Title: POSTPRANDIAL CHANGES IN GASTROINTESTINAL FUNCTION AND TRANSIT IN CYSTIC FIBROSIS ASSESSED BY MAGNETIC RESONANCE IMAGING

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Highlights

- Gastrointestinal symptoms are experienced by the majority of CF patients.

- Oro-caecal transit time was 2 hours longer in CF than controls.

- Absent postprandial drop in small bowel water suggests partial ileal obstruction.

- Using MRI as an endpoint may facilitate future gut research in CF.

Abstract

Background

Cystic fibrosis (CF) is a multi-system genetic disorder affecting >72,000 people worldwide. Most CF patients experience gastrointestinal symptoms and can develop complications. However, the mechanisms of CF gut disease are not well understood. We evaluated gut function and transit in CF using magnetic resonance imaging (MRI). We hypothesised oro-caecal transit time (OCTT) is longer in CF; with lower small bowel water content (SBWC).

Methods

Twelve CF patients aged 12-40 years and 12 age and sex-matched controls underwent serial MRIs over 1 day with standardised meals. The primary endpoint was OCTT, assessed by the appearance of a food bolus in the caecum. Other measures included corrected SBWC and corrected colonic volume (both area under the curve, AUC), gastric half-emptying time and gastrointestinal symptoms.

Results

OCTT was longer in CF (CF 330 mins [270, >360] vs. controls 210 mins [173, 315], p=0.04), with no difference in gastric half-emptying times. Corrected SBWC was higher in CF (CF 62 L.min/m^2 [36, 80] vs. controls 34 L.min/m^2 [28, 41], p=0.021); minimal postprandial decrease between T240 and T300 (CF 13mL/m^2 [-13, 57] vs. controls 102 mL/m^2 [67, 108], p = 0.002) suggests impaired ileal emptying. Corrected colonic volumes were higher in CF (CF 186 L.min/m^2 [167, 206] vs. controls 123

L.min/m^2 [89, 146], p=0.012). There were no differences in gastrointestinal symptoms.

Conclusions

MRI provides novel insights into CF pathophysiology. Sub-clinical ileal obstruction may be more prevalent than previously thought. Gastrointestinal MRI shows promise as an investigational tool in CF.

ClinicalTrials.gov NCT03566550

Keywords

Cystic fibrosis; MRI; gastrointestinal function; gastrointestinal symptoms.

Abbreviations

cystic fibrosis (CF); cystic fibrosis transmembrane conductance regulator (CFTR); distal intestinal obstruction syndrome (DIOS); patient assessment of constipation symptoms (PAC-SYM); patient assessment of gastrointestinal disorders (PAGI-SYM; gastrointestinal symptoms rating scale (GSRS); magnetic resonance imaging (MRI); small bowel water content (SBWC); oro-caecal transit time (OCTT).

Background

Cystic fibrosis (CF) is an autosomal recessive disorder, affecting over 72,000 people worldwide.[1-3] The mutation with the highest prevalence is p.Phe508del and between 85-90% of CF patients have at least one copy of this gene mutation.[2,3] The mutation leads to a dysfunctional CF transmembrane conductance regulator (CFTR) protein which disrupts the passage of chloride ions and bicarbonate, causing increased viscosity of epithelial mucus. CFTR is expressed on many epithelia; including the gastrointestinal mucosa.[1]

In 2018, a James Lind Alliance Priority Setting Partnership involving patients, their families and clinicians identified the relief of gastrointestinal symptoms as one of the top priorities for CF research.[4]

Almost every individual with CF experiences gastrointestinal symptoms.[5,6] Nearly half report constipation.[7] The most severe gastrointestinal complication, distal intestinal obstruction syndrome (DIOS), is seen in 5% of patients annually.[3] Efforts to describe comprehensively the burden of gastrointestinal symptoms in CF, such as the North American GALAXY study,[8] have used questionnaires derived for use in functional bowel disorders such as the Patient Assessment of Constipation-Symptoms (PAC-SYM).[9] In the United Kingdom, use of the Gastrointestinal Symptoms Rating Scale (GSRS) has identified a high symptom burden in a large proportion of patients.[5] A CF-specific questionnaire (CFAbd-Score) has also been derived and validated[6,10]. Such work describes the prevalence and severity of symptoms but does not inform understanding of the underlying mechanisms.

