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Nottingham**

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MAGNETIC RESONANCE IMAGING OF COELIAC DISEASE

**Thesis submitted to the University of Nottingham for the Degree of
Doctor of Philosophy**

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LIST OF ABBREVIATIONS

a-DGP	anti-Deamidated Gliadin Peptides
AD	Anno Domini
BMI	Body mass index
BRC	Biomedical Research Centre
B ₀	Magnetic field
CSF	Cerebrospinal fluid
CT	Computed Tomography
CONSORT	Consolidated Standards of Reporting Trials
DGP	Deamidated gliadin peptides
DNA	Deoxyribonucleic acid
EATL	Enteropathy associated T cell lymphoma
EMA	Endomysium antigen
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology, and Nutrition
EPI	Echo Planar Imaging
FBH	Fasted breath hydrogen
FFE	Fast Field Echo
fMRI	Functional Magnetic Resonance Imaging
FOV	Field of view
FSPGR	Fast Spoiled Gradient Echo
GCP	Good Clinical Practice
GFD	Gluten free diet
GI	Gastrointestinal

GLP-2	Glucagon-like peptide-2
H ₂	Hydrogen gas
HADs	Hospital anxiety and depression score
Hb	Haemoglobin
HC	Healthy control
HLA	Human leukocyte antigen
IBS	Irritable Bowel Syndrome
IEL	Intraepithelial lymphocytes
IgA	Immunoglobulin A
IgA-DGP,	Immunoglobulin A deamidated gliadin peptide
IgA-TG2	Immunoglobulin A Transglutaminase2
IgG-DGP,	Immunoglobulin G deamidated gliadin peptide
MMC	Migrating motor complex
MRI	Magnetic Resonance Imaging
MRC	Medical Research Council
NDDC	Nottingham Digestive Diseases Centre
MRCP	Magnetic resonance cholangiopancreatography
NIHR	National Institute for Health Research
NUH	Nottingham University Hospitals
NREC	National Research Ethics Committee
NMR	Nuclear Magnetic Resonance
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PHQ	Personal Health Questionnaire
PI	Principal Investigator at a local centre

PIS	Patient Information Sheet
QMC	Queen's Medical Centre
R&D	Research and Development
RCD	Refractory coeliac disease
REC	Research Ethics Committee
RF	Radio-frequency
SAE	Serious Adverse Event
SCFA	short chain fatty acids
SEM	standard error of mean
SBE	small bowel enema or enteroclysis
SBFT	small bowel follow through
SBWC	Small Bowel Water Content
SIBO	Small Bowel Bacterial Overgrowth
SPMIC	Sir Peter Mansfield Imaging Centre
SNR	Signal to Noise Ratio
SSFSE	Single Shot Fast Spin Echo
T1	Spin-lattice relaxation time
T2	Transverse relaxation time
TE	Echo time
TFE	Turbo field echo
TSE	Turbo Spin Echo
TMF	Trial Master File
TR	Repetition time
TTG	Tissue transglutaminase
UJ	Ulcerative jejunoileitis

WAPS	Weighted Average Position score
WGTT	Whole Gut Transit Time
VCE	Video capsule endoscopy

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RELATED PUBLICATIONS AND AWARDS

Publications

Lam, C., Sanders, D., Lanyon, P., Garsed, K., Foley, S., Pritchard, S., Marciani, L., Hoad, C., **Costigan, C.**, Gowland, P., Spiller, R. Increased fasting small-bowel water content in untreated coeliac disease and scleroderma as assessed by magnetic resonance imaging. United European gastroenterology journal, 2019. 7(10): p. 1353-1360.

Costigan, C. and Marciani, L. Tools/Methods for Quantifying Digestion: Medical Imaging Aspect, in Interdisciplinary Approaches to Food Digestion, S.F. Carbone, Editor. 2019, Springer, Cham.

Conference abstracts

Abnormalities of whole gut transit in newly diagnosed coeliac disease and effect of gluten free diet treatment demonstrated by magnetic resonance imaging. Federation of Neurogastroenterology & Motility (FNM 2020) Adelaide, Australia (March 2020).

MRI of whole gut transit time in newly diagnosed coeliac disease. International Society for Magnetic Resonance in Medicine, Paris, France 2018.

MRI imaging of coeliac disease. Contemporary practice in MRI session UK Radiological Congress, Liverpool, UK 2018

Using MRI to improve our understanding of coeliac disease. Coeliac UK Research Conference London, UK 2017.

Is MRI the future for the measurement of gastrointestinal function? UK Radiological Congress, Liverpool, UK, 2016.

Awards and prizes

College of Radiographers Overseas Conference Grant 2019 (£1000) to present at the Federation of Neurogastroenterology & Motility (FNM 2020) Adelaide, Australia March 2020.

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Developing Radiographer Research & Audit Skills Allied Health Professionals ICS Conference November 2019 (first prize).

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ABSTRACT

Background

Coeliac disease is an autoimmune disease affecting primarily the small bowel mucosa. Diagnosis is by blood test and duodenal biopsy, with the patient on a gluten-containing diet. The only treatment currently available is a gluten free diet (GFD). Further work is required to understand macroscopic gastrointestinal (GI) function and mechanisms of symptoms in coeliac disease. Magnetic Resonance Imaging (MRI) offers a unique tool to study GI fluid volumes, organ volumes and gut transit non-invasively.

Objectives

This study aimed to test the main hypotheses that:

In adults newly diagnosed with coeliac disease, treatment with a GFD will reduce the water content of the fasting small bowel, the volume of the fasting colon and whole gut transit time (WGTT).

This work also validated a new method for calibrating small bowel water content (SBWC) measurement on different MRI scanners.

Methods

36 newly diagnosed patients and 36 healthy controls were recruited. They attended for an MRI visit having fasted overnight. SBWC, colonic volumes and WGTT were measured. Symptoms, expired breath hydrogen, stool diaries and stool samples were also collected. This was then repeated at 12 months follow-up.

A validation study in healthy volunteers was carried out to calibrate and optimise the MRI scanner measurement of SBWC.

Results

No significant difference in SBWC was seen for the coeliac patients between baseline and 12 month follow up although there was significant difference in fluid volumes compared to the controls. Significant reductions were seen in the overall colonic volumes, and the ascending colon volumes in coeliac patients after treatment with a GFD. Ascending colon volumes and overall colonic volume were also larger in coeliac patients than in the controls both before and after treatment initiation with a GFD.

A significant decrease in whole gut transit time was seen in coeliac patients between baseline and 12 month follow up. Transit time between coeliac patients and healthy controls was significantly slower both at baseline (44%) and at 12 month follow up (29%).

Quality of life reported by the incident coeliacs was poorer than that in the healthy controls, even following GFD treatment. GI symptoms were significantly greater at baseline than at follow-up for the coeliac patients and although had improved following the GFD were still significantly higher than the controls at both time points. There was also a correlation seen between the severity of study day GI symptoms, and poorer PHQ_15 and overall HADS scores.

Conclusion

This study in patients with incident coeliac disease has shown for the first time that treatment with a gluten free diet for a year reduces the small bowel water, significantly reduces the overall colon volume and decreases the whole gut transit time.

The coeliac patients also have greater SBWC than healthy controls at baseline and at follow up. Furthermore, the colon volumes are also larger at the time of diagnosis of coeliac disease and reduce over 12 month follow up on a gluten free diet.

Finally, there is a significant reduction in whole gut transit time following a gluten free diet in the coeliac patients.

The increased SBWC in coeliacs before and after treatment compared to healthy controls may represent an imbalance between absorption and secretion due to the damaged villi.

These results support the underlying hypotheses of this work and show that MRI can be a useful non-invasive tool in evaluating coeliac patients and this work can add to our understanding of coeliac disease. This work provided also a new method to calibrate and standardise such small bowel water volume quantitation between scanners and laboratories.

1 INTRODUCTION

The primary aim of this work was to develop non-invasive MRI techniques in a single, fasting short scanning session to provide novel insights into coeliac (celiac) disease, the effect of a gluten free diet (GFD), and the relationship of gastrointestinal function and symptoms in patients with this disease.

In this chapter the background to coeliac disease and its diagnosis is introduced and then the radiological investigation of this condition is discussed in more detail.

1.1 INTRODUCTION TO COELIAC DISEASE

Coeliac disease is a chronic auto immune mediated disorder which demonstrates an increased immunological response to dietary gluten, an alcohol-soluble protein from cereals such as wheat, barley, or rye, in genetically predisposed individuals who carry one or both of the HLA-DQ2 or -DQ8 genotype [5]. It results in inflammation and enteropathy of the small bowel and requires a life- long commitment to GFD treatment. Aretaeus Of Cappadocia, a Greek physician from Cappadocia described the disease in the first century AD, when he defined “The Coeliac Affection” : “If the stomach be irretentive of the food and if it pass through undigested and crude, and nothing ascends into the body, we call such persons coeliacs”, - “koiliakos” from a Greek word “koelia” for abdomen

[6]. He also noted that this disease seemed to affect more women than men.

Centuries later the characteristic fatty stools of coeliac patients were documented by Mathew Baillie in 1814 in his "Observations On a Particular Species of Purging" [6]. These observations described 'a chronic diarrheal disorder of adults causing malnutrition' and characterised by 'a gas-distended abdomen'. He even suggested a dietary treatment, saying "Some patients have appeared to derive considerable advantage from living almost entirely upon rice."

Until the mid-20th century, coeliac disease was known as Gee-Herter disease – after Christian Herter, a physician in the United States who reported the characteristic malnutrition and growth retardation in children with this condition in 1908 and from the United Kingdom Samuel Gee who described the success of a 'farinaceous' free diet in 1887 [7].

In the 1920s in the United States a new successful (albeit for a mistaken belief) dietary treatment was introduced - 'the banana diet' when Sidney Haas described a successful treatment of eight children diagnosed with coeliac disease. Based on a successful treatment of a case of anorexia with a banana diet, he experimented with the same diet treatment, which excluded many carbohydrates, in these eight children who were also anorexic. He published a series of ten cases, eight of them 'clinically cured' with this diet and the two untreated died. Although ethically dubious by modern standards and based on a false premise, Haas' insistence that carbohydrates were the culprit in this disease, and the exclusion of bread, crackers, potatoes and all cereals, probably saved

some lives. With hindsight it is evident that his success was because of elimination of gluten-containing grains but Haas continued to be resistant to any dissenting opinions and insisted for many years despite new evidence from Europe that on his banana diet regime “all patients are cured by the specific carbohydrate diet, a cure which is permanent without relapse”

The breakthrough that Haas chose to downplay that was to change radically the treatment of coeliac disease was when wheat was identified as a trigger in the 1930s by the Dutch paediatrician Dr Willem Dicke [6, 8]. He wrote his thesis on a remarkable series of observational clinical studies which resulted in a breakthrough in the understanding of the toxic effects of wheat protein in coeliac disease.

He first started his experiments with a wheat-free diet between 1934 and 1936 [9]. He then observed during periods of food shortage during the Dutch famine of 1944, the symptoms of his patients improved when bread was replaced by different non-cereal-containing substitute foods [7, 9, 10]. Subsequently when bread was reintroduced after the wartime food shortages, he noticed symptoms recurring.

Together with J H van de Kamer, another Dutch researcher who was a biochemist at the Netherlands Central Institute for Nutritional Research TNO in Utrecht Dicke developed a test to measure faecal fat content in wet faeces. The pair together with H A Wyers, a paediatrician, then experimented with standardised diets to give children cereal challenges and measure faecal fat content to give a faecal fat coefficient. They subsequently concluded that ‘the alcohol soluble or the gliadin

component of the water insoluble protein or gluten moiety of wheat, was responsible for the fat malabsorption in patients with coeliac disease'. The development of the gluten free diet was based on these discoveries [9].

A new method for diagnosis this disease appeared in the 1950s when German-British gastroenterologist and medical researcher Margot Shiner developed a new jejunal biopsy tube to reach and biopsy the distal duodenum. A more useable and less cumbersome capsule (which bears his name) was developed shortly after by the United State Lieutenant Colonel Crosby [11]. Shiner's innovative use of biopsy tubes to diagnose coeliac disease and lymphoma in children was credited with launching paediatric gastroenterology as its own distinct subspecialty [12]. Access to these biopsy tools finally allowed doctors to see a specific, recognisable pattern of damage to the proximal small intestinal mucosa previously only observable at surgery or post mortem.

So, at the beginning of the 1960s it was known that gluten was the trigger for coeliac disease, that there was associated characteristic damage to the proximal small bowel mucosa and there was now an instrument that allowed biopsies of the region without open surgery.

However, as there are other causes of small bowel mucosal atrophy, diagnosis was not considered definitive unless complete remission of the atrophy on a gluten free diet, and its recurrence once gluten was reintroduced into the diet, was seen. These criteria were formalized in 1969 by a panel of experts in the emergent European Society for Paediatric Gastroenterology (now ESPGHAN) as the "Interlaken criteria"

which for more than 20 years were the gold standard diagnostic criteria internationally [13].

The guidelines required for a definitive diagnosis of coeliac disease 'at least three small bowel biopsies demonstrating severe villous atrophy at diagnosis, remission on a gluten-free diet and relapse after gluten provocation'. As these guidelines were being implemented discoveries were also being made related to the blood antibodies being seen in the coeliac population. Berger detected and then reported the first anti-gliadin antibodies in 1964 in coeliac children, followed by Seah and his team who identified the auto-antibody anti-reticulins seven years later [14]. It was to take several years from this for their diagnostic usefulness to be properly recognised in clinical use. In 1997 Detlef Schuppan discovered tissue transglutaminase (TTG2) as an autoantigen in coeliac disease [15] and this has been described as a paradigm shift in coeliac disease research.

Studies such as the large Italian multi-centre trial in the 1980 led to the ESPGHAN [16, 17] guidelines being revised in 2012 where the recognition of the role in diagnosis of coeliac-specific antibodies - autoantibodies against Transglutaminase 2 (TTG), including endomysial

antibodies (EMA), and antibodies against deamidated forms of gliadin peptides (DGP) was recognised (Figure 1-1).

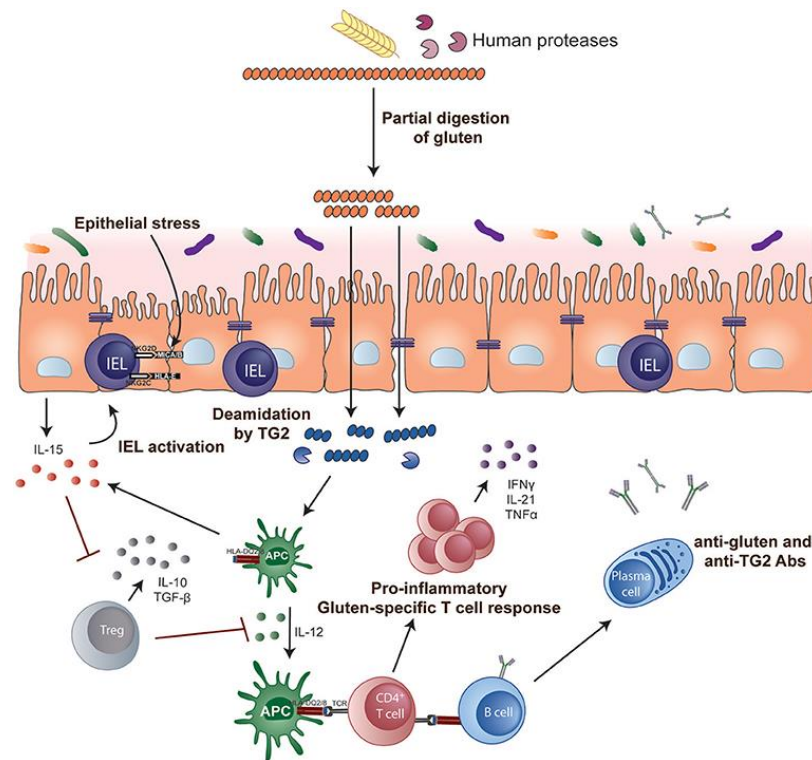


Figure 1-1 Key steps in coeliac disease pathogenesis [3].

The Marsh classification was introduced in 1992 by Michael Marsh, a pathologist at the University of Manchester, as a way to describe the stages of the histological changes as seen in the small intestinal mucosa under a microscope [18]. The initial classification system of four stages of damage, type I to type IV, has been modified since then but remains a foundation for diagnosing the disease [19, 20]. Marsh understood that there is a spectrum of inflammatory changes in coeliac disease – up to then it was thought that only the most advanced changes (i.e. Marsh III-IV) characterised the disease.

So these detailed descriptions allowed clinicians to look at the severity of the small bowel damage as well as monitor response to the gluten-free diet treatment. Rostami et al [21] argued for greater emphasis on IEL numbers rather than Oberhuber's sub grading based on degree of atrophy as his commentary with Marsh in 2016 [21, 22] on the difficulties of characterising mucosal biopsies argued that '*villi are progressively amalgamated into raised hypertrophic (mosaic) plateaux, well above the arbitrary crypt-villus junction*' which makes them difficult to accurately classify.

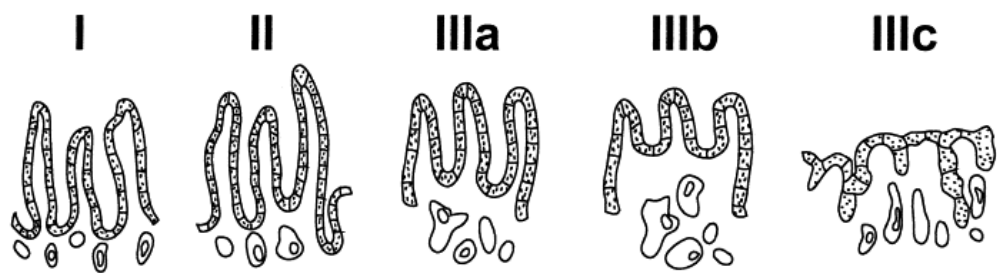


Figure 1-2 Marsh Grading of histologic findings in coeliac disease [2].

1.2 CONTEMPORARY UNDERSTANDING OF COELIAC DISEASE:

In coeliac disease symptoms occur when a person who is genetically predisposed develops an immunological response to an alcohol-soluble fraction of gluten called gliadin. In these individuals, when gluten-containing food is consumed tissue transglutaminase (TTG) is then released. TTG is an intracellular enzyme that is released from endothelial cells (among others) as a result of inflammation or mechanical irritation. It also functions as an antigen. Even though the exact pathophysiological role of its antibody Immunoglobulin A (IgA) is unknown, the detection is central to the diagnosis of coeliac disease [16]. Figure 1-2 describes the gluten trigger pathway

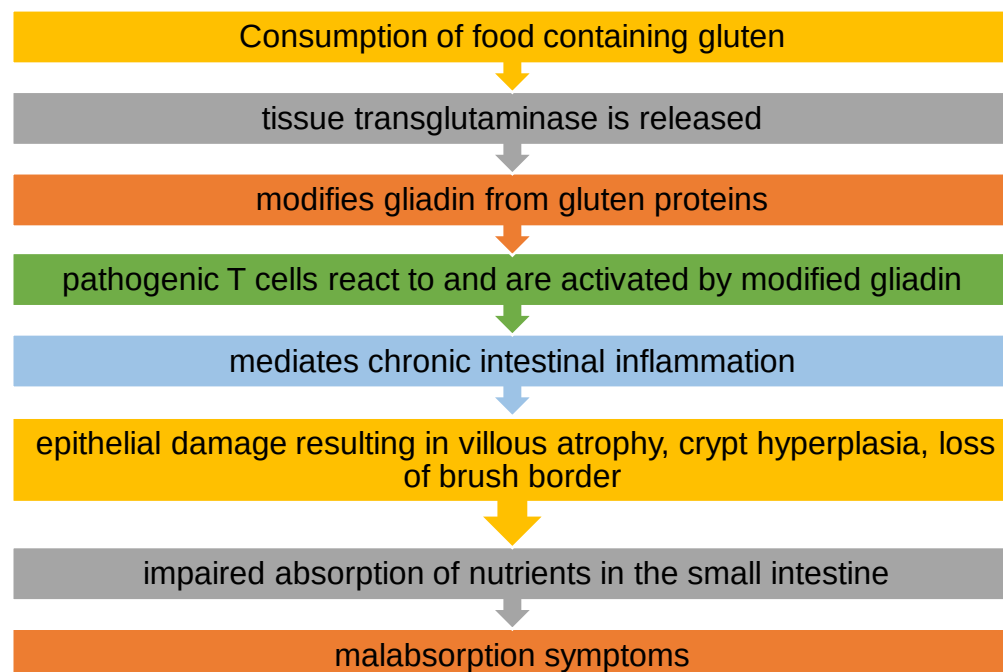


Figure 1-2 The gluten trigger pathway in coeliac disease.

The only available treatment currently is a strict lifelong avoidance of dietary sources of gluten. The aim of this gluten free diet (GFD) is recovery of the small bowel mucosa and reversal of the enteropathy. Despite a long-term adherence to a GFD, a significant proportion of coeliac patients have been reported to have persistent gastrointestinal (GI) symptoms [23-25]. For example, 25% of 596 people with treated disease had persistent symptoms such as abdominal pain and bloating despite claiming to adhere to a strict GFD [26].

Failure to improve symptomatically on a gluten-free diet is most commonly due to exposure to gluten, but may be due to other concurrent GI disorders such as Small Bowel Bacterial Overgrowth (SIBO) and Irritable Bowel Syndrome (IBS) (there is a reported IBS prevalence of 22% in patients with coeliac disease [27]) and complications of the disease such as ulcerative jejunitis, refractory coeliac disease (RCD), and enteropathy-associated T-cell lymphoma.

RCD is defined using the Oslo classification as *'persistence or recurrence of Malabsorptive symptoms and signs, together with villous atrophy on small intestinal biopsy, despite maintenance of an adequate gluten-free diet for over 12 months'* (**Appendix 1 Accepted nomenclature for Coeliac Disease [166]**).

