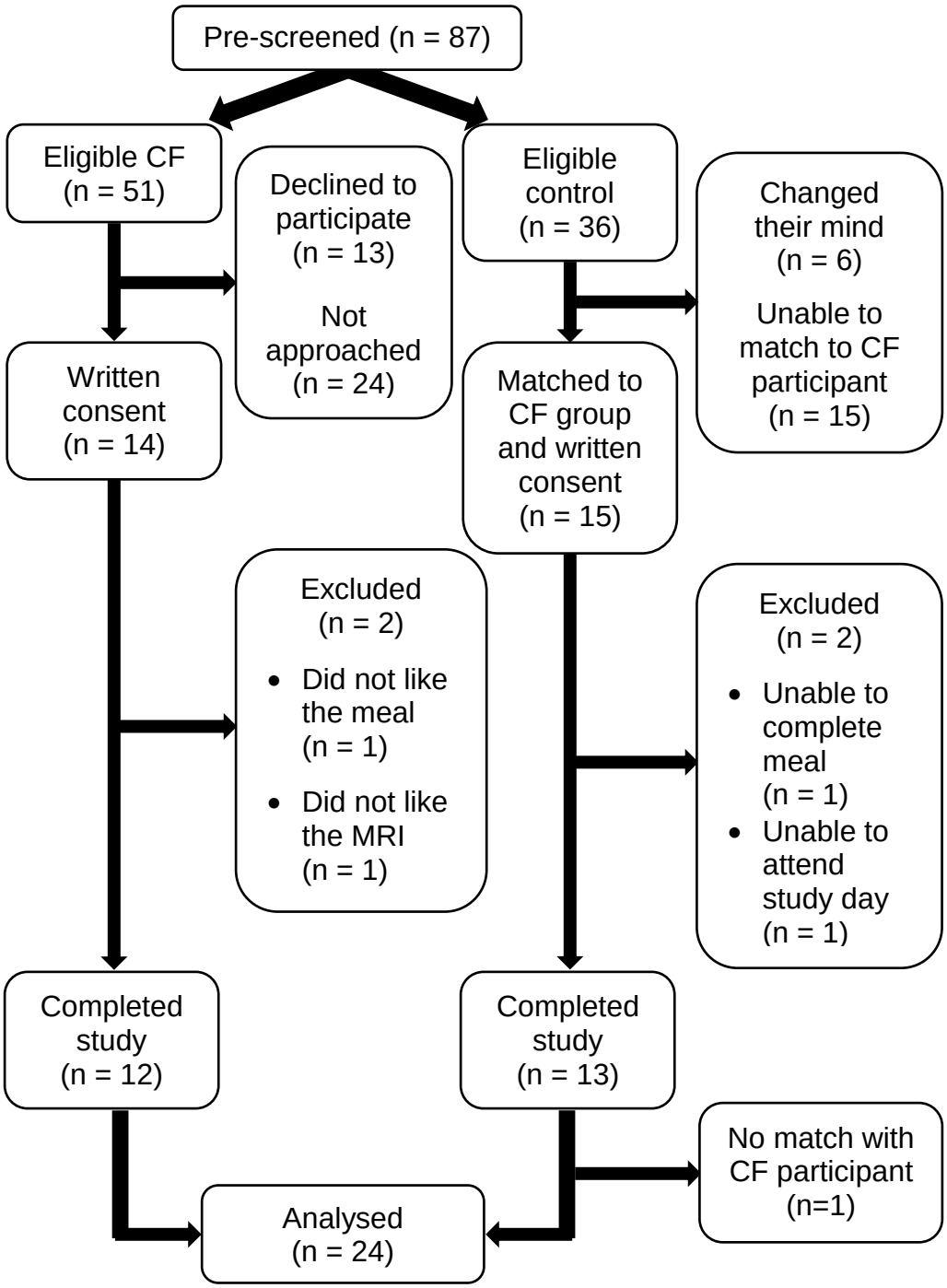



*Figure 4.4. CONSORT diagram to show flow of study participants*

*Table 4.4. Participant demographics. Q25 = lower quartile. Q75 = upper quartile.*

| *Median (Q25-Q75)* | **Control (n = 12)** | **CF (n = 12)** | **p-value** |
|---|---|---|---|
| Age (years) | 19 (15, 25) | 19 (15, 24) | 1 |
| Gender (Male : Female) | 7 : 5 | 7 : 5 | 1 |
| Height (cm) | 166 (160, 176) | 166 (158, 175) | 1 |
| Weight (kg) | 62 (59, 75) | 56 (53, 59) | 0.005 |

### 4.4.2. Participant feedback

Feedback collected from participants at the end of the study visit were not analysed statistically. The first control participant, who was not matched to a CF participant, provided feedback regarding fluid intake during the study day. They reported that 100 mL orange juice would not be sufficient hydration until the second meal. For subsequent participants (all 24 who were included in the final analysis), the first meal also included 240 mL water.

Other feedback from participants included questions regarding the scan itself, the test meals and waiting area. The feedback questionnaire revealed that 83% (20 out of 24 participants) of all participants scored more than 8 out of 10 for both the comfort of scans and changeover between scans, where a score of 10 was very comfortable or amazing respectively. Some participants with CF (67%, 8 out of 12 participants) struggled with breathing sometimes but 58% (14 out of 24 participants) of all participants did not struggle at all. Most participants (96%, 23 out

of 24 participants) were not worried about their breathing problems and 83% (20 out of 24 participants) did not struggle with the breath holds. In spite of the anxieties reported, MR images were not compromised and the participants completed the study visits without interruption. All the images were adequate for the planned analysis.

In terms of the test meals given, half of participants enjoyed the meals and 83% (20 out of 24 participants) found the portion sizes just right. Following the increase in volume of water after the first control participant, 42% (10 out of 24 participants) of participants found the volume too little but none found the volume too much.

No participants requested any extra items to be provided in the volunteer lounge in the future.

The majority of participants provided positive free-text feedback. Examples include:

- “the food was lovely – better than imagined” from an adult participant with CF aged 36 years;
- “food was tasty” from a young person with CF aged 13 years
- “ran smooth, non-stressful, if I had a problem I know I could stop at any time” from an adult participant with CF aged 19 years;
- “the process and organisation was very good” from an adult control participant aged 19 years;
- “good explanations of procedure” from an adult control participant aged 24 years; and

- “fun, never had a MRI scan before” from a young person control participant aged 15 years.

Common themes in regards to participants not enjoying the day included providing a stool sample, the waiting time in between MRI scans and length of the study visit.

### 4.4.3. Primary MRI outcome: OCTT

The median OCTT was longer in CF than controls (CF 330 minutes [270, >360] vs. controls 210 minutes [173, 315], p=0.04, Figure 4.5). In four people with CF, the meal had not reached the caecum by 360 minutes after the first meal. This suggested the OCTT was greater than 360 minutes, which was consistent with a high MR signal intensity of the meal still seen within the small bowel. All controls had an OCTT of 360 minutes or less.

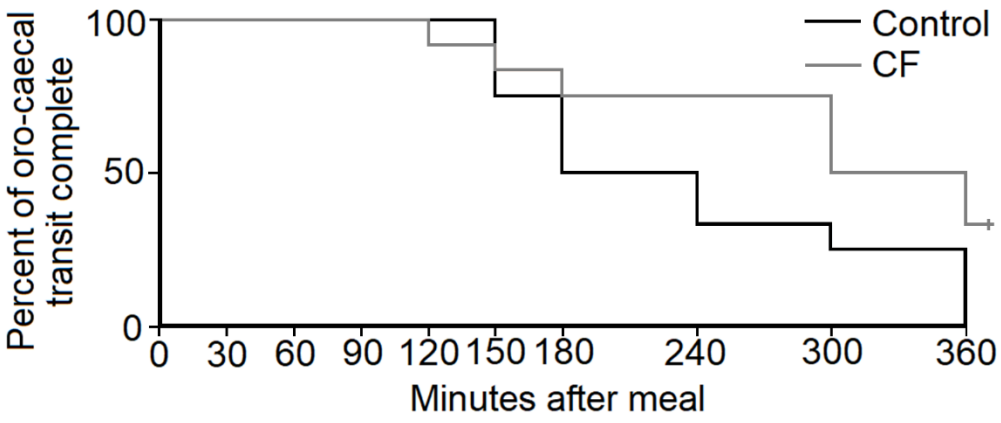



*Figure 4.5. Graph to show the time taken for a meal to reach the caecum*

### 4.4.4. Secondary outcomes

#### *4.4.4.1. Gastric half-emptying times*

There was no detectable difference in the median gastric half-emptying times between the two groups (CF 97 minutes [71, 128] vs. controls 80 minutes [66, 88], p=0.3).

#### *4.4.4.2. Corrected SBWC*

The median AUC for corrected SBWC was higher in CF than controls (CF 63 L·min/m2 [36, 80] vs. controls 34 L·min/m2 [28, 41], p=0.021, Figure 4.6), with a minimal reduction in small bowel water after the second meal which suggests impaired ileal emptying (normal physiology described in Chapter 1, Section 1.5). This change in corrected SBWC before and after the second set meal (at the time points 240 and 300 minutes) will be referred to as the delta TI from this point forwards. The median corrected delta TI was lower in the CF group than controls (CF 13 mL/m2 [-13, 57] vs. controls 102 mL/m2 [67, 108], Figure 4.7).

A second observer (ND) reviewed about 10% (24/264) of scans at random, and the limit of inter-observer variability was less than 20% for the majority of scans reviewed. There was a mean inter-observer difference of SBWC was 19 mL (Figure 4.8).

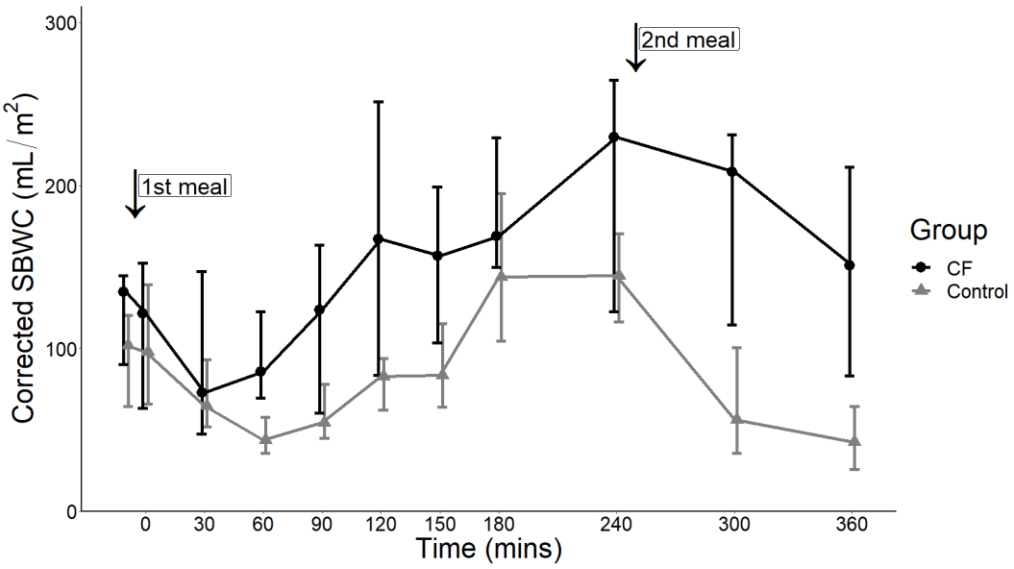



*Figure 4.6. Graph to show the corrected SBWC (median, inter-quartile range [IQR]) over the scanning day.*

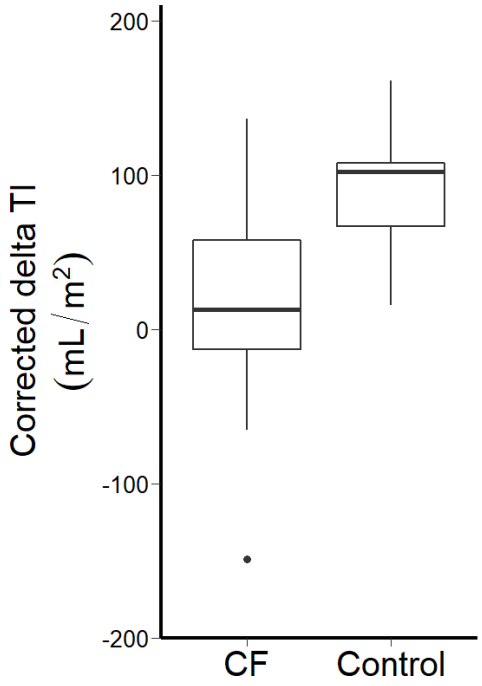



*Figure 4.7. Boxplot of corrected delta TI for the CF and control group. Box to show the median and IQR of corrected delta TI. Whiskers show 1.5 times the IQR.*

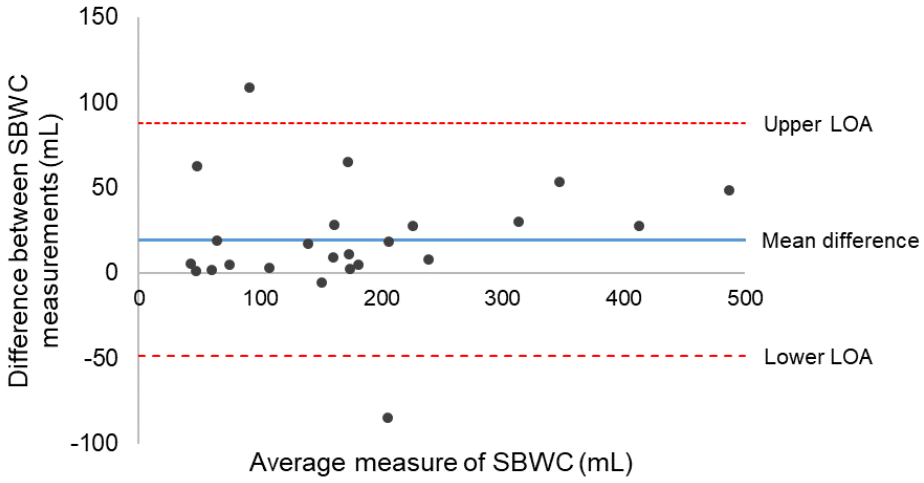



*Figure 4.8. Bland-Altman plot to show the inter-observer variability in 24 out of 264 SBWC measurements. Blue line = mean difference (19 mL). Red dashed line = 95% confidence interval (-49 mL, 88 mL). LOA = limit of agreement.*

#### *4.4.4.3. Corrected colonic volumes*

The median AUC for corrected colonic volumes were significantly higher in CF compared to controls (CF 186 L·min/$m^2$ [166, 206] vs. controls 123 L·min/$m^2$ [89, 146], p=0.012, Figure 4.9).

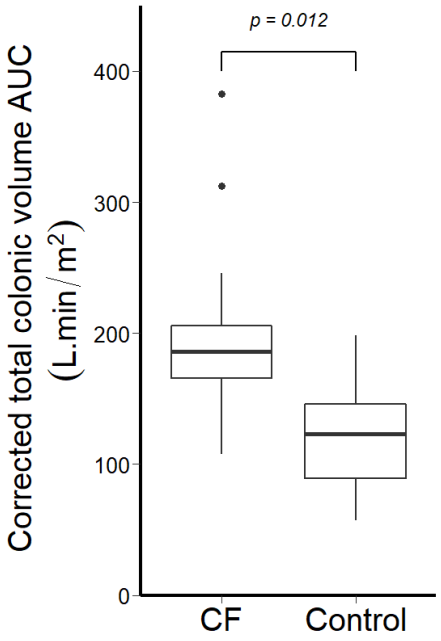



*Figure 4.9. Boxplot of corrected total colonic volume AUC for the CF and control group. Box to show the median and IQR of corrected total colon volume (AUC). Whiskers show 1.5 times the IQR.*

Drawing around a region of interest for colonic volumes from MR images can be affected by subjective interpretation of the images. Therefore, a second observer (ND) reviewed about 10% (24/264) of scans at random, and showed minimal inter-observer variability. The limit of inter-observer variability was less than 5% and mean difference 0.9 mL for the ascending, transverse and descending colons (Figure 4.10).

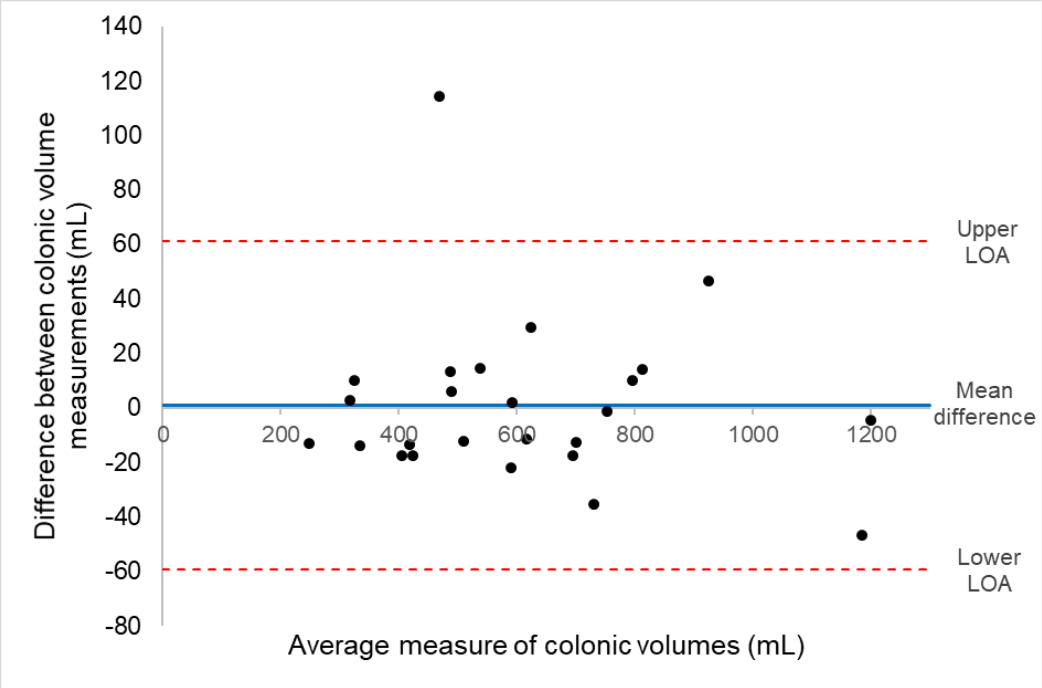



*Figure 4.10. Bland-Altman plot to show the inter-observer variability in ascending, transverse and descending colonic volumes for 24 out of 264 measurements. Blue line = mean difference (0.9 mL). Red dashed line = 95% confidence interval (-59 mL, 61 mL). LOA = limit of agreement.*

There was up to 10% inter-observer variability (with the exception of two cases where there was 16% and 29% variability due to difficulties in differentiating between small and large bowel) and mean difference of 6 mL when taking into account the recto-sigmoid colon (Figure 4.11). This

was due to some difficulty in selecting the recto- sigmoid for the following reasons:

1) A collapsed recto-sigmoid colon;

2) Difficulty in identifying the colon when there is little water or gas;

3) Heterogeneous contents (faecalisation) within the small bowel, which causes difficulty in differentiating the small bowel from colon, especially in the pelvis;

4) Inexperience of researchers reviewing recto-sigmoid colons in CF.