The association between gastrointestinal mechanisms and symptoms in CF is not fully understood. It has been proposed that altered intestinal fluid secretions,[11]

5

dysmotility, dysbiosis and intestinal inflammation contribute to gut dysfunction.[12] Investigations to address this knowledge gap must avoid undue increase in the high procedural burden already experienced by CF patients. Endoscopy is invasive (although sometimes clinically indicated); radiation exposure should be limited as patients are already subject to chest radiographs and CT scans to monitor their chest disease; capsule technologies for physiological assessment are not approved for use in children.

Magnetic Resonance Imaging (MRI) has been used in research to study gut function non-invasively for over 20 years. Validated MRI measures of gastric emptying,[13] small bowel water content (SBWC),[14] oro-caecal transit time (OCTT),[15] and colonic volumes[16] have been applied to gut diseases such as irritable bowel syndrome,[16] inflammatory bowel disease and constipation.[17]

Aims

The aim of the of the Gut Imaging for Function and Transit in CF (GIFT-CF) study was to evaluate the feasibility and utility of MRI in assessing gut function and transit in CF patients. Our principal hypothesis was: OCTT is slower in CF patients based on previous report.[18] Our secondary hypotheses were CF patients have:

- less SBWC than controls due to CFTR dysfunction;
- larger colonic volumes because of the prevalence of constipation in CF;
- symptoms correlating with underlying physiological abnormalities.

Methods

We conducted a prospective study, using MRI to compare the postprandial changes in gut function and transit in people with and without CF.

Study population

All participants with CF were homozygous for the p.Phe508del mutation and aged between 12 and 40 years. CF patients were recruited from the tertiary service at Nottingham University Hospitals NHS Trust. CF patients were approached in order of clinic or ward attendance to reduce recruitment bias. CF patients were not required to have a history of gastrointestinal symptoms or complications. Controls, who had no clinical evidence or suspicion of CF, were recruited by open advertisement in the Nottingham area and were matched to cases by age and gender. Participants were excluded at screening if they met the exclusion criteria (Supplementary protocol).

Procedures

Participants spent one day at the Sir Peter Mansfield Imaging Centre, Nottingham, UK. They fasted from 20:00h the previous evening, other than a glass of water for essential medicines. On the day before scanning, participants avoided strenuous exercise and food and drink known to have laxative effects or contain highly fermentable carbohydrates. Participants stopped taking any laxatives or anti-diarrhoeals on the day of MRI scanning.

Firstly, participants completed the PAC-SYM[9] and CFAbd-Score[10] questionnaires and then underwent a fasting MRI scan, followed by their first meal. Serial MRI scans were performed after the meal, at set intervals (Figure 1), giving 11 scans in total. After each scan, participants scored their degree of abdominal pain, bloating

and flatulence on a Likert scale ranging between 0 (not at all) and 3 (severe/disabling), as used in the GSRS.[19] A second meal was eaten after the scan at 240 minutes.
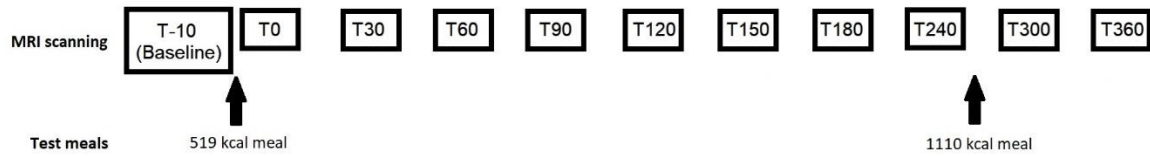
Figure 1. MRI study day schedule. Test meals are set standardised meals given to each participant. "T" is the time related to when the first test meal was eaten. For example, T0 is the MRI scan taken at 0 minutes after the first meal was eaten. Post-prandial MRI scans were performed immediately after the first test meal, then at 30 minute intervals for 180 minutes with further scans at 240, 300 and 360 minutes. The second test meal was given immediately after the T240 scan.

No food or drink was permitted during the scan day other than the meals provided. The first meal (519 kcal, fat 19 g, carbohydrate 77g) consisted of 300 g creamed rice pudding mixed with 25 g seedless raspberry jam and 30 g double cream, and a drink of 100 mL orange juice with 240 mL water (all Sainsbury's®, UK). The second meal (1,110 kcal, fat 54 g, carbohydrate 116 g) consisted of 400 g macaroni cheese (Sainsbury's®, UK), 100 g strawberry cheesecake (Rhokett®, UK) and 240 mL water. Participants with CF took their prescribed pancreatic enzyme replacement therapy with meals.