1.3 EPIDEMIOLOGY

The reported prevalence of coeliac disease is increasing around the world [28, 29] and 1 in 4 individuals with the disease are undiagnosed. Non-Coeliac Gluten sensitivity is also on the increase with individuals who do not have coeliac disease but report an intestinal or non-intestinal symptoms from ingesting gluten which resolve on gluten withdrawal from their diet [30].

The female predominance in adult coeliac disease prevalence is also well documented with up to almost twice as many women diagnosed as men [31], with the average age at diagnosis also lower for women [24]. This matches the sex distribution that is characteristic of other autoimmune diseases such as Sjogren's syndrome, scleroderma, lupus, Hashimoto's thyroiditis and Grave's disease [32].

The discovery of antigliadin antibodies led to the identification of the first serological marker for coeliac disease in 1961 [33]. Although serological screening is now widely used for detection, in adults duodenal biopsies whilst on a diet containing gluten are still considered essential for diagnosis [5, 34].

Any non-invasive or less invasive diagnostic tool would be beneficial, especially for follow up of refractory disease. Traditional radiological small bowel imaging investigations such as barium fluoroscopic studies (small bowel enteroclysis and barium follow through) are being replaced by Computed Tomography (CT) and Magnetic resonance Imaging (MRI).

1.4 INVESTIGATION OF COELIAC DISEASE

Demonstration of villous atrophy in the small bowel is required to definitively diagnose coeliac disease in adults [35]. This small bowel damage may result in malabsorption of essential nutrients such as folic acid, iron, and fat soluble vitamins resulting in a variety of clinical signs, some of which are non-specific. Reports from England [36], Scotland [37], and North America [38, 39] have shown that almost half of patients with newly diagnosed coeliac disease do not present with gastrointestinal (GI) symptoms.

The incidence of coeliac disease is much higher in patients who have a first-degree family member diagnosed with the disease [40]. The European Society of Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) concluded that coeliac disease meets the criteria of a true autoimmune disease for which the genetic susceptibility (HLA), external trigger (gluten), and autoantigen (TTG) are known [35].

In countries where most of the population is of European genetic background, the prevalence of coeliac disease is about 0.5%–1% of the population (1). The epidemiological changes of coeliac disease are described using the 'iceberg' model (Figure 1-3), originally described by Richard Logan in 1991 [41] and refined subsequently as more is discovered about this disease. In this analogy the peak above the water level represents the clinical manifestations of coeliac disease, all the patients will have villous atrophy, elevation of their blood antibodies and at least one of the genetic markers known to be necessary for coeliac

disease, HLA-DQ2 or DQ8. Beneath the waves lies the patients with 'silent' disease, they will show the same markers of coeliac disease as the patients above the waterline but will have minimal or no symptoms. The lowest, and largest part of the iceberg has the largest number of coeliac patients, who will have the genetic markers, and may have elevated antibodies but will have no symptoms and little or no intestinal damage [42].

Patients with coeliac disease have an overall reduced quality of life, increased risk of haematinic deficiencies, reduced bone mineral density and modest increased risk of certain malignancies. More recently other comorbidities such as non-alcoholic fatty liver disease (NAFLD) [43] chronic pancreatitis [44] and autoimmune hepatitis [45] have been reported. Reilly et al ([43] in a population based cohort study described a higher risks of NAFLD in the first year after coeliac disease diagnosis which persisted through 15 years after diagnosis. There are potential non-dietary therapies being developed for coeliac disease but currently none of them have progressed to Phase III trials [46].

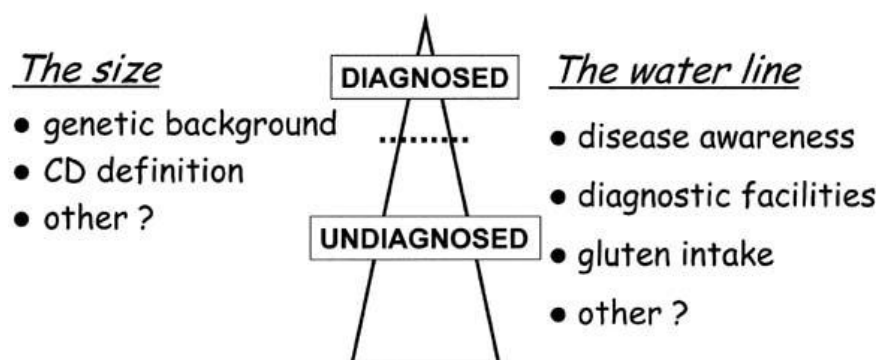


Figure 1-3 Factors influencing the 'coeliac iceberg' [42].

1.5 WHOLE GUT TRANSIT IN COELIAC DISEASE.

Observational studies involving manometric, breath test and camera pill measurements have observed an underlying gastrointestinal dysmotility in coeliac disease. Oesophageal transit and oro-coecal transit times [47-49] are delayed, and colonic transit is faster [49]. In untreated coeliacs these GI motor disorders tend to resolve on gluten free diet treatment [49, 50] suggesting that inflammatory mechanism is related to the motor dysfunction [48]. This generalised small bowel hypomotility with abnormal jejunum dilatation [51] is thought to be functional due to impaired motor response to jejunal distension [52].

In 1987, Spiller *et al* [47] described how the steatorrhea of coeliac disease is often paradoxically associated with slow small bowel transit despite normal gastric emptying times [47]. In the early 1980s the concept of fat, normally absorbed proximal to the ileum [53], passing through to the ileum in untreated coeliacs inhibiting jejunal transit -the 'ileal brake'- was suggested [54, 55]. In untreated disease transit times may also be related to Body Mass Index (BMI), with lean patients showing more small bowel transit delay [50]. Patients with untreated coeliac disease also have been shown to have larger fasting gallbladder volumes, delayed gall bladder emptying [56] and slower gastric emptying [57, 58]. Fraquelli *et al*'s study attributed delayed gall bladder emptying in untreated coeliac disease to elevated somatostatin level and the delayed gastric emptying seen to decreased cholecystokinin secretion- these were observed to reverse on GFD treatment [56].

1.6 HOW IS COELIAC DISEASE DIAGNOSED?

In adults coeliac disease is usually diagnosed firstly by a blood test for serologic testing of coeliac-specific antibodies followed by endoscopy to obtain duodenal mucosal biopsies. Both these investigations should be performed on a gluten-containing diet [35]. In 1970 the ESPGHAN guidelines required for a definitive diagnosis of coeliac disease performing at least 3 small bowel biopsies demonstrating severe villous atrophy at diagnosis, remission on a gluten-free diet and relapse after gluten provocation. This challenging regime was revised in 2012 and now guidelines from the same body suggest in symptomatic children positive coeliac serology may be sufficient for diagnosis [16]. This is further defined in the most recent 2019 ESPGHAN guidelines for diagnosing coeliac disease “*The no-biopsy approach for CD diagnosis is safe in children with high TGA-IgA values (≥ 10 times the upper limit of normal) with appropriate tests and positive endomysial antibodies (EMA-IgA) in a second serum sample*” [59].

However in the adult population recommendations from the British Society of Gastroenterology in 2014, based on a comprehensive review of the literature by a multidisciplinary panel of experts from the USA and Europe, (including the collaborators for this study at Department of Medicine and Surgery, University of Salerno, Italy) state that ‘*Biopsy remains essential for the diagnosis of adult CD and cannot be replaced*

by serology' [34]. One drawback in coeliac disease research has been the lack of a reliable and reproducible animal model, the only exception being the Irish (red) setter dog, which may develop a gluten-related enteropathy [60].

1.7 COELIAC DISEASE SEROLOGY TESTING

Serologic tests, such as assays for anti-tissue Transglutaminase IgA antibodies (anti-TTG), anti-Deamidated Gliadin Peptides IgA and IgG antibodies (a-DGP IgA/IgG) and anti-Endomysium IgA antibodies (EMA) are used to screen for coeliac disease [14, 61]. Most untreated coeliacs will have both IgA anti-TTG and endomysial antibodies. However no antibody test currently available provides a sensitivity and specificity of 100% [61].

The ESPGHAN Criteria 2019 suggest using exclusively total serum IgA and anti-tissue Transglutaminase IgA antibodies (anti-TTG) in paediatric coeliac disease screening [59]. They recommend that if anti-TTG is raised – but less than 10 x upper limit of normal for assay then 4 biopsies from the distal duodenum and at least 1 from the bulb should be taken for a diagnosis. Strict adherence to a GFD in most patients will lead to a significant reduction in antibodies in twelve to twenty four months but this does not necessarily reflect the regrowth of the intestinal villi [62].

1.8 ROLE OF HUMAN LEUKOCYTE ANTIGENS (HLA) IN THE DIAGNOSIS OF COELIAC DISEASE

Coeliac disease is very strongly associated with HLA-DQ2 and/or HLA-DQ8 genotypes. Patients who do not have these HLA genotype pairs are very unlikely to have the disease however the positive predictive value is very low, as many of the population may carry either of these genes and do not have coeliac disease [63].

Genetic testing may be useful in some circumstances- if a patient has commenced a GFD, conventional testing will not be useful but if HLA-DQ2 and/or –DQ8 are not present, the individual does not have and will never develop coeliac disease [64].

Testing (case finding) might be also be useful to determine if individuals in higher risk groups (such as family members, type I diabetics, autoimmune thyroiditis, and other autoimmune disorders) should be screened for coeliac disease.

HLA-DQ2 is associated with most cases of coeliac disease, whereas HLA-DQ8 is present in just a minority of patients [65].

Furthermore, studies in siblings and identical twins suggest that the contribution of HLA genes to the genetic component of coeliac disease is less than 50% [66].

1.9 ENDOSCOPIC BIOPSY AND COELIAC DISEASE

Although there are characteristic features of villous atrophy on endoscopy, a normal appearance does not exclude villous atrophy so the endoscopic appearance of the duodenum alone should not determine whether biopsy is performed. The pattern of villous atrophy may be patchy in coeliac disease; therefore multiple specimens from the bulb and the distal duodenum are essential. A large US study on 132,352 patients demonstrated that obtaining at least four biopsy specimens doubles the diagnostic rate compared with patients who have less than four biopsies [67].

The progression of mucosal damage was first described by Marsh *et al* [18] and has been modified by Oberhuber (Table 1) amongst others into a grading of histologic damage that reflects the degrees of villous atrophy and inflammatory change [18, 68]. The degree of crypt hyperplasia and the villous structure are used to give a grading from 0 to 3c.

Counting intraepithelial lymphocytes (IEL) was considered central to the histological diagnosis of coeliac disease but no definitive 'normal' IEL range had ever been published until the 2018 multi-centre study by Rostami *et al* which demonstrated no differences between IEL count in Marsh III a, b and c, indicating that the subdivision of Marsh III may have no clinical value [21].

Grade Histological features	
0	Normal Mucosa
1	Increased number of IELs, usually exceeding 20 per 100 enterocytes
2	Proliferation of the crypts of Lieberkühn
3	Variable villous atrophy
3a	Partial villous atrophy
3b	Subtotal villous atrophy
3c	Total villous atrophy

Table 1 Modified Marsh Oberhuber grading of histologic damage for coeliac diagnosis [69]

Recently Corazza and Villanacci have attempted to simplify the Marsh–Oberhuber classification from five to three stages as seen in **Figure 1-4**.

- Type 0: Normal; celiac disease highly unlikely.
- Type 1: Seen in patients on gluten free diet (suggesting minimal amounts of gluten or gliadin are being ingested); patients with dermatitis herpetiformis; family members of celiac disease patients, not specific, may be seen in infections.

- Type 2: Very rare, seen occasionally in dermatitis herpetiformis.
- Type 3: Spectrum of changes seen in symptomatic celiac disease.

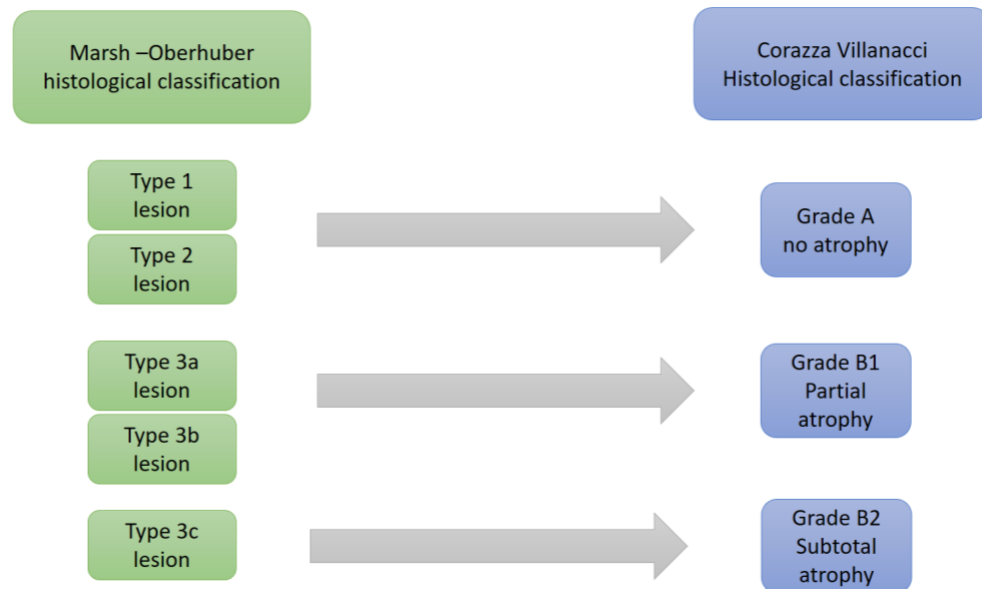


Figure 1-4 Comparison between two classifications for the grading of duodenal biopsy in coeliac disease [61].

In patients with coeliac disease who are on a gluten- containing diet, the abnormalities observed include:

- partial to total villous atrophy
- elongated crypts
- increased mitotic index in the crypts
- increased intraepithelial lymphocytes (IELs)
- infiltrations of plasma cells and lymphocytes as well as mast cells, eosinophils and basophils in the lamina propria
- flattened, cuboidal epithelium

Orientation of the biopsy is very important as only a correctly oriented biopsy allows proper evaluation of the villi/crypt ratio and a correct count of intraepithelial CD3+T lymphocytes [34]. It is very important that the specimens are reviewed by an experienced pathologist familiar with the spectrum of mucosal changes in coeliac disease [68]. Histologic recovery in patients who have coeliac disease usually takes several months but can take years, even if the patient adheres to a strict gluten-free diet [70].

1.10 VIDEO CAPSULE ENDOSCOPY

Video capsule endoscopy (VCE) uses a small easy to swallow wireless video camera to endoscopically image the lining of small bowel. The capsule (about 23 mm long) (Figure 1-5) contains a light source, a camera, a radio transmitter, and a battery. After swallowing the capsule the camera transmits about two images per second as it passes through the GI tract. The images are stored on a data recorder with small sensor pads worn by the patient and subsequently downloaded onto a workstation for viewing and evaluation [71].

The recording takes about 8 hours to complete. VCE and MR enteroclysis are considered complementary modalities. MR enteroclysis is more specific than VCE, while both produced the same sensitivity (52), although VCE cannot be used in patients with swallowing disorders, or bowel stenosis (due to the risk of bowel obstruction), and may only be

used in patients with implanted devices such as cardiac pacemakers under certain conditions.

As with MRE, if the dietary preparation has not worked or the bowel is not clean enough, the examination images may be inadequate.

It is also worth noting that patients with retained capsules must not undergo MR examinations as the capsules are not MR compatible.



Figure 1-5 A Wireless video capsule for Video Capsule Endoscopy investigation (orange arrow).

VCE has been used in coeliac disease diagnosis and follow-up, and although it is not as accurate as endoscopic biopsy in a recent meta-analysis the overall VCE sensitivity was 89% and its specificity 95% [72] and it is found to be more acceptable to patients than conventional endoscopy.

1.11 HYDROGEN BREATH TESTS AND COELIAC DISEASE

Measurement of fasting breath hydrogen (H_2) excretion gives an estimation of H_2 production in the bowel [73]. The only source of H_2 in the breath is from bacterial fermentation in the bowel. Bacteria will only

produce H₂ gas in the large bowel if dietary carbohydrates are not absorbed in the small bowel.

Simple non-invasive breath tests can detect H₂ gas produced by bacterial fermentation of unabsorbed carbohydrate in the intestines and may be used in the diagnosis of malabsorption of carbohydrates, the presence of small intestinal bacterial overgrowth (SIBO), and for measuring transit time [74]. Higher levels of fasting breath hydrogen have been seen in untreated coeliacs in 1987 with the levels reduced after a GFD, although this may be due to a concomitant SIBO. In the patients in this study who did not demonstrate a reduction in fasting breath H₂ after a GFD, a degree of villous atrophy was still present on biopsy [75]. A high prevalence of SIBO in coeliac patients with persistence of gastrointestinal symptoms after gluten withdrawal has also been reported [76].

A sorbitol breath test in which breath H₂ levels were measured both at baseline and after ingestion of a 2% sorbitol solution at 30 minute intervals for four hours was shown to reliably detect impaired GI function in children with coeliac disease [77]. Comparing H₂ breath tests with EMAs in subclinical coeliac patients showed both a correlation with abnormal fasting breath H₂ and histological lesions and that the prevalence of EMA in undiagnosed coeliac disease is lower than expected [78].

1.12 FAECAL MICROBIOTA

Studies have demonstrated intestinal dysbiosis in untreated coeliac disease and in patients with the disease following a GFD treatment [79, 80] and there are suggestions that abnormal intestinal microbiota may contribute to GI symptoms in coeliac disease, especially in those with persisting symptoms despite following a GFD.

It is also thought that their microbiota differ to those whose symptoms resolve on this diet treatment, with a reduced microbial richness and Proteobacteria-rich microbiota [80]. The role of microbiota in treated coeliac disease patients whose symptoms persist despite adherence to a GFD is unknown and may be accounted for by concomitant irritable bowel syndrome or SIBO [81].

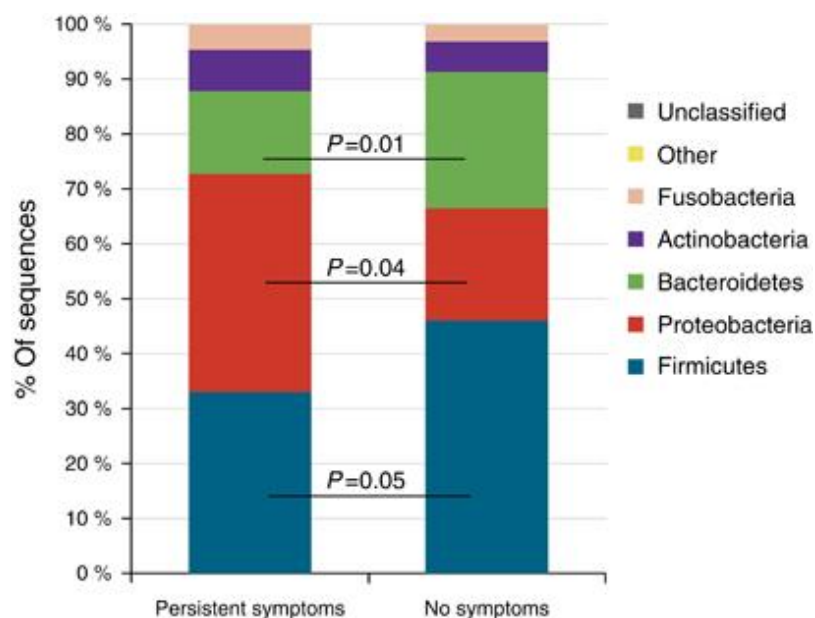


Figure 1-6 Average abundances of bacterial phyla in treated coeliac disease with persistent symptoms and treated patients without symptoms [80].

In a Swedish study [82] of the gut flora of 36 children diagnosed with coeliac disease together with 46 coeliac children who had been on a GFD for at least three months and with healthy controls showed significant differences in the short chain fatty acids (SCFAs) between the three groups. This indicates that there may be a difference in the metabolic activity of intestinal microbial flora in children with coeliac disease compared to those without the disease, and this difference may not be affected by diet treatment.

A review of adults with coeliac disease concluded that coeliac patients have a reduction in beneficial species and an increase in potentially pathogenic compared to non-coeliac healthy individuals [83]. Lactobacillus counts were found to be significantly reduced in children with coeliac disease compared to the healthy individuals by Girón Fernández-Crehuet et al [84] with the overall microbiota composition in these children influenced by the degree of enteropathy noted.

1.13 RADIOLOGICAL INVESTIGATION OF COELIAC DISEASE.

The small bowel is a difficult and challenging area to image accurately due to its overlapping loops and peristaltic movement [85]. The ideal imaging method for this area should be non-invasive, use non-ionising radiation and show the small bowel wall, lumen and its surrounding structures. In coeliac disease, pathological changes are seen mainly in the duodenum and the proximal jejunum; although the extent of the disease varies and changes may involve the whole of the small bowel.

Increased IELs have also been seen in the terminal ileum of those with villous atrophy [86].

1.13.1.1 Barium sulphate studies

Historically, barium sulphate studies were the first line radiological examinations in the investigation of small bowel disease [87]. They were available in most radiology departments, reproducible, easy to perform, well tolerated by patients and relatively inexpensive. There were two main techniques used: the 'small bowel follow through' (SBFT) and the 'small bowel enema or enteroclysis' (SBE). Bowel preparation such as a low residue diets plus or minus the use of a laxative the day before and fasting on the day of the examination were commonly used.



Figure 1-7 Small bowel barium study demonstrating 'flocculation' of the barium within the proximal small bowel (small arrows) and 'cod-fish' thoracic vertebrae (large arrow).

The most specific sign of coeliac disease in the small bowel barium study is fold pattern abnormalities [88], other findings include small bowel dilation, seen mostly in the jejunum, and segmentation, fragmentation and flocculation of the barium. The flocculations represent lumps of disintegrated barium caused by the increase in resting fluids in coeliac [89] which is seen in about 65% of coeliac patients [1].

Osteoporosis associated with poor calcium absorption in untreated coeliac disease may occasionally be seen in the vertebrae giving a characteristic 'cod-fish' appearance (**Figure 1-7**). With the fold pattern abnormalities described in coeliac disease the mucosal folds may be thickened or changed in number. The proximal jejunum may have a decrease in the number of folds and the distal ileum a raised number of folds.