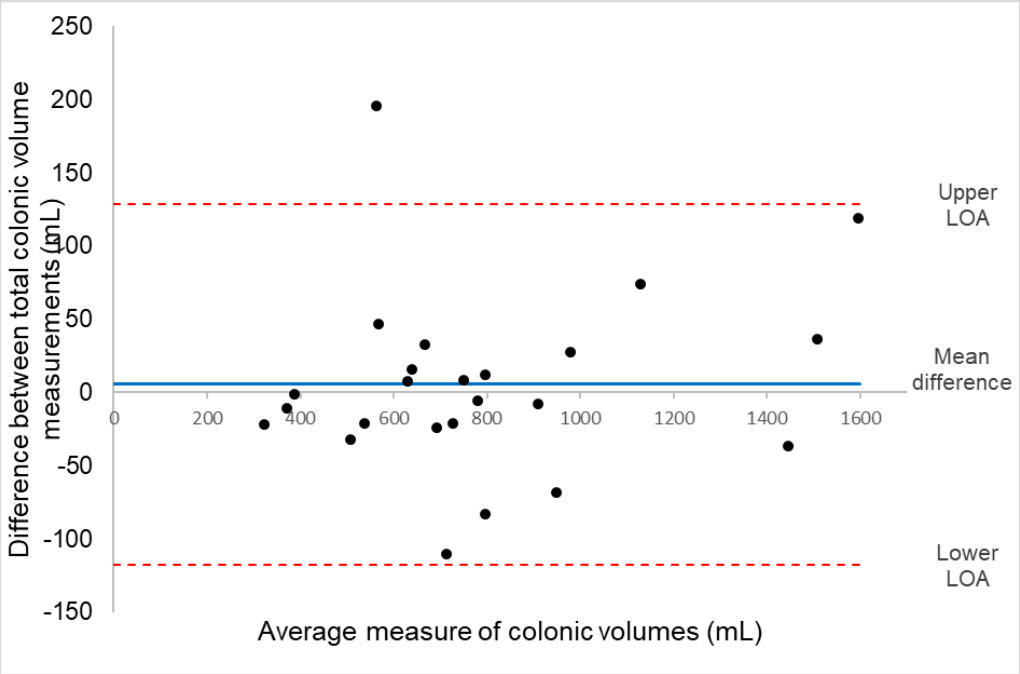



*Figure 4.11. Bland-Altman plot to show the inter-observer variability in total colonic volumes (ascending, transverse, descending and recto-sigmoid colon) in 24 out of 264 measurements. Blue line = mean difference (6 mL). Red dashed line = 95% confidence interval (-118 mL, 129 mL). LOA = limit of agreement.*

#### *4.4.4.4. GI symptoms*

***PAC-SYM***

There was no statistical difference in median PAC-SYM score between the two groups (CF 0.38 [0.16, 0.54] vs. control 0.33 [0.07, 0.66], p = 1).

PAC-SYM score was not associated with OCTT (p = 0.77), corrected colonic volumes AUC (p = 0.10) or corrected SBWC AUC (p = 0.97).

***CFAbd-Score***

The median CFAbd-Score in the CF group was higher than the control group, however this was not statistically significant (CF 16 [5, 25] vs. control 7 [3, 14], p = 0.13). Each of the domains within the CFAbd-Score were also higher in the CF group compared to the control group (Figure 4.12), but not statistically significant. There were no relationships between CFAbd-Score and OCTT (p = 0.12), corrected colonic volumes AUC (p = 0.72) or corrected SBWC AUC (p = 0.72).

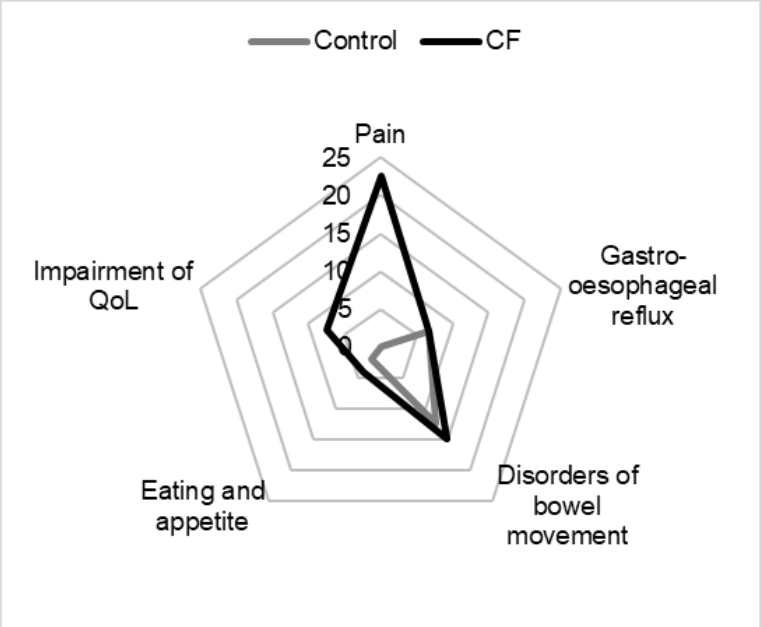



*Figure 4.12. Chart to show the median domain scores of the CFAbd-Score between the control and CF group.*

### *3-point Likert-scale*

The 3-point Likert-scale assessed three GI symptoms: flatulence, bloating and abdominal pain. Both the individual symptom score and total symptom scores at each time point were low for the control and CF group, meaning that no individual reported more than mild symptoms during the study days (Figure 4.13). There were no patterns seen between the symptoms and MRI metrics during the scanning period.

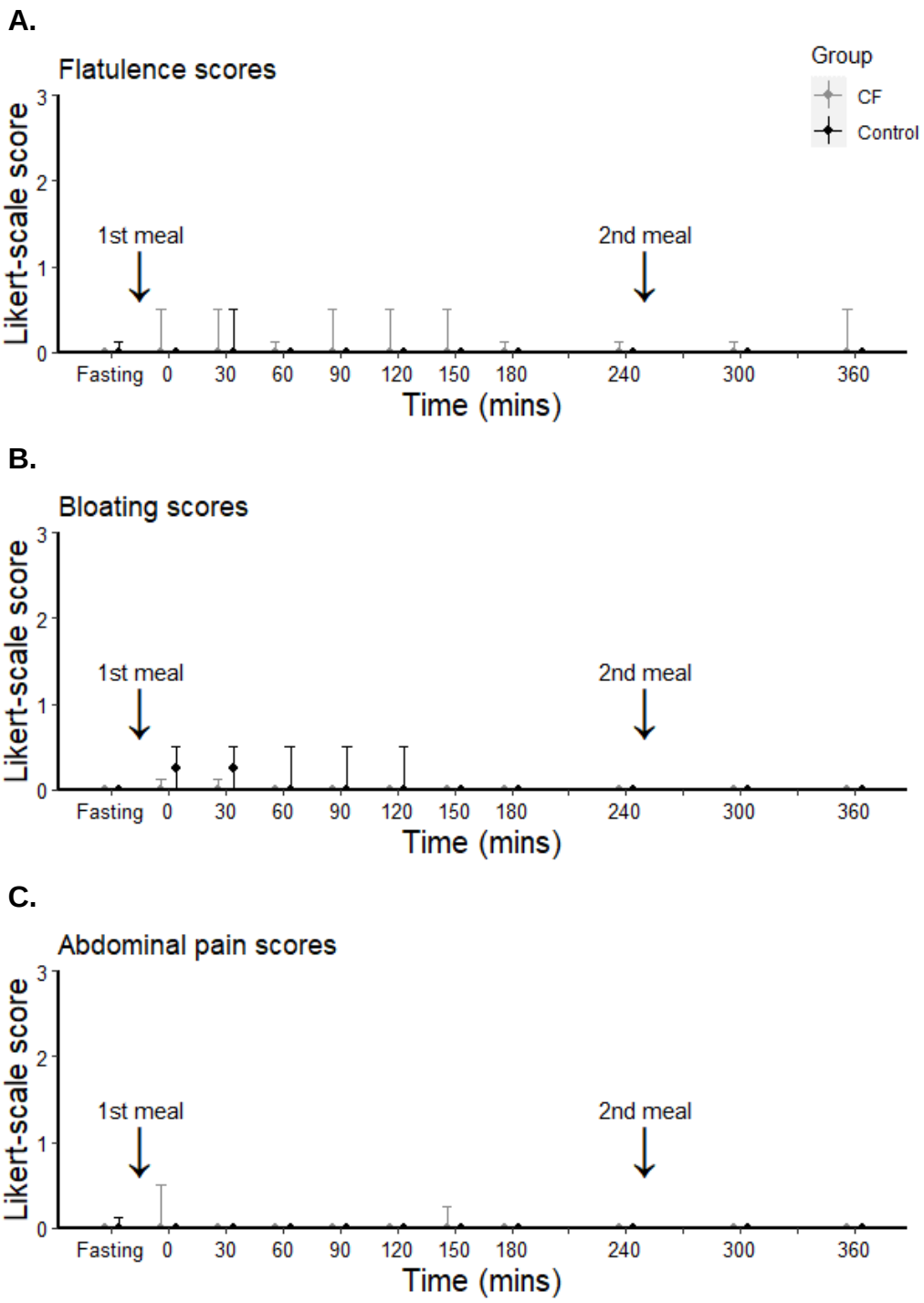



*Figure 4.13. Graphs to show reported GI symptoms for A. flatulence, B. bloating and C. abdominal pain during the study day in response to a test meal. Data points are median and IQR. The Likert-scale scores refer to the following descriptions: 0 = not at all, 1 = mild, 2 = moderate, 3 = severe.*

### 4.4.5. Exploratory outcomes

#### *4.4.5.1. Small bowel motility*

A higher motility score represents more motility. In the fasting state, the small bowel motility was significantly lower in the CF group than in controls (Table 4.5). After consuming a meal, at scans T0 and T300, there was no significant difference between the two groups (Table 4.5).

*Table 4.5. Post-prandial small bowel motility in arbitrary units (au).*

| *au, Median (Q25, Q75)* | **Control** | **CF** | **p-value** |
|---|---|---|---|
| **Fasting** | 86 (52, 106) | 40 (31, 46) | 0.034 |
| **T0** | 115 (74, 208) | 82 (69, 102) | 0.084 |
| **T300** | 98 (86, 137) | 86 (65, 132) | 0.433 |

#### *4.4.5.2. $T_1$AC*

The $T_1$AC was significantly lower in the CF group compared to controls in the fasting state (Table 4.6) reflecting less water content in the ascending colon in the CF group. Before and after consuming the second meal, there was no difference in the $T_1$AC between the two groups (Table 4.6).

*Table 4.6. Pre and post-prandial $T_1$AC in seconds*

| *secs, Median (Q25, Q75)* | **Control** | **CF** | **p-value** |
|---|---|---|---|
| **Fasting** | 0.79 (0.55, 1.31) | 0.59 (0.38, 0.77) | 0.010 |
| **T240** | 0.67 (0.54, 0.96) | 0.54 (0.37, 0.93) | 0.182 |
| **T300** | 0.70 (0.62, 0.85) | 0.61 (0.39, 1.02) | 0.594 |

#### *4.4.5.3. Faecal elastase*

The median faecal elastase concentration was significantly lower in the CF group compared to controls (CF 4 µg/g [1, 5] vs. control 758 µg/g [619, 767], p = 0.008). This confirms the CF group were all pancreatic insufficient and the control group were all pancreatic sufficient.

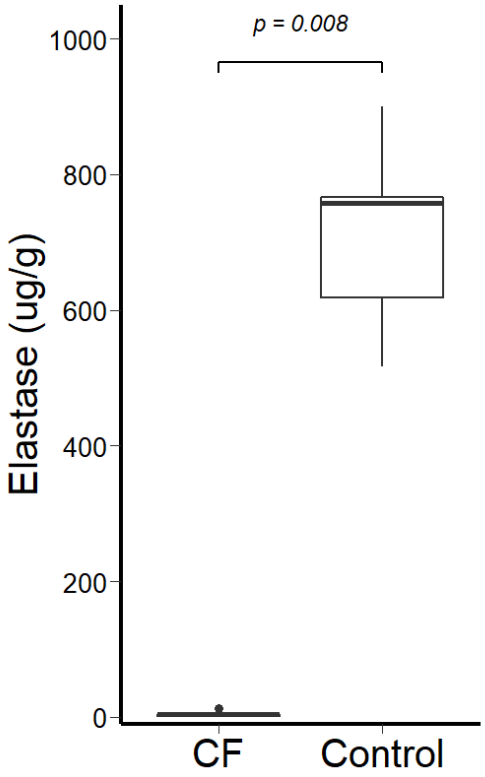



*Figure 4.14. Boxplot to show the median and IQR of faecal elastase between control and CF groups. Whiskers are 1.5 times the IQR.*

#### *4.4.5.4. Faecal calprotectin*

The median faecal calprotectin concentration were significantly higher in the CF group compared to controls (CF 18 µg/g [10, 28] vs. control 4 µg/g [3, 5], p = 0.008).

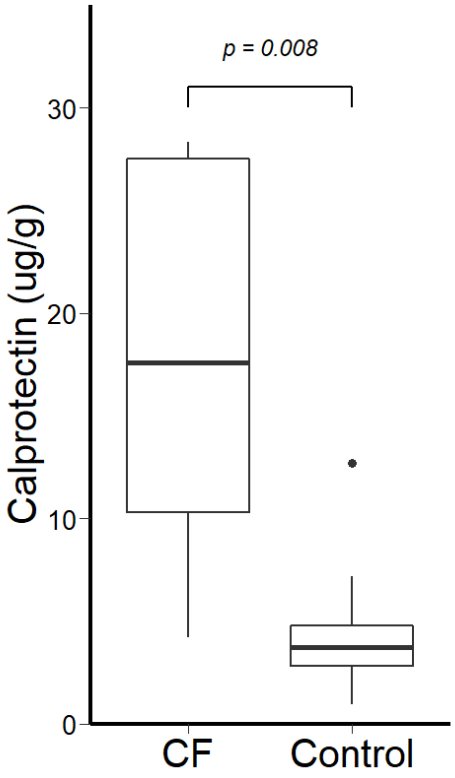



*Figure 4.15. Boxplot to show median and IQR of faecal calprotectin between control and CF groups. Whiskers are 1.5 times the IQR for control and the largest value for the CF group.*

## 4.5. Discussion

The primary aim of the study was to test the feasibility of using MRI methodologies to assess gut function and transit in CF. Over half of eligible people with CF were given the study information and half of these people provided written consent. A review evaluating trials funded by the UK National Institute for Health Research (NIHR) under

the health technology assessment (HTA) programme showed that recruitment rates ranged from 0.04 to 57.75 participants per centre per month, with a median recruitment rate of 0.92 participants per centre per month and the 80$^{th}$ percentile was 4.4 participants per centre per month. The review also reported the median retention rates of the trials to be at 89% (IQR 79-97%)[283]. The study described in this chapter showed that I managed to recruit 100% of the original target sample size with a recruitment rate of 4.2 participants per month and a retention rate of 86%, similar to the rates reported by Walters *et al*[283]. The reasons eligible people did not wish to participate were expected. The length of the study protocol meant that unless study visits occurred at the weekend or during school holidays, eligible people would miss school or work. For people with CF, this could be seen as unfavourable since they already miss school or work due to hospital visits or illness and a research study adds to the burden when they have a choice. Although the test meals catered for people who were vegetarian, it did not cater for people who were vegan or dairy free and therefore, this group of eligible people declined to participate.

Another assessment of feasibility was to see whether people with CF could tolerate the study protocol[284]. In the CF group, one paediatric participant chose to withdraw after consenting because they decided they would not like the MRI scanner; the participant did not disclose specific details. In each of the CF and control groups, one participant tried and disliked the first test meal after consenting to the study. However, the majority of people recruited to the study found the study

protocol acceptable, including the ease of access to the imaging centre since this study was undertaken at a single centre with MRI scanners dedicated for research purposes, and the data was adequate for analysis. Taking into account the recruitment rate, retention rate, acceptability by study participants and successful MRI analysis, the study was feasible[284]. Furthermore, this study was completed to time (took less than the 12 months predicted), cost (did not go over budget given) and target (pre-specified sample size achieved). In order to assess whether this study is feasible in other centres, which do not necessarily have dedicated research scanners, the protocol and time taken to analyse parameters needs to be reduced.

OCTT, SBWC and colonic volumes have all shown statistically significant and potentially clinically meaningful differences between the control and CF groups. Furthermore, the exploratory outcomes demonstrate a difference in small bowel motility, $T_1$AC, and faecal calprotectin between the two groups. These data allow the mechanisms of gut dysfunction in CF to be explored further.

OCTT was delayed by 2 hours in the CF group compared to the control group, which is consistent with previous data using a wireless motility capsule (SmartPill®)[64] and capsule endoscopy (Pillcam™)[285].

The MRI and SmartPill® methods for measuring gastric emptying time differ. The MRI method measures gastric volumes at sequential time points and calculates the gastric half-emptying time by fitting the data to a curve to determine when a reduction of 50% of gastric volume has

been reached. In comparison, the SmartPill® collects a continuous measurement of intestinal pH and the point at which the pH rises is the gastric emptying time. Each method calculates the gastric emptying time through different approaches but both show there is no difference in the gastric emptying time between CF and control groups.

The absolute values for gastric emptying time and small bowel transit from Gelfond's data[64] (4 hours for gastric emptying and 6 hours for small bowel transit in CF) are different compared to the study presented in this chapter (97 minutes for gastric half-emptying time and 330 minutes for OCTT). However, the use of the wireless capsules and small sample sizes could also account for this. In the stomach, a process called gastric sieving occurs. Smaller particles are propelled more quickly (by the migrating motor complex) than larger particles[37]. Therefore, since the cylindrical shaped wireless capsule is approximately 27 mm long and 12 mm in diameter[64,286], its propulsion from the stomach into the duodenum will occur in the later stages of gastric emptying. Malagelada *et al*[285] also report delays of nearly 2 hours in small bowel transit in CF compared to healthy controls. Over fifty percent in their CF group sample had an OCTT of more than 8 hours[285]. The authors interpret the turbid nature of small bowel contents, as well as bowel hypomotility[285] identified on Pillcam™ data, as potential causes of the delay. In contrast to the SmartPill® and Pillcam™ where the capsules are subject to gastric sieving, the MRI method uses the head of the rice pudding meal as a marker for the OCTT[202]. The OCTT is determined by a change in signal intensity at the

caecum at a given time point. Both the MRI and SmartPill® demonstrated a 2-hour difference in small bowel transit, with the delay being in the CF group. Partial digestion of the rice pudding meal mean the particles are smaller than the capsules and therefore could explain the absolute differences in transit times between the different methodologies. The OCTT data from the control group are also similar to data from previous healthy volunteer MRI studies[202]. In the GIFT-CF 1 study, the median (Q25, Q75) ascending, transverse and descending colon volumes were 209 (134, 239) mL, 221 (164, 280) mL and 166 (91, 201) mL, respectively. In Chaddock's study, the mean (SD) ascending, transverse and descending colon volumes were 203 (75) mL, 199 (79) mL and 159 (85) mL, respectively.

The AUC for corrected SBWC was higher in CF as well as each of the median SBWC at all scanning time points in CF. This was contrary to my initial hypothesis, where I hypothesised that GI secretions would be thick and viscous, similar to the respiratory tract due to abnormally functioning CFTR proteins and therefore reducing the hydration of epithelial cell surfaces.