Scanning protocol

A 3T Philips Ingenia MRI scanner (Philips Healthcare, Best, The Netherlands) was used. Participants were positioned supine with the DS anterior coil over the abdomen. A plastic guard held the coil away from the participant's chest to allow for optimal breathing and a respiratory belt was used to monitor breathing patterns. An

initial survey scan determined the location of abdominal organs, followed by these sequences (see also supplementary MRI protocol):

1) Identifying OCTT and measure colonic volumes: dual fast field gradient echo sequence.

2) Aiding identification of the head of the meal at the caecum for OCTT analysis: high resolution balanced turbo field echo sequence.

3) Assessing gastric volumes: half Fourier turbo spin echo sequence.

4) Assessing SBWC: strongly T2-weighted turbo spin echo sequence.

All sequences required a series of breath holds, with the maximum breath hold time of 10 s, and acquired at 11 time points throughout the day (Figure 1). Participants spent up to 15 minutes inside the magnet for each time point and spent the rest of the study day upright in an adjacent room.

Data analysis

MRI analyses were performed using Medical Image Processing, Analysis and Visualisation (MIPAV, NIH, Bethesda)[20] and in-house software written in IDL® 6.4 (Research Systems Inc. Boulder, Colorado, USA). MRI data were relabelled by an independent researcher to ensure blinding to participants' group during MRI analyses.

Outcome measures

The primary outcome measure was OCTT, defined by the scan time when the meal was first detectable in the caecum (Supplementary figure 1).[15] OCTTs were analysed by two researchers, with review by a third researcher to resolve any disagreements.

Secondary outcomes included: SBWC corrected for body surface area (area under the curve, AUC); colonic volume corrected for body surface area (AUC); gastric volumes for gastric half-emptying time; and gastrointestinal symptom scores. Body surface area was calculated using Mosteller's formula ($\sqrt{[(\text{height (cm)} \times \text{weight (kg)})/3600]}$).[21]

Gastric[13] and colonic volumes[16] were determined as described previously by using MIPAV. Gastric volumes were measured at each scanning time point until the second meal (Figure 1). Time taken for the stomach to half-empty was calculated (gastric half-emptying time) from sequential volumes, until the volume dropped below 100 mL.[13]

Colonic volumes were determined for ascending, transverse, descending and recto-sigmoid segments.[16] Where segments were fully collapsed and not visualised on MIPAV, a volume of 0 mL was assigned. A random 10% of scans were reviewed by a second researcher. The limit of inter-observer variability was no more than 5%, in keeping with previous data.[17]

SBWC was analysed using in-house software as previously described.[14] Corrected colonic volume and corrected SBWC were calculated by the colonic volume or SBWC AUC over the scan time period divided by the body surface area.

CFAbd-Score[10] and PAC-SYM[9] questionnaires assessed gastrointestinal symptoms over the 2 weeks (low scores correlate with low symptom burden) preceding the scan day. Total CFAbd-Score[10] and PAC-SYM[9] score were calculated as previously described.

Symptom scores after each MRI time point were calculated from the sum of Likert scale component scores for abdominal pain, bloating and flatulence.[19]

Statistical analysis

Little prior data to base a power calculation were available so an informative pilot sample size was chosen. For OCTT, Kaplan-Meier curve and Log rank test were used. Non-parametric analyses (Wilcoxon signed-rank test) were planned for the secondary outcomes because of the matched study design and small sample size. Statistical analysis was carried out using RStudio, Inc (version 1.1.463, Boston). A p-value of less than 0.05 was considered significant.

Ethical Considerations

Approval was obtained from the UK National Research Ethics Committee (18/WM/0242). All participants gave written informed consent. Participants under 16 years gave written assent and parental consent. The study was registered on a publicly accessible trials database prior to commencement (ClinicalTrials.gov NCT03566550, Supplementary protocol).

Results

Study Progress

All procedures took place between August 2018 and February 2019. Fifty-one CF patients were eligible. CF patients were approached in order of their hospital attendance and the first 12 to provide written consent were enrolled into the study. Thirteen declined to participate and 2 withdrew consent as they did not like the standardised meals or MRI. None of the participants with CF had previous gut surgery and 1 had previous history of DIOS. Twelve age and gender-matched controls were also enrolled.