Figure 1-8 Small bowel barium study demonstrating the 'Moulage' sign - dilated loop of bowel with effaced folds (white circle) [4].

Typical histological coeliac features of villous atrophy are demonstrated radiologically as a type of smooth featureless mucosa known as 'moulage' [90] which occurs where there is a dilated loop of bowel with effaced folds. It looks like a tube into which wax has been poured (**Figure 1-8**).

SBE is considered more useful than SBFT in the investigation and evaluation of the disease yielding an accuracy of 75% [87]. Although barium studies are inexpensive and relatively non-invasive they carry a significant radiation dose and are not specific enough for a definitive diagnosis of coeliac disease without additional investigations.

Also, these examinations did not allow simultaneous examination of structures outside the small intestine. Ultrasound has been used to demonstrate fluid filled small bowel loops in coeliac disease, and is also used to demonstrate adenopathy and ascites but is not considered particularly useful in the disease workup unless the patients have an atypical presentation [91]. Intestinal scleroderma may also present with malabsorption and have a dilated and fluid filled small bowel [4].

1.13.1.2 *Cross-sectional imaging*

Although in recent years cross sectional imaging such as Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) have now largely replaced barium imaging of coeliac disease [85], abdominal imaging is not considered necessary for the diagnosis [92, 93].

Adult patients are often referred for radiological imaging such as CT with nonspecific abdominal symptoms such as loss of appetite, weight loss and abdominal pain. CT is particularly useful for imaging coeliac complications such as transient non-obstructing intussusceptions which are seen on CT for up to 20% of patients with coeliac disease [1] and 25% of children in a prospective paediatric coeliac ultrasound study [94]. (Intussusception is the “telescoping” or sliding of one portion of the intestine (the intussusceptum) into another (the intussusciens) (Figure 1-9).

Malabsorptive indicators such as tonic, flaccid, dilated small bowel loops with the obliteration of inner loop mesenteric fat are also seen on CT [93].

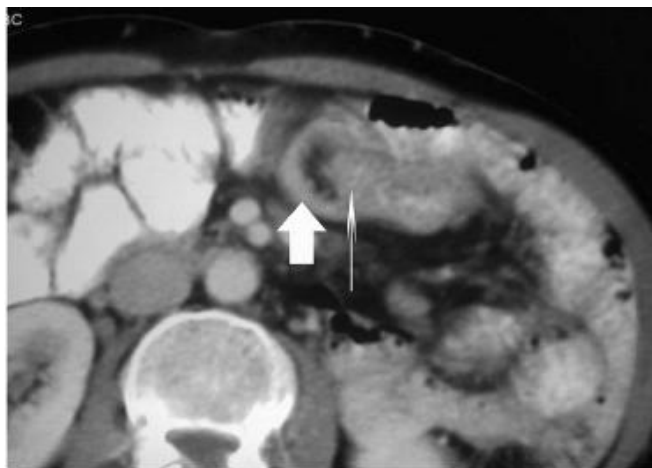


Figure 1-9 Transverse CT scan of the pelvis in a female patient. The intussusception appears ‘sausage shaped’ and the scan gives clear visualization of the intussusceptum (thin arrow) as well as the intussusciens (wide arrow) [1].

Malignancies are the most serious complications that may occur in patients with coeliac disease, with lymphadenopathy and malignant transformation clearly demonstrated by CT. Ulcerative jejunoileitis (UJ),

which is seen in older coeliac patients, is considered a premalignant condition and a precursor of enteropathy associated T cell lymphoma (EATL). The thickening of the small bowel wall and benign-appearing inflammatory ulcers seen in UJ make it difficult to distinguish from EATL [95]. In coeliac patients, biopsy should always be considered if adenopathy is seen on CT [1].

Ulcerative jejunoileitis shows the same abnormal IELs seen in type II refractory celiac disease, but unlike type II, they are only found in the ulcerated parts [96]. Conversely, as the majority of patients with coeliac disease remain undiagnosed and therefore untreated patients presenting with intestinal T-cell lymphoma, without a history of coeliac disease, should be tested for the disease [97].

Although more costly and not as widely available, MRI has many advantages over CT, including better soft-tissue contrast (which can be achieved using a variety of pulse sequences), better resolution, multiplanar imaging, and no ionising radiation exposure, that allows sequential data acquisitions over time. As patients with coeliac disease may require repeated follow up scans the lack of ionising radiation exposure for this modality is important. Due to radiation exposure concerns, CT cannot be repeated over many time points like MRI which prevents dynamic imaging.

CT Enteroclysis or CT Enterography are superior to conventional abdominal CT in demonstrating small mucosal and wall lesions [98] but still often fail to show fine mucosal changes and an area of peristaltic

contraction during a CT scan acquisition can be misdiagnosed as a small bowel lesion.

As with barium studies, the most specific signs of coeliac disease on MRI is fold pattern abnormalities. These can be described as a reduction in the number of jejunal folds [99], which are considered reduced in number if less than 3 per inch. An increased number of ileal folds (more than 5 per inch) are another specific sign of coeliac disease. This increased number of ileal folds represents an adaptation of the ileum to the reduced absorptive capacity of the jejunum. Jejunal dilatation (greater than 3 cm), is often found in coeliac disease and is secondary to intestinal hypomotility. Small bowel dilatation was seen on MRI in 61.3% of adult coeliac patients in a study by Tomei et al [100].

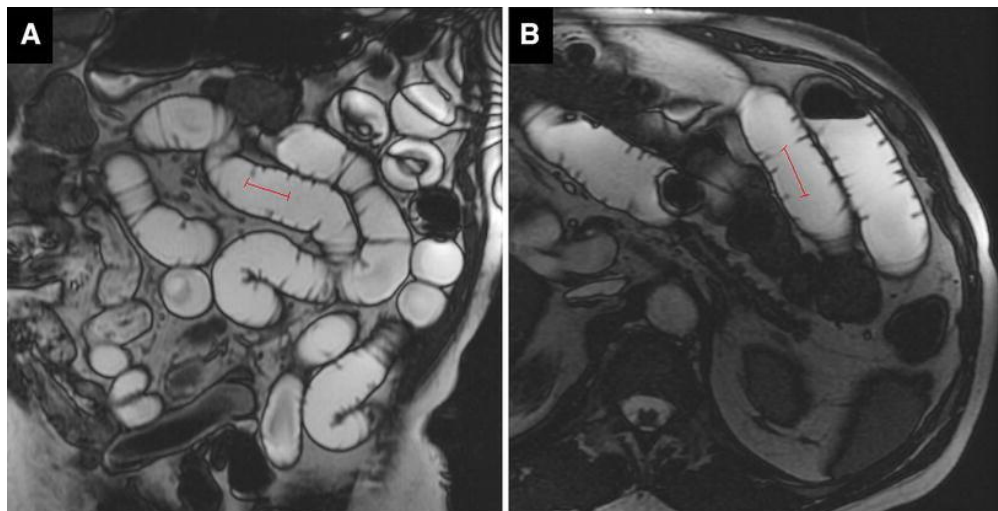


Figure 1-10 Decrease in number of jejunal folds. Coronal (A) and transverse (B) images from MR enteroclysis demonstrate both a decrease in the number of, and blunting of the jejunal folds (intraluminal red lines in images **A** and **B** each measure 1 inch) [93].

The *valvulae conniventes* show a number of distinct patterns, from normal folds to squared ends where the ends at the margin are squared off rather than rounded, reversed jejunal fold pattern: decreased jejunal folds with increased ileal folds and the “Moulage sign”, characteristic of coeliac disease due to total villous atrophy.

Transient intussusception may be seen in MR enterography and this is usually self-limiting but must be examined carefully as it is also associated with malignant GI tumours such as lymphoma and adenocarcinoma [101]. The relative risk of adenocarcinoma in the coeliac population is 60 to 80 times higher than the general population and 300 for the development of lymphoma [29] .

Life expectancy in patients with RCD I is similar to patients with uncomplicated coeliac disease however RCD II has a much poorer prognosis, mainly due to the greater likelihood of developing enteropathy-associated T-cell lymphoma (57). Although MR enteroclysis can be used to investigate the presence of RCD II or malignancy in symptomatic coeliac patients, it is not always possible using this technique to distinguish between uncomplicated coeliac disease and RCD (47).

Summary of radiological signs of coeliac disease

- Dilatation of small bowel
- Segmentation
- Moulage sign: rare
- Transient intussusceptions (coiled spring)

- Decreased fold pattern of jejunum
- Prominent ileal fold pattern (ileum appears like jejunum)
- Increased secretions: flocculation with older barium suspensions
- Benign mesenteric lymphadenopathy
- Increased incidence of malignancy, aggressive lymphoma, carcinoma

1.14 SUMMARY

The diagnosis and management of coeliac disease relies on a combination of results including medical history and physical examination, serology and endoscopic biopsy. Radiological investigations such as CT and MRI, although not essential for diagnosis are useful in evaluation of the disease and its complications. In particular MR enterography is considered useful in detecting complications in patients with known coeliac disease and a poor response to GFD, or in patients with recurrent symptoms despite gluten withdrawal, and shows some correlation with the severity of the disease but must be confirmed by positive serology and biopsy (36, 47, 48). To date no radiological investigation can match the sensitivity and specificity of endoscopic biopsy for the diagnosis of coeliac disease.

A study evaluating patient's perception of the treatment burden of seven chronic health conditions in 2014 reported that coeliac patients, despite their good overall health status were second only to those on dialysis for

end-stage kidney disease for disease burden, with a quarter of patients surveyed expressing regret at being diagnosed. This is considered due to the 'significant burden' of the gluten free diet therapy [102].

Due to the many limitations of gluten-free diet, including cost and difficulty especially when eating outside the home, studies have shown only 42-92% for people diagnosed with coeliac disease achieve strict adherence to a GFD [120, 121, [103].

Factors that influenced poor dietary adherence were young age at diagnosis, low standard of formal education and long duration of treatment [121].

Patients and their carers want less invasive test for diagnosing and monitoring coeliac disease [104]. Duodenal biopsies are invasive and expensive and may be tolerated poorly by patients and are not therefore suitable for serial monitoring of the disease and its response to treatment. With the prevalence of the disease increasing worldwide [5] and the development of novel non-dietary therapies such as enzyme therapy to break down gluten and therapeutic vaccines [105] new non-invasive markers of coeliac disease severity and progression/regression are required to help progress the development of these new treatments [46].

1.15 AIMS AND HYPOTHESIS

Based on the background knowledge and literature briefly reviewed above the aims of this thesis were to use newly developed non-invasive MRI techniques combined in a single short scanning session to provide novel insights into coeliac disease, the effect of GFD, and the relationship of gastrointestinal function and symptoms.

Based on our pilot data and on the literature available, this hypothesis underpinning this proposed work is that in adults newly diagnosed with coeliac disease treatment with GFD will:

1. Reduce the water content of the fasting small bowel
2. Reduce the volume of the fasting colon
3. Decrease whole gut transit time

The specific objectives of the study were therefore to quantify any change following treatment with a gluten-free diet (GFD) in:

- The water content of the fasting small bowel
- Fasting colon volume
- Gastrointestinal transit time

in adults newly diagnosed with coeliac disease using non-invasive MRI techniques and in a matched cohort of healthy controls who do not follow a gluten-free diet to provide descriptive statistics on a likely reference range for the healthy population to inform the design of future studies.

1.16 THESIS OUTLINE

All the work described in this thesis was conducted by the author, except where specifically credited to collaborators, at the Sir Peter Mansfield Imaging Centre, the School of Medicine, University of Nottingham and the Nottingham Digestive Diseases Centre (NDDC) and the National Institute for Health Research (NIHR) Biomedical Research Centre (BRC) from 2015 to 2019. The research described in this thesis is original, unless otherwise stated.

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

- Chapter one:** Introduction to coeliac disease, its diagnosis and its radiological investigation
- Chapter two:** MRI methods for measuring GI anatomy and physiology
- Chapter three:** MRI development and optimisation study for the MR validation of small bowel water content quantification sequences used in the main study
- Chapter four:** The main patient study: Magnetic Resonance Imaging of Coeliac Disease (MARCO), configuration and protocol.
- Chapter five:** MARCO study results
- Chapter six:** Discussion, overall conclusions and possible future directions

2 MRI FOR MEASURING GI ANATOMY AND PHYSIOLOGY

2.1 INTRODUCTION

This chapter discusses the anatomy and physiology of the digestive system. The MRI principles of image formation are then briefly considered, followed by a summary of MRI methods for measuring GI anatomy and function.

2.2 ANATOMY AND PHYSIOLOGY OF THE DIGESTIVE SYSTEM

The digestive system in humans consists of the GI tract and the accessory organs of digestion, the salivary glands, liver, gall bladder and pancreas. Its function is the ingestion of food and liquids, the breakdown of these foods by mechanical and chemical processes, the subsequent absorption of nutrients into the body and elimination of the waste products.

The GI tract is essentially a long tube that begins at the oral cavity (mouth) and extends distally with the oesophagus, the stomach, the small bowel (small intestine), the large bowel (colon) and ends at the anus (**Figure 2-1**).

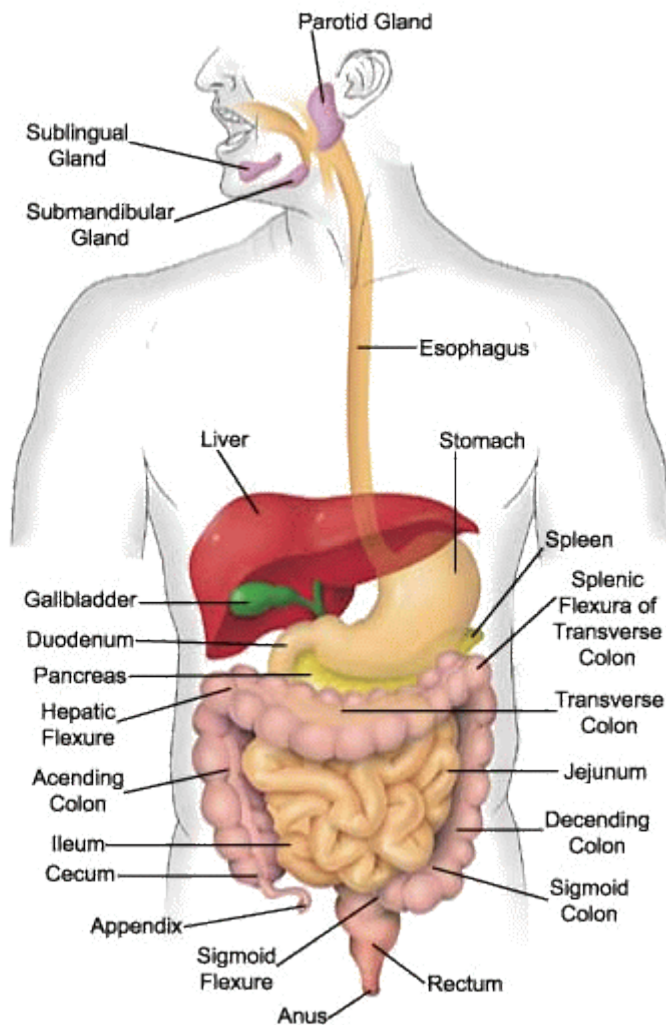


Figure 2-1 Schematic representation of the gastrointestinal tract and the accessory organs of digestion accessed 1/10/1.
<https://www.britannica.com/science/gastrointestinal-tract>

Digestion begins in the mouth where amylase, a digestive enzyme in saliva, starts to break down carbohydrates. The food is mixed and softened by a process of mastication, creating a bolus of food mass. When swallowed, a bolus of food crosses the oesophagus and then reaches the stomach by peristalsis, a wavelike muscular contraction generated to propel onwards the contents of the digestive tract. The

transit through the oesophagus is quite fast, taking only a matter of seconds. In the presence of food and drink the proximal part of the stomach relaxes and acts as a reservoir before a tonic contraction redistributes the food toward the distal part of the stomach. The repeated contractions of the antrum enables the bolus of food to be further broken as it mixes with gastric juices [106]. Transit of food through the stomach may take up to two/three hours depending principally on energy content and type of macronutrients.

From the stomach the partially digested food (chyme) reaches the small bowel. This is about six to seven meters long and consists of three segments, the duodenum, jejunum, and ileum.

The small bowel is so called because its luminal diameter is smaller than the large bowel, even though its length is longer. The surface area is increased by villi, which are finger –like projections involving the epithelium and lamina propria, which project into the lumen of the small bowel (**Figure 2-2**). These folds increase the absorption surface area substantially in the small intestine. Each villus has many microvilli (microscopic cellular membranes) projecting from their epithelial surface collectively forming a brush border which further increases the absorptive surface.

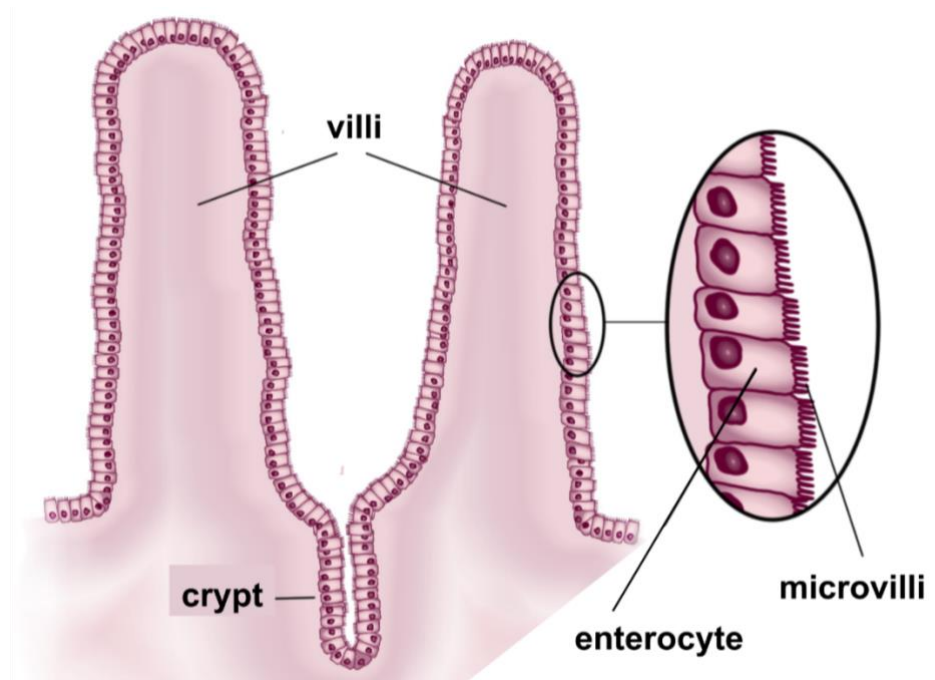


Figure 2-2 Lumen of the small bowel: villi and microvilli (accessed 8/10/19 (<https://biologydictionary.net/simple-columnar-epithelium/>))

When the contents of the stomach progress into the small bowel (**Figure 2-3**), the pancreatic enzymes then start to work on this semi liquid chyme. Bile from the liver is also secreted into the small bowel. The bile is important for emulsifying fats and it also enhances the activity of pancreatic and intestinal lipases. Ninety five percent of the absorption of nutrients occurs in the small bowel and one of the important factors that regulate the amount of nutrients absorbed by the intestine is the intestinal transit time [107].

Small intestinal transit to the caecum takes about 90 minutes with a range of 15 minutes to up to five hours in the normal bowel [108]. The chyme then passes into the colon via the ileocecal valve, a one-way valve between the ileum and the cecum, which helps control the passage of

contents into the colon and also increases the contact time of nutrients in the small bowel. It also prevents the colonic contents from refluxing back up into the ileum, thus reducing or preventing the movement of bacteria from the colon back into the small bowel.

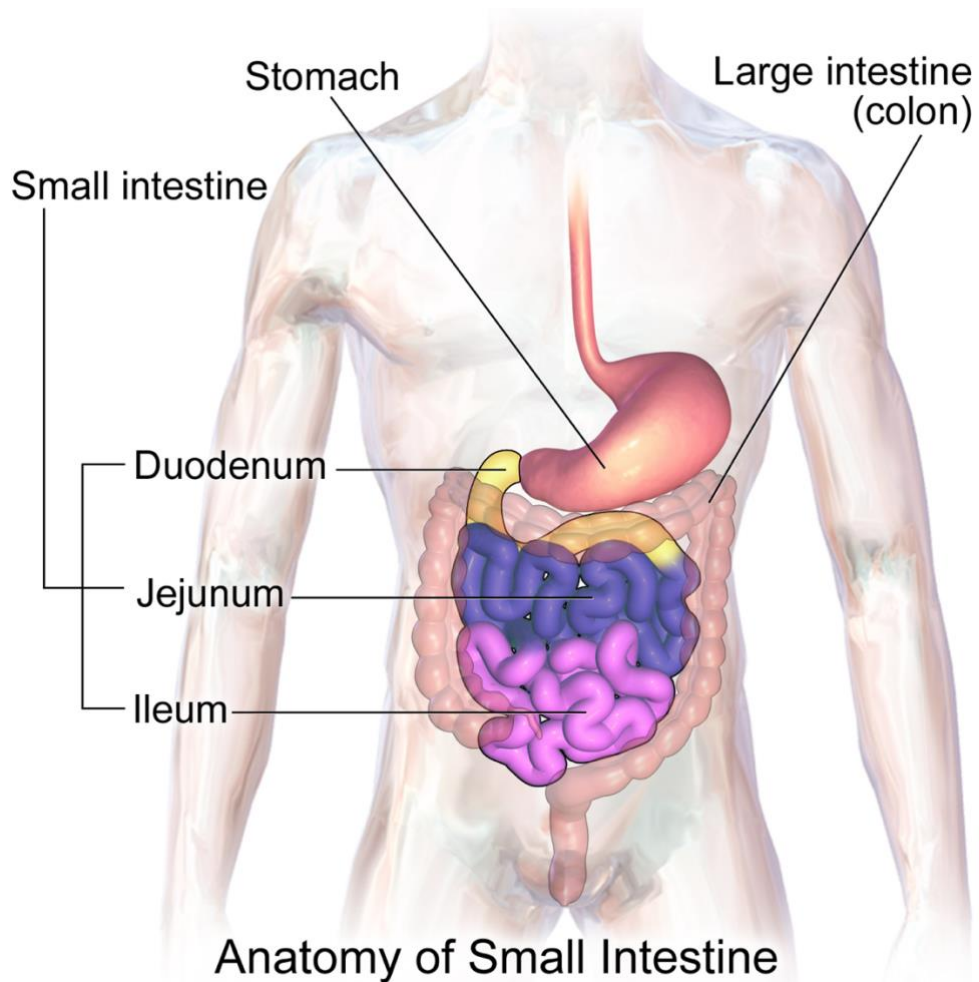


Figure 2-3 Small intestine anatomy. Blausen.com staff (2014). "Medical gallery of Blausen Medical 2014".