Previous studies[287,288] have demonstrated in people without GI disease that the SBWC responds to meals in two phases: gastric and intestinal. During the gastric phase, following a morning meal, there is a drop in SBWC due to glucose absorption. It is likely that although the CF group have exocrine pancreatic insufficiency, the use of PERT and potential residual pancreatic function is sufficient to aid the digestion of food

molecules and therefore allow glucose absorption in the small intestine, as also shown in the control group.

After 1 hour, SBWC is seen to recover until a second meal is ingested. Following the second meal, the SBWC falls in the intestinal phase due to the small bowel contents emptying from the terminal ileum into the caecum.

Following the second test meal in this GIFT-CF 1 study, it was noticeable that the SBWC did not fall immediately in CF compared to the controls. The postprandial drop in SBWC (delta TI), previously seen in healthy volunteers[115], is due to the gastro-ileal reflex where ileal contents efflux into the ascending colon in response to a high calorie meal entering the stomach (as described in Chapter 1). There are several mechanisms which might explain why the overall corrected SBWC was higher in CF. One possible mechanism could be due to the disruption of fluid absorption and secretion in the small bowel, where a reduction in water absorption in the duodenum could indirectly lead to overall increase in SBWC[93]. Russo et al[93] proposed there to be disruption in paracellular chloride absorption within the jejunum in people with CF, which leads to a reduction in water absorption in the jejunum. These increased SBWC measured by MRI could be reflective of the abnormal chloride and water transport and could support the hypothesis that disruptions in jejunal paracellular mechanisms exist in people with CF in addition to dysfunctional CFTR channels. In addition to alternative pathways, turbid luminal GI fluid has been reported by Malagelada et al[285] using capsule endoscopy, similar to in the thick

viscous mucus in the respiratory tract. This inspissated intestinal mucus can form a layer on the GI epithelial surface[98] and become trapped within the intestinal crypts[97], subsequently preventing water absorption within the small intestine which leads to an overall increase in SBWC. An alternative mechanism could be due to inadequate intake of pancreatic enzymes so there is incomplete fat digestion. Subsequently, this could lead to an exaggerated ileal brake, where ileal fat delays jejunal transit[43]. However, the differences in the delta TI between CF and controls suggest there could be restricted flow at the terminal ileum preventing the efflux of small bowel water in response to the gastro-ileal reflex[289]. It is plausible that the restricted flow is due to an inflammation at the ileo-caecal junction, and reflected by the difference in faecal calprotectin between the CF and control group. Faecal calprotectin concentrations were significantly higher in CF than in controls, which suggest there may be an underlying subclinical gut inflammation[99,290]. Previous studies using wireless capsule endoscopy[99] and ultrasound imaging[291] have observed signs of intestinal inflammation within the CF small bowel, in particular in the ileum, similar to inflammatory bowel disease. However, it is worth noting that the faecal calprotectin concentrations were less than 50 µg/g, the upper limit of normal calprotectin concentrations. In addition, there are some studies that correlate high faecal calprotectin concentrations with poor lung function[292] and *Pseudomonas Aeruginosa* colonisation[293]. There are some suggestions that higher faecal calprotectin concentrations are due to the inflammatory process caused by recurrent chest infections,

which could increase systemic inflammatory markers and be released into the intestine; or that swallowed sputum with high concentrations of inflammatory cells and bacteria could pass into the intestine[292]. Therefore, it is possible that the faecal calprotectin concentrations within normal range in the CF group reflects the absence of an acute respiratory exacerbation and the faecal calprotectin results should be approached with caution. Although further research is needed to determine the exact cause of the proposed subclinical ileal outflow obstruction, the delta TI measure could be a surrogate marker of CF gut disease in the future.

The exploratory outcomes (small bowel motility and $T_1$AC) further contribute to our understanding of the delayed OCTT and increased SBWC results. Firstly, the differences in small bowel motility support the delays seen in small bowel transit. The factors affecting transit time can be structural, affecting the peristaltic action (e.g. bowel wall thickening or inflammation), or luminal, affecting the fluidity of intestinal contents, as reported by Malagelada *et al*[285]. The present study data shows that in the CF group at fasting, with little intestinal content flowing through, there was reduced small bowel motility compared to matched controls. Reduced small bowel motility is suggestive of the presence of inflammation in other patient groups[267]. In addition to Malagelada's report on intestinal inflammation seen by video endoscopy, Dhaliwal *et al*[294] demonstrated that faecal calprotectin was significantly raised in CF, although not as high as in inflammatory bowel disease.

The small bowel motility, however, recovered to near normal levels after a meal and had similar postprandial response to the control group, and a previous healthy volunteer study[295]. This provides further support for the proposition that there may be obstruction or abnormalities within the small bowel wall that reduces small bowel motility. The stimulus of a meal may be sufficient to allow recovery of small bowel motility. It is unclear the exact mechanism that contributes to reduced small bowel motility in CF, nevertheless, it is possible that small bowel motility could be used as a marker of GI disease in CF.

The $T_1$AC measurements support the hypothesis that there is an element of obstruction at the terminal ileum. Despite the large amount of SBWC in the CF group, the ascending colonic contents remain drier than the control group at fasting. Drier ascending colonic contents are reported in chronic constipation[115], which some people with CF also report[112,113]. The increased AUC in corrected colon volume in the CF group further supports the hypothesis that constipation exists in a large proportion of people with CF since, the volumes are similar to previous reports of those with constipation[296]. People with functional constipation have increased colonic volumes[115,254,297]. The raw colonic volume (not corrected for body surface area) data from the present study are similar to studies of healthy individuals[254].

Every effort was made to reduce confounders between the CF and control group, in particular matching the age and gender of participants in each group. Previous studies have demonstrated differences in colonic volumes with height[254] and total intestinal length with height and

weight[51], since the GI tract continues growing until adulthood[49]. In the present study, there was a difference between the two groups in the median weight. This was expected since people with CF experience nutritional deficiencies due to factors affecting digestion and absorption[282]. The continuous variables (colonic volume, SBWC) were corrected for body surface area in order to mitigate the differences expected in weight measurements given the wide age range of participants. Correcting for body surface area is a recognised approach in paediatrics[279,298,299]. People who had previous extensive gut surgery or inflammatory bowel disease were excluded to reduce the risk of confounding, in addition to age and gender.

This study did not find any difference in symptoms between the groups, despite other studies reporting 65%[3] to 100%[100] of people with CF having GI symptoms. The lack of any difference in symptoms between the CF and control groups may also reflect the small sample size in this study. Previous studies[3,100] evaluating symptoms require a large sample size of over 100 participants to show a difference. This study demonstrates that the use of an objective outcome measure has less variability and therefore requires a smaller sample size to show a difference in gut pathology between people with CF and control participants. This study has highlighted the possibility that people with CF may not report GI symptoms but could have an underlying subclinical GI pathology, detectable by objective measures such as MRI. These findings are similar to a study by Wilschanski *et al*[290] who observed intestinal changes by ultrasonography in people with CF

without GI symptoms. For people with CF who report more frequent or more severe GI symptoms, for example those who participated in the CFAbd-Score studies, it could be speculated that that larger differences in objective GI outcome measures are seen compared to those with lesser symptoms. Similarly, people with CF who have recurrent DIOS may also have larger differences in GI outcome measures.

Using MRI has the advantage that it uses a non-invasive and non-radiation imaging technique that is able to evaluate both structure and function. In particular, this study demonstrates that it is possible to assess gut function and transit in the undisturbed CF bowel. Despite concomitant chest disease, people with CF are able to tolerate breath-hold sequences during scanning.

### 4.5.1. Strengths and weaknesses

A major strength of this study is the low burden of using MRI as an investigative tool because it is completely non-invasive and radiation-free. Furthermore, at the University of Nottingham, this MRI study was possible because there are multiple MRI scanners dedicated solely for research purposes. With careful organisation and foresight, it was possible to book the scanner for a whole day to allow protocol completion. MRI is appealing to people with CF who already have a high investigation and treatment burden, especially if their lung function is poor and they require repeated CT scans of their chest. Furthermore, the design of the study is such that the protocol did not require participants to be cannulated for hyoscine butylbromide or to drink large volumes of bowel preparation. The scanning protocol was also adapted

to reduce the time required for participants to hold their breath. This was a breath hold reduction from 20 seconds to 10 seconds and tolerated by all participants with CF.

To reduce the participant variation of the GI physiological response to a meal, the test meal was the same for all participants. This was a limitation to the recruitment process because some people chose not to participate in the study due to being lactose intolerant or vegan. I achieved the target sample size despite this challenge, as there was a large pool of eligible participants. In attempt to control the GI physiological response, a pre-specified exclusion criterion was people who were unable to stop medications directly prescribed to alter bowel habit on the study day. The reason for the exclusion was to ensure there were no confounders for the MRI outcome measures. However, this could be seen as a limitation to the study since it may bias the demographic of enrolled participants to those with less severe GI disease. In spite of this, none of the potential participants declined to participate due to the inability to stop medications that altered bowel habit.

One weakness of the study design was the study visit length and number of MRI scans required to obtain the study outcomes. Not only is this a time burden for the participants but MRI scanning over a period of 7 hours incurs a large cost. It was necessary to perform the series of scans to begin to understand the underlying physiology of the CF gut as measured by MRI. Future studies will be able to use these data and understanding of CF GI physiology to influence the study design. The

study has been a success in terms of study completion and participant acceptability to assess gut function in CF using MRI. However, in order for the protocol to be time and cost effective for future studies, the protocol requires further refinement. Adaptations to the protocol for use on different brands of scanners would also make this tool more acceptable and accessible to other research and clinical centres. Further work to identify and refine clinically meaningful MRI GI outcomes is necessary before MRI is used as a surrogate marker for GI outcomes both in research and in clinical practice.

## 4.6. Summary

This is the first study to demonstrate that gut pathologies can exist with few GI symptoms in CF using MRI. The methodology has provided a platform and a potential surrogate outcome measure (delta TI) for investigating GI complications in CF. It has highlighted that there may be predisposing factors in the small bowel, particularly at the terminal ileum, that could be the precursor to CF gut complications. Future research will be important in evaluating the MRI differences between people with CF who are report GI symptoms to those who have no GI symptoms, and in evaluating the GI MRI differences in new and existing CF therapies.

The next chapter will transfer the methods used in Chapter 4 to evaluate the GI effects of a CFTR modulator (tezacaftor-ivacaftor), a newly licensed CF drug.

# Chapter 5 A double-blind, placebo-controlled, randomised crossover trial of the effects of tezacaftor-ivacaftor on gastrointestinal function, using MRI parameters in people with CF

This chapter will describe the GI effects of the CFTR modulator tezacaftor-ivacaftor (TEZ/IVA) in CF using MRI: the GIFT-CF 2 study.

The US CF Foundation funded part of the clinical research fellow salary, Vertex Pharmaceuticals Incorporated funded the study visit costs (including study drug provision and part of the clinical research fellow salary) through an investigator initiated study grant and Nottingham University Hospitals Charity funded the stool analysis. Vertex Pharmaceuticals Incorporated did not design the study protocol.

## 5.1. Background

The introduction of a CFTR modulator ivacaftor (a potentiator of CFTR function) over a decade ago transformed clinical care for people with mutations affecting the gating function of the CFTR protein. Since then, new combination CFTR therapies have been developed which improve lung function and lead to fewer exacerbations of pulmonary symptoms. Trials of CFTR modulators have primarily used respiratory outcome measures because bronchiectasis and respiratory failure are the leading causes of morbidity and mortality in CF[4,15]. Phase 3 CFTR

modulator clinical trials frequently report GI effects as adverse events and at most, report GI symptoms as part of the CFQ-R questionnaire. The CFQ-R questionnaire assesses the overall health and quality of life of a person with CF; within the questionnaire there only three questions relating to GI symptoms[109]. Thus, the CFQ-R does not fully identify the extent of GI symptoms in comparison to GI specific questionnaires[3].

There are limited studies that evaluate the effects of a CFTR modulator on different aspects of GI physiology, such as pancreatic exocrine function[162,163], gut pH[154], inflammation and microbiome[155]. None of the studies used placebo as a comparator for CFTR modulators and the effect size of the GI effects of CFTR modulators remain unknown. Therefore, it was necessary to undertake a pilot study to inform future study design.

As discussed in Chapter 4, I have demonstrated the feasibility of using MRI to measure gut function and transit in CF[272]. This study applied those methods to evaluate the GI effects of TEZ/IVA in CF. It also evaluated the effects of TEZ/IVA on GI symptoms and stool markers for inflammation and pancreatic sufficiency.

## 5.2. Study aims and hypothesis

The first aim of the work presented in this chapter was to inform future CFTR modulator study designs and sample size calculations; and the second aim was to evaluate the GI effects of TEZ/IVA in CF assessed by MRI, symptom questionnaires and stool biomarkers.

The hypotheses tested the GI effects of 3 weeks treatment with TEZ/IVA compared to placebo. The hypotheses were that:

I. OCTT would be shorter after TEZ/IVA than placebo due to improved hydration of GI secretions with TEZ/IVA;
II. SBWC would be reduced with TEZ/IVA compared to placebo due to a reduction in viscous mucus lining of the intestinal lumen and reduced inflammation at the terminal ileum;
III. GI symptoms would be less severe after TEZ/IVA compared to placebo due to the improvements in CFTR function.

## 5.3. Methods

### 5.3.1. Study design

GIFT-CF 2 was a pilot double blind, placebo-controlled, two-period randomised crossover trial conducted at the University of Nottingham and Nottingham University Hospitals NHS Trust.

The trial was prospectively registered on clinicaltrials.gov (NCT04006873) and received ethical approval from the West Midlands – South Birmingham Research Ethics Committee (19/WM/0130). The study was carried out according to the principles of Good Clinical Practice in accordance with the Declaration of Helsinki.

### 5.3.2. Study population

Potential participants with CF (who received their clinical care from the paediatric or adult CF services at Nottingham University Hospitals NHS Trust) aged between 12 and 40 years, homozygous for p.Phe508del

and eligible for TEZ/IVA were pre-screened by their usual clinical team. A member of the clinical team first approached potential participants during a clinical interaction (by hospital attendance or outpatient appointment) before the research team provided further information. All potential participants had a minimum of 48 hours to read the participant information sheets before giving written consent. A member of the research team (Dr. H. Barr or myself) obtained written informed consent from all participants. Participants aged 16 years and under were given the option to provide written assent alongside written parental consent. Participants who completed the GIFT-CF 1 study and still met the inclusion and exclusion criteria were also invited to participate.

Table 5.1 provides a summary of the inclusion and exclusion criteria for this study. The one difference between this study and the study described in Chapter 4 was the exclusion of people who were already prescribed a CFTR modulator. This was because the GI effects of modulators are not well-described and could introduce confounding variables to the study.

*Table 5.1. Inclusion and exclusion criteria for GIFT-CF 2*

| Inclusion criteria | Exclusion criteria |
|---|---|
| – Age 12 – 40 years<br>– Capacity to consent, or understand the requirements of the study where parental consent is needed<br>– Confirmed diagnosis of CF (homozygous p.Phe508del) | – Currently taking a CFTR modulator drug or contra-indication to use of TEZ/IVA<br>– $FEV_1$ <40% predicted using the Global Lung Initiative criteria[274]<br>– Contra-indication to MRI scanning, such as embedded metal or pacemaker<br>– Pregnancy<br>– Unable to stop medications directly prescribed to alter bowel habit such as laxatives or anti-diarrhoeals on the study day<br>– Previous resection of any part of the GI tract apart from appendicectomy or cholecystectomy. Surgical relief of meconium ileus or DIOS were permitted unless clinical records showed excision of intestine >20 cm in length<br>– Intestinal stoma<br>– Diagnosis of inflammatory bowel disease or coeliac disease confirmed by biopsy<br>– GI malignancy<br>– Unable to comply with dietary restrictions required for the study |

### 5.3.3. Study procedures

All participants attended the Sir Peter Mansfield Imaging Centre (SPMIC), University of Nottingham, for a series of baseline MRI scans and GI symptom questionnaire completion. They also provided a stool sample. Participants who had participated in the GIFT-CF 1 study were not required to attend for the baseline study visit as their GIFT-CF 1 study data were used for baseline data.

After the baseline study visit, participants were randomised to a treatment sequence. The Nottingham University Hospital trials pharmacy used an online computer program (Randomization.com[300]) to generate a list by simple randomisation, with balanced permutations for 20 subjects, and ensure allocation concealment. The treatment sequences were AB or BA (where A was TEZ/IVA and B was placebo) with a 28-day washout period in between each treatment arm. Each treatment period was 28 days. Vertex Pharmaceuticals Incorporated provided visually identical TEZ/IVA and placebo medication and packaging. The participants and all study staff (except the trials pharmacy) were blinded to treatment allocation. Participants were invited to attend for a study visit at the SPMIC between day 19 and day 23 of each treatment arm to allow for flexibility in coordinating the logistics for the study visit (i.e. MRI scanner and scanner operator availability in addition to researchers and participant availability).

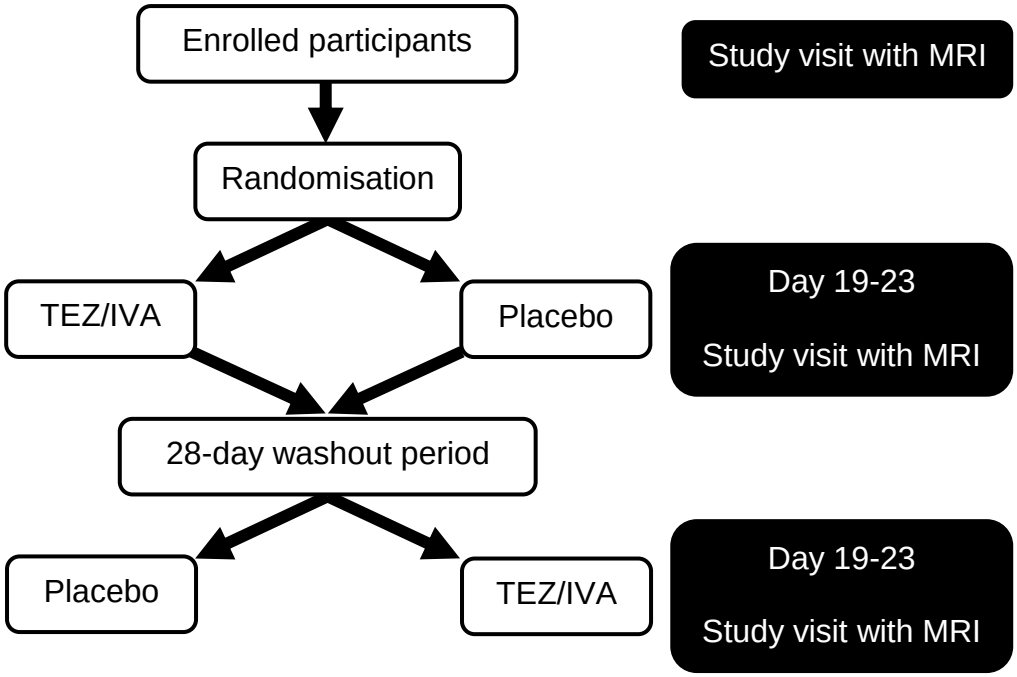



*Figure 5.1. Flowchart to show the two-period crossover trial design. Each treatment period (TEZ/IVA or placebo) was 28 days. The study visits are described in Figure 5.2.*

For each of the study visits at baseline and in each treatment period, the participants underwent similar procedures (Figure 5.2) as those described in Chapter 4: a series of 11 MRI scans and completion of GI symptom questionnaires (CFAbd-Score, PAC-SYM, 3-point Likert scale). During the study visits in each treatment period, blood tests for renal and liver function, together with blood pressure were taken for safety monitoring in line with pharmaceutical precautions[149]. Participants gave a stool sample within 24 hours of the study visit for faecal calprotectin and faecal elastase analysis.