Twelve CF patients and 12 controls completed the protocol without any adverse events. One further control participant was withdrawn and replaced as the participant was unable to complete the first standardised meal and the remaining 10 MRI scans.

Although participants were age and gender matched, controls had a higher body weight than CF patients, reflecting the nutritional challenges in CF patients (Table 1).

Median (Inter-quartile range [IQR])	Control (n=12)	Cystic Fibrosis (n=12)	p-value
Age, years	19 (15, 25)	19 (15, 24)	0.42
Male, n (%)	7 (58)	7 (58)	
Weight, kg	62 (59, 75)	56 (53, 59)	0.005
Height, cm	166 (160, 176)	166 (158, 175)	0.33

Table 1. Baseline characteristics for control and CF groups at the time of MRI scanning.

Primary Outcome

Oro-Caecal Transit Time (OCTT)

OCTT was significantly longer in CF than controls, with a median difference of 2 hours (CF 330 mins [IQR 270, >360] vs. controls 210 mins [IQR 173, 315], p = 0.04). An OCTT above 360 minutes was not quantified as the last MRI scan was at 360 minutes postprandial. The meal had not reached the caecum in 4 CF patients, confirmed by a high intensity signal in the small bowel at 360 minutes; suggesting an OCTT greater than 360 minutes. All controls had an OCTT of 360 minutes or less.

Secondary outcomes

Gastric half-emptying time

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

Corrected Small Bowel Water Content (SBWC)

Median corrected SBWC AUC was higher in CF than controls. This was contrary to our *a priori* hypothesis. There was no difference in median baseline corrected SBWC (CF 135 mL/m^2 [IQR 90, 145] vs. controls 102 mL/m^2 [IQR 65, 120], p = 0.73, Wilcoxon). However, the median corrected SBWC AUC was higher in CF (CF 62 L.min/m^2 [IQR 36, 80] vs. controls 34 L.min/m^2 [IQR 28, 41], p = 0.021, Wilcoxon, Figure 2).
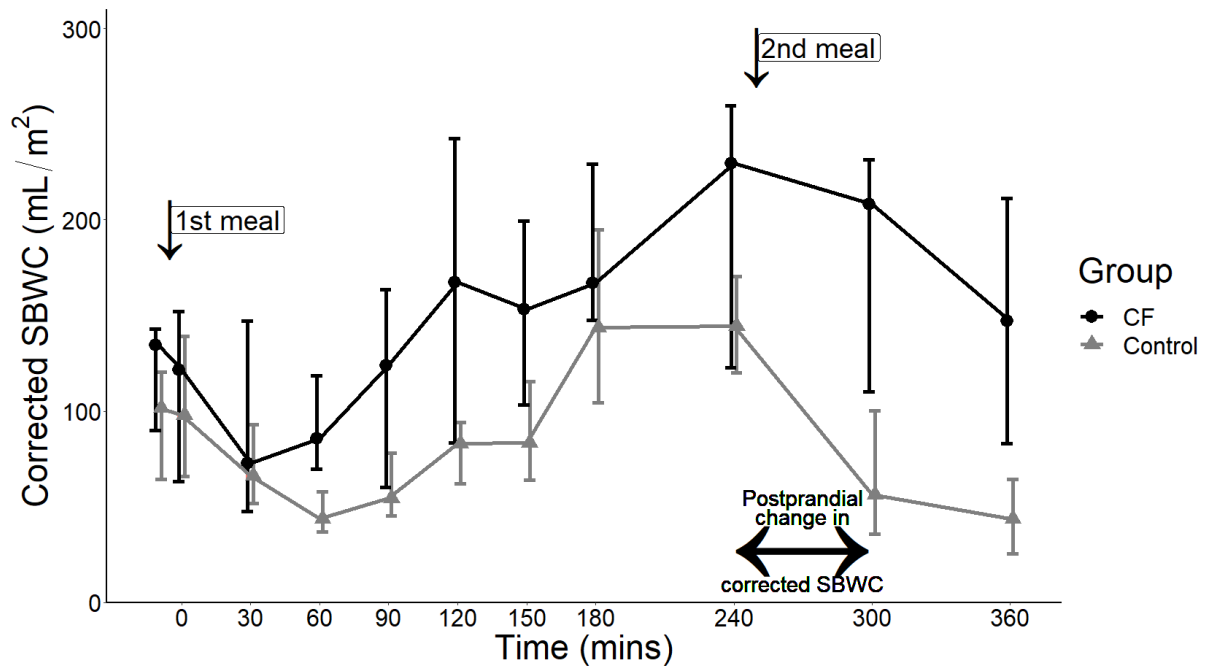
Figure 2. Corrected SBWC at each scanning time point for control and CF group. Error bars show median and IQR. Between 240 and 300 minutes, the drop in corrected SBWC (postprandial change in corrected SBWC) is reduced in the CF group compared to the control group.