The colon or large bowel is about 1.5 meters long and made up of the caecum, ascending (right) colon, the transverse colon, the descending (left) colon and the sigmoid colon, which connects to the rectum.

In the colon the transit of the digesting food is much slower. The gut flora or microbiota ferment the remaining undigested food and the resulting

semi-solid waste is eliminated from the body by episodic contractions that propel the faeces forward to reach the rectum. Here also electrolytes continue to be absorbed while the stool is thickened with mucus. When the stool reaches the rectum it causes rectal distension, which cause the stretch receptors in the wall of the anus to be activated and the sphincters to relax giving the impulse and urge to defecate.

The consistency of the digestive contents at different part of the gut is determined by the physiology of the GI tract and the mechanisms of digestion- as the fluid contents move into the caecum and ascending colon water is absorbed and the stool becomes semi solid. If the digestive transit time is too slow, too much water may be absorbed and the stool becomes hard and dry, resulting in constipation.

Conversely, a short transit time may lead to loose watery stools as insufficient water is absorbed in the colon. The usual number of bowel movement varies from person to person, most healthy individuals have 0-4 bowel movements per week but this varies from three times a day to three times a week.

Constipation is usually defined (Rome IV criteria [109]) as fewer than three spontaneous bowel movement per week (with other symptoms such as of straining, lumpy or hard stools, sensation of incomplete evacuation, sensation of anorectal blockage, use of manual manoeuvres to facilitate defecation) and functional diarrhoea as more that 25% of stools being loose [109].

2.3 MOVEMENT OF THE DIGESTIVE CONTENTS:

Following the ingestion of food, the movement of the digestive contents is facilitated by peristalsis, involuntary movements of the longitudinal and circular muscles which causes a series of wave-like muscle contractions. This starts in the oesophagus when a bolus of food is swallowed and the strong wave-like motions carry the food to the stomach, where it is churned into a chyme using segmentation contractions of the smooth muscles of the stomach. Then peristalsis continues in the small bowel where it mixes and shifts the chyme back and forth, allowing nutrients to be absorbed into the bloodstream. In the colon the peristaltic wave, or mass movement, is progressive; it advances the faeces steadily toward the end of the GI of tract, pushing waste material in front of the wave as water from the undigested food material is absorbed into the bloodstream and the waste matter subsequently expelled.

2.4 THE ROLE OF THE MIGRATING MOTOR COMPLEX

The migrating motor complex (MMC) is a cyclic, recurring motility pattern that occurs in the stomach and small bowel during fasting. It may be thought of as an 'intestinal housekeeper' as it flushes undigested matter and everything else into the colon. Divided into three phases, starting in the stomach and traveling through to the small bowel and moving down to the ileocecal valve it clears solids that may have remained in the stomach and residual content of the small bowel into the colon. It is

activated by having an empty stomach and occurs naturally every 90-120 minutes.

Phase one is described as the quiescent period lasting from 40% to 60% of total length of the cycle. Then phase two, which last about 20%-30% of the cycle, has strong but irregular contractions. The strongest contractions occur in phase three, when these strong contractions propagate caudally. This can start in the stomach or the small intestine.

Motilin and ghrelin and the vagus nerve are considered important regulators for phase three contractions originating in the antrum, while somatostatin and serotonin seem to be involved in the regulation of phase three contractions from the duodenum.

The narrow junction between the terminal ileum and the colon produces a region of high pressure that resists movement of material from the colon back into the ileum. The MMC pushes matter through the ileocecal junction and thus evokes colonic contractions [110].

The activity of the MMC is considered a clinical marker for the functionality of the GI tract, and disorders linked to a disturbed MMC include gastroparesis, delayed gastric emptying and intestinal pseudo-obstruction [111] Its exact physiological role is incompletely understood although diseases such as scleroderma which impair the MMC are often associated with SIBO, particularly the absence or reduced phase three MMC is strongly associated with SIBO. It is thought that this bacterial proliferation may be increased by resting secretions from impaired MMC function [112]. An altered MMC can also be associated with obesity as it has a role in controlling hunger sensations [113].

2.5 MAGNETIC RESONANCE IMAGING

2.6 INTRODUCTION TO MRI PRINCIPLES

This section introduces briefly the theory behind the formation of an MR image. The hardware used to produce an MR image is described together with the formation of image contrast in MRI. The main MRI parameters measured during this work are then introduced together with the MRI techniques developed and used in this thesis to assess small bowel water content, transit and colon size.

2.7 BACKGROUND

Nuclear Magnetic Resonance (NMR) was simultaneously discovered by two physicists- Felix Bloch and Edward Mills Purcell, following the end of the Second World War. This work led to the use of NMR (later renamed MRI) to produce 2D images. MRI was discovered independently by Sir Peter Mansfield in Nottingham and Paul Lauterbur, an American chemist based in Illinois in 1973. They were jointly awarded the Nobel Prize in Physiology or Medicine in 2003 '*for their discoveries concerning magnetic resonance imaging*' (www.nobelprize.org). The first cross-sectional MRI image of a live human abdomen was reported in 1978 in Nottingham by Mansfield using a home-built scanner. The first commercial MRI systems were developed soon afterwards in the 1980s.

Since then advances in the hardware used, magnets, radiofrequency coils and gradients, together with advances in software for improved pulse sequence design and image reconstruction has allowed this imaging modality to flourish for both clinical and research applications.

2.8 BASIC PRINCIPLES OF MRI

Due to its abundance in the human body and its large magnetic moment the hydrogen nucleus is the main MR active nucleus used in MR imaging. The proton contains an intrinsic magnetisation called spin. The spin magnetisation rotates (precesses) around the magnetic field at a frequency known as the Larmor (or resonant) frequency, which is proportional the strength of the magnetic field.

When a patient is placed in the magnet of the MR scanner, the protons in the hydrogen atoms start to align parallel to the magnetic field B_0 .

When a radiofrequency (RF) signal tuned to the Larmor frequency of the hydrogen atoms is emitted from the scanner it creates a phase coherence in the precession of the proton spins.

The duration of the pulse can be set to tilt the spin magnetisation perpendicular to the magnetic field. When the pulse transmission is finished, the receiver coil picks up the transverse magnetisation changes which generate an electric current in the coil by Faraday induction – this is the Nuclear Magnetic Resonance (NMR) signal.

The signal is attenuated by two relaxation processes identified by a respective time constant, T_1 the spin-lattice relaxation time and T_2 the transverse relaxation time.

Contrast in the MR images originates for the fact that different tissues, especially soft tissues, have different T_1 and T_2 relaxation times. The T_1 longitudinal (spin-lattice) relaxation time is a measure of the time taken for the net magnetic vector to recover to its ground state in the direction of B_0 .

At the same time, spins are decaying from their aligned precession in the transverse plane. Differences in this decay are captured by allowing a specific T_2 (spin-spin) weighting in the MRI imaging sequence. The repetition time TR is the time between the applications of the RF excitation pulse to the application of the next pulse.

The echo time TE is the time between the application of the RF excitation pulse and the peak of the induced signal in the receiver coil (**Figure 2-4**).

If the TE in a T_2 sequence is very long, only signal tissues with very long T_2 relaxation times such as fluid will be measured, this is the basis of the MRCP sequence [114] used for demonstrating bright fluid in the structure being imaged.

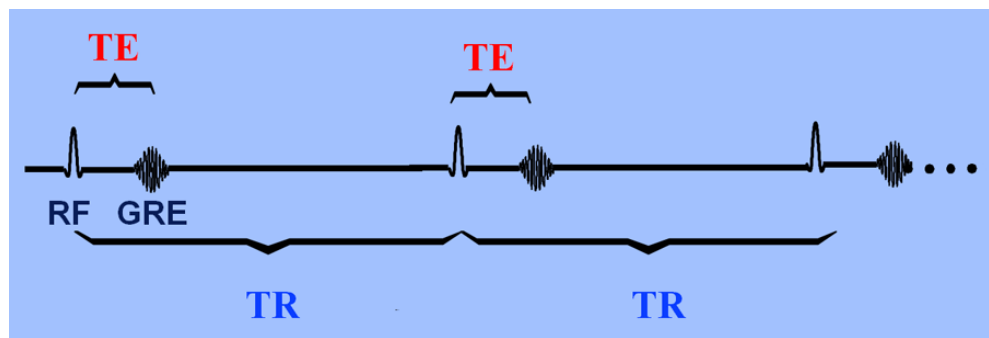


Figure 2-4 Simplified pulse sequence diagram showing TR (repetition time) and TE (echo time) [96]

2.9 MRI HARDWARE

The principal parts of an MR scanner are the magnet, the RF coils, the gradient coils and the control computer. MR image quality is contingent on having a uniform strong static magnetic field. Early MRI systems used resistive electromagnets with a field strength of less than 0.3 Tesla (T). Permanent magnets were too heavy to be useful above the field strength of about 1 T, although they did not require the cooling systems the resistive magnets needed.

When superconducting magnets were introduced, they gave a larger, more stable magnetic field. In 1985 1.5 T magnets became commercially available and still remain the most common field strength for diagnostic MR imaging.

The increased use of spectroscopy and functional imaging in brain research required higher field strength to maximise signal to noise ratio

in order to demonstrate the small signal changes seen and 3T systems were marketed commercially in the late 1990s. Since then 'ultra-high field' research scanners are available at 7T and even 9.4T. GI diagnostic imaging is usually restricted to 1.5T or 3T due to the high power deposition and B1 field inhomogeneities and restricted field of view seen at these higher magnetic field strengths.

2.10 RADIOFREQUENCY (RF) SYSTEMS

RF systems transmit radiofrequency energy into the patient and then detect and process the resulting MR signal from the patient's anatomy using transmit and receive RF coils. Modern scanner design has now separated transmit and receiver RF-coil functions.

The main body RF coil which, is a fixed part of the scanner, around the bore, is generally used as the transmitter. The receive coil is usually placed as close as possible to the part of the body being imaged. Receive coils can be just a simple surface coil which comprises a loop of wire placed over the region under examination. These give excellent signal to noise ratio, but have the inherent design disadvantage of the loss of signal intensity with distance from the coil, which results in signal intensity variation and a limited field of view. The development of phased array coils allowed large areas to be imaged with the signal to noise quality of the smaller coils making up the array. RF coils may also be

transmit –receive such as volume coils used for head or extremity imaging.

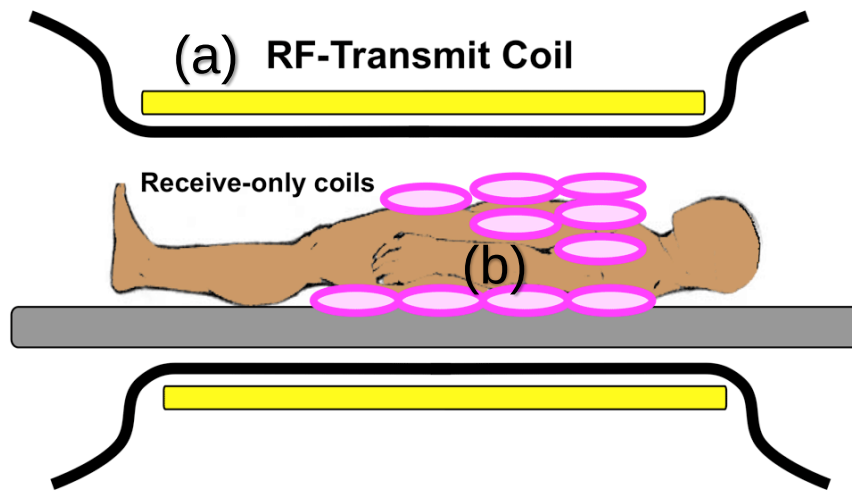


Figure 2-5 Radio-frequency coil location example showing (a) the RF transmit in the scanner bore and (b) a receive-only body array coil around the patient. Adapted from <http://mriquestions.com/receive-only-coils.html> (accessed 11/09/2019).

2.11 GRADIENTS AND SLICE SELECTION

Magnetic field gradients are fundamental for the determination of spatial information of the MR signal. They modify the main magnetic field so that the field varies with spatial position in a linear manner within the bore. By passing high currents (~hundreds of Ampere) very rapidly (kiloHertz frequency) through coils of wire (gradient coils) the magnetic field strength is altered in a controlled way. Gradients exist in the z, y and x

axes with the isocentre at the centre of all three gradients. These are called G_x , G_y and G_z - for the three orthogonal directions. Because of these gradients, the same nuclei at different positions along the gradient direction will experience a slightly different total magnetic field, and therefore precess with a slightly different frequency.

The MRI system knows which gradient is allocated for slice-selection by the plane of the scan. When the scan plane is selected in the scan protocol, it determines which gradient (G_x , G_y , G_z or a combination of these) is applied during the RF excitation pulse (and during the RF rephasing pulse in spin-echo pulse sequences).

When slice thickness and gap between the slices is selected in the scan protocol, it determines the amplitude of current applied through the slice-select gradient during the pulse sequence. Thin slices are produced by passing a large current through the slice-select gradient and thick slices by passing less current [115].

So to spatially encode an image, three separate functions are necessary, with each gradient performing one of the tasks (**Figure 2-6**) [115]:

- slice select: locates a slice in the scan plane selected
- phase encoding: locate a signal along the short-axis of the image
- frequency encoding: locate a signal along the long-axis of the image

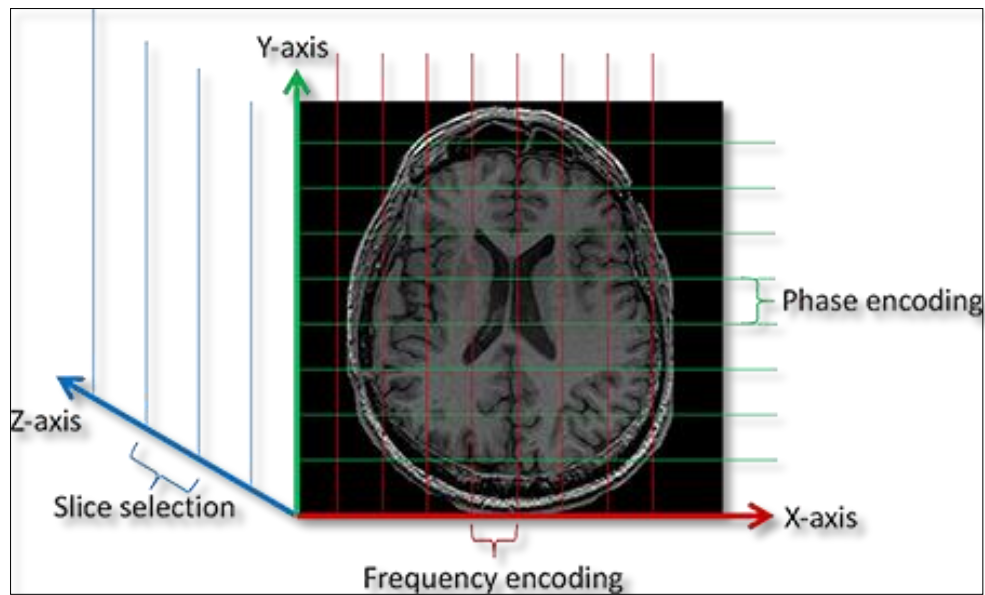


Figure 2-6 Spatial encoding of the MR image, showing G_x the frequency encoding gradient, G_y the phase-encoding gradient and G_z the slice selection gradient [116].

Image sequences – gradient echo and chemical shift artefact.

Gradient echo sequences differ from spin echo sequences in that they use a flip angle (α) of less than 90 degrees, (over a wide range from 5-80 degrees) and they use gradient fields to generate transverse magnetisation. The main advantage of gradient echo imaging is faster acquisitions. Larger flip angle, (45-90 degrees) produce T_1 contrast. T_2^* contrast is achieved by a small flip angle, a long TE and a long TR.

Another way of creating contrast using gradient echo techniques within the abdomen is derived from the chemical shift in resonant frequency between fat and water, as the phases of these protons relative to each

other change after the initial excitation. This is known as chemical shift artefact [117] and is also known as the 'india ink' or 'black line' artefact as it looks like the anatomical structures have been outlined with a black pen.

Immediately after the initial excitation the fat protons and water protons are in phase but the water protons precess faster than the fat protons. It is therefore possible to take images of the fat and water protons in and out of phase by varying the TE. Two images are created, the first has an echo time of 2.3ms, designed such that the echoes from fat and water are out of phase (at 1.5T) and the second has twice this echo time so that the echoes from fat and water are in phase. The out of phase image creates a black border at the fat/tissue interface and this may help to highlight anatomical details within the abdomen. This form of chemical shift artefact or misregistration is known as chemical shift artefact of the second kind. It only occurs in gradient echo imaging and is due to the phase – cancellation effect. It is not limited to the frequency encoding direction like the first kind of chemical shift found in spin echo imaging, but can be seen in all pixels along a fat/water interface [115].

2.12 SIGNAL TO NOISE RATIO AND RESOLUTION

Signal to noise ratio (SNR) is defined as the ratio of the amplitude of the signal received by the radiofrequency (RF) coil to the amplitude of the

noise. The signal (which is the voltage induced in the receiver coil) varies and the noise is a constant value, related to resistive elements in the system such as the equipment and the patient in the scanner. Signal is dependent on, amongst other things, the strength of the magnetic field and the quality of the RF coils used. SNR is also proportional to voxel volume and the square root of the acquisition time.

As improved signal to noise and high resolution increases image quality and also scan time, there is therefore a trade-off between scan time and the image quality in order to provide adequate anatomic visualisation for image analysis and detection of pathology.

2.13 PARALLEL IMAGING

Parallel imaging uses multiple receive coils to scan faster, making it suitable for us in GI imaging where breath-hold scans are used to reduce motion. The GE (General Electric Medical Systems, Milwaukee, WI) parallel imaging method ASSET (Array coil Spatial Sensitivity Encoding) is used in this study to speed up image acquisition. This involves 4 steps:

1. Generate coil sensitivity maps
2. Acquire partial k-space MR data
3. Reconstruct partial images from each coil
4. Unfold and combine these images using matrix inversion.

For a parallel imaging acceleration factor of 2, alternate lines of k-space are skipped, resulting in a $\frac{1}{2}$ -Field Of View (FOV) images obtained from

each coil with aliasing (wrap-around). A matrix inversion process is used to unfold and combine the aliased images from each coil.

2.14 MRI OF THE GI TRACT

MRI of the GI tract was initially very limited in clinical application for both anatomical and physiological reasons and technical issues. The GI tract is a complex three dimensional structure which not only varies between individuals but also varies in an individual according to physical activity and position and between fasted and non-fasted states. As well as this, peristaltic movement and breathing means that the structure is constantly moving and therefore may be challenging to image.

MRI is also relatively costly and due to technical and safety restrictions is not suitable for all patients. However advances in MR hardware and software in the past decade have allowed many of these restrictions to be overcome and now the advantages of MRI in the GI tract such as the ability to visualise exquisite soft tissue detail of the entire tract without the use of ionising radiation and its multi planar capabilities to demonstrate the varied anatomy of the GI tract, and also the ability to look at function as well as structure – for instance using rapid ‘cine’ MRI sequences to look at motility have led this modality to be the modality of choice for many GI diseases.

3 OPTIMISATION- THE SMALL BOWEL WATER CONTENT VALIDATION STUDY

3.1 INTRODUCTION: QUANTIFYING INTESTINAL FLUID VOLUMES.

Small Bowel Water Content (SBWC) is an important parameter in the study of normal GI physiology and disease, and the gut's response to stimuli such as feeding. Factors that could contribute to the amount of free fluid in the small bowel water are gastric emptying, small bowel secretions, water absorption and progress to the colon. During digestion, the interaction between gastro-intestinal and pancreaticobiliary secretions and the absorption of water in the small bowel is complex as virtually all of the chemical digestion and absorption of protein and lipids happens here.

Initial attempts in Nottingham to estimate water absorption and secretion in the small bowel using MRI were hampered by the low field imaging (0.5T) used [118, 119] however improvements in field strength, coil and sequence design have improved spatial and temporal resolution since this work in the mid-1990s.

Schiller *et al* used what he called 'water-sensitive MRI' to measure intestinal fluid content and transit of capsules in 2005 [120] but no validation or error analysis was reported. As water in the small bowel can be widely distributed accurate quantification is challenging. Small pockets of water can be missed due to partial volume effects- this occurs

in a voxel that contains more than one type of tissue with different signal intensities. This is due to the finite spatial resolution of MRI sequences, especially breath hold sequences used for GI scanning. The signal in the voxel will therefore be a combination of the contributions of the different tissues [121]. This means only larger pockets of fluid will be measured in the small bowel.

A method for estimation of fluid volumes in the stomach for gastric emptying studies using MRI has been previously validated in a study in Nottingham where an oro-gastric tube with a bag attached was passed into the stomach of five volunteers, so that the bag resided in the stomach. Water was then infused into the bag in 60 mL aliquots and volume measurements were undertaken using an echo-planar imaging (EPI) scanning sequence [122]. This showed excellent correlation between infused volumes and those measured by the EPI sequence. Although this validation was for fluid volumes in the stomach, SBWC may also be estimated using similar fast MR imaging sequences that are sensitive to fluid.