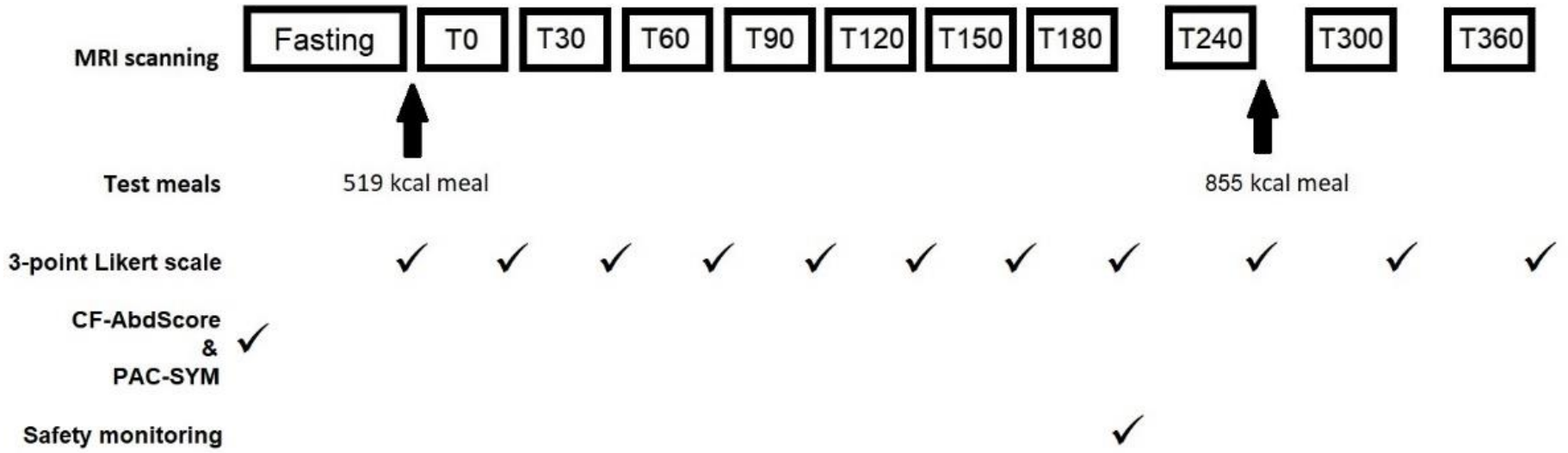



*Figure 5.2. GIFT-CF 2 MRI study day schedule. MRI scans performed at fasting then at half hourly intervals until T180 and at hourly intervals until T360. Set test meals were given after the fasting and T240 scans. T0 relates to the MRI scan taken at 0 minutes after the first meal is eaten. The CFAbd-Score and PAC-SYM questionnaires were completed prior to the fasting scan. The 3-point Likert scale questionnaires were completed after every scan. Safety monitoring was completed after the T180 scan where there was a large gap between scans.*

As before, participants fasted for at least 12 hours prior to the study visit except for drinking water until midnight the night before and a small amount of water with essential medications on the morning of the study visit. The first set test meal was the same as in GIFT-CF 1 but the second set test meal contained reduced calories due to a change in Sainsbury's supermarket recipe for macaroni cheese (see Table 5.2 for summary of nutritional contents). Participants took their usual prescribed dose of PERT with their meals.

*Table 5.2. Summary of nutritional contents of GIFT-CF 2 set meals.*

| | **Energy (kcal)** | **Carbohydrate (g)** | **Protein (g)** | **Fat (g)** |
|---|---|---|---|---|
| 300 g Sainsbury's creamed rice pudding | 282 | 52.5 | 8.1 | 4.2 |
| 30 g Sainsbury's double cream | 132 | <0.5 | <0.5 | 14.3 |
| 25g Sainsbury's seedless raspberry jam | 62.75 | 15.4 | <0.5 | <0.5 |
| 100 mL Sainsbury's pure orange juice from concentrate | 42 | 8.6 | 0.6 | <0.5 |
| **Set meal 1** | **519** | **77** | **9** | **19** |
| 375 g Sainsbury's macaroni cheese | 500 | 52.9 | 23.3 | 20.1 |
| 100 g Rhokett® strawberry cheesecake | 355 | 37.8 | 3.7 | 21.2 |
| **Set meal 2** | 855 | 90.7 | 27 | 41.3 |

#### *5.3.3.1. MRI protocol and analysis*

For each scanning time point, the MR sequences as described in Chapter 4, Section 4.3.3.1, were performed using the 3 T Philips Ingenia MRI scanner. The only difference from the MR sequences described previously was the cine MR motility scan. The cine MR sequence for the GIFT-CF 1 study included the overall small bowel motility measurements. In contrast, the study described in this chapter focused on terminal ileum motility measurements. This was to investigate further the observation, in the GIFT-CF 1 study, that there was less small bowel water efflux from the terminal ileum to the caecum following the second meal (an attenuated gastro-ileal reflex). The adaptation to focus on terminal ileum motility meant that the number of slices were reduced from six to two, resulting in a reduction by 1 minute per scanning time point.

An independent researcher re-labelled all the MRI data collected to ensure blinding of the participants' treatment period during analysis. This ensured that, when I analysed the MRI scans to measure MRI endpoints, I was not only blind to the identity of each participant, I was also blind to the treatment period when each scan was taken. This meant I could not inadvertently unblind through pattern recognition. MR data were analysed as described in Chapter 3, Section 3.8.4 and Chapter 4, Section 4.3.3.2.

### 5.3.4. Adaptations after study commencement due to unforeseen circumstances

This study met several challenges during its course, including issues with drug supply, the announcement of funding for the study drug in routine clinical practice in the UK and recruitment during the COVID-19 pandemic. Despite these difficulties, I found solutions to minimise disruptions to the study.

#### *5.3.4.1. Drug supply*

The initial agreement with the pharmaceutical company was to use lumacaftor-ivacaftor and placebo for the study. However, at the time of study commencement, the pharmaceutical company had completed their commercial trials using lumacaftor-ivacaftor, and therefore were not able to provide the visually identical placebo and study drug as agreed. Alternative agreements were arranged to use, instead of lumacaftor-ivacaftor, its successor drug TEZ/IVA. Imported drugs from the US to the UK needed to pass through the Medicines and Healthcare products Regulatory Agency (MHRA) and customs regulatory approval. In spite of receiving ethics approval in May 2019, the study did not receive Nottingham University Hospitals sponsor approval to commence recruitment until September 2019 due to delays from importing the study drugs.

In January 2020, a further issue regarding the short expiry date of the study drug and placebo supply was noted when a newly enrolled participant randomised to their first treatment arm was potentially not able to complete the second treatment period if they were enrolled from

February 2020. This affected five potential participants. The typical delivery time after request to the pharmaceutical company was six to eight weeks. I mitigated unnecessary dropouts, due to study drug supply, through using effective communication with the pharmaceutical company and importers to ensure a delivery of a new supply of study drug and placebo. I sent the initial correspondence to the pharmaceutical company on 22nd January 2020. A delivery of the study drugs and placebo with new expiry dates were received on 24th February 2020 at Nottingham University Hospitals NHS Trust.

#### *5.3.4.2. Study drug funding for routine clinical practice*

The study design described was possible because the study drug was licenced in the UK by the MHRA for people with CF, with specific mutations, but not funded by the National Health Service (NHS). Therefore, participants would not have received the drug as part of routine UK clinical practice. This meant that it was ethically justifiable to offer participation in a placebo-controlled trial of the drug, as they would not need to stop its routine use.

However, on the 24th October 2019, NHS England announced the agreement with the pharmaceutical company for funded access to medicines for CF including TEZ/IVA within 30 days. This announcement came without warning and required a substantial amendment to the ethics application in order to ensure potential participants were aware of the situation as continued participation in the trial could delay their receipt of routine treatment with TEZ/IVA.

This led to a drop in the number of people who expressed an interest in study participation. I approached other tertiary CF centres in the UK (Leeds, Birmingham, Leicester and Sheffield) with the intention to open participant identification centre. However, the majority of the Leeds CF centre adult patients had already started TEZ/IVA and the other centres did not respond. Therefore, I did not pursue opening a new participant identification centre any further. The only other option was to approach patients who were nearly 12 years old and due to become eligible for TEZ/IVA.

#### *5.3.4.3. Coronavirus (COVID-19) pandemic*

The COVID-19 pandemic has seen over 16 million positive cases[301] and over 176, 000 deaths[302] associated with COVID-19 in the UK as of the 25th January 2022. The severity of the virus led to the closure of schools and universities campuses and the government stopped all non-essential travel.

Temporary closure of the University of Nottingham from the 17th March 2020 and redeployment of academic clinicians to clinical areas resulted in a 21-week study pause. At this point, all the enrolled participants had completed their baseline study visits but not all had completed the first and second treatment period. During the study pause period, when there was uncertainty of when participants would complete the study per protocol, some participants opted to start TEZ/IVA as part of their routine CF clinical care. The University allowed on-site research to re-commence on 1st August 2020. After discussion with the principal investigator (Prof. A. Smyth) and protocol amendment, the remaining

four study participants underwent observer-blind study visits whilst taking TEZ/IVA. An observer-blind study visit was defined as the observer analysing the data collected being blind to the treatment status but the participant not being blind to their treatment.

### 5.3.5. Outcomes

The primary endpoint was OCTT. The secondary endpoints were:

- gastric half-emptying times;
- corrected small bowel water content (SBWC);
- post-prandial change in corrected small bowel water after a large meal (delta TI);
- total corrected colon volumes; and
- GI symptoms.

SBWC, delta TI and colon volumes were corrected for body surface area, as previously discussed (see Section 4.3.4.3).

Exploratory outcomes were:

- terminal ileum motility;
- $T_1$ of the ascending colon ($T_1$AC);
- faecal elastase; and
- faecal calprotectin.

### 5.3.6. Sample size and statistical analysis

This study was a pilot study because there were insufficient previous data upon which to base a sample size calculation. A sample size of 16, based upon the mean GIFT-CF 1 OCTT difference (60 minutes)

and within-subject standard deviation in the GIFT-CF 1 CF group (93 minutes), was calculated with 0.8 power and 0.05 significance. However, this calculation assumes the OCTT in a CF group returns to a non-CF state. The data from the GIFT-CF 1 study did not provide sufficient data to determine a clinically meaningful difference in the GI outcomes measured for within-CF participants. Therefore, the rationale for using a sample size of 12 was chosen for both its feasibility of recruitment and because there is little gain in precision about the mean and variance within a population with a sample size more than 12[280]. A pre-specified maximum number of 15 eligible people were recruited to allow for dropouts and ensure the planned sample size of 12 was achieved.

The mean half-life of tezacaftor is 156 hours (or 6.5 days) and the mean half-life of ivacaftor is 16 hours[147]. A 28-day washout period was sufficient to minimise period and carry-over effects of the drug and therefore within-participant analysis was undertaken. Within-participant differences for OCTT to compare TEZ/IVA with placebo were analysed using the Kaplan-Meier method and log-rank test. The Kaplan-Meier survival analysis was used to visually present the percentage of participants whose OCTT was complete by the time of the final scan at 360 minutes. Participants whose OCTT was not identified by 360 minutes were censored.

In view of the small sample size, within-participant analysis of the secondary and exploratory outcomes used the non-parametric test Wilcoxon matched-pairs sign-rank paired test.

Due to the COVID-19 disruptions described above, a proportion of the participants were unable to complete the study per protocol. I therefore conducted a secondary post-hoc analysis. For this analysis, the placebo results from the participants who completed the protocol (n = 8) were combined with the baseline (off-treatment) results for the participants who did not complete the full protocol but had observer-blind scans both on and off treatment (n=4). The TEZ/IVA results used for these 4 participants were collected during the observer-blind treatment visit. This adaptation allowed a secondary post-hoc analysis, which included 12 participants.

All data are presented as median (Q25, Q75). All statistical tests were completed using RStudio and a p-value of less than 0.05 was considered statistically significant.

#### 5.3.7. Test-retest observation

The test-retest observations compared baseline and placebo scans using linear regression analysis, intra-class correlation coefficient (ICC) and a Bland-Altman plot. Comparisons included OCTT, corrected colon volumes, corrected SBWC and corrected delta TI. Exploratory outcomes were not included in the test-retest comparison.

## 5.4. Results

All study procedures took place between September 2019 and October 2020.

### 5.4.1. Recruitment and sample size for analysis

I recruited 15 participants with CF (see Figure 5.3 for CONSORT diagram), of whom 3 dropped out, leaving the planned sample size of 12. The reasons for dropout included one non-attendance for study visits, one inability to complete the first test meal and one experienced claustrophobia when positioned in the MRI scanner.

Four participants were unable to complete the study per protocol due to the COVID-19 pandemic. Of these participants, two completed the first treatment period (one received placebo and one received TEZ/IVA). The within-participant comparison for these two participants were observer-blind to TEZ/IVA or baseline from the GIFT-CF 1 study, respectively. The remaining two participants underwent observer-blind study visits off (baseline scan) and on TEZ/IVA. Therefore, the final primary per protocol analysis included 8 participants and the secondary post-hoc analysis included all 12 participants.

Participant demographics and baseline results are summarised in Table 5.3.

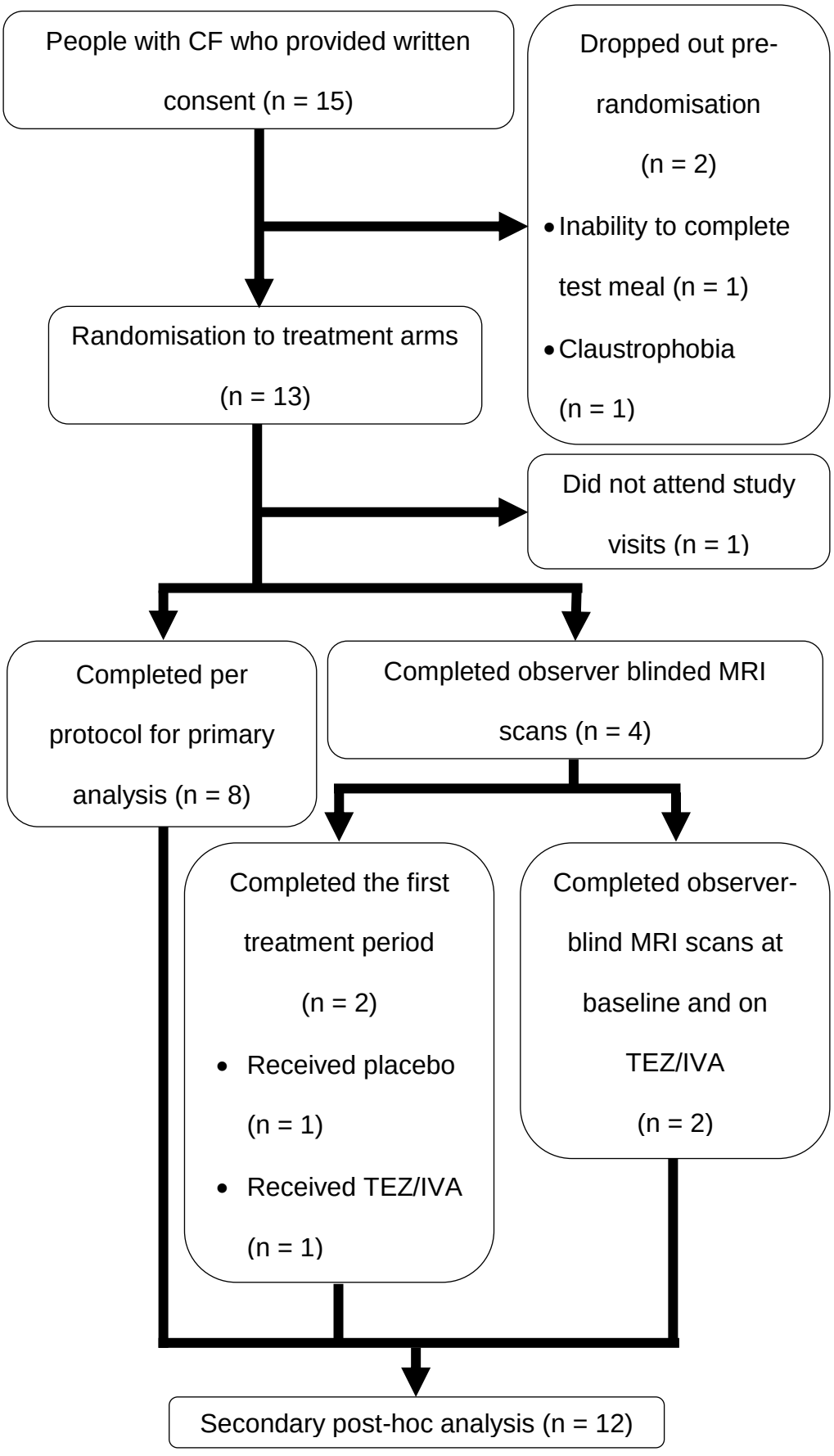



*Figure 5.3. CONSORT diagram to show participant flow.*

*Table 5.3. Participant demographics at baseline and baseline study visit measurements.*

| ***Characteristic, Median (Q25, Q75)*** | **N = 12** |
|---|---|
| Gender (M:F) | 8 : 4 |
| Age (years) | 20 (17, 26) |
| $FEV_1$ (%) | 80 (59, 100) |
| Height (cm) | 167 (161, 176) |
| Weight (kg) | 59 (55, 65) |
| ***Baseline visit, median (Q25, Q75)*** | N = 12 |
| OCTT (mins) | 330 (300, >360) |
| Corrected SBWC AUC ($L \cdot min/m^2$) | 64 (53, 75) |
| Corrected Delta TI ($mL/m^2$) | 19 (11, 60) |
| Fasting corrected colon volume ($mL/m^2$) | 291 (190, 437) |
| CFAbd-Score | 16.2 (13.8, 21.7) |
| PAC-SYM | 0.38 (0.25, 0.5) |

### 5.4.2. Primary outcome

For the primary per protocol analysis (n = 8), the median OCTT after TEZ/IVA was >360 mins [225, >360] compared to 330 mins [285, >360] after placebo, with no statistically significant difference between treatments (p = 0.8, Figure 5.4).

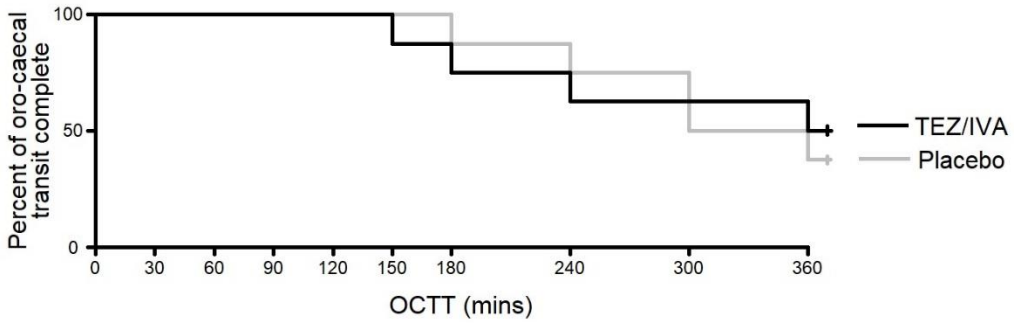



*Figure 5.4. Graph to show the time taken for a meal to reach the caecum in the primary analysis (n = 8). Number censored TEZ/IVA = 4, placebo = 3.*

For the secondary post-hoc analysis (n = 12), there was no significant difference in OCTT with TEZ/IVA compared to off treatment (TEZ/IVA >360 minutes [225, >360] vs. off treatment 360 minutes [300, >360], p > 0.99, Figure 5.5).