The large drop in corrected SBWC after the second meal (T240 to T300 scans) seen in the control group was not observed in the CF group. Exploratory analysis showed only minimal decrease in median corrected SBWC in the CF group (CF 13 mL/m^2 [IQR -13, 57] vs. controls 102 mL/m^2 [IQR 67, 108], p = 0.002, Wilcoxon).

Corrected Colonic Volumes

No difference in median corrected baseline total colonic volumes was seen (CF 541 mL/m^2 [IQR 454, 688] vs. controls 387 mL/m^2 [IQR 351, 482], p = 0.092, Wilcoxon). The baseline recto-sigmoid colonic volume for one control was not visualised and so was assigned 0 mL. There was a median difference of 63 L.min/m^2 in corrected total colonic volume AUC between the two groups (CF 186 L.min/m^2 [IQR 166, 206] vs. controls 123 L.min/m^2 [IQR 89, 146], p = 0.012, Wilcoxon, Figure 3, Supplementary table 1). The transverse colonic volume at T360 for one control and recto-sigmoid

colonic volume at 6 time-points for another control were not visualised and so were
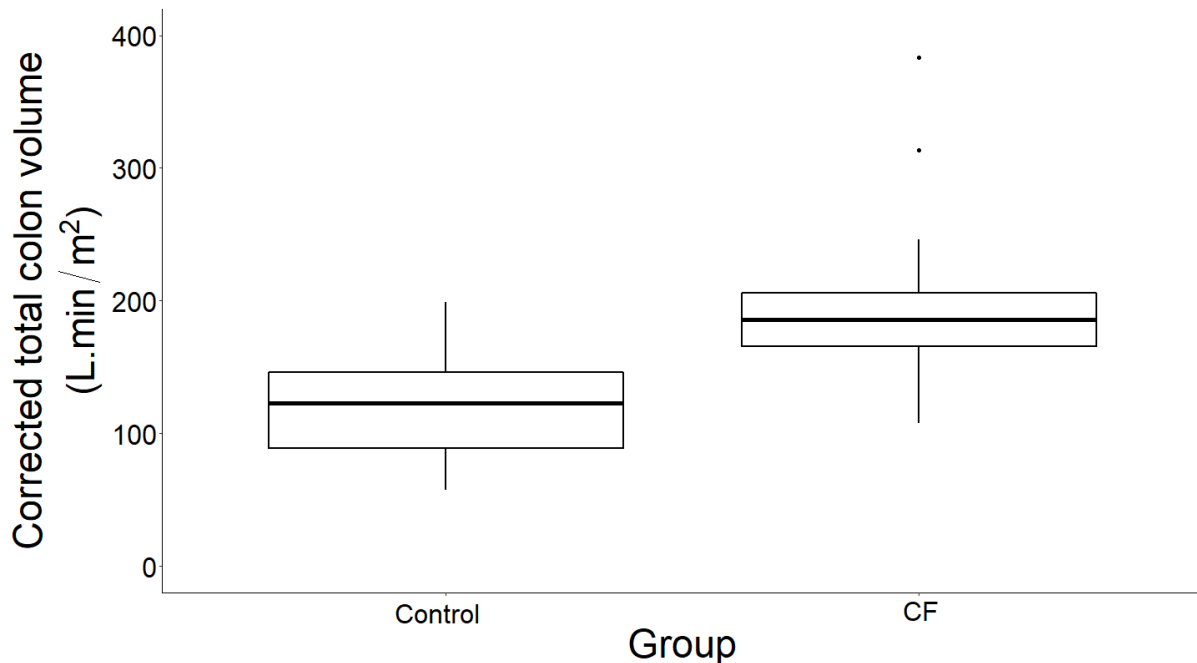assigned 0 mL.

Figure 3. Box and whisker plot indicating the median, IQR and outliers of the corrected total colon volumes (AUC)
for the control and CF group.