The magnetic resonance cholangiopancreatography (MRCP) sequence was originally devised to non – invasively image the biliary system using heavily T2 weighted Turbo spin echo (TSE) sequences. These sequences obtain images in which the static fluid in the biliary tree appears hyper intense against a dark background without the use of contrast media [123]. Adapting this sequence, a non-invasive MRI method for measuring SBWC using a heavily T2 weighted sequence was

validated using naso-duodenal bolus infusion of a non-absorbable solution of saline and mannitol [124].

As well as giving insights into gut function in a diseased state this technique has been used to investigate the effects of drugs on gut function. Measurement of intraluminal cross-sectional diameter changes in selected loops of bowel distended with oral contrast in healthy volunteers has allowed the inhibitory properties of hyoscine butylbromide and glucagon [125] to be demonstrated. Using the same technique a reduction in net gut water volumes, either by stimulation of absorption or inhibition of secretion with Loperamide was demonstrated where previous invasive intubation methods had concluded Loperamide acts solely via change in intestinal transit [126]

This method worked very well on the specific Philips (Philips Medical Systems, Best, Netherlands) 1.5T MRI scanner platform it had been developed on. With the evolution of the Sir Peter Mansfield Imaging Centre (SPMIC) MRI centre a new 3T MRI scanner was installed. It was apparent that the different field strength would require changes to the modelling behind the small bowel water content image analysis and quantification. This was done by repeating the intubation study (unpublished) on that machine.

When the MARCO study was being set up the imminent decommissioning of the old Philips 1.5T scanner led the group to explore the use of the other 1.5T MRI scanner available, which is from a different manufacturer platform General Electric (GE) (General Electric Medical Systems, Milwaukee, WI). It quickly became clear that equivalent heavily

T2 weighted sequences on this platform provided different signal characteristics and post-processing parameter needs compared to the original validated work. This raised again the issue of how to calibrate the analysis without resorting to another onerous and invasive intubation study, for MARCO but also for any future studies on any platform. Further refinements were made to this sequence as the study progressed to improve the resolution of the image. From that point onwards, to preserve the continuity of data collection for the MARCO study, both the original 'old' sequence on the GE scanner and the tweaked 'new' sequence on the GE scanner were then used for each MARCO participant scanned. The following paragraphs will describe firstly the details of the original image analysis method, the new idea of a non-invasive calibration protocol, the corresponding study and its results and secondly an analysis using the new calibration factors on the 'old' and 'new' sequences to further validate these factors.

3.2 THE ORIGINAL METHOD TO QUANTIFYING SMALL BOWEL WATER

Imaging freely mobile, fluid water using MRI is not difficult, the main problem is how to quantify the volume of the fluid imaged as the edges of the regions of interest that the operator can define in the bowel depend heavily on the signal intensity, so an appropriate threshold must be chosen in an objective and reproducible way.

The method used for quantifying water content assumes that in the heavily T2 weighted MR image of the abdomen (from the diaphragm to

the symphysis pubis) any pixel with a signal intensity about a pre-set threshold (S_{th}) is filled with 'free' water. 'Free' water means in this context water molecules which are highly mobile as opposed to water molecules which have restricted mobility (e.g. water bound to gel molecules or trapped in mucous). The latter become invisible to heavily T2-weighted sequences.

The signal intensity threshold will vary because of intra- and inter-subject variation in signal produced due to inherent inhomogeneity of the magnet, scanner instabilities, subject repositioning and coil loading, thus some objective normalisation must be obtained. The use of an external marker is not suitable as the marker would be on the outer part of the subject and too close to the receiver coil, thus allowing for increased variation.

An internal marker is desirable and the participant's cerebrospinal fluid (CSF) was chosen to set the threshold as it is very fluid, visible on the scan, is near the area of interest and has a constant composition. Schiller [120] used the gallbladder to set this threshold but the cholesterol in bile does not make a good intrinsic marker for water, the gallbladder also may empty during the day with feeding for example. The CSF marker choice is more reliable, and the CSF marker method was validated against known volumes of infused fluid for the original imaging sequence on a Philips 1.5 T scanner [122] to define the multiplication factor to the CSF marker value needed to derive accurate volumes.

The algorithm to generate the CSF marker value from the images is made up of three parts as shown in Figure 3-1. Firstly the region of the

CSF (including surrounding background tissue) is defined on the images. Secondly a histogram of this data is generated using a fixed bin size and thirdly the second derivative of the data is calculated to find where this value tends towards zero. In other words the first derivative represents the slope of the signal changes in the histogram, and the second derivative represents the rate of change of this slope.

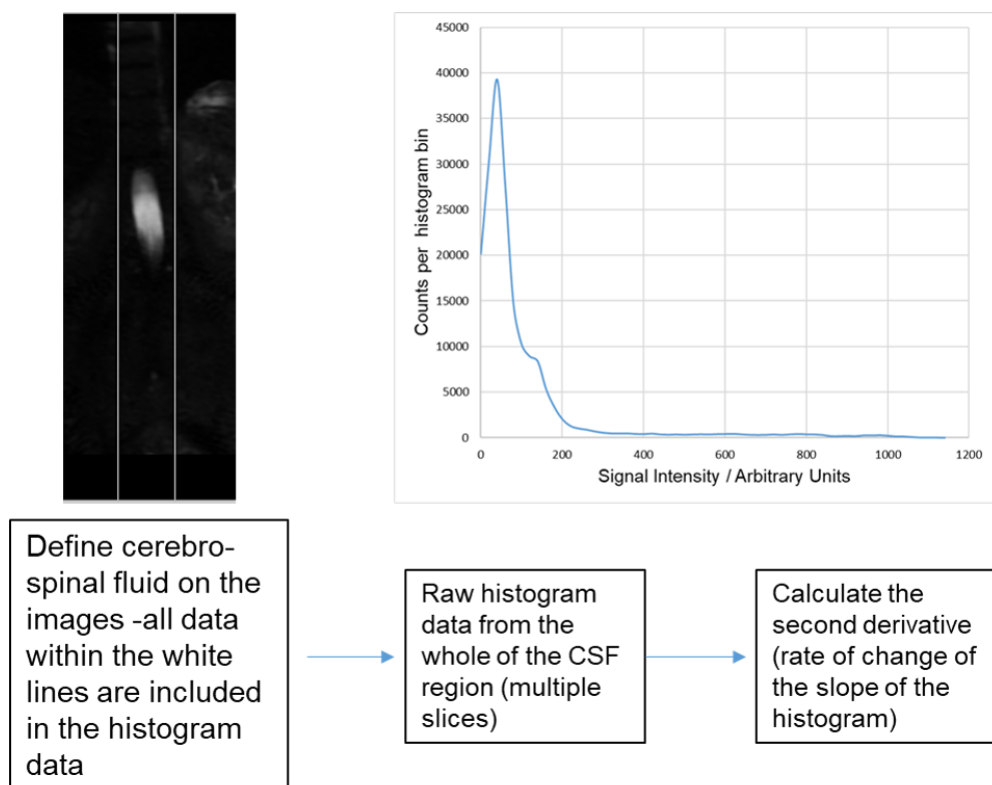


Figure 3-1 Defining the Cerebrospinal Fluid (CSF) on the images and using the raw data for histogram level calculation.

Custom written IDL® software (Research Systems Inc., Boulder, Colorado, USA) is used for this image analysis with a specialised programme developed by Dr Caroline Hoad at the SPMIC.

Firstly the threshold is set using the CSF as a marker as described above (See **Figure 3-1** left panel). Regions of interest are loosely drawn manually around each loop of the small bowel that contains bright fluid signal, in every slice of the set.

For small bowel water bright fluid signal from organs other than small bowel (e.g. stomach, biliary system, major blood vessels) are segmented out manually (**Figure 3-2**). The volume of mobile free water is then calculated by including all regions of fluid in the small bowel, then summing the volume of all image pixels in the area of interest with a signal greater than this threshold.

As already mentioned, for the MARCO study moving to a new scanner made by a different manufacturer and consequently using a new MR sequence has implications for this method to generate the correct threshold level for accurate volume measurements for the following technical reasons:

Firstly the dynamic range of the image data may be different because of the different hardware used (different receivers and amplifiers for example), hence the signal interval steps (called bin size) of the CSF histogram data may need to be altered. Secondly the multiplication factor from the CSF marker to the threshold level for free water may be different, as different RF pulses and timing are used to generate the images.

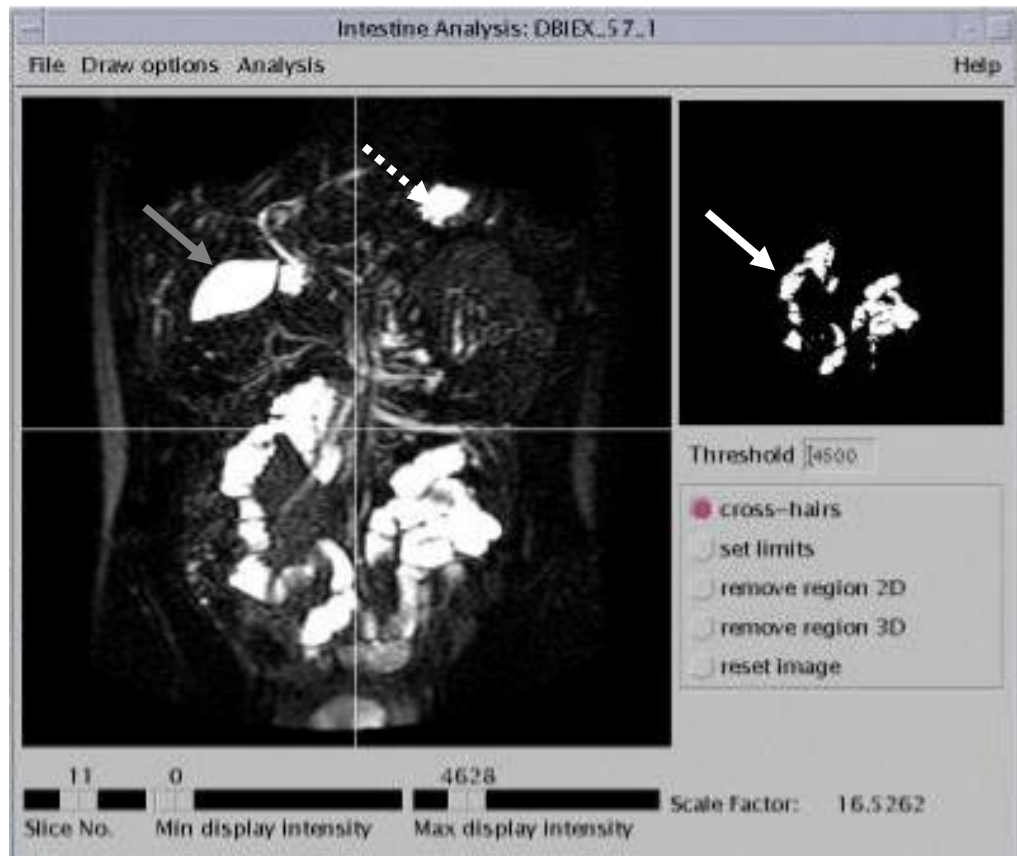


Figure 3-2 Using the software to segment out manually the small bowel (white arrow) from other abdominal fluid-filled structures such as stomach (dashed arrow) and gall bladder (grey arrow).

This chapter describes the methods employed to resolve these issues for quantifying the small bowel free water content using the 1.5 T GE scanner and the MRI sequence used in the MARCO study.

3.3 SMALL BOWEL WATER CONTENT VALIDATION STUDY

3.3.1 Aim

The aim of this validation study was to find the new conversion factor for the *in-house* software used for measuring fluid for the scanner to be used in the MARCO study.

This is the multiplication factor from the CSF marker to the threshold level for free water) and previously validated against naso-duodenal infusions [5] on a Phillips 1.5T not on the GE 1.5T scanner used in the MARCO study. In order to avoid an invasive intubation study, an alternative way of comparing known fluid volumes in the abdomen to the Small Bowel Water Content (heavily T2 weighted 'MRCP' sequence) measured fluid volumes had to be found. Stomach fluid volume measurements made with moderately T2 weighted, anatomical scans have long been accepted as accurate and have been previously validated [124]. Therefore we planned to compare stomach fluid volumes obtained with these anatomical sequence acquired very rapidly before and after the Coronal SBWC sequence to calibrate.

3.3.2 Validation study phase one

The first part of this study carried this out using healthy volunteers. The optimised threshold value was then used to set the threshold value for the SBWC sequence on the 1.5T GE scanner.

3.3.3 Method

The study was approved by the local ethics committee of the University of Nottingham (Ethics number B14112016 *MR imaging to assess small bowel water content volume: calibration and generalisation of methods to different MRI scanners*) and written informed consent was obtained from all participants.

Six healthy volunteers were recruited, with a variety of BMIs: three females age range 41-52 years, and three males age range 20-57 years. Standard MRI exclusion criteria were applied.

The participants attended the MRI centre at 8 am following an overnight fast, with the exception of sips of water for essential medication.

3.3.4 Study design

All imaging was carried out in the SPMIC University of Nottingham Medical School Campus on a GE 1.5 T MRI scanner using a 12 Channel 12 element body array coil.

After a 15 second 3 plane localising scan, three MRI sequences were used to image the abdomen at each time point, an axial SSFSE (single shot fast spin echo) sequence (the moderately T2 weighted, 'anatomical' scan of the stomach water), a coronal heavily T2 weighted single shot Turbo Spin Echo (TSE) sequence (the 'MRCP' sequence) and then the axial SSFSE echo sequence is repeated (the moderately T2 weighted, 'anatomical' scan of the stomach water again). All sequences used a 40 cm field of view (FOV) to ensure full anatomical coverage of the area.

Each image set was acquired on an expiration breath-hold with duration of 19 and 21 seconds, respectively. Baseline scans were obtained with the patient lying supine in the scanner. Participants then drank 300 millilitres of water after the baseline scans and were then scanned sequentially (**Figure 3-3**) using the sequences outlined below until the stomach was almost empty of fluid.

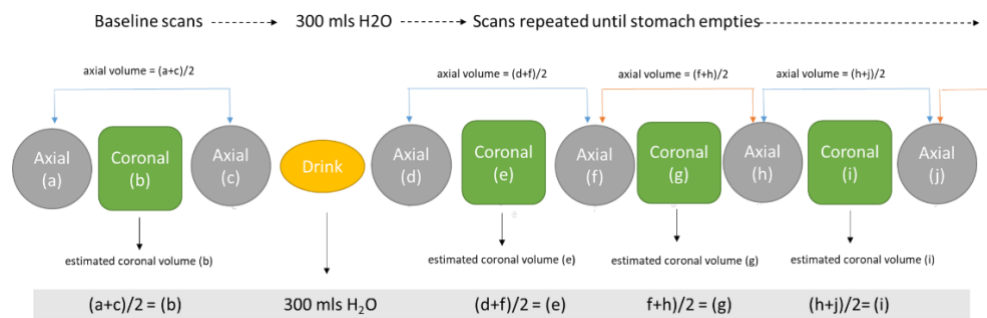


Figure 3-3 Measured axial volumes of the liquid in the stomach from the anatomical scan either side of the coronal sequence are averaged to find a value that should best correspond to the interleaved coronal gastric liquid volumes.

3.3.5 Imaging parameters

Firstly an axial 2D Single Shot Fast Spin Echo (SSFE) sequence was used. Thirty six interleaved 5mm slices were obtained, with contiguous slices used for accurate volume estimation and thin slices to reduce partial volume effects. A TE of 60 milliseconds, TR of 495 milliseconds and a flip angle of 90° gave good contrast between the fluid contents of the stomach and surrounding gastric walls. The interleaved slices were obtained in a 19 second breath hold and an ASSET (Array Coil Spatial Sensitivity Encoding) factor of 2.

Coronal small bowel sequence used a coronal single shot fast spin echo sequence with fat saturation. A TE of 320 milliseconds and a TR of 1154 milliseconds ensured a heavily T2 weighted sequence. This ensured high intensity signals from areas with freely mobile fluid and little signal from body tissues. Slice thicknesses of 7 mm were used.

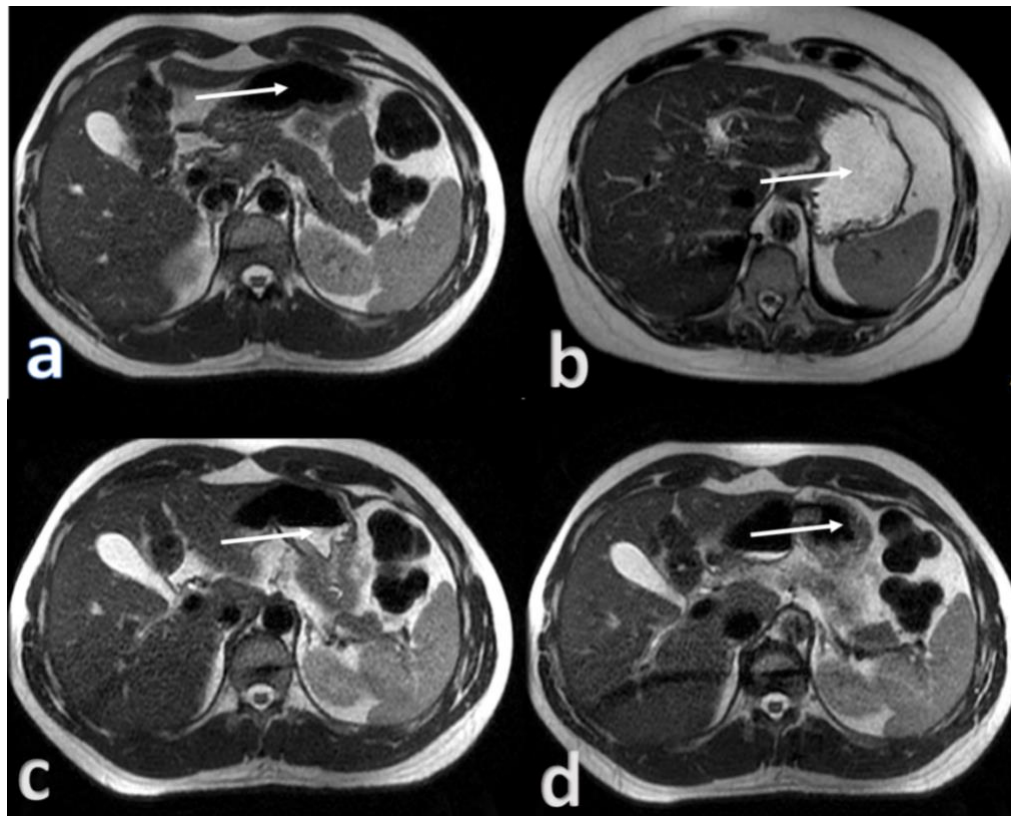


Figure 3-4 The white arrows point at the stomach in these axial, moderately T2 weighted, 2D Single Shot Fast Spin Echo (SSFE) sequence (a) baseline empty stomach (b) post drink -fluid in stomach (c) 30 min post drink – almost empty stomach (d) last scan- emptied stomach

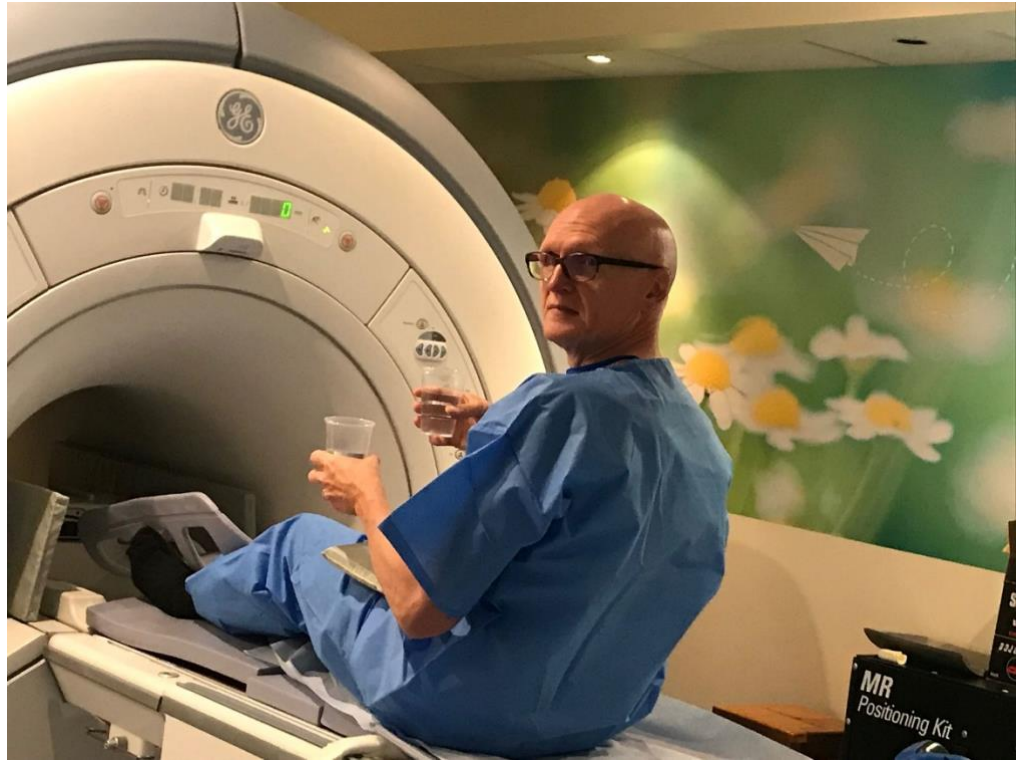


Figure 3-5 Study participant ingesting 300 ml water after the baseline scan during the validation study (permission given).

3.3.6 Image analysis

Post-processing analysis algorithm for measurement of gastric volumes. Axial volumes: Using an intensity-based, semi-automatic method [127], volumes of the gastric contents were calculated by drawing around the bright liquid in the stomach shown on each axial slice. Custom written Interactive Data Language (IDL®) software (Research Systems Inc., Boulder, Colorado, USA) is used for this image analysis with a specialised programme developed by Dr Caroline Hoad at the SPMIC.