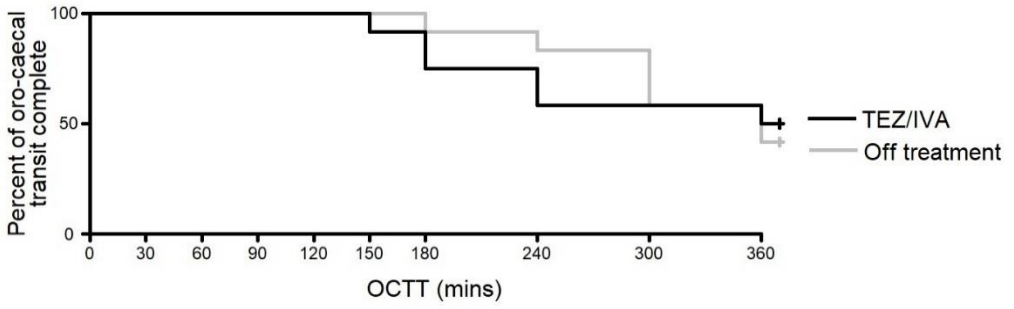



*Figure 5.5. Graph to show the time taken for a meal to reach the caecum in the secondary analysis (n = 12). Number censored TEZ/IVA = 6, off treatment = 5.*

As per the GIFT-CF 1 methods, an OCTT longer than 360 minutes was defined by no arrival of the head of the meal in the caecum by the final scan and the meal contents still visible as high signal intensity regions within the distal small bowel. These results were incomplete and subsequently censored for the Kaplan-Meier curve.

In the primary analysis, there were two participants whose OCTT was longer than 360 minutes in both treatment periods. There was one

participant whose OCTT was incomplete during the placebo period only and two participants whose OCTT was incomplete during the TEZ/IVA period only.

In the secondary post-hoc analysis, there were four participants whose OCTT was longer than 360 minutes in both treatment periods. There was one participant whose OCTT was incomplete during the placebo period only and two participants whose OCTT was incomplete during the TEZ/IVA period only.

### 5.4.3. Secondary outcomes

There were no significant differences between TEZ/IVA and placebo in the secondary outcomes for the primary per protocol analysis (n = 8) and are described in the following sections. The secondary post-hoc analyses are summarised in Table 5.4 and displayed in Figure 5.7 and Figure 5.9.

#### *5.4.3.1. Gastric half-emptying times*

Gastric half-emptying times were 96 minutes [70, 105] for TEZ/IVA compared to placebo 95 minutes [70, 110], p = 0.57.

#### *5.4.3.2. Corrected SBWC and corrected delta TI*

The median corrected SBWC AUC were not statistically significant between TEZ/IVA and placebo (TEZ/IVA 49 L·min /$m^2$ [39, 69] vs. placebo 35 L·min/$m^2$ [29, 42], p = 0.08, Figure 5.6A). The median corrected delta TI was not statistically significant between TEZ/IVA and placebo (TEZ/IVA 35 mL/$m^2$ [-67, 64] vs. placebo 3 mL/$m^2$ [-8, 33], p > 0.99, Figure 5.6B). Of note, there was an outlier participant in the

corrected delta TI secondary post-hoc analysis. This participant had a much larger corrected delta TI change than other participants and underwent surgery for meconium ileus as a neonate.

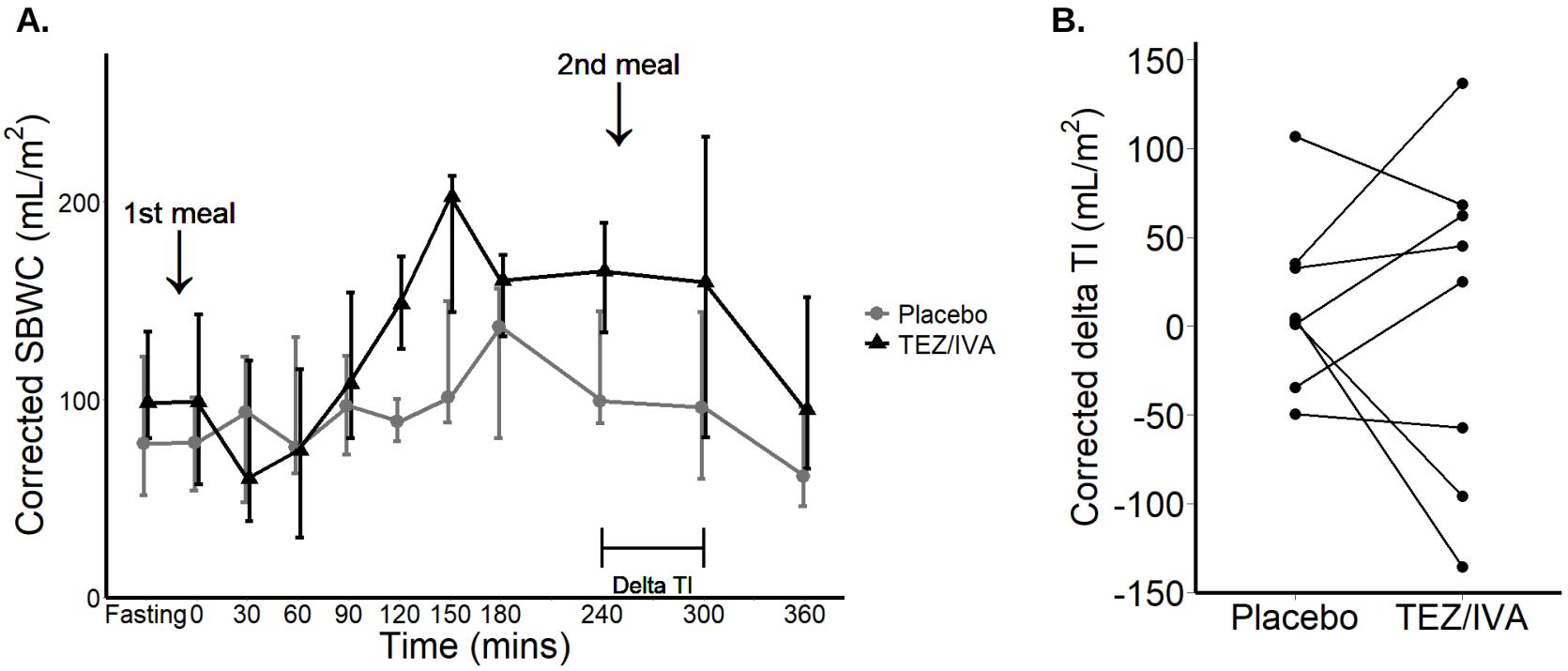



*Figure 5.6. Graphs for primary analysis (n = 8) of corrected SBWC and corrected delta TI. (A) Median (dot) and IQR (whiskers) changes in corrected SBWC during a study day. (B) Change in corrected delta TI between placebo and TEZ/IVA for each participant.*

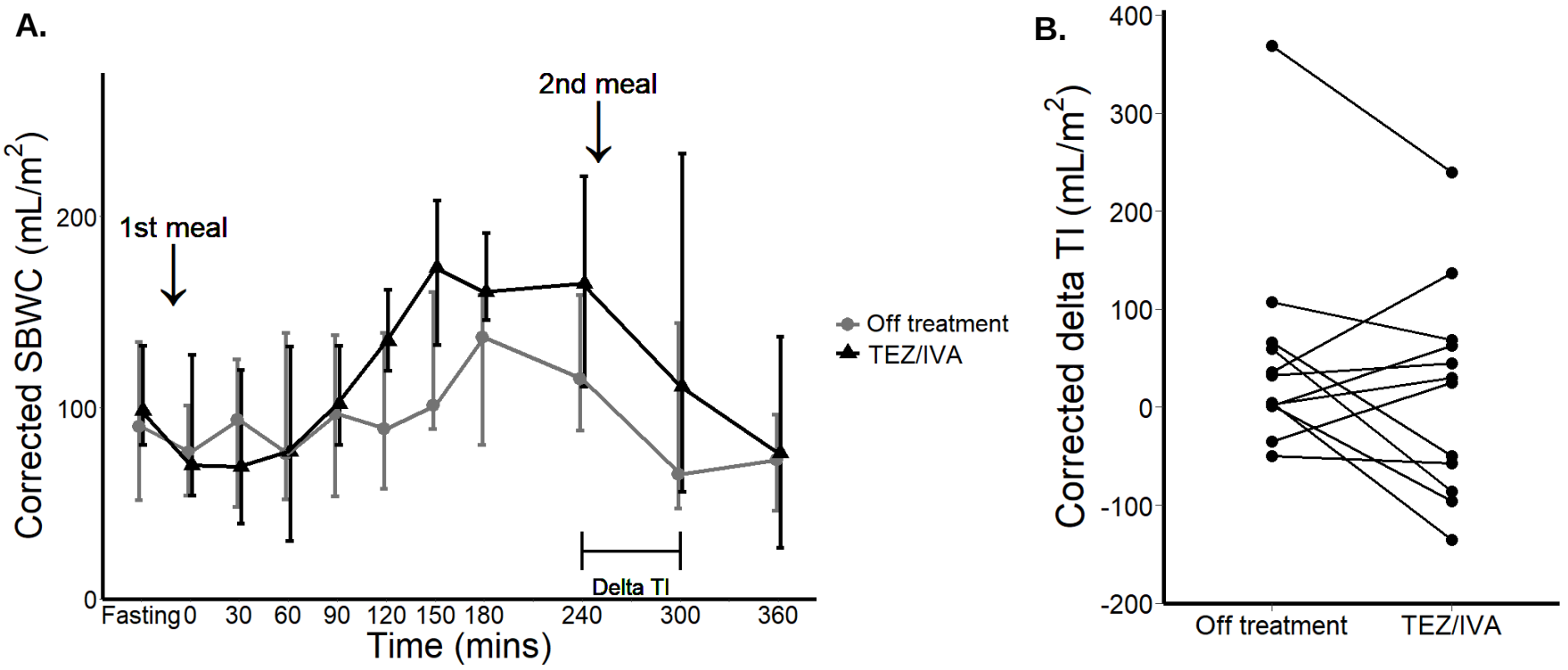



*Figure 5.7. Graphs for secondary analysis (n = 12) of corrected SBWC and corrected delta TI. (A) Median (dot) and IQR (whiskers) changes in corrected SBWC during a study day. (B) Change in corrected delta TI between placebo and TEZ/IVA for each participant.*

#### *5.4.3.3. Corrected colonic volumes*

The corrected colonic volumes were not statistically different between TEZ/IVA and placebo for AUC (TEZ/IVA 179 L·min /$m^2$ [156, 232] vs. placebo 167 L·min /$m^2$ [144, 211], p = 0.46, Figure 5.8A) and at fasting (TEZ/IVA 565 mL/$m^2$ [490, 732] vs. 566 mL/$m^2$ [416, 614], p = 0.64, Figure 5.8B).

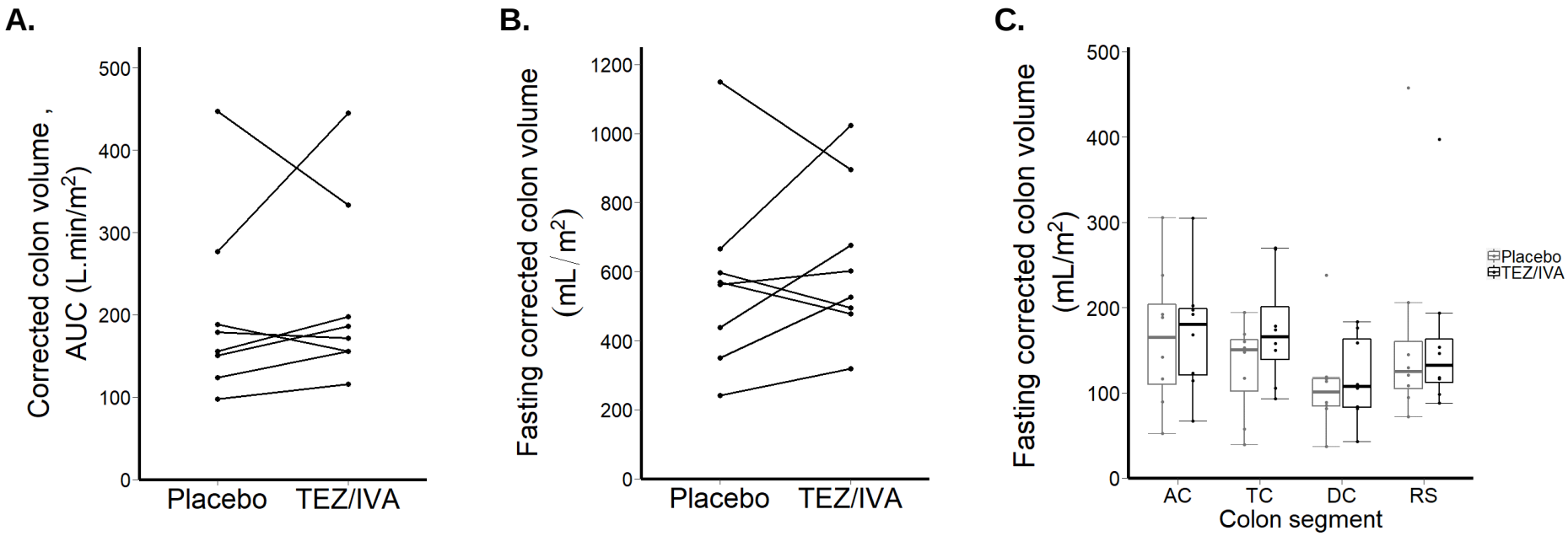



*Figure 5.8. Graphs for primary analysis (n = 8) of corrected colon volumes. (A) Corrected colon volume area under the curve (AUC) after 21 days treatment. (B) Fasting corrected colon volume after 21 days treatment. (C) Fasting corrected colon volumes for ascending (AC), transverse (TC), descending (DC) and recto-sigmoid (RS) colon. Boxes display the median and IQR of fasting corrected colon volumes, whiskers indicate 1.5 times the IQR, dots indicate individual corrected colon volume values.*

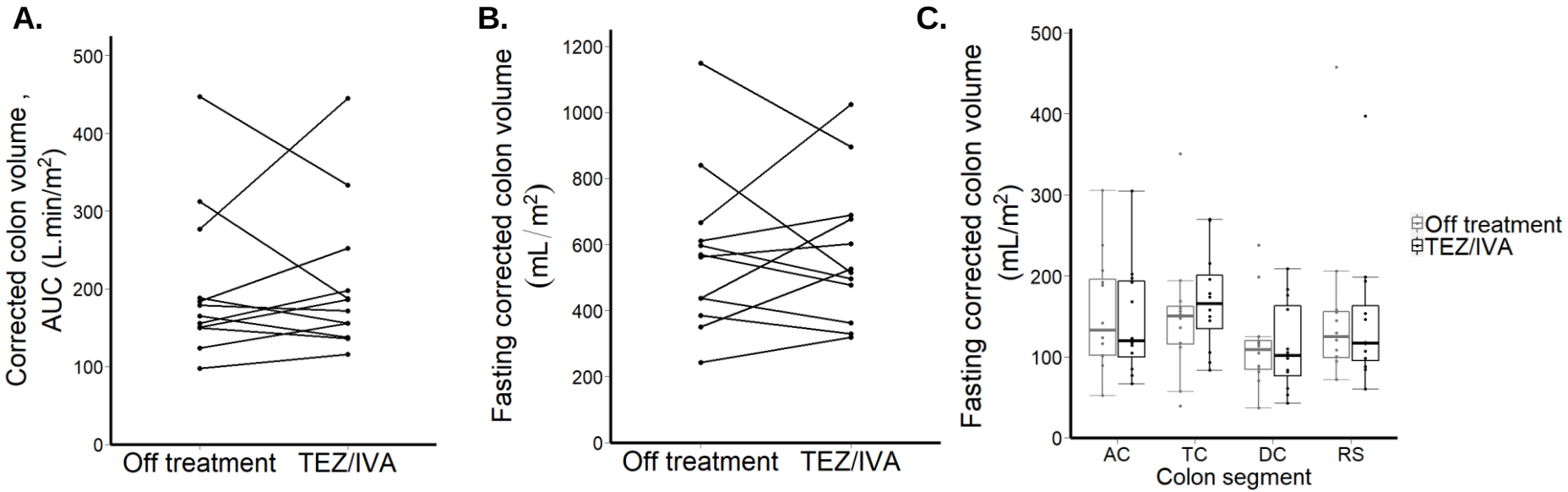



*Figure 5.9. Graphs for secondary analysis (n = 12) of corrected colon volumes. (A) Corrected colon volume area under the curve (AUC) after 21 days treatment. (B) Fasting corrected colon volume after 21 days treatment. (C) Fasting corrected colon volumes for ascending (AC), transverse (TC), descending (DC) and recto-sigmoid (RS) colon. Boxes display the median and IQR of fasting corrected colon volumes, whiskers indicate 1.5 times the IQR, dots indicate individual corrected colon volume values.*

#### *5.4.3.4. GI Symptoms*

The median scores for the GI symptom questionnaires relating to the GI symptoms in the preceding two weeks showed participants to experience GI symptoms although they were not significantly different after taking TEZ/IVA. A high PAC-SYM or CFAbd-Score relates to worse GI symptoms. The median score for the PAC-SYM questionnaire was 0.63 [0.08, 0.88] on TEZ/IVA compared to 0.71 [0.13, 0.83] on placebo, and were not significantly different (p = 0.68). The median score for CFAbd-Score was 17.2 [12.1, 27.4] on TEZ/IVA compared to 21.2 [16.9, 27.8] on placebo and were not significantly different (p = 0.45). The highest scoring domain within the CFAbd-Score was the disorders of bowel movement and the lowest scoring domain was for eating and appetite (Figure 5.10). For the 3-point Likert scale, the scores for flatulence, bloating and abdominal pain were low at each scanning time point in both treatment periods. The total scores for flatulence, bloating and abdominal pain were also low over the course of the study visit day for both TEZ/IVA and placebo.