Symptoms

CFAbd-Score

Median total CFAbd-Score tended to be higher in CF, but this was not statistically
significant (CF 16 [IQR 5, 25] vs. controls 7 [IQR 3, 14], p=0.13, Wilcoxon). There
was no correlation between the CFAbd-Score and primary or secondary MRI
outcomes. The domains pain, disorders of bowel movement and impairment of
quality of life scored numerically higher in the CF group but this was not statistically
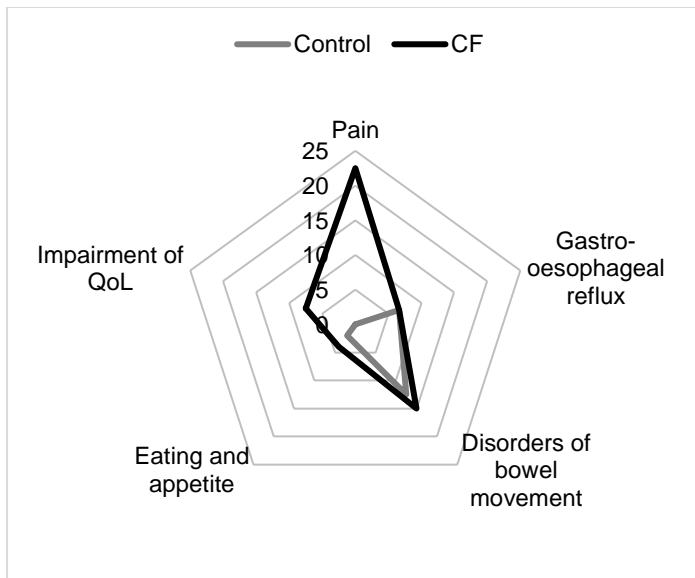significant compared to the control group (Figure 4).

Figure 4. Median domain scores for CFAbd-Score between control and CF group

PAC-SYM

No difference in median PAC-SYM score was seen (CF 5 [IQR 2, 7] vs. control 4 [IQR 1, 7], p=1, Wilcoxon), nor were there any correlations with MRI metrics.

Likert scale for flatulence, bloating and abdominal pain

Individual symptom scores and total scores using the Likert scale were low for both groups during the study day and not significantly different.

Exploratory outcome

Qualitative observation of the MR images identified appearances consistent with faecal material (usually seen in the large bowel) within the distal small bowel of CF patients. It was not seen in healthy controls or previously in similar studies (Supplementary figure 2).[16]

Discussion

Our study has shown, for the first time, that MRI can elucidate gut function and transit in CF. We demonstrated a prolonged OCTT in the CF group, as well as an increase in both corrected SBWC and corrected colonic volume compared to matched controls.

Our observation that OCTT is significantly longer in CF, whilst gastric half-emptying time is similar to controls, suggests that the delay in OCTT occurs in the small bowel and not the stomach. This is consistent with a previous report using the wireless motility capsule (SmartPill®).[18] Transit time in the control group was similar to a previous study of healthy individuals using MRI.[15] Our method assesses the normal physiological process by tracking the arrival of food in the caecum. This non-invasive observation contrasts with the SmartPill® which, due to its size, only leaves the stomach when propelled by a migrating motor complex and in association with gastric sieving.[18,22] Gastric sieving refers to the separation of aqueous and solid components of a meal and the two components are expelled from the stomach at different rates; the larger the particle, the slower the emptying.[23,24]

European guidelines[11] for DIOS in CF recommend the osmotic laxative polyethylene glycol (PEG) as first line treatment, although no randomised controlled trials have tested this approach. Treatment with PEG is consistent with the principle of hydrating viscous secretions and correcting reduced intestinal water content.

Contrary to our expectations, we observed a higher corrected SBWC AUC in CF. Higher SBWC volumes suggest an altered balance between intestinal secretion, absorption and motility. One plausible hypothesis would be that people with CF have an impaired capacity to absorb intestinal water because of their hyper-viscous

epithelial mucus.[25] However, the combination of increased SBWC and prolonged OCTT could be explained by two mechanisms: a disrupted gastro-ileal reflex and exaggerated ileal brake.

We observed a fall in SBWC following a second meal in the controls, in line with our previous MRI work.[26] This is due to the gastro-ileal reflex where ileal contents are discharged into the colon on the arrival of calorific material in the stomach.[27] The drop was markedly reduced in the CF group which may reflect restricted flow at the terminal ileum, a recognised site of pathology in CF.[11] The images which suggest faecal material in the terminal ileum support this interpretation.