A 'seed point' was selected in the bright signal corresponding to the water in the stomach and then a minimum signal level was selected using a slider as illustrated in **Figure 3-6** below. All voxels above this level were included to define a binary mask. The software found the edges of the stomach content region easily because of the good contrast against the stomach walls and gas in this MRI sequence and this was displayed. The software allowed the ability to further refine the intensity levels if necessary.

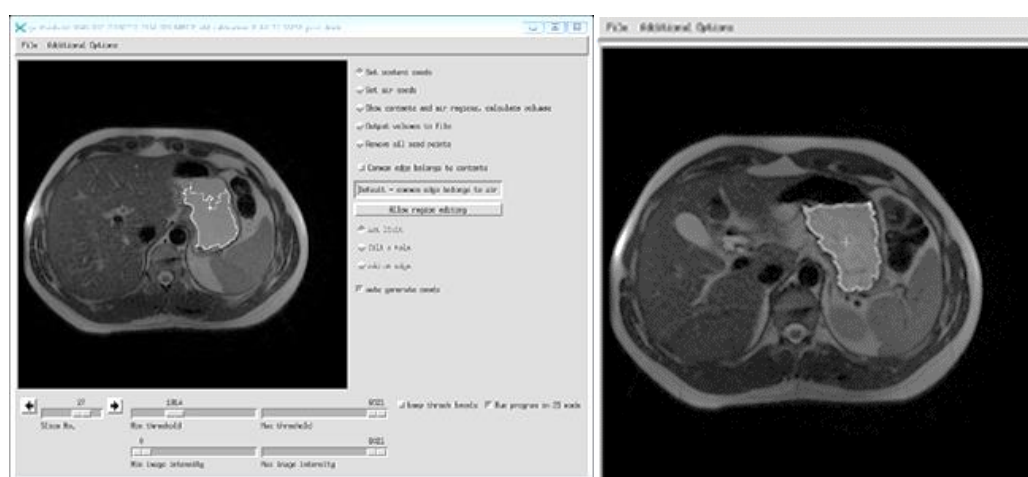


Figure 3-6 Seed point (white cross) and minimum threshold selection shown on axial anatomical slice outlining the stomach contents post ingestion of water.

3.3.7 Coronal volume calculation.

At this point the small bowel water content software previously described was used to define the stomach fluid volumes on the coronal images, using the same procedure as for the small bowel. Using a similar

intensity-based, semi-automatic method, an intensity value was set using the CSF (**Figure 3-7**) in the same way as for the small bowel analysis.

Then non-stomach data was excluded from each coronal slice by drawing around the bright liquid in the stomach (**Figure 3-8**). The software then calculated the number of voxels (a 3-dimensional dataset) in each of the regions of interest drawn across the dataset with an intensity above the threshold calibrated using the subject's cerebrospinal fluid. The bin size used was varied and the multiplication factor was changed to generate different threshold levels, which subsequently generated different stomach volumes.

By working in this way now both stomach 'true' fluid volume data and 'stomach fluid volume data obtained with the sequence in need of calibration were available and could be compared for calibration.



Figure 3-7 Setting the spine region (white arrow) on the coronal scans for threshold calculation.

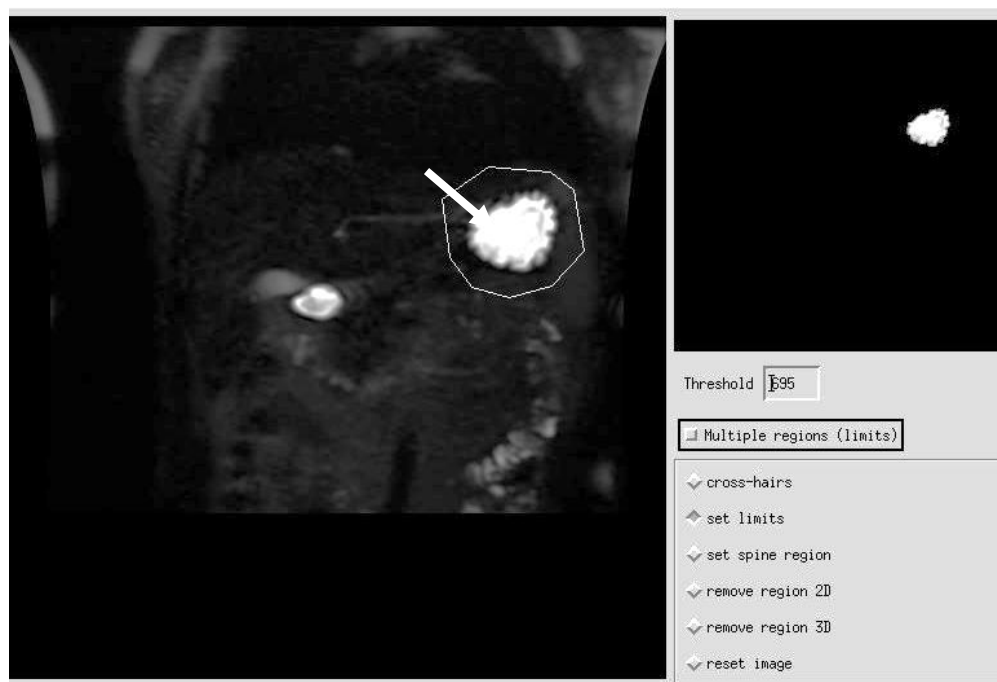


Figure 3-8 Segmenting the stomach contents (white arrow) on each coronal image.

3.3.8 Results

All participants tolerated the scanning and completed the study. The images were analysed and the measured axial volumes of the liquid in the stomach from the anatomical scan either side of the coronal SBWC sequence were then averaged to find a value that should best correspond to the interleaved coronal gastric liquid volumes. Each sequence was acquired in a breath-hold. A new sequence could be acquired approximately every minute including scanner preparation and reconstruction time; the stomach would have emptied a small amount of fluid in that time, and the average of the two anatomical scans either side of the SBWC sequence compensate for that emptied volume.

The coronal sequence gastric volumes were calculated using thresholds with two bin sizes (20 and 30) multiplication factors 1.1, 1.2, 1.3, 1.5, 1.7, 2. These initial values are not arbitrary but were chosen around the expected possible range of values based on previous experience.

On the x-y scatter plot below (Figure 3-9) the axial anatomical volumes are on the x axis and the estimated coronal volumes are on the y axis, with each CSF multiplier displayed as a different dataset (just 4 of the multipliers shown for clarity).

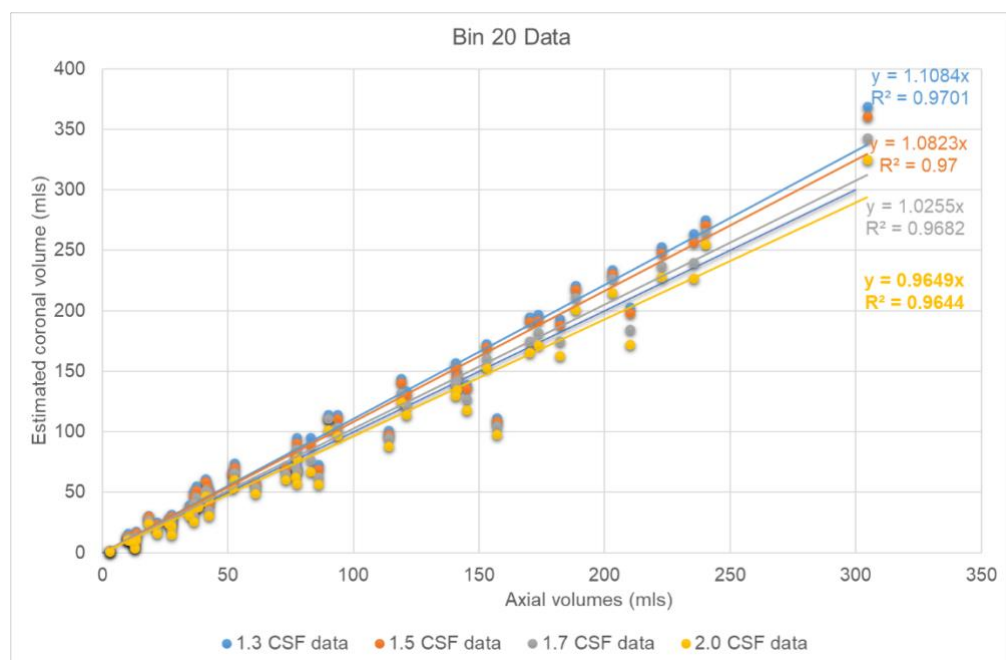


Figure 3-9 Graph to show estimated coronal volume in millilitres versus measured axial volumes. Linear fits to the data are shown in the legend.

Each dataset was then fitted to a linear fit to get the gradient of the line – the aim was to get a gradient of 1 which means the same stomach fluid volume was being measured with both techniques. The data fits were repeated for a range of bin sizes and a range of CSF marker multipliers

and the best combination providing a gradient closer to 1 and the higher R^2 fit was chosen (

Table 2 Gradients for a range of bin sizes and threshold values.)

This was true for a threshold 1.83 at bin size 20 (**Figure 3-10**). These values were carried forward for the rest of the study.

Threshold	Gradient	Gradient
	Bin 20	Bin 30
1.3	1.1084	1.0304
1.5	1.0823	1.0049
1.7	1.0255	0.9428
2	0.9649	0.8715
Gradient	-0.2122	-0.2354
Intercept	1.3901	1.3449
	CSF	CSF
So for y=1	1.83836	1.465166

Table 2 Gradients for a range of bin sizes and threshold values.

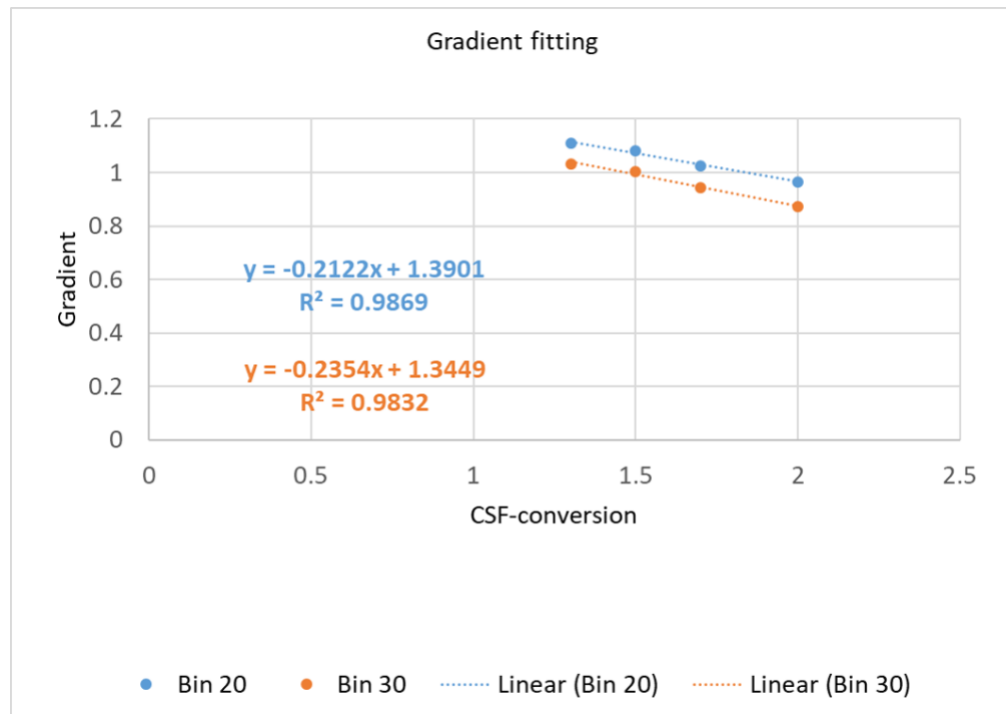


Figure 3-10 Graph showing the gradient fitting for bin size 20 and bin size 30.

3.3.9 Validation study phase two

The first phase of calibration work described above took place while the first few MARCO study participants were being recruited and scanned. Upon initial analysis and following a manufacturer's software upgrade on the scanner it was realised that further improvement in the SBWC sequence on the 1.5T GE itself were possible. These small changes in the sequence parameters yielded sharper images with much better contrast. This new improved SBWC sequence was therefore

implemented in the MARCO protocol whilst keeping in the scan protocol the previous version of the SBWC sequence too for consistency and comparison.

The improvements in the SBWC sequence parameters were carried out on the same 1.5T GE platform and same imaging sequence. Therefore much of the phase one validation work still applied and only a simple comparison between the same data sets acquired with the 'old' and 'new' SBWC parameters would be sufficient to calibrate the analysis software. The graph below describes the direct comparison of the data analysis made using 23 sets of 'old' and 'new' SBWC sequence parameters. The correlation between the two parameter sets is very good ($R^2=0.9716$) (**Figure 3-11**). Forcing the intercept through zero as done for phase one a gradient of 1.03 is again obtained, only 3 ml out for every 100 ml measured.

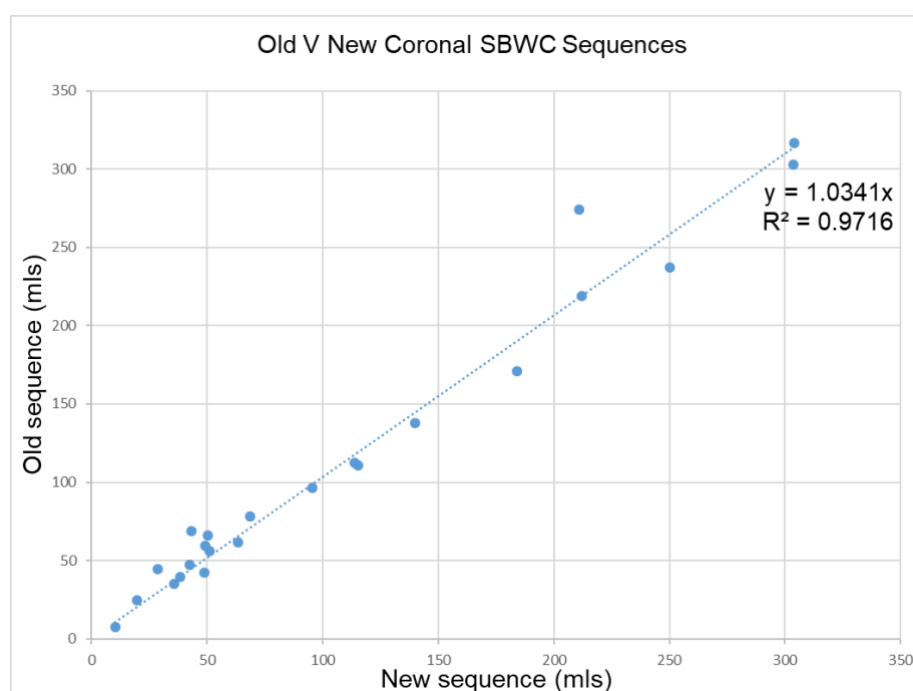


Figure 3-11 Graph showing the values in millilitres for the old versus new coronal Small Bowel Water Content (SBWC) sequences.

3.3.10 Discussion

Reproducibility of quantitative measurements when moving between different scanner platforms and field strengths is crucial for clinical translation of MRI research. In GI MRI studies, measuring fluid volumes, such as gastric emptying which measures the reduction in volume of gastric contents over time, and small bowel water measurement, accuracy and repeatability is essential and to gain widespread use the technique must be user friendly, non-time consuming and suitable for use on data from all major scanner manufacturers.

This is the first time the measurement of mobile water in the abdomen using the SBWC sequence has been validated moving from one manufacture's scanner to another. Although only one experienced observer measured the volumes in this validation study inter observer variability for the semi-automatic algorithm for measuring stomach contents and air volumes has been previously reported (for similarly experienced observers) at less than 10 mls per 400 mls [127] and the coefficient of variation of the volume measurements was below 5% for volumes above 100ml in a similar MRI gastric emptying study [128].

The technique does have some limitations - due to partial volume effects as the resolution used means that voxels with very sub-voxel small amounts of water are excluded from the analysis, and we are only measuring 'mobile' free water.

Notwithstanding these limitations, the findings in this validation study demonstrate a new method for validating mobile fluid measurement

across different MRI field strengths and manufacturers which will expand the research and clinical potential of this technique.

3.3.11 Conclusion

I have described firstly a new method to calibrate quantification of small bowel free water content and secondly used the result to set the threshold value for quantification analysis of the SBWC sequence used in the MARCO study using a different scanner platform and software. The new method, for the first time, does not require invasive intubation procedures for validation.

This method may be used to set a new threshold value for SBWC image analysis to ensure reproducibility when moving quantitative experiments between coils, scanners of different field strengths and/or manufacturers and, therefore, between laboratories, therefore making the SBWC measurement and analysis more translational.

4 MAGNETIC RESONANCE IMAGING OF COELIAC DISEASE (MARCO)

4.1 INTRODUCTION

The literature review presented in Chapter 1 of this thesis has reviewed the usefulness of conventional diagnostic imaging in the evaluation and monitoring of coeliac disease. Previous studies in Nottingham developed new MRI techniques to image the undisturbed bowel and to quantify novel parameters of GI function. This work led to this study to investigate if MRI could provide new insights into the mechanisms of coeliac disease, the effect of a gluten free diet on the bowel of coeliac patients and its relationship with symptoms. The overall aim was to develop new non-invasive measurements that could be useful to follow-up the response to gluten-free diet treatment and future therapies.

4.2 STUDY DESIGN

4.2.1 Rationale

A recent pilot study from our group assessing SBWC in untreated patients with coeliac disease, patients with scleroderma and irritable bowel syndrome with diarrhoea (IBS-D) showed that MRI measurements of fasting SBWC and of colonic volumes may relate to clinical features of

these diverse gastrointestinal diseases [129]. This provided not only an initial novel insight into bowel features but also raised the question of whether this could lead to new and useful non-invasive MRI techniques to evaluate the effects on the bowel of a gluten free diet on incident coeliac disease.

4.2.2 MRI of small bowel water content.

Figure 4-1 shows a coronal ‘small bowel water content’ MRI image of the abdomen of a healthy volunteer and one of an untreated coeliac patient taken from Lam *et al* [130]. These MRI images are strongly ‘T2 weighted’ which means they show very little signal from tissue and very bright signal from freely mobile fluid. The image from the coeliac patient shows a substantial volume of fluid resting in the fasted small bowel.

As discussed in previous chapters, the main factors contributing to the amount of fluid present in the bowel at a given time point are gastric emptying, water absorption, small bowel secretions (including from the gallbladder and pancreas) and propulsion to the colon. Accordingly, fasting SBWC reflects the underlying balance between secretion, absorption and motility without the additional contributions of the fed state motor and secretory responses.

The increased fasting SBWC in coeliac disease is hypothesised to be due to a combination of impaired motility, impaired absorption due to the villous atrophy and increased secretions due to the crypt hyperplasia.

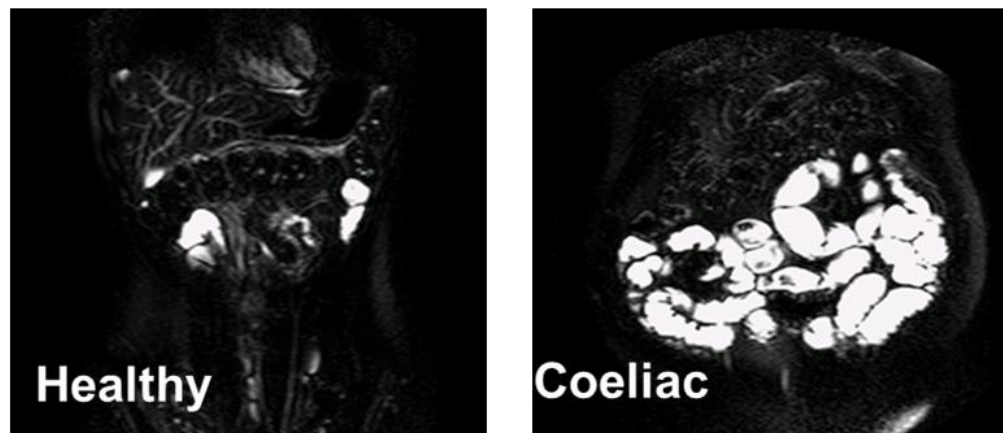


Figure 4-1 Coronal ‘small bowel water content’ MRI images of the abdomen taken from Lam *et al* [130].

4.2.3 MRI of colonic volumes

MRI is also widely used to measure organ volumes. Within research conducted during a study of IBS patients and on the mode of action of MoviPrep, a low-volume polyethylene glycol (PEG) osmotic bowel cleansing preparation (10/H0906/50) [131], our research group at the University of Nottingham has recently developed protocols to measure regional colonic volumes from coronal dual-Fast Field Echo imaging [132] as illustrated in (**Figure 4-2**). Pilot data from our laboratory also indicates that the volume of the fasting colon in adults newly diagnosed with coeliac disease may correlate with disease severity [129].

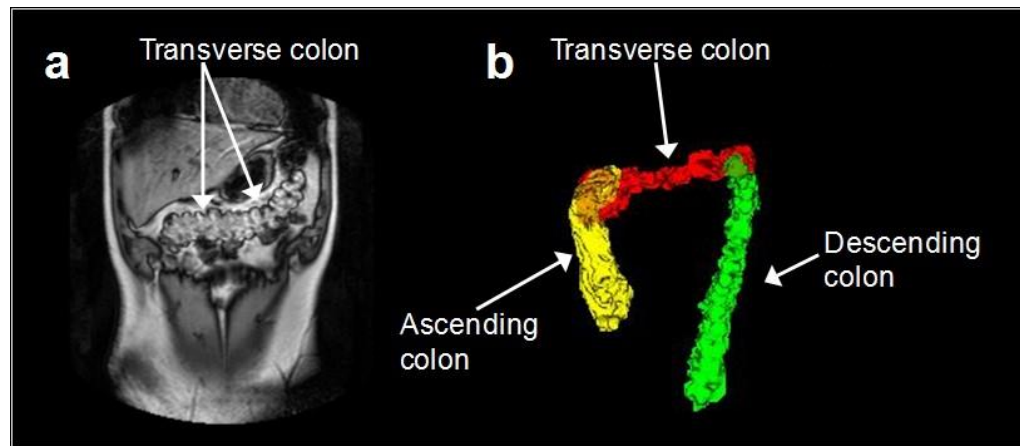


Figure 4-2 shows on the left (a) one coronal ‘dual FFE’ MRI image of the transverse colon of a fasting healthy volunteer and on the right (b) a three-dimensional reconstruction of the different regions (ascending, transverse and descending) of the colon of another healthy volunteer.