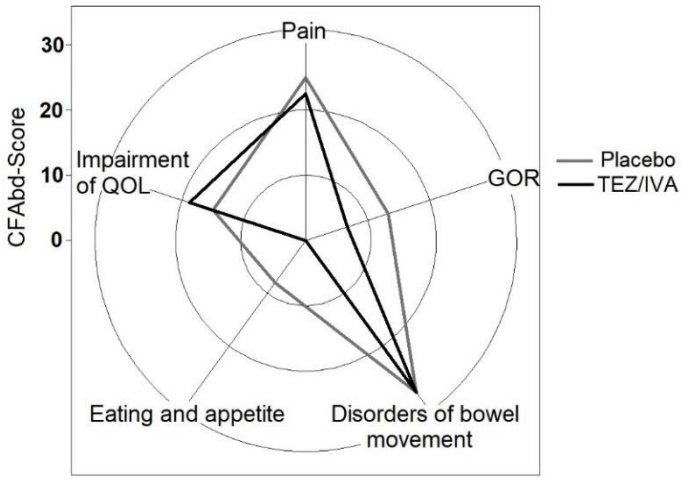



*Figure 5.10. Diagram to show median scores for each domain of the CFAbd-Score in the primary analysis (n = 8).*

*Table 5.4. Summary of secondary outcomes.*

| Outcome<br>Median (Q25, Q75) | Primary per protocol analysis (n = 8) | | | Secondary post-hoc analysis (n = 12) | | |
|---|---|---|---|---|---|---|
| | **TEZ/IVA** | **Placebo** | **p-value** | **TEZ/IVA** | **Placebo** | **p-value** |
| Gastric half-emptying time *(mins)* | 96 (70, 105) | 95 (70, 110) | 0.57 | 92 (70, 105) | 86 (67, 101) | 0.48 |
| Corrected delta TI *(mL/m$^2$)* | 35 (-67, 64) | 3 (-8, 33) | >0.99 | 28 (-64, 64) | 19 (2, 50) | 0.27 |
| Corrected SBWC AUC*(L·min /m$^2$)* | 49 (39, 69) | 35 (29, 42) | 0.08 | 45 (36 ,62) | 34 (29, 49) | 0.12 |
| Corrected colonic volumes AUC *(L·min /m$^2$)* | 179 (156, 232) | 167 (144, 211) | 0.46 | 179 (151, 211) | 172 (150, 186) | 0.73 |
| CFAbd-Score | 17.2 (12.1, 27.4) | 21.2 (16.9, 27.8) | 0.45 | 12.2 (7.2, 21.8) | 18.9 (13.2, 22.9) | 0.20 |
| PAC-SYM | 0.63 (0.08, 0.88) | 0.71 (0.50, 0.90) | 0.68 | 0.25 (0.08, 0.71) | 0.67 (0.13, 0.83) | 0.41 |
| Total Likert scores | 3.5 (0, 9.63) | 2.75 (0.88, 9.50) | 0.50 | 3.5 (0, 5.25) | 2.75 (0.38, 8.75) | 0.64 |

### 5.4.4. Exploratory outcomes

There were no statistically significant differences in the exploratory outcomes for the primary per protocol or secondary post-hoc analysis (Table 5.5).

The terminal ileum motility scores were low at fasting and postprandial for both TEZ/IVA and placebo groups. Since the cine MR sequence for motility had changed from the GIFT-CF 1 study, for the secondary analysis, it was not possible to evaluate the terminal ileum motility scores for 2 participants at fasting and T300. In some participants, it was not possible to visualise the terminal ileum to obtain a motility score and therefore these were missing data.

The fasting $T_1$AC was low for both TEZ/IVA and placebo groups.

The faecal elastase and faecal calprotectin were low for all participants after TEZ/IVA and placebo. A low faecal elastase is indicative of pancreatic insufficiency. A faecal calprotectin outside of the normal range (>50µg/g) indicates there may be active intestinal inflammation.

*Table 5.5. Summary of exploratory outcomes. TI = terminal ileum. [a]Two participants were excluded from the motility analysis due to changes in the MR sequence between baseline and treatment scans. [b]A few participants were excluded from the motility analysis due to the terminal ileum not being visualised. [#] n = 10, [†] n = 8, * n = 7, [††] n = 6.*

| Outcome<br>Median (Q25, Q75) | Primary per protocol analysis (n = 8) | | | Secondary post-hoc analysis (n = 12) | | |
|---|---|---|---|---|---|---|
| | **TEZ/IVA** | **Placebo** | **p-value** | **TEZ/IVA** | **Placebo** | **p-value** |
| TI motility *(au)* *Fasting* | 0.15 (0.13, 0.23)[b,*] | 0.17 (0.11, 0.21) [b,*] | 0.94 | 0.15 (0.11, 0.22)[#,a] | 0.15 (0.10, 0.19) [#,a] | 0.92 |
| *Postprandial T0* | 0.20 (0.16, 0.21) [b,††] | 0.23 (0.14, 0.26) [b,††] | >0.99 | 0.20 (0.15, 0.24) [†,a,b] | 0.23 (0.19, 0.27) [†,a,b] | 0.74 |
| *Postprandial T300* | 0.16 (0.13, 0.19) [b,†] | 0.19 (0.18, 0.22) [b,†] | 0.38 | 0.16 (0.14, 0.21) [#,a] | 0.19 (0.15, 0.21) [#,a] | 0.21 |
| Fasting $T_1$AC *(secs)* | 0.66 (0.61, 0.82) | 0.49 (0.41, 0.97) | 0.74 | 0.65 (0.51, 0.80) | 0.55 (0.43, 0.95) | 0.97 |
| Faecal elastase *(µg/g)* | 4.9 (2.0, 6.5) | 2.5 (1.2, 7.7) | 0.84 | 1.9 (<1.0, 5.6) | <1.0 (<1.0, 7.0) | 0.42 |
| Faecal calprotectin *(µg/g)* | 13.7 (5.2, 24.9) | 16.4 (8.0, 23.6) | 0.64 | 13.7 (5.2, 25.8) | 12.6 (8.0, 21.5) | 0.73 |

### 5.4.5. Safety monitoring

All blood pressure and blood tests (including liver function tests) for safety monitoring were within normal limits or stable for the participants. No participants had elevations in liver transaminases above the pharmaceutical precaution limit[149]. There were no adverse events during the study.

### 5.4.6. Test-retest observation

Figure 5.11 to Figure 5.14 show the observations of MRI measures between the baseline and placebo scans. The baseline scans for six participants were from the GIFT-CF 1 study and therefore obtained between September 2018 and February 2019. The baseline scans for three participants were from the GIFT-CF 2 study and therefore obtained between September 2019 and March 2021.

There is a positive correlation for the OCTT, corrected colon volumes, corrected SBWC and corrected delta TI between baseline and placebo scans. All the observations made lie within the upper and lower limits of agreement, except for the OCTT of one participant.

The ICC using a two way mixed effects with absolute agreement showed that there was moderate reliability for OCTT (ICC = 0.59, p = 0.036) and corrected colon volume AUC (ICC = 0.90, p = 0.001). However, there was poor reliability for SBWC AUC (ICC = 0.261, p = 0.23) and delta TI (ICC = 0.48, p = 0.078).

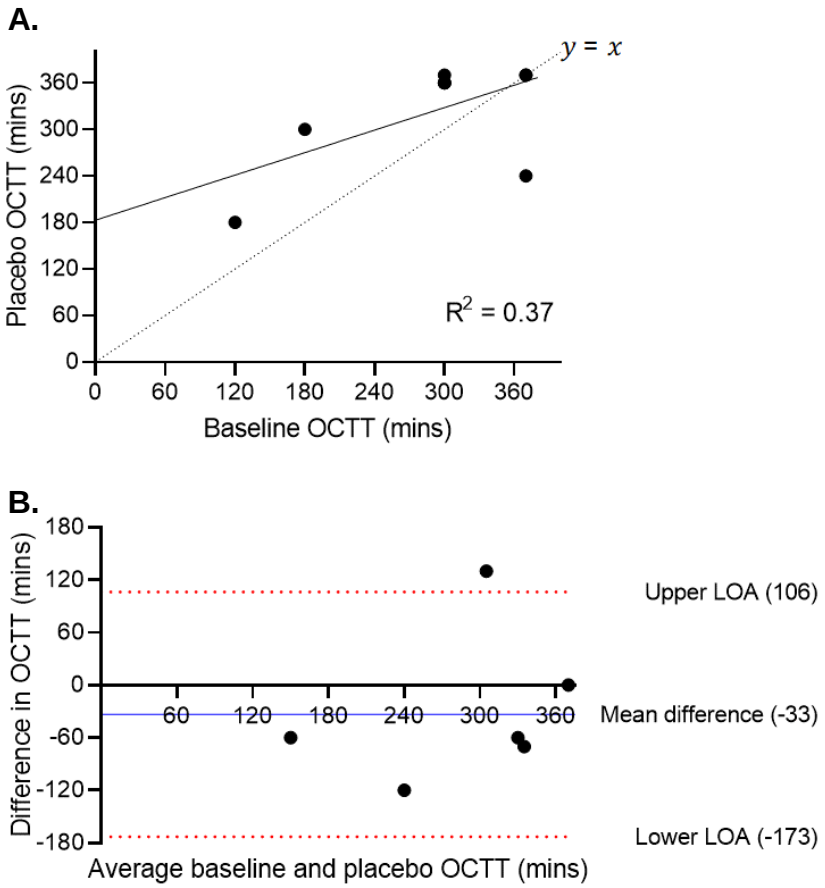



*Figure 5.11. Test-retest comparison for OCTT. (A) Correlation of baseline and placebo OCTT showing $R^2$ = 0.367, p=0.08. (B) Bland-Altman plot to show agreement between baseline and placebo OCTT. The x-axis shows the average OCTT measurement at baseline and on placebo, and the y-axis shows the difference between the OCTT at different study visits. This plot shows there was a mean difference in OCTT of -33 minutes between study visits with limits of agreement ranging from 106 to -173.*

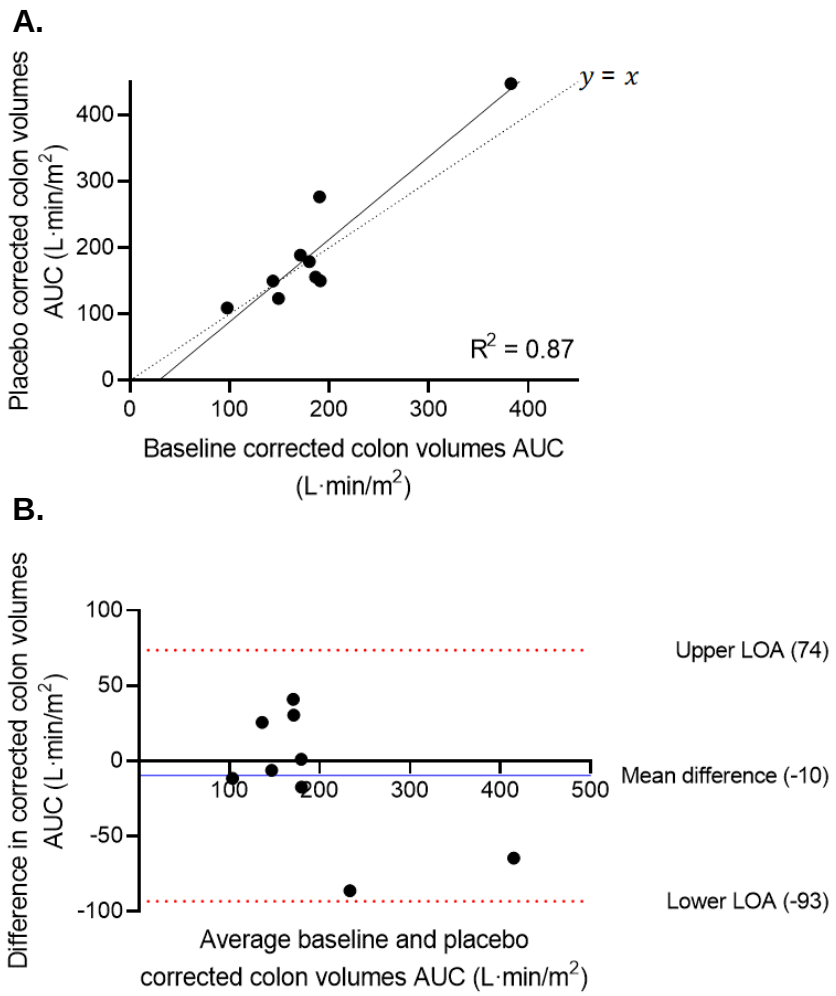



*Figure 5.12. Test-retest comparison for corrected colon volumes AUC (A) Correlation of baseline and placebo corrected colon volumes showing $R^2$ = 0.87, p=0.0002. (B) Bland-Altman plot to show agreement between baseline and placebo corrected colon volumes AUC. The x-axis shows the average corrected colon volumes AUC at baseline and on placebo, and the y-axis shows the difference between the corrected colon volumes at different study visits. This plot shows there was a mean difference in corrected colon volumes of -10 L·min/m² between study visits with limits of agreement ranging from 74 to -93.*

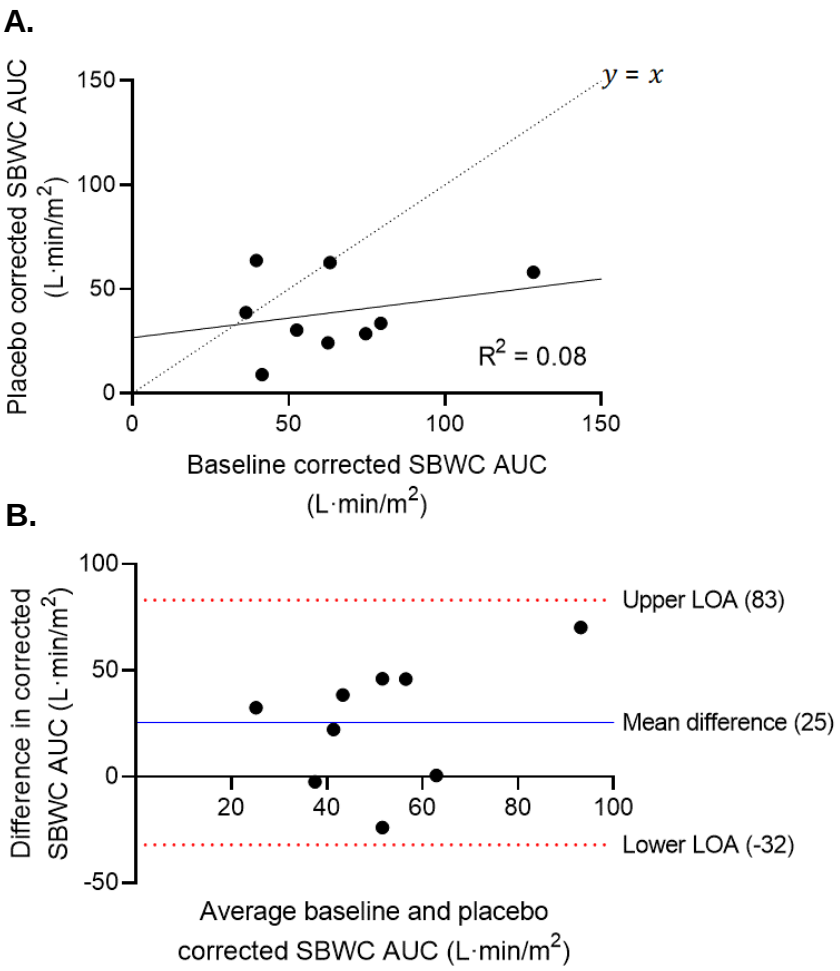



*Figure 5.13. Test-retest comparison for corrected SBWC AUC. (A) Correlation of baseline and placebo corrected SBWC AUC showing $R^2$ = 0.08, p=0.46. (B) Bland-Altman plot to show agreement between baseline and placebo corrected SBWC AUC. The x-axis shows the average corrected SBWC AUC at baseline and on placebo, and the y-axis shows the difference between the corrected SBWC at different study visits. This plot shows there was a mean difference in corrected SBWC AUC of 25 L·min/m$^2$ between study visits with limits of agreement ranging from 83 to -32.*

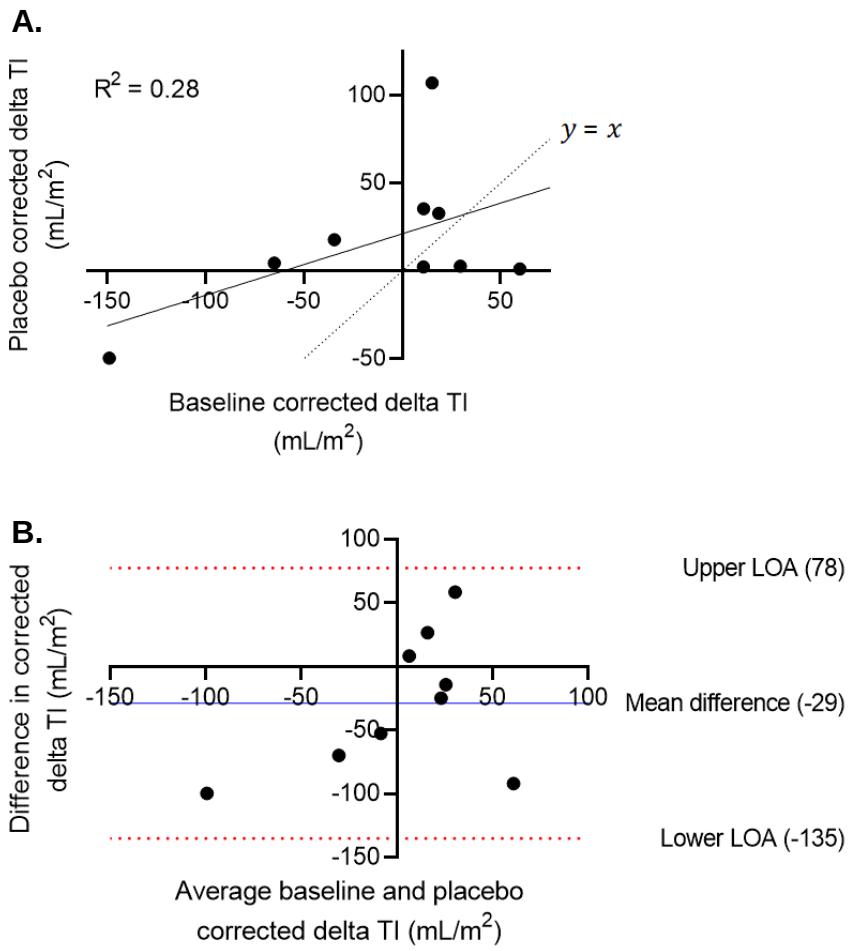



*Figure 5.14. Test-retest comparison for corrected delta TI. (A) Correlation of baseline and placebo corrected delta TI showing $R^2$ = 0.28, p=0.15. (B) Bland-Altman plot to show agreement between baseline and placebo corrected delta TI. The x-axis shows the average corrected delta TI at baseline and on placebo, and the y-axis shows the difference between the corrected delta TI at different study visits. This plot shows there was a mean difference in corrected delta TI of -29 L·min/m$^2$ between study visits with limits of agreement ranging from 78 to -13*

## 5.5. Discussion

This is the first study to evaluate the GI effects of a CFTR modulator in a randomised, placebo-controlled trial using both objective and subjective outcome measures. Although these data did not demonstrate any change in GI function after 21 days of TEZ/IVA, they do provide further information on study design and sample size, so that any future studies could be better powered to detect any difference caused by treatment.