The second possible mechanism is an exaggerated ileal brake, where ileal fat delays jejunal transit.[28] Inadequate pancreatic enzymes could increase fat in the terminal ileum and release gut-derived peptide YY and glucagon-like peptide-1 hormone.[28] This would further slow expulsion of small bowel contents into the caecum.

Both mechanisms raise questions about current treatment options for DIOS and the possibility that sub-clinical terminal ileum pathology exists. Stasis and prolonged transit times in the small bowel may lead to bacterial overgrowth and inflammation.[12]

Increases in colonic volumes in our CF group could reflect the increased prevalence of constipation as a similar increase is found in functional constipation with slow transit.[17] Our raw colonic volume data (Supplementary table 2, Supplementary table 3) are consistent with other studies for healthy controls[16] and constipation groups.[17]

The gastrointestinal tract continues to develop and grow until adulthood.[29] Although we matched the study groups for age and gender, we expected differences in nutritional status.[2,3] Previous studies have described increasing colonic volumes with height[16] and total intestinal length with height and weight.[30] Correcting data by body

surface area, rather than body mass index, is a recognised approach in paediatric care, such as to determine appropriate drug doses[31] and estimate glomerular filtration rates.[32] We therefore opted to adjust SBWC and colonic volumes by body surface area.

We assessed gastrointestinal symptoms as recent studies have shown significant differences between CF and control groups.[10] This was not evident in our study and may be because we excluded people with previous extensive gut surgery and unable to stop drugs affecting bowel habit. It may also reflect the small sample size; since studies demonstrating a difference in gastrointestinal symptoms generally require larger sample sizes.[5,6,9,10] Our study illustrates objective markers of CF gut function may be more sensitive in detecting an abnormality than subjective symptom report.

Strengths and weaknesses

Our completely non-invasive and radiation-free imaging technique allows a better understanding of the mechanism of disease in the undisturbed gastrointestinal tract. Despite concomitant lung disease, CF patients tolerated multiple breath-holds.

We have captured the dynamic changes of the CF gastrointestinal tract. Such a protocol is, however, time consuming and costly; and this may limit widespread uptake. Future research using MRI should refine protocols to aid implementation in clinical practice. In comparison, the wireless motility capsule can measure only transit and not luminal volumes. However these systems cost much less than MRI and do not require the patient to spend many hours in the research facility.

Another limitation is the need for a trained researcher to analyse OCTT. Future studies could use a less labour-intensive protocol to measure OCTT by employing

emerging MRI tools, such as indigestible MRI-visible mini-capsules which leave the stomach in the same phase as food particles during physiological gastric emptying and are more readily detectable in the intestine.[24]

Future directions

Future MRI studies should compare individuals with CF who have known gastrointestinal pathology with those who report minimal symptoms, to further explore the spectrum of CF gut pathophysiology. The same approach can then be applied to evaluating therapies for gastrointestinal symptoms or complications. MRI metrics may allow the evaluation of gastrointestinal effects of new CFTR modulator therapies.

Conclusions

This is the first study to demonstrate that investigating underlying gut pathologies in CF, using MRI, is achievable.

Our methodology has provided a platform for studying CF gut disease and demonstrated the potential mechanisms of underlying CF gut pathology. We have identified novel areas for future research on gut function in CF, in particular the reduced postprandial drop in SBWC which could reflect terminal ileum obstruction.

Further collaboration between the gastroenterology and CF communities are required to improve treatment and quality of life for CF patients.

Acknowledgements

Conflict of Interest Statement

NSD, CLD, LM, LB, HLB, AJ, PG have nothing to disclose.

CN, JGM and GM report grants and speaker honorarium from Vertex, outside the submitted work.

APP reports grants from Thrasher Research Fund, grants from Action for A-T, grants from Health Education East Midlands, outside the submitted work; these grants are for the development of lung MRI in children and infants.

RS reports grants from Zespri International Ltd and Sanofi-Aventis, as well as lecturing fees from Menarini and Alfawasserman, outside the submitted work.

ARS reports grants from Vertex, as well as speaker honoraria and expenses from Teva and Novartis and personal fees from Vertex, outside the submitted work. In addition, ARS has a patent issued "Alkyl quinolones as biomarkers of *Pseudomonas aeruginosa* infection and uses thereof".

Funding

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