4.2.4 MRI of whole gut transit

A novel method to measure whole gut transit time using MRI localisation of MRI transit marker capsules was developed and validated thanks to Medical Research Council (MRC) funding (NREC approval 11/EM/0245). The MRI markers are capsules made of polyoxymethylene, a biologically inert substance [99]. The capsules consist of two half-shells which are glued together using cyanoacrylate superglue. A small hole is drilled in the top of one half-shell, through which the capsules are hand filled with 0.4 mL water solution containing Dotarem™ (15 μ M Gadoteric acid Gd-DOTA).

A small plastic screw is then glued into position in the hole with the superglue to prevent leakage. Spectrophotometry is then used to assess leakage on one fifth of each batch of capsules produced.



Figure 4-3 MRI Transit capsules size: 20 x 7 mm

The day before the study day, study participants swallow 5 inert plastic MRI transit markers. The capsules are inert, the size of a typical adult vitamin pill and do not interact with the body (**Figure 4-3**). Twenty – four hours after ingestion the participants attend for an MRI scan to locate the capsules in the gut. The whole gut transit time is calculated by scoring the position of the markers in the gut according to a simple position scoring card and calculating a weighted average position, as validated against conventional X-ray methods in our previous study looking at oro-coecal transit time in healthy volunteers [99].

The MRI transit capsules have had NRES approval for various studies with our GI imaging group including the MIMIC study (Sponsor 14001 – NRES 14/EM/0075). Although there is no pilot data on gastrointestinal transit in coeliac disease, data available in the literature suggests that gastrointestinal transit is delayed in coeliac disease in the colon [43] and increased in the untreated coeliac small bowel [44]. These markers

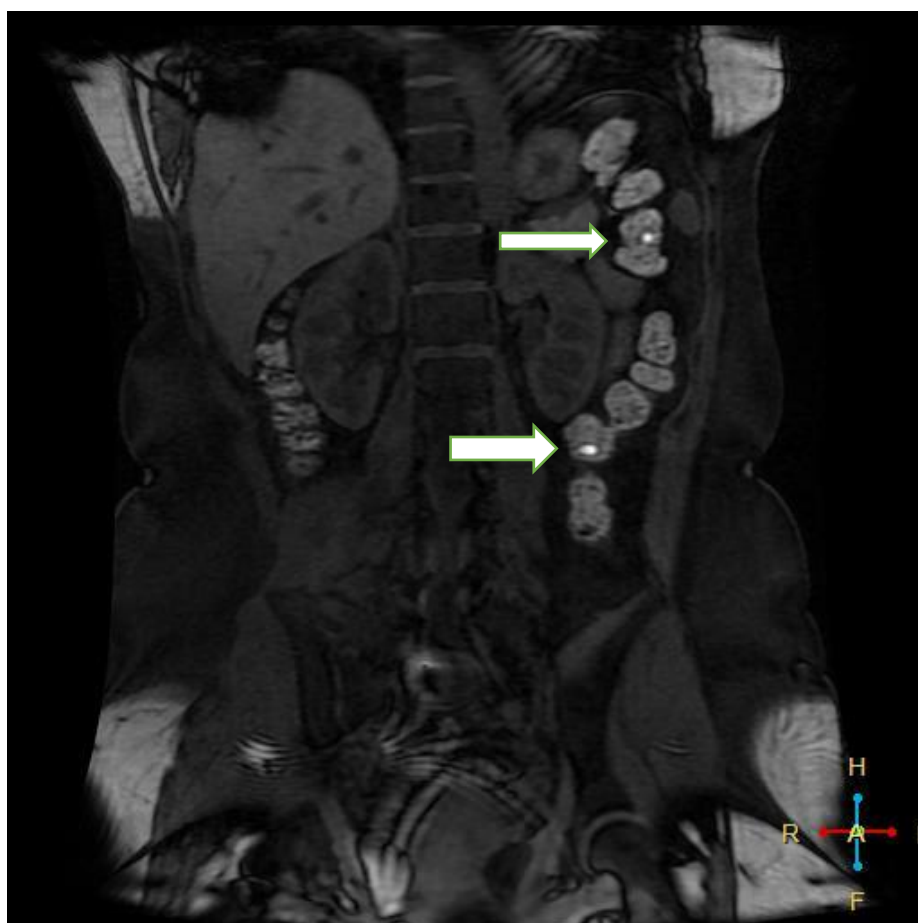


Figure 4-4 T1-weighted TFE sequence images of the abdomen of a study participant taken 24 hours after swallowing the MRI transit marker capsules showing location of transit pills in the gut (white arrows).

allowed evaluation of coeliac transit times both in the untreated and treated disease. The image of a T1-weighted TFE sequence images of

the abdomen of a study participant taken 24 hours after swallowing the MRI transit marker capsules is shown above (**Figure 4-4**).

On this MRI sequence the marker capsules appear bright. Capsules are visible here (indicated also by the arrows) in the descending colon.

The whole gut transit time is calculated by scoring the position of the markers in the gut according to a simple position scoring card (**Figure 4-5**) and calculating a weighted average position, as validated against conventional X-ray methods in our previous study looking at oro-coecal transit time in healthy volunteers [133] .

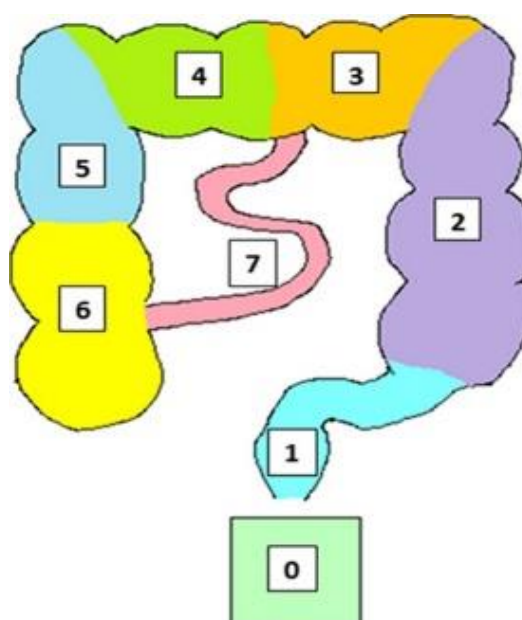


Figure 4-5 Segmented Colon. Showing the segmented colon used to score the MRI marker capsules at 24 hours, where 0 = not found (presumed to be excreted), 1 = sigmoid and rectum, 2 = descending colon, 3 = left transverse colon, 4 = right transverse colon, 5 = upper ascending colon.

4.3 HYPOTHESES

The findings from the initial pilot study on SBWC described above [130] led to the main hypotheses that SBWC is increased in patients with incident coeliac disease and that this would reduce after gluten free diet treatment.

It is also hypothesised that assessing fluid volumes in the small bowel, colonic volumes and whole gut transit, their relationship to symptoms and changes induced by GFD could provide new understanding of mechanism of coeliac disease and effects of treatment. Doing this has proven difficult so far due to limitations of previous methods. MRI could provide these parameters, noninvasively in a single patient acceptable scanning session and with no difficult and unpleasant bowel preparation. Combining together all the above, the overarching hypothesis underpinning this work was that non-invasive MRI techniques can provide novel insights into coeliac disease, the effect of GFD, and the relationship of gastrointestinal function and symptoms.

4.4 AIMS AND OBJECTIVES

Based on pilot data and on the literature available, this study aimed to test the main hypotheses that in adults newly diagnosed with coeliac disease treatment with GFD will:

1. reduce the water content of the fasting small bowel
2. reduce the volume of the fasting colon

3. decrease whole gut transit time

4.4.1 Primary Objectives

The specific objectives of the study were therefore to quantify any change following treatment with a gluten-free diet (GFD) in:

- the water content of the fasting small bowel
- fasting colon volume
- Whole gut transit time

in adults newly diagnosed with coeliac disease using non-invasive MRI techniques.

4.4.2 Co-variables of interest

This study also measured a series of exploratory co-variables of interest at diagnosis and 12 months follow-up:

- Body Mass Index (BMI)
- Gastrointestinal symptoms (Bristol Stool diary for a week, HAD, PHQ-15, PHQ-3, GI symptoms Visual Analogue Scale (VAS))
- Marsh grading of duodenal biopsies (for coeliac patients, at diagnosis only)
- Fasting breath hydrogen
- Serology values
- Faecal microbiota and short chain fatty acids

- In a subgroup analysis, associations between the MRI outcomes and these co-variables: (a) before treatment, (b) after treatment and (c) differences between the two are described.

4.5 STUDY CONFIGURATION

This pilot study in 36 newly diagnosed coeliac disease patients before and after a gluten-free diet took place at the Nottingham Digestive Diseases Centre (NDDC) and NIHR Biomedical Research Centre (BRC) at Nottingham University Hospitals NHS Trust for screening and follow up visits, and the University of Nottingham's Sir Peter Mansfield Imaging Centre (SPMIC for MR imaging).

A parallel group of 36 healthy controls matched for age and sex were recruited for pilot comparison data. They were scanned twice and approximately 12 months apart, as the patients were, to allow investigating temporal effects in the endpoints measured and to assess also the impact of any seasonal change.

4.5.1 Patient Participant recruitment

Patient participants were recruited through specialist clinics at Nottingham University Hospitals NHS Trust. The initial approach was from a member of the patient's usual care team (Gastroenterologists and specialist dieticians), and information about the trial was also on display in the relevant clinical areas. All patients who expressed an interest in taking part were sent a detailed information sheet, which also contained

the contact information of the study team and this gave them an opportunity to raise any questions regarding the trial before and during the first screening visit. Patients were also recruited via the specialist dietician coeliac group sessions for newly diagnosed patients.

Within the study period 36 patients were recruited, 24 females and 12 males with a mean age of 46 years, range 20-69 years. The mean age of the females was 40 years, and the male patients mean age was 59 years. Overall the mean BMI of the patients was 25.6 kg/m² (SD 6.6) at recruitment, 24.26 kg/m² for females and 28.6 kg/m² for the males. (See consort diagrams (**Figure 4-6**)).

This study intercepted most newly diagnosed coeliac adult patients referred to the specialist coeliac dietician service at Nottingham University Hospitals NHS Trust during the recruitment period by the attending the group sessions and presenting details of the MARCO study. In this way one third of those attendees were recruited.

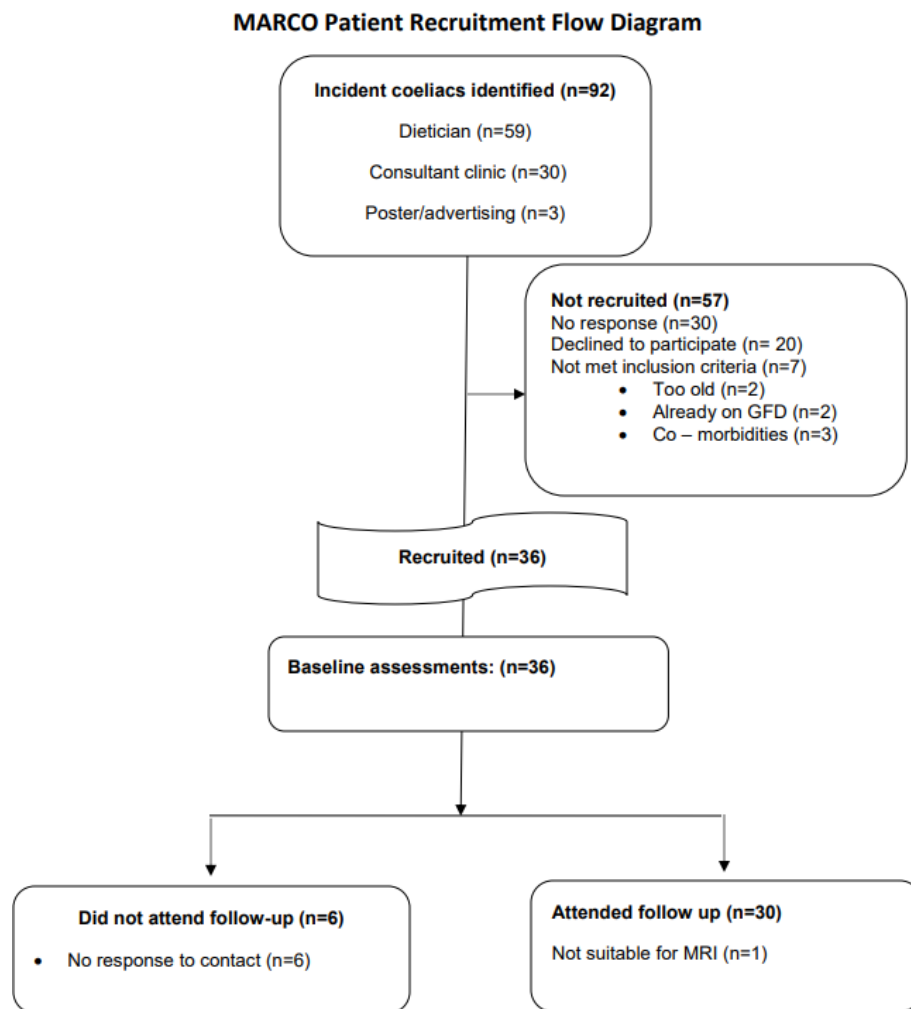


Figure 4-6 CONSORT diagram for MARCO patient recruitment

4.5.2 Healthy volunteer participant recruitment

This study also included a parallel pilot study on healthy controls frequency matched for age and sex to provide descriptive statistics on a likely reference range for the healthy population to inform the design of future studies. General advertisement for participants in the healthy

volunteer group took place in parallel, on the campuses of the University, including the Cripps Health Centre (primary care), and Nottingham University Hospitals, by poster. Healthy controls were also recruited using social media such as the Nottingham Digestive Diseases Unit Facebook and Twitter accounts.

Fifty-three healthy controls were assessed for eligibility via email or telephone and 24 females and 12 males were recruited and underwent baseline assessments and MRI scan. The healthy participants at recruitment had a mean age of 43 years with a range of range 20-65 years and mean BMI was 25.97 (SD 4.4) kg/m². The mean age of the female healthy controls was 26 years, and the male controls mean age was 50 years

Thirty-three attended follow up assessment 12 months later, 1 participant did not respond to follow up contact, 1 became pregnant and 1 had viral gastroenteritis so was ineligible for follow up (see consort **diagram Figure 4-7**).

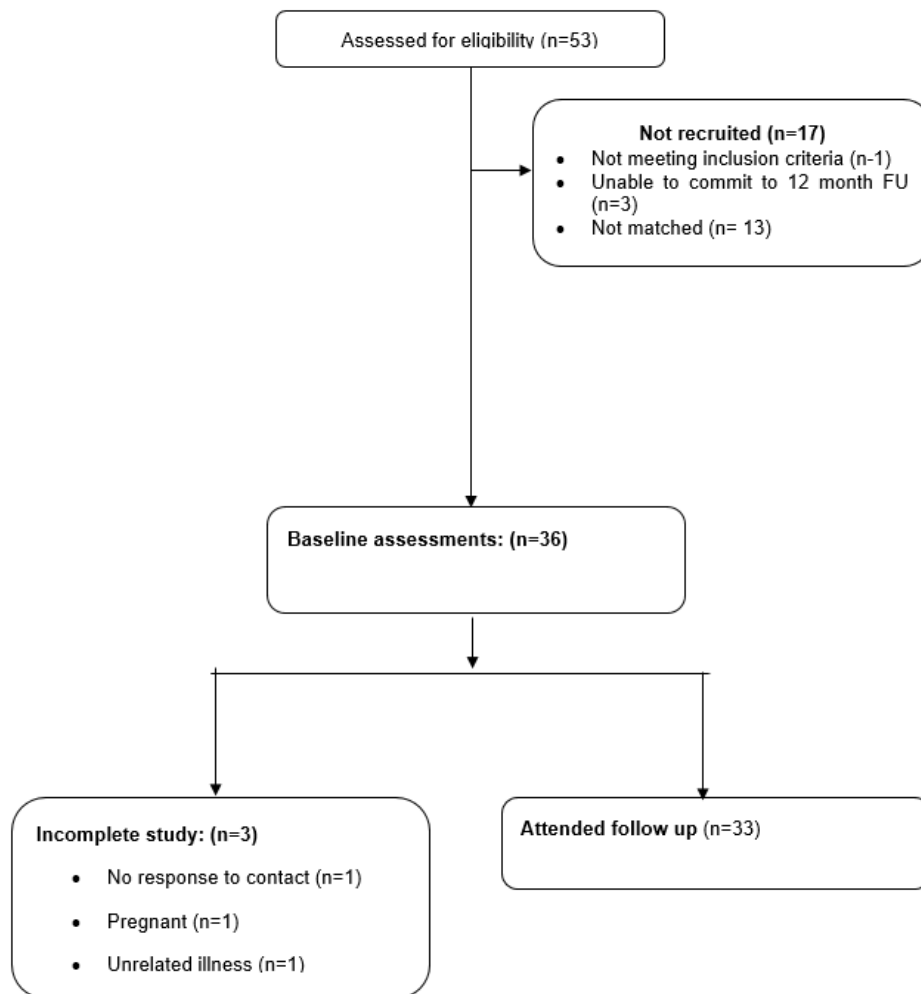


Figure 4-7 Consort diagram of healthy control recruitment for the MARCO study

4.5.3 Eligibility

Those who expressed interest in the study were initially screened by the author via email or telephone, then if deemed eligible were invited to a screening session to establish whether they met the study inclusion criteria and could comply with the study regime.

Thirty six participants newly diagnosed with Coeliac disease before starting treatment with a gluten free diet and thirty six participants who do not meet criteria for a clinical diagnosis of coeliac disease (healthy controls) as determined by screening blood sample were recruited.

Criteria for diagnosis

Patient on a gluten-containing diet, IgA-TG2 or IgA-DGP, or IgG-DGP, and duodenal biopsy confirming coeliac disease.

Patient study inclusion criteria

- Patients newly diagnosed with coeliac disease
- Aged 18-70
- Male or female
- Able to give informed consent
- Able to schedule the first MRI scan (Visit 2 of the study) within a month of having had a duodenal biopsy.

Healthy controls inclusion criteria

- Healthy controls (without any co-morbidities)
- Aged 18-65
- Able to give informed consent

Patient study exclusion criteria

- Any past serious, unstable medical condition, unstable/uncontrolled diabetes mellitus, major psychiatric diagnosis
- Any reported history of gastrointestinal surgery that could affect gastrointestinal function (colectomy, small bowel resection)
- Pregnancy declared by candidate

- Contraindications for MRI scanning i.e. metallic implants, pacemakers, history of metallic foreign body in eye(s) and penetrating eye injury
- Reported alcohol dependence
- Unable to stop drugs known to alter GI motility including mebeverine, opiates, monoamine oxidase inhibitors, phenothiazines, benzodiazepines, calcium channel antagonists during or in the 2 weeks prior to the test. The following medications were permitted during the course of the study, as long as they had been used at a constant dosage and were commenced at least 1 month prior to the start of the study: birth control pill, or intramuscular contraceptive preparation, oestrogen–progesterone replacement therapy, L-thyroxine, low-dose antidepressants (up to 25 mg day⁻¹ of amitriptyline, nortriptyline, or selective serotonin reuptake inhibitor), or antihypertensive in the diuretic, angiotensin-converting enzyme inhibitor or angiotensin II inhibitor classes.
- Antibiotic or probiotic treatment in the past 4 weeks
- Inability to lie flat or exceed scanner limits of weight <120kg
- Poor understanding of English language
- Participation of any medical trials for the previous 3 months
- Judgement by the PI that the candidate who will be unable to comply with the full study protocol e.g. severe COPD

Healthy control study exclusion criteria

- Serology positive test for coeliac disease markers
- Any reported history of gastrointestinal surgery (including appendicectomy or cholecystectomy)
- Presence of an intestinal stoma
- Pregnancy declared by candidate
- Contraindications for MRI scanning i.e. metallic implants, pacemakers, history of metallic foreign body in eye(s) and penetrating eye injury
- Reported alcohol dependence
- Unable to stop drugs known to alter GI motility including mebeverine, opiates, monoamine oxidase inhibitors, phenothiazines, benzodiazepines, calcium channel antagonists during or in the 2 weeks prior to the test. The following medications were permitted during the course of the study, as long as they had been used at a constant dosage and were commenced at least 1 month prior to the start of the study: birth control pill, or intramuscular contraceptive preparation, oestrogen–progesterone replacement therapy, L-thyroxine, low-dose antidepressants (up to 25 mg day⁻¹ of amitriptyline, nortriptyline, or selective serotonin reuptake inhibitor), or antihypertensive in the diuretic, angiotensin-converting enzyme inhibitor or angiotensin II inhibitor classes.
- Proton Pump Inhibitor (PPI), antibiotic or probiotic treatment in the past 12 weeks
- Inability to lie flat or exceed scanner limits of weight <120kg

- Poor understanding of English language
- Participation of any medical trials for the past 3 months

Removal of participants from assessments/Participant Withdrawal

Participants could be withdrawn from the trial either at their own request or at the discretion of the Investigator. Reasons for the investigator withdrawing a participant included non-adherence, or safety reasons (including pregnancy).

Given the pilot study nature of the study, participants withdrawn from the study were not replaced. The participants would have been made aware that this did not affect their future care. Participants were made aware (via the information sheet and consent form) that, if they had withdrawn, the data collected to that date was not erased and may have been be used in the final analysis.

Once a participant was withdrawn, or been withdrawn, from the study, no further data about them was entered. There were no risks to the participant of withdrawing from the study as participation did not affect standard care pathways.