After 21 days of TEZ/IVA, OCTT remained delayed with a median OCTT over 6 hours. In Chapter 4, I described that OCTT, as identified through MRI, was 2 hours longer in people with CF than in matched healthy controls. These data were similar to those from other research groups[64,285] who used wireless capsules. Further research by Gelfond *et al*[154] using wireless capsules found that ivacaftor alone increased small intestine pH after 1 month of treatment. Whilst their data suggest that the underlying defect causing a reduction in bicarbonate secretions improves with ivacaftor, they did not detect other changes in the GI tract such as transit times. It could be that, despite ivacaftor treatment being associated with a normalising of small intestinal pH, the intestinal chyme remained viscous, which could contribute to the prolonged OCTT. Another reason for the persistence of prolonged transit time could be continued maldigestion and malabsorption of fat despite pancreatic enzyme supplements. Two studies have reported recovery of pancreatic exocrine function in children under 5 years old after 24 weeks of ivacaftor[162,163]. However, in the current study, all the

participants had a low faecal elastase concentration. This suggests that, whilst in younger children with CF pancreatic function may be recoverable; this may not be possible in people where pancreatic destruction has passed the point of reversibility so their faecal elastase concentration remains low. Another alternative explanation would be that 3 weeks of CFTR modulator therapy is not long enough for pancreatic exocrine recovery, if that were possible, or that the dual therapy used for this clinical trial was not efficacious.

Another reason for prolonged OCTT could be due to a reduction in overall small bowel motility. Although this was not a measured outcome in the present study, it is worth noting that the focused motility measurement on the terminal ileum was low in all participants. Previous data by Menys *et al*[267] demonstrated that a low terminal ileum motility score correlated with active inflammation in inflammatory bowel disease. Other research groups have documented an inflammatory process in the CF small bowel by video endoscopy[99,285]; however, active inflammation is less likely in the cohort described in this chapter given the low faecal calprotectin concentration. It is possible that subclinical chronic inflammation was present at the terminal ileum, which subsequently led to fibrosis. This in turn could have led to a reduction in motility and partial obstruction to small bowel water outflow into the ascending colon. In the present study, TEZ/IVA did not change terminal ileum motility.

Corrected SBWC AUC was unchanged after a period of TEZ/IVA. As discussed in Chapter 4, SBWC was significantly different in people with

CF compared to healthy controls, with the peaks and troughs of SBWC over time likely relating to glucose absorption and the gastro-ileal reflex[37]. The data presented in this chapter demonstrates the similarities to previous studies[272,287] in small bowel water movement in response to meals, despite the lack of difference after TEZ/IVA. Furthermore, the study in Chapter 4 highlighted there may be a possible partial obstruction at the terminal ileum reflected by the lower delta TI values in the CF group (13 mL/$m^2$ [-13, 57]). Likewise, this study also demonstrates a low delta TI in the presence of TEZ/IVA (35 mL/$m^2$ [-67, 64]) and with placebo (3 mL/$m^2$ [-8, 33]) and suggests that the suspected partial obstruction at the terminal ileum still exists. The participant who underwent surgical treatment for meconium ileus as a neonate showed as an outlier in the delta TI dataset for secondary analysis (Figure 5.7B). This participant's medical notes did not document the exact length of small bowel surgically removed and since the participant did not have any issues with growth and nutrition, there was no reason to suspect the participant experienced short gut syndrome. The large drop in SBWC after the second meal in this participant suggests there was little or no obstruction compared to others with CF and further supports this participant did not have short gut syndrome. It is possible that the surgery included removal of parts of the small bowel that could cause obstruction, in particular at the terminal ileum, and therefore the absence of partial obstruction, thought to occur at the terminal ileum in CF, allowed the free flow of small bowel water into the caecum for this participant.

Since corrected SBWC has remained high despite TEZ/IVA treatment, it is unsurprising that the water content within the ascending colon chyme measured by $T_1$AC also remained low. A low $T_1$AC signifies there is less water within the ascending colon chyme and is consistent with people who experience constipation, as previously described[115]. The high colonic volumes with and without TEZ/IVA also support the link with constipation[254,272]. The PAC-SYM data demonstrated that both study groups report some symptoms of constipation. A score of zero indicates there are no symptoms relating to constipation. There were 7 (88%) participants in the primary analysis and 10 (83%) participants in the secondary analysis who reported some constipation symptoms. This could reflect the reports of increased prevalence of constipation in CF compared to the general population[112,113].

On both the CFAbd-Score and PAC-SYM questionnaires, participants reported GI symptoms regardless of whether they are taking TEZ/IVA or placebo. There were no differences in the two scores after TEZ/IVA, but this could be a false negative finding due to the sample size and should be interpreted with caution.

In keeping with the study hypothesis, *in vitro* studies have shown return of CFTR function in intestinal organoids in CF after double and triple combination CFTR therapies[303]. However, the data presented in this chapter show no significant difference between “no modulator” treatment and TEZ/IVA in either MRI metrics, biochemical markers of inflammation (calprotectin) or pancreatic exocrine function (elastase) or reported GI symptoms. This may be because there is truly no difference

or because the small sample size was not sufficient to statistically show such a difference. Furthermore, it is possible that TEZ/IVA is not sufficiently efficacious in improving CFTR function, which may have contributed to the findings described. It is also possible that future clinical trials could demonstrate an effect on GI health with longer treatment periods or a more effective CFTR modulator therapy.

### 5.5.1. Strengths and weaknesses

This study supports the use of MRI to evaluate GI physiology in CF and the data demonstrated the test-retest capability of MRI. These data are similar to my previous findings and work by others. There are positive correlations between the MRI measurements at two different study visits (described in section 5.4.6) and all results, except for one participant, were within the limits of agreement. The mean differences for each measurement were acceptable. For the OCTT, the mean difference -33 minutes reflects the timing of MRI scans where there was a minimum gap of 30 minutes between scans. For corrected colon volumes and corrected SBWC, a mean difference of -10 $L \cdot min/m^2$ and 25 $L \cdot min/m^2$ respectively, are small values in comparison to the final measurements and therefore would cause little effect on the overall result. Although the ICC for SBWC was poor, a recent publication shows high variability within the healthy volunteer group across different studies[304]. In addition, the study described in this chapter was not designed to look at test-retest specifically and the results could be due to the small sample size or the long time interval between test and retest.

Another strength of the study was the use of placebo as a comparator. There have been studies that evaluated the respiratory effects of a CFTR modulator compared to placebo and some studies that have evaluated the GI effects before and after the introduction of ivacaftor. However, no other studies, prior to the present study protocol registration, were placebo-controlled using MRI to evaluate the GI effects of a CFTR modulator. The introduction of combination CFTR modulators, such as elexacaftor-tezacaftor-ivacaftor, for the majority of people with CF means that with time, future trials of new CF agents are unlikely to be placebo-controlled as it will be unethical to ask potential participants to stop modulator therapy. Instead, future trials are likely to compare against current standard therapy that includes CFTR modulators.

There were also limitations to the study, including the announcement of funding of the study drug a month after opening the study for recruitment and the COVID-19 pandemic. The decision made by NHS England to fund the study drug as part of routine clinical practice limited the pool of potential participants, especially since approximately 30% of people with CF eligible for the study drug in the Nottingham area did not wish to delay their access to treatment. The additional disruption by the COVID-19 pandemic not only limited the recruitment but also restricted the number of participants able to complete the study per protocol, as described in section 5.3.4.3. Although the enrolled participants who had not yet completed the protocol were able to attend observer-blind study visits, it was not possible to control the length of

time participants were prescribed TEZ/IVA before their study visit and there was potential for reporting bias in the GI symptom questionnaires.

In addition to the limitations above, the time required for MRI scans in the present study could limit further application of this MRI protocol. Participants reported they were comfortable during the study visits and continued their participation but incorporating this protocol into clinical practice would be burdensome for patients and expensive for the healthcare system. Whilst the present protocol is lengthy, a better understanding of the use of MRI in CF GI disease will help inform which measurements could be surrogate markers of disease. This would allow reduction of the protocol length and reduce patient burden.

The reason for measuring outcomes between 19 and 23 days of treatment was to provide a buffer for the timing of study visit, which had the potential to be disrupted by scanner bookings, weekends and participant availability. This ensured that participants would not complete their treatment period before attending the study visit. However, the short duration of study treatment is a limitation. Given that studies using wireless capsules[154] and quantifying inflammatory markers[155] showed significant differences after 1 month of ivacaftor, albeit with participants mostly with the gene mutations p.Gly551Asp/p.Phe508del, it seemed reasonable to design a study to detect changes in GI health approximately 3 weeks of TEZ/IVA. In spite of a lack of difference seen after TEZ/IVA, the present study may help to inform future study design such as longitudinal study of newer CFTR modulators.

## 5.6. Summary

In summary, this study is the first randomised placebo-controlled trial to use both objective and subjective GI outcome measures to compare the effects of a CFTR modulator. There was no difference in GI function observed after a 3 week period of TEZ/IVA. This study has therefore suggested that that future studies may need larger sample sizes which are adequately powered to detect a minimal clinically important difference; longer treatment periods to allow for potential GI changes to take effect; or more effective triple combination CFTR modulators such as elexacaftor-tezacaftor-ivacaftor which have shown significant changes in lung function. This study further demonstrates the potential use of MRI for assessing CF gut disease and provides a valuable dataset with which to compare future studies.

The next chapter will draw conclusions from the original research I have presented in this thesis and propose directions for future research in this field.

# Chapter 6 Discussion

## 6.1. Summary of original research

### 6.1.1. The use of pancreatic enzyme supplements to optimise efficacy

Approximately 90% of people with CF have pancreatic insufficiency and need to take PERT with fat-containing foods[134]. The introduction of PERT significantly improved nutrition and growth in this group of people with CF and linked to better lung function[111]. Since lung disease is the leading cause of mortality in CF[4,15], PERT indirectly links with lengthening life expectancy in pancreatic insufficient CF[111].

The aim of PERT is to improve maldigestion and malabsorption in CF by providing the enzymes required for digestion of fat, protein and starch[171]. Although clinical trials have shown the positive effects of PERT on overall growth and nutrition, people with CF continue to report GI symptoms that affect their quality of life[32].

The systematic review presented in this thesis identified only two studies[197,198] with sufficient treatment periods despite having insufficient washout periods. However, the studies did not have sufficient data to be included in the review. The systematic review has demonstrated there are a lack of evidence, within the randomised controlled trial literature, available to draw conclusions about how to optimise the timing of PERT administration relative to a meal.

The review raised some issues that could limit clinical trial design, for example: the ethical dilemma of stopping PERT to have a PERT-free

washout period or to use a placebo treatment; and the practical issues of participant blinding to intervention, which could introduce performance bias or reporting bias by participants. In the era of new CF therapies, it will be important to address whether GI symptoms persist or if people with pancreatic insufficient CF need PERT to maintain a healthy nutritional status. Nevertheless, the question regarding optimisation of PERT timings remains unanswered due to the lack of evidence available.

### 6.1.2. Gastrointestinal MRI in CF

The GIFT-CF 1 study adds to the existing literature about the gut physiology in CF. Tools to investigate GI problems in CF can be invasive or expose people with CF to radiation[6]. MRI has the advantage that it is both non-invasive and radiation-free, making it more acceptable for people with CF who already have a high investigational burden for their multi-organ disease. The use of MRI for assessing GI function and transit in CF is feasible at a dedicated MRI research facility. Specifically, I have shown that people with CF will tolerate holding their breath for up to 10 seconds during MR sequences, to obtain the images. More than half of the participants were satisfied with the meals provided, comfort of MRI scans and scanning location.

This was the first study to document the changes seen in the GI tract in CF using MRI. The study demonstrated statistically significant differences in OCTT, corrected colon volumes, corrected SBWC, $T_1$AC and small bowel motility in people with CF compared to matched controls.

The median OCTT was 2 hours longer for the CF group than controls. Viscous GI secretions and reduced small bowel motility may be contributing factors for the significant difference seen.

The increased corrected colonic volumes in the CF group compared to matched controls could be associated with the higher prevalence of constipation seen in people with CF[112,113] compared to the general population[114]. A similar observation of higher colonic volumes has been reported in people with chronic constipation compared to those with a normal bowel habit[115].

The volume of small bowel water did not reflect the presence of viscous secretions that is true to the respiratory tract where CFTR dysfunction dehydrates the mucus layer. Contrary to expectations, the volume of SBWC was significantly higher in the CF group compared to controls. A higher volume of water within the small bowel suggests that several mechanisms alongside dehydrated GI secretions may be associated with the disruption of ion transport and water within the GI small bowel and contribute to GI pathology in CF. The initial SBWC drop during the gastric phase seen in both study groups could reflect the presence of PERT and subclinical pancreatic function, which contribute to food digestion and subsequently glucose absorption. However, alongside a prolonged OCTT, the lack of post-prandial drop in small bowel water (delta TI) and the reduction in water content in the ascending colon ($T_1$AC) in CF compared to controls support the theory that a partial obstruction near the ileo-caecal valve could exist. Further research is required to deepen our understanding of the underlying mechanisms

that cause a potential partial and subclinical obstruction at the terminal ileum.

### 6.1.3. Application of gastrointestinal MRI in evaluating tezacaftor-ivacaftor in CF

The GIFT-CF 2 study was the first randomised double-blind placebo-controlled trial to evaluate the GI effects of TEZ/IVA in CF using MRI. The application of MRI as a tool for assessing GI function in a randomised controlled trial was feasible but did not demonstrate a statistically significant difference in MRI, stool or patient reported outcomes with TEZ/IVA.

There were no changes seen in the MRI outcomes with TEZ/IVA. The OCTT and corrected colon volume results were similar to those seen in the CF group in the GIFT-CF 1 study. The OCTT was >360 minutes after TEZ/IVA and 330 minutes after placebo, compared to >360 minutes in the GIFT-CF 1 CF group. The corrected colon volumes AUC values were 179 L·min /$m^2$ after TEZ/IVA and 167 L·min /$m^2$ after placebo, compared with 186 L·min/$m^2$ in the GIFT-CF 1 CF group. The similarity of the OCTT and corrected colon volumes between the two studies suggest that no measurable physiological changes take place in the gut following 3 weeks treatment with TEZ/IVA.

The median corrected SBWC AUC values in the GIFT-CF 2 study were midway between those seen in the CF group and the non-CF control group in GIFT-CF 1. However, it is important to note that after an overnight fast, SBWC in healthy individuals can range between 2 to 373

mL[304]. Delta TI is the fall in SBWC, seen after a large second meal in the MRI protocol. It represents the movement of small bowel water from the ileum into the caecum due to the gastro-ileal reflex. The corrected delta TI measurement was similar between the GIFT-CF 1 CF group and the GIFT-CF 2 study, compared to the non-CF controls in GIFT-CF 1. These results suggest that the delta TI measurement could be a surrogate marker of CF gut disease, but would need to undergo further validation prior to its use.

The GIFT-CF 2 study also found novel evidence to suggest the terminal ileum motility is similar to that of active inflammation in inflammatory bowel disease. A terminal ileum motility score of less than 0.3 au is considered low. The faecal calprotectin concentration does not reflect active inflammation in both treatment arms, but the low motility scores (at fasting: 0.15 au with TEZ/IVA and 0.17 au with placebo) could be due to chronic inflammation and fibrosis. Small bowel or terminal ileum motility may be a more indicative marker of inflammation as opposed to faecal calprotectin in this study group.

It is possible that the GIFT-CF 2 results are due to the small sample size or due to the use of a less effective CFTR modulator in this study. Others have previously documented GI changes, such as increases in small intestine pH[154] and decreases in stool calprotectin[155], in response to ivacaftor.

## 6.2. Future directions

At the beginning of the thesis, I set out to answer three specific questions. My original research has answered these questions, as detailed below, but like many other studies, it has posed the need to answer further questions.

**Is there sufficient evidence to support the administration timings of PERT?**

One way of further optimising PERT is to have tighter control on when the PERT is administered, to keep as close to normal physiology as possible. However, the systematic review found there to be a lack of studies designed to answer this question. This means that a gap in the current literature remains and further studies with careful design are needed if this question is to be answered.

**Is there a tool that could be used to further understand the GI pathophysiology of CF without adding burden to people with CF?**

The GIFT-CF 1 has demonstrated the feasibility and acceptability of using MRI to assess gut function and transit in people with CF. This study proved that MRI is a novel approach in demonstrating significant changes in gut physiology between people with CF and matched controls. Although MRI for the CF gut has provided novel insights, the current MR protocol is associated with high cost and time usage. Refinement of the MR protocol, such as reducing the number of outcome measures and scanning time, is required before translation to clinical practice. If the outcome measure delta TI is used as a surrogate

marker of small bowel disease in CF, this could markedly reduce the amount of MRI scans required. However, further understanding of the CF pathophysiology at the terminal ileum should be explored at a higher resolution.

Other non-CF studies have used different MRI parameters (e.g. $T_2$ relaxometry) to assess inflammation[305] and permeability[306] of the small bowel. However, these studies require the use of oral contrast and intravenous medication to allow assessment of small bowel contents and small bowel wall. In contrast to the GIFT-CF 1 and GIFT-CF 2 protocol, the gut physiology is disturbed and it is a more invasive protocol.

The GIFT-CF 1 study has raised the following questions:

- If there is a difference in GI pathophysiology, could this affect or be affected by the gut microbiome?
- Can MRI to assess gut physiology in children under 12 years?
- Can we combine GI MRI with MR imaging of other organs?
- How could the MR protocol be applied to a multi-centre randomised controlled trial?

Stool samples were collected as part of the GIFT-CF 1 study and the gut microbiome were evaluated as part of another PhD thesis at Manchester Metropolitan University, supervised by Prof. van der Gast. These data show a difference in the gut microbiome in CF compared to matched controls and have been published in the Journal of Cystic Fibrosis[278].

Work has already begun to assess the feasibility of combining a shortened GI MRI protocol alongside MR protocols evaluating liver and lung disease in children aged 6 to 11 years old with and without CF.

Further work and validation needs to be undertaken to further understand the physiology of CF SBWC and the delta TI metric before the MR protocol can be applied to larger clinical trials. Specifically, the reasons behind the increased volume of small bowel water and the possible pathology at the terminal ileum need to be explored further.

Reducing the quantity of MRI scans will make this study design more feasible for multi-centre clinical trials both for time and cost to participants and researchers.

**Do new CF therapies (CFTR modulators) improve GI function and symptoms?**

The data from the GIFT-CF 2 study demonstrated that after 3 weeks of TEZ/IVA there was no difference in GI MRI outcomes or GI symptoms compared to placebo, unlike the significant differences seen in lung function after TEZ/IVA. This could be due to a type 2 error since the sample size was small. The GIFT-CF 2 data could be used for future sample size calculations to ensure studies are adequately powered. The GIFT-CF 2 study has offered up further research questions to the field including:

- Will CFTR modulators normalise GI physiology if CFTR modulators are taken for longer?

- What are the effects of triple combination CFTR modulators such as elexacaftor-tezacaftor-ivacaftor?

Further MRI data has been collected as part of the 6-month cohort study of elexacaftor-tezacaftor-ivacaftor in people with CF (the GIFT-CF 3 study) to attempt to answer the questions above. Once the analysis is complete for these data, the results will further our understanding of CFTR modulators on GI function. It is important to note that the introduction of CFTR modulators as routine clinical care for a large proportion of people with CF means that future trials are unlikely to compare underlying pre-CFTR modulator gut pathophysiology. The GIFT-CF 2 study is the first, and at present the only, placebo-controlled trial that objectively evaluates the GI effects of a CFTR modulator.

Other future studies should also evaluate the GI effects of CFTR modulators in children under 12 years old, since there is anecdotal evidence that the single CFTR modulator ivacaftor can reverse pancreatic insufficiency in children under 5 years old.

Capturing the longitudinal GI effects of CFTR modulators is essential in the era of triple combination CFTR modulator therapies becoming available for the majority of people with CF, since data on newer CFTR modulators have previously focussed on respiratory outcomes.

## 6.3. Final remarks

The body of work presented in this thesis has occurred during a pivotal moment in CF research and clinical care resulting from the rollout of combination CFTR modulator therapies. Now it has been even more important to address the GI issues within CF since the GI effects of the new therapies are unclear. This thesis identified gaps in the literature for optimising PERT administration and highlighted that MRI can be used in assessing GI physiology in CF. Furthermore, these studies have provided novel insights into the GI physiology in CF compared to matched controls and demonstrated that more effective CFTR modulator, longer treatment periods or larger sample sizes may be required to observe GI effects. Future work will focus on deepening the understanding of CF GI pathophysiology and long-term effects of new CF therapies.

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# List of appendices

**Appendix A:** **GIFT-CF 1 raw data**

**Appendix B:** **GIFT-CF 2 raw data**

**Appendix C:** **Example GI symptom questionnaires used in the GIFT-CF 1 and GIFT-CF 2 studies**

Patient Assessment of Constipation Symptoms (PAC-SYM)

3-point Likert-scale for MRI day

**Appendix D:** **Rights and permissions**

**Appendix E:** **Presentations arising from work done during this PhD**

**Appendix F:** **Publications arising from work done during this PhD**

## Appendix A: GIFT-CF 1 raw MRI and questionnaire data

| ID | Group | Age (years) | Sex | BSA ($kg/m^2$) | Weight (kg) | Height (cm) | Colonic volume AUC (L.min) | SBWC AUC (L.min) | OCTT (mins) | Gastric half - emptying Time (mins) | Small bowel motility, fasting (au) | $T_1$AC, fasting (secs) | PAC-SYM | CFAbd-Score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | CONTROL | 27 | F | 1.61 | 63.2 | 148.5 | 123 | 32 | 360 | 93.2 | 108 | 0.52 | 1.18 | 34.4 |
| 2 | CF | 27 | F | 1.69 | 59 | 175 | 416 | 104 | 300 | 107.3 | 43 | 0.41 | 0.09 | 4.4 |
| 3 | CF | 13 | M | 1.31 | 40.78 | 151.7 | 141 | 44 | 150 | 102 | 28 | 1.02 | 0.91 | 31.9 |
| 5 | CF | 15 | M | 1.62 | 56.84 | 165.2 | 309 | 101 | 180 | 137.8 | 42 | 0.79 | 0.14 | 1.4 |
| 6 | CF | 23 | M | 1.69 | 58.4 | 177 | 530 | 136 | >360 | 58.1 | 34 | 0.61 | 0 | 0.7 |
| 7 | CONTROL | 15 | F | 1.72 | 60 | 177 | 242 | 36 | 180 | 71.3 | 46 | 0.73 | 0.64 | 4.4 |
| 8 | CONTROL | 13 | M | 1.63 | 60 | 160 | 264 | 87 | 240 | 54.7 | 256 | 1.56 | 0.09 | 0 |
| 9 | CONTROL | 15 | M | 1.62 | 57 | 165 | 190 | 66 | 150 | 66.7 | 90 | 0.51 | 0.73 | 3.5 |
| 10 | CF | 21 | F | 1.53 | 54 | 157 | 229 | 81 | 120 | 131.1 | 40 | 0.66 | 0.67 | 13.8 |
| 11 | CF | 30 | M | 1.75 | 62.6 | 176 | 337 | 50 | 360 | 61.4 | 35 | 0.35 | 0.17 | 5.3 |
| 12 | CF | 19 | M | 1.77 | 63.9 | 176 | 337 | 64 | >360 | 74.6 | 17 | 0.71 | 0.50 | 24.8 |
| 13 | CONTROL | 24 | M | 1.91 | 75 | 175 | 178 | 92 | 300 | 92.4 | 76 | 0.84 | 0 | 5.2 |
| 14 | CF | 16 | F | 1.55 | 51.8 | 167.7 | 280 | 123 | 300 | 151.8 | 47 | 0.40 | 0.50 | 19 |

| ID | Group | Age (years) | Sex | BSA ($kg/m^2$) | Weight (kg) | Height (cm) | Colonic volume AUC (L·min) | SBWC AUC (L·min) | OCTT (mins) | Gastric half-emptying Time (mins) | Small bowel motility, fasting (au) | $T_1$AC, fasting (secs) | PAC-SYM | CFAbd-Score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 | CONTROL | 29 | M | 1.95 | 75 | 182 | 250 | 63 | 180 | 64.7 | 146 | 0.58 | 0.09 | 2.7 |
| 16 | CONTROL | 19 | M | 1.75 | 63 | 176 | 325 | 51 | 360 | 87.1 | 85 | 1.11 | 0.17 | 9.5 |
| 17 | CONTROL | 35 | F | 1.53 | 52 | 163 | 88 | 36 | 240 | 79.6 | 88 | 1.37 | 0.83 | 13.1 |
| 18 | CONTROL | 21 | F | 1.66 | 59.3 | 167 | 153 | 69 | 180 | 64.1 | 19 | 0.72 | 0.58 | 17.3 |
| 19 | CF | 13 | M | 1.55 | 54.5 | 158 | 265 | 98 | >360 | 126.8 | 30 | 0.37 | 0.25 | 13.8 |
| 20 | CONTROL | 13 | M | 1.95 | 76.1 | 180 | 156 | 68 | 150 | 80.1 | 30 | 0.54 | 0 | 3.6 |
| 21 | CF | 36 | F | 1.54 | 54.2 | 158 | 590 | 198 | 300 | 40.1 | 40 | 0.90 | 0.50 | 27 |
| 22 | CONTROL | 19 | F | 1.91 | 81.7 | 160 | 266 | 71 | 360 | 81.4 | 100 | 1.40 | 0.50 | 18.6 |
| 23 | CF | 12 | M | 1.17 | 33.92 | 144.8 | 212 | 104 | >360 | 90.9 | 55 | 0.57 | 0.67 | 16.4 |
| 24 | CONTROL | 12 | M | 1.40 | 44.8 | 158 | 278 | 46 | 150 | 120.5 | 70 | 0.86 | 0 | 0 |
| 25 | CF | 19 | F | 1.69 | 60.3 | 170 | 247 | 35 | 360 | 75.9 | 137 | 0.33 | 0.25 | 32.3 |

## Appendix B: GIFT-CF 2 raw MRI and questionnaire data

** denotes participants underwent observer-blind study visits whilst on treatment*

| ID | Group | Age (years) | Sex | BSA ($kg/m^2$) | Weight (kg) | Height (cm) | OCTT (mins) | Gastric half-emptying Time (mins) | Colonic volume AUC (L·min) | SBWC AUC (L·min) | Delta TI (mL) | TI motility, fasting (au) | $T_1$AC, fasting (secs) | PAC-SYM | CFAbd-Score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1A | Placebo | 20 | M | 1.80 | 66.2 | 176 | 240 | 128 | 498 | 70 | -89 | 0.22 | 0.29 | 1.08 | 43 |
| 1B | Treatment | 20 | M | 1.78 | 64.9 | 176 | >360 | 122 | 793 | 98 | -102 | 0.11 | 1.14 | 0.83 | 45.1 |
| 1C | Placebo | 20 | M | 2.20 | 91.3 | 190 | 300 | 71 | 214 | 87 | -76 | 0.20 | 0.96 | 0.67 | 22.1 |
| 2A | Placebo | 14 | M | 1.74 | 65.1 | 167 | >360 | 91 | 328 | 109 | 2 | 0.20 | 0.38 | 0.83 | 25.3 |
| 2B | Treatment | 14 | M | 1.71 | 63.7 | 166 | >360 | 70 | 267 | 116 | 107 | 0.14 | 0.51 | 0.083 | 13 |
| 2C | Baseline | 17 | M | 1.79 | 64 | 177 | >360 | 88 | 332 | 133 | 19 | No data | 0.59 | 0.25 | 15.5 |
| 3A | Placebo | 22 | F | 1.53 | 53.6 | 158 | 180 | 109 | 189 | 47 | 164 | 0.34 | 0.43 | 0.67 | 17.4 |
| 3B | Treatment | 22 | F | 1.54 | 53.9 | 158 | 360 | 98 | 239 | 36 | 105 | 0.34 | 0.40 | 0.67 | 14.2 |
| 3C | Baseline | 31 | M | 1.83 | 72 | 169 | 300 | 74 | 263 | 76 | 54 | No data | 0.43 | 0 | 7.9 |
| 4A | Treatment | 20 | M | 2.20 | 91.7 | 190 | >360 | 94 | 254 | 81 | 55 | No data | 0.87 | 0.08 | 9.3 |
| 4B | Baseline | 20 | M | 2.20 | 92 | 190 | 360 | 103 | 241 | 140 | 39 | No data | 0.77 | 2.33 | 43.1 |
| 5A | Placebo | 36 | F | 1.56 | 56 | 157 | 360 | 66 | 699 | 91 | 7 | 0.14 | 1.00 | 0.75 | 20.3 |

| ID | Group | Age (years) | Sex | BSA ($kg/m^2$) | Weight (kg) | Height (cm) | OCTT (mins) | Gastric half-emptying Time (mins) | Colonic volume AUC (L·min) | SBWC AUC (L·min) | Delta TI (mL) | TI motility, fasting (au) | $T_1$AC, fasting (secs) | PAC-SYM | CFAbd-Score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5B | Treatment | 36 | F | 1.59 | 57.7 | 157 | 150 | 70 | 529 | 132 | -215 | 0.23 | 0.80 | 1 | 29.6 |
| 6A | Treatment | 17 | F | 1.58 | 53.5 | 168 | 240 | 121 | 271 | 67 | 216 | 0.24 | 0.64 | 1 | 26.6 |
| 6B | Placebo | 17 | F | 1.58 | 53.6 | 168 | >360 | 115 | 283 | 53 | 56 | 0.07 | 0.55 | 1.08 | 35.3 |
| 7A | Treatment | 25 | M | 1.69 | 58 | 177 | >360 | 80 | 317 | 140 | -145 | 0.09 | 0.50 | 0 | 1.4 |
| 7B | Treatment* | 25 | M | 1.75 | 62.6 | 177 | >360 | 73 | 356 | 147 | -120 | 0.10 | 0.58 | 0 | 1 |
| 8A | Treatment | 17 | M | 1.69 | 61 | 168 | 180 | 99 | 315 | 76 | 76 | 0.15 | 0.67 | 0 | 1.4 |
| 8B | Placebo | 17 | M | 1.68 | 61.2 | 166 | 300 | 99 | 253 | 41 | 55 | 0.17 | 0.42 | 0 | 1.4 |
| 9A | Baseline | 29 | F | 1.60 | 57 | 162 | >360 | 89 | 265 | 50 | 106 | 0.16 | 0.43 | 0.83 | 21.7 |
| 9B | Treatment* | 29 | F | 1.59 | 56.1 | 162 | >360 | 122 | 220 | 44 | -80 | 0.11 | 0.49 | 0.25 | 11.4 |
| 10A | Placebo | 31 | M | 1.82 | 70.6 | 169 | 360 | 43 | 273 | 16 | 5 | 0.10 | 0.55 | 0.17 | 6.4 |
| 10B | Treatment* | 31 | M | 1.90 | 76.8 | 169 | 240 | 58 | 258 | 66 | 57 | 0.22 | 0.50 | 0.25 | 7.5 |
| 11A | Treatment* | 13 | M | 1.11 | 29.4 | 150.6 | 180 | 89 | 302 | 58 | 287 | No data | 0.81 | 0.17 | 6.1 |
| 11B | Baseline | 13 | M | 1.20 | 36 | 146 | 300 | 83 | 221 | 83 | 442 | No data | 0.94 | 0.25 | 16.8 |
| 12A | Treatment | 17 | M | 1.79 | 65.4 | 177 | >360 | 43 | 355 | 144 | -172 | 0.08 | 0.65 | 0.58 | 20.2 |
| 12B | Placebo | 17 | M | 1.73 | 61.1 | 177 | >360 | 67 | 270 | 50 | 4 | 0.06 | 1.15 | 0 | 15.4 |

## Appendix C: Example GI symptom questionnaires used in the GIFT-CF 1 and GIFT-CF 2 studies

### PAC-SYM

This questionnaire asks you about your constipation in the **past 2 weeks**. Answer each question according to your symptoms, as accurately as possible. There are no right or wrong answers.

For each symptom below, please indicate **how severe** your symptoms have been during the **past 2 weeks**.

If you have not had the symptom during the past 2 weeks, check 0. If the symptom seemed mild, check 1. If the symptom seemed moderate, check 2. If the symptom seemed severe, check 3. If the symptom seemed very severe, check 4.

Please be sure to answer every question.

| **How severe have each of these symptoms been in the last 2 weeks** | **Absent** | **Mild** | **Moderate** | **Severe** | **Very Severe** |
|---|---|---|---|---|---|
| | **0** | **1** | **2** | **3** | **4** |
| Discomfort in your abdomen | | | | | |
| Pain in your abdomen | | | | | |
| Bloating in your abdomen | | | | | |
| Stomach cramps | | | | | |
| Painful bowel movements | | | | | |

SAMPLE COPY, DO NOT USE WITHOUT PERMISSION

| **How severe have each of these symptoms been in the last 2 weeks** | **Absent** | **Mild** | **Moderate** | **Severe** | **Very Severe** |
|---|---|---|---|---|---|
| | **0** | **1** | **2** | **3** | **4** |
| Rectal burning during or after a bowel movement | | | | | |
| Rectal bleeding or tearing during or after a bowel movement | | | | | |
| Incomplete bowel movement, like you didn't "finish" | | | | | |
| Bowel movements that were too hard | | | | | |
| Bowel movements that were too small | | | | | |
| Straining or squeezing to try to pass bowel movements | | | | | |
| Feeling like you have to pass a bowel movement but you couldn't (false alarm) | | | | | |

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**3-point Likert-scale**

The questions below are an example of the questionnaire that the participant completed after each scan time point.

**Time point: FASTING** **Time is now:** H H : M M

| check **ONE** box that **best** describes how bad each symptom is **right now:**<br>**0 = not at all; 1 = mild** (distinct but negligible)**;**<br>**2 = moderate** (annoying)**; 3 = severe** (disabling) | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Flatulence (Wind):** | **0** | **0.5** | **1** | **1.5** | **2** | **2.5** | **3** |
| | | | | | | | |
| **Bloating:** | **0** | **0.5** | **1** | **1.5** | **2** | **2.5** | **3** |
| | | | | | | | |
| **Abdominal Pain:** | **0** | **0.5** | **1** | **1.5** | **2** | **2.5** | **3** |
| | | | | | | | |

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Prof. Jochen Mainz (author of the CFAbd-Score questionnaire), granted the use of the CFAbd-Score questionnaire. However, reproduction of the questionnaire in this thesis was not permitted.

## Appendix E: Presentations arising from work done during this PhD

Ng C. GIFT-CF: Gut imaging for function and transit in cystic fibrosis. Oral presentation at the Cystic Fibrosis Trust community event, 2019.

Ng C, Smyth AR. Abdominal MRI in CF patients. Oral presentation at the European Cystic Fibrosis Society Regional Educational Activities Platform (ECFS-REAP) International Gastroenterology Meeting, 2019.

Ng C, Dellschaft NS, Hoad C, Marciani L, Gowland P, Major G, Smyth AR. Gut Imaging for Function and Transit in Cystic Fibrosis: Can we use MRI as a measure of gut problems in people with cystic fibrosis? Oral presentation at the European Cystic Fibrosis Society conference, 2019.

Dellschaft N, Ng C, Hoad C, Marciani L, Spiller R, Gowland P, Smyth AR, Major G. Characterising digestive disorders in Cystic Fibrosis using MRI: an exemplar condition to drive clinical application. Oral presentation at the International Society for Magnetic Resonance in Medicine British Chapter Meeting, 2019.

Ng C, Dellschaft NS, Hoad C, Barr H, Marciani L, Spiller R, Gowland P, Major G, Smyth AR. GastroIntestinal Function & Transit using Magnetic Resonance Imaging: The GIFT-CF Study. Oral and poster presentation at the North American Cystic Fibrosis Conference, 2019.

Ng C, Major G. MRI for Gastrointestinal Function and Transit: GIFT-CF. Oral presentation at the Lectures Education Awareness and Discussion (LEAD) in Cystic Fibrosis Meeting: core outcomes for gastrointestinal problems in CF, 2019.

Dellschaft N, Ng C, Hoad C, Marciani L, Spiller R, Gowland P, Smyth AR, Major G. Digestive disorders in Cystic Fibrosis: Transit, Motility and MRI Signs of Small Intestinal Bacterial Overgrowth. Oral presentation at the International Society for Magnetic Resonance in Medicine annual meeting (virtual), 2020.

Ng C, Dellschaft NS, Hoad C, Marciani L, Mainz JG, Hill T, Crooks C, Barr HL, Menys A, Spiller R, Gowland P, Major G, Smyth AR. Novel insights into the effect of tezacaftor/ivacaftor on gut function and transit in cystic fibrosis using MRI. Oral presentation at the European Cystic Fibrosis Society virtual conference, 2021.

Ng C, Dellschaft NS, Hoad C, Marciani L, Mainz JG, Hill T, Crooks C, Barr HL, Spiller R, Gowland P, Major G, Smyth AR. Effects of tezacaftor/ivacaftor on gut function and transit in CF: a randomised, double blind, placebo controlled, crossover trial. Oral and poster presentation at the North American Cystic Fibrosis virtual conference, 2021.

Marsh R, Gavillet H, Hanson L, Ng C, Major G, Smyth A, Rivett DW, van der Gast C. Intestinal function and transit relates to microbial dysbiosis in the CF gut. Poster presentation at the North American Cystic Fibrosis virtual conference, 2021.

Dellschaft N, Hoad C, Ng C, Marciani L, Spiller R, Smyth AR, Major G, Gowland P. Using texture analysis to detect changes in intestinal contents in people with cystic fibrosis. Oral presentation at the International Society for Magnetic Resonance in Medicine annual meeting (virtual), 2021.

## Appendix F: Publications arising from work done during this PhD

Ng C, Major G, Smyth AR. Dosing regimens for pancreatic enzyme replacement therapy (PERT) in cystic fibrosis (protocol). Cochrane Database of Syst Rev. 2019(12): CD013488.

Ng C, Dellschaft NS, Hoad CL, Marciani L, Ban L, Prayle AP, Barr HL, Jaudszus A, Mainz JG, Spiller RC, Gowland P, Major G, Smyth AR. Postprandial changes in gastrointestinal function and transit in cystic fibrosis assessed by Magnetic Resonance Imaging. J Cyst Fibros. 2021 Jul; **20**(4):591-597. Epub 2020 Jun 16.

Ng C, Major G, Smyth AR. Timing of pancreatic enzyme replacement therapy (PERT) in cystic fibrosis. Cochrane Database Syst Rev. 2021 Aug 2; 8(8):CD013488.

Dellschaft NS, Ng C, Hoad C, Marciani L, Spiller R, Stewart I, Menys A, Barr H, Gowland PA, Major G, Smyth AR. Magnetic resonance imaging of the gastrointestinal tract shows reduced small bowel motility and altered chyme in cystic fibrosis compared to controls. J Cyst Fibros. 2021 Dec 15; S1569-1993(21)02166-4. Online ahead of print.

Marsh R, Gavillet H, Hanson L, Ng C, Mitchell-Whyte M, Major G, Smyth AR, Rivett D, van der Gast C. Intestinal function and transit associate with gut microbiota dysbiosis in cystic fibrosis. J Cyst Fibros. 2021 Dec 8; S1569-1993(21)02155-X. Online ahead of print.