4.5.4 Study regimen and visit schedule

4.5.4.1 Newly diagnosed coeliac patients

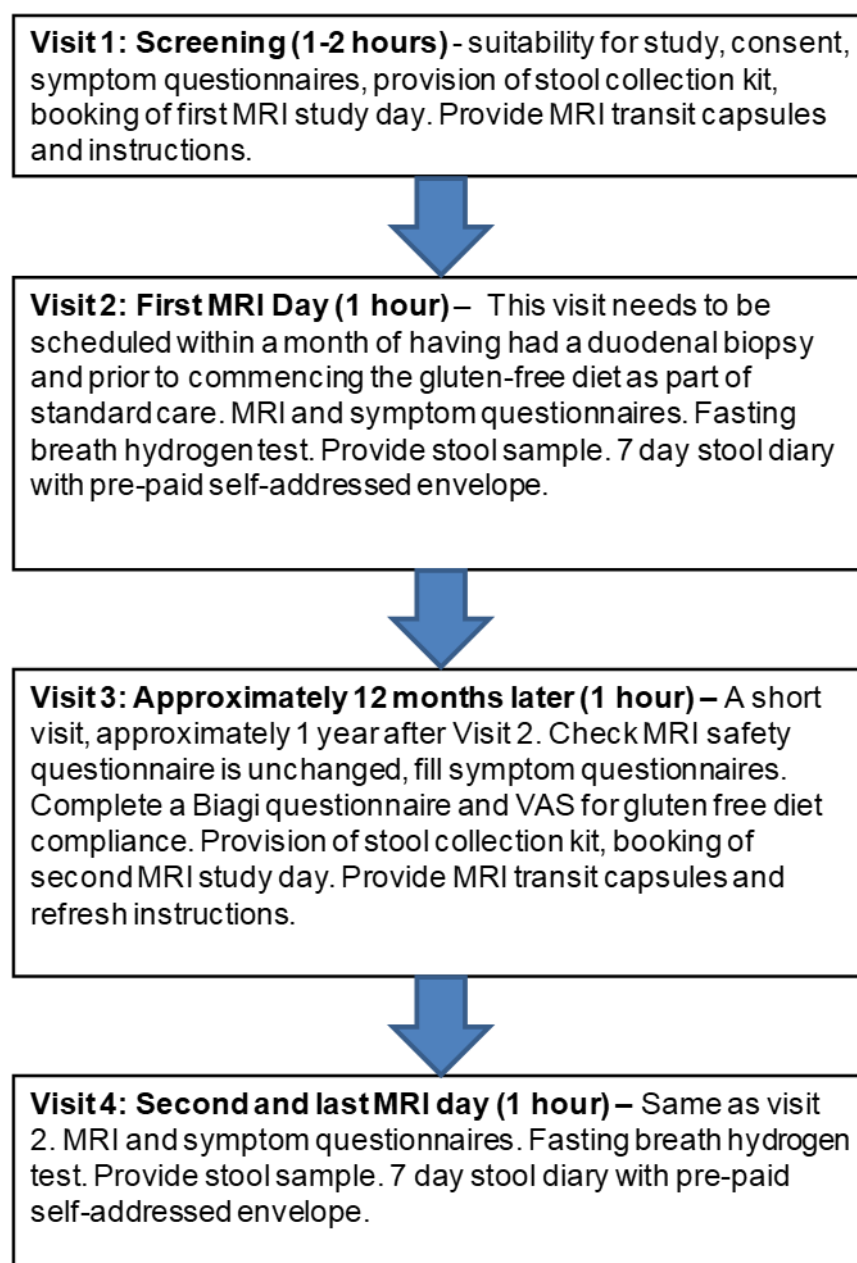


Figure 4-8 Schematic diagram of MARCO study involvement for coeliac patients

Visit 1

This was a screening visit at the NIHR Nottingham Digestive Disease Biomedical Research Unit, QMC Hospital, Nottingham. This first study visit took place after the patients have been through diagnosis under their normal care. At this visit they were given more explanations and the long form of the Information Sheet which was discussed with them to ensure understanding. The visit lasted 1/2 hour-1 hour and if the patients were happy to do so the investigator checked suitability for the study, and went through the process of consent, including completion of the Hospital Anxiety and Depression Questionnaire and the PHQ 15 questionnaire, as well as brief medical history. All controls completed an MRI safety questionnaire, to exclude persons with contraindications to MRI. If they were eligible and consented to be in the study, the patients were also given 7-day Bristol stool diaries, to be filled in during the week preceding each subsequent study day, and a stool collection kit in order to bring a stool sample to visit 2. MRI transit capsules and instructions were also provided. At the end of Visit 1 the first MRI study day (Visit 2) was booked.

The instructions for the MRI transit capsules were as follows: the patients were instructed to swallow the 5 MRI transit capsules at 8am before their normal breakfast on the day before Visit 2 with a large glass of water (at least half a pint or 350 millilitres). No lifestyle changes were required during that day. In the evening the patients were asked to have their main meal by 8pm. After that, they were asked not to eat, and only drink water. The patients were then asked not to eat anything on the morning of the

MRI test so that they had fasted for 12 hours. They were allowed a small sip of water to take any medicines, but this had to be before 7am.

Visit 2: First MRI Day (1 hour) – at the Sir Peter Mansfield Imaging Centre 1.5T MRI unit – This visit was scheduled for the patients within a month of having had a duodenal biopsy and before starting the gluten free diet as part of their standard care. A fasting breath hydrogen test was carried out. The patients provided a stool sample, and underwent a short (approximately 15 minutes) set of MRI scans. The patients were given a 7-day stool diary with a pre-paid, self-addressed envelope to return it. They were asked to change their clothing into disposable scrubs provided and to remove all metal objects (e.g. watches, jewellery). They then underwent the MRI scanning protocol as detailed in section 4.2. All procedures including the MRI scanner were carried out by the author.

In between patient Visit 2 and patient Visit 3 the patients followed their gluten free diet treatment regime and had a follow up visit at approximately six months including repeat serology with the specialist dietician, as part of their standard clinical care.

Visit 3: Approximately 12 months later (1/2 hour to 1 hour) – at the NIHR Nottingham Biomedical Research Centre, QMC Hospital, Nottingham – At this point the patients had undergone their clinical follow up visit as part of their standard care as described above. After this the patients were contacted and they were invited to attend this short Visit 3. This involved checking that the MRI safety questionnaire was unchanged, filling in again the same symptom questionnaires as in Visit 1. A Biagi questionnaire and VAS for gluten free diet adherence was completed. A

stool collection kit was provided again. The second and last MRI study day was then booked as soon as possible. MRI transit capsules and instructions were also provided as before.

Visit 4: Second and last MRI day (1 hour) – at the Sir Peter Mansfield Imaging Centre 1.5T MRI unit – This was exactly as Visit 2. A fasting breath hydrogen test was carried out. The patients provided a stool sample, changed their clothing into disposable scrubs and underwent a short (approximately 15 minutes) set of MRI scans. A VAS symptom score was also taken.

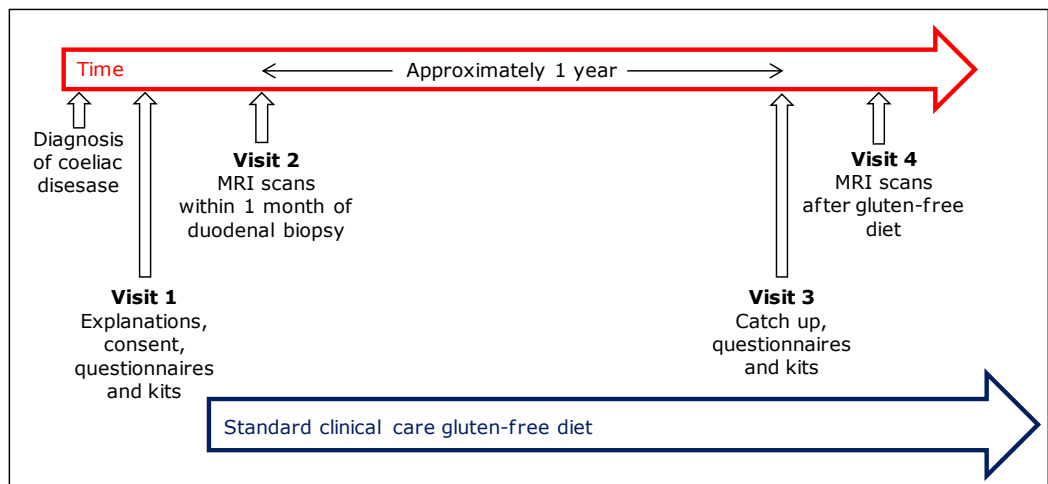


Figure 4-9 MARCO study workflow for coeliac patients

4.5.4.2 Healthy controls:

The healthy controls do not follow a gluten-free diet (GFD).

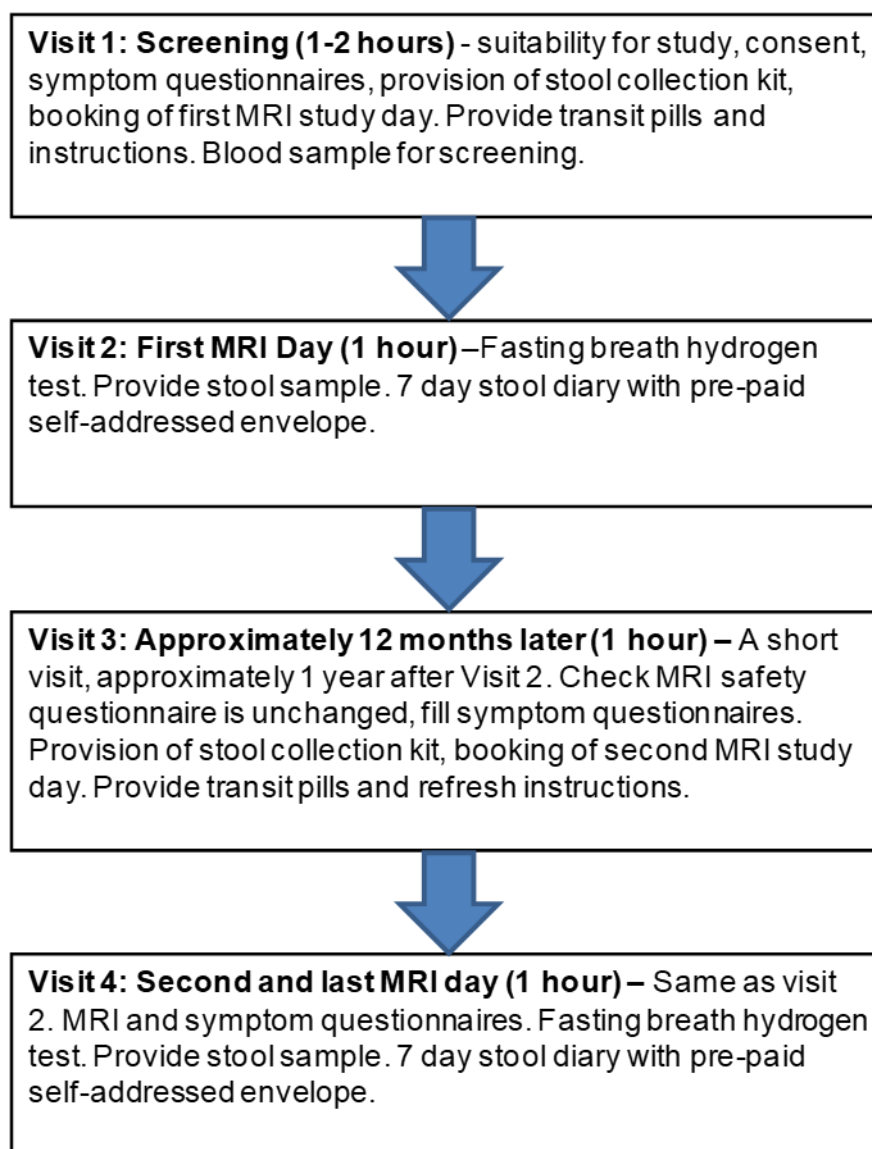


Figure 4-10 Schematic diagram of MARCO study involvement for healthy controls

Visit 1:– screening visit at the NIHR Nottingham Digestive Disease Biomedical Research Unit, Queen’s Medical Centre Campus,

Nottingham University Hospitals NHS Trust. This was the first visit, after the healthy controls had answered an advert for the study. At this visit they were given more explanations and the long form of the Information Sheet which was discussed to ensure understanding. The visit lasted 1 hour and if the healthy controls were happy to do so the investigator checked suitability for the study, and went through the process of consent, as well as brief medical history and drug history. All controls completed an MRI safety questionnaire, to exclude persons with contraindications to MRI.

If they were eligible and consented to be in the study, the healthy controls were then given 7-day stool diaries, to be filled in during the week preceding each subsequent study day, and a stool collection kit in order to bring a stool sample to visit 2. Hospital Anxiety and Depression Questionnaires and the PHQ 15 questionnaires were also completed. A blood sample was taken for serology to confirm inclusion criteria. MRI transit capsules and instructions were also provided.

At the end of Visit 1 the first MRI study day (Visit 2) was scheduled. This was provisionally booked pending serology confirmation of inclusion criteria.

The instructions for the MRI transit capsules were as for the coeliac patients above. Once a negative coeliac serology result was obtained, visit two booking was confirmed.

Visit 2: First MRI Day (1 hour) – at the SPMIC 1.5T MRI unit.

A fasting breath hydrogen test was carried out. The healthy controls also provided a stool sample, then they were asked to change into disposable

scrubs and to remove all metal objects (e.g. watches, jewellery) and underwent a short (approximately 15 minutes) set of MRI scans.

Visit 3: Approximately 12 months later (1 hour) – at the NIHR Nottingham Digestive Disease Biomedical Research Unit, Queen's Medical Centre Campus, Nottingham University Hospitals NHS Trust –The healthy controls were contacted approximately one year after Visit 2. They were invited to attend a short visit. This involved checking that the MRI safety questionnaire was unchanged, and filling out again the same questionnaires (HADS, PHQ and Biagi scores,) as in Visit 1. A stool collection kit was provided again. The second and last MRI study day was then scheduled with the MRI transit capsules and instructions provided again.

Visit 4: Second and last MRI day (1 hour) – at the Sir Peter Mansfield Imaging Centre 1.5T MRI unit – This was exactly as Visit 2. A fasting breath hydrogen test was carried out. The healthy controls again provided a stool sample and stool diary, and underwent a short (approximately 15 minutes) set of MRI scans.

Similarly to the patient arm of the study, all the procedures and study visits were carried out by the author.

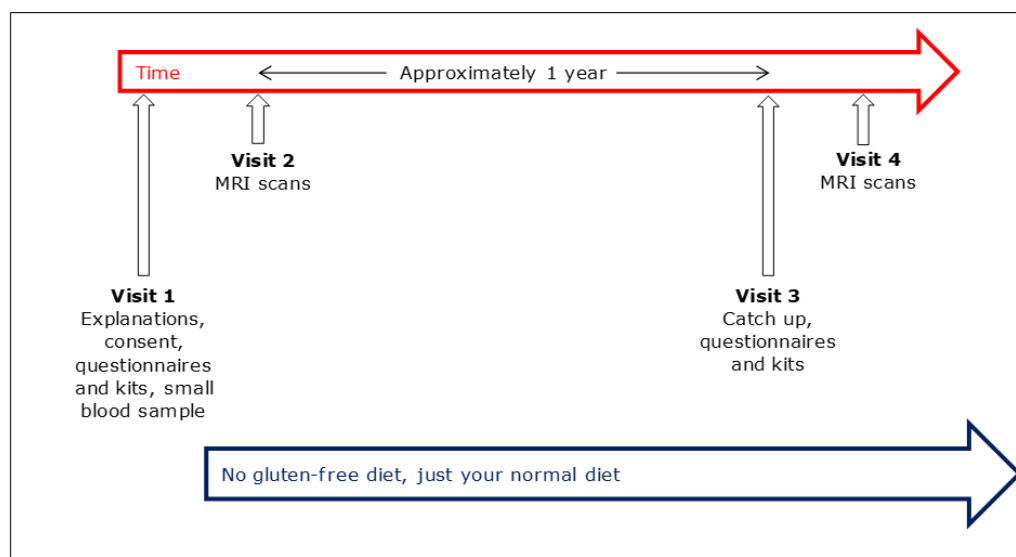


Figure 4-11 MARCO study workflow for healthy controls.

4.5.5 Case report forms (CRFs)

Each participant was assigned a study identity code number at randomisation, for use on CRFs other study documents and the electronic database. The documents and database also used patient initials and date of birth (dd/mm/yy). CRFs were held securely in accordance with regulations. Computer held data including the study database was held securely and password protected. All data was stored on a secure dedicated web server.

4.5.6 Data collection

The data were recorded on paper Case Report Forms/ Study Forms and then transcribed to an EXCEL spreadsheet and then imported into Graph Pad Prism (v.8; San Diego, CA, USA) which was used for statistical

analysis. Each participant was assigned a trial identity code number for use on study forms, other trial documents and the excel database. The documents and database also used their initials (of first and last names separated by a hyphen or a middle name initial when available) and date of birth (dd/mm/yy).

Study forms were treated as confidential documents and held securely in accordance with regulations. A separate confidential record of the participant's name, date of birth, local hospital number or NHS number where available, and Participant Trial Number (the Trial Recruitment Log), to permit identification of all participants enrolled in the trial, in accordance with regulatory requirements and for follow-up as required was also held. Study forms were restricted to those personnel approved by the Principal Investigator and recorded on the 'Trial Delegation Log.' All data was recorded in the trial master file (TMF). All CRF, database input entries and also subsequent data analysis was carried out by the author.

4.5.7 Data management

The data set for each subject were stored on a password-protected server and processed as previously described using either software written in house in IDL (version 6.4; Research Systems Inc., Boulder, CO, USA); for analysis of SBWC by Dr Caroline Hoad at the SPMIC, or commercial software (Analyze v.9, Biomedical Imaging Resources, Mayo Clinic, Rochester, MN, USA;) for analysis of colon volumes.

4.5.8 Statistical analysis

This study benefitted from methodological and statistical expert support provided centrally by our NIHR Nottingham Biomedical Research Centre GI and liver theme. Dr Lu Ban helped with the initial study design and Dr Colin Crooks and Dr Trevor Hill helped with the concluding statistical analyses and interpretations.

On the advice of the statistician, a formal power calculation was not performed, as there was no data available to estimate the size of the change in water content of the fasting small bowel in patients after a GFD diet, hence this is inherently a pilot study.

It is possible however to draw some inference on fasting SBWC in newly diagnosed patients. Our own MRI pilot data [129] in $n=20$ untreated, newly diagnosed coeliac patients showed a fasting small bowel water content of (mean $\pm$ SD) 202 ± 115 ml volume. From this it was predicted that we should be able to detect a change of 40% (or a reduction of 80 ml volume) after gluten free diet with a power of 90% and a Type I error probability of 0.05 using $n=24$ patients in a paired study design. This would be considered a clinically significant change after gluten free diet; this change reflects twice the level of normal control values of 65 ± 43 ml volume[134]. A recruitment target of $n=36$ was planned to allow for dropouts and to increase power for secondary outcomes.

Descriptive and statistical analyses were undertaken using Graphpad Prism (v.8.4; San Diego, CA, USA)

Data were assessed for normality using the D'Agostino & Pearson normality test: normally distributed data were analysed using parametric methods; non-normally distributed data were analysed using non-parametric methods. All data are presented as mean $\pm$ SEM unless otherwise indicated. $P \leq 0.05$ was used as the threshold for statistically significant outcomes.

For the primary endpoints, having defined adherence to the protocol the initiation of a GFD diet (for patients) and returning for the follow up visit (for both patients and controls) the results in this chapter are given as analysis of the full data set available for participants who attended both visits

For participants with baseline and follow up studies: Normally distributed data were analysed using the paired t-test, while non-normally distributed data were analysed using Wilcoxon matched pairs sign-ranked test.

For unmatched pairs: Normally distributed data were analysed using the unpaired t-test, while non-normally distributed data were analysed using Mann–Whitney test. $p = 0.05$ was used as the threshold for statistically significant outcomes. Means are quoted as mean $\pm$ standard error of mean (SEM).

Possible correlations of the SBWC, colon volumes and transit ; and participants' age, BMI, Marsh grading and symptoms and somatisation, HBTs, stool form and frequency, and Biagi scores were assessed using Spearman non-parametric correlation analysis and Multiple linear regression (least squares) analysis.

4.5.9 Adverse events

Participants were asked to contact the study site immediately in the event of any serious adverse event. In concordance with Good Clinical Practice all SAEs would have been recorded and reported to the MHRA and REC as part of the annual reports. Definitions of an Adverse Event (AE), Serious Adverse Event (SAE) and Suspected Unexpected Serious Adverse Reactions (SUSARs) are as defined by the NHS Health Research Authority [135].

SUSARs would have been reported within the statutory timeframes to the MHRA and REC. The Chief investigator was responsible for all adverse event reporting. There were no SAEs or SUSARs during this study.

4.5.10 Adherence

The newly diagnosed coeliac patients, following a face to session with the specialist coeliac dietician, all commenced their gluten free diet treatment immediately after their baseline visit. Adherence was measured by repeating the coeliac serology testing at 12 month follow up, as well as using the Biagi scoring (**Appendix 3 Biagi score**) [136, 137], a validated reliable and simple questionnaire based on four questions to verify patients' adherence to a GFD, giving a possible score of 0-5 with 5 being the highest adherence.

The Biagi score is not based on the food the patients eat, but rather the strategies the patient uses to avoid gluten regardless of the food eaten. This allows us to look at the patients' adherence to a GFD over time and also make comparison between different groups irrespective of cultural or dietary preferences.

The results are divided into three levels:

Level 1: those with a score of 0 or 1 do not follow a strict GFD,

Level 2: those with a score of 2 followed a GFD but with 'imperfect understanding that requires adjustment' and finally

Level 3: those with a score of 3 or 4 observed a strict GFD [109].

4.6 METHODOLOGY

4.6.1 Magnetic Resonance Imaging

All participants were scanned in the supine position on a GE 1.5 Tesla (1.5T) MRI scanner using a 12 channel, 12 element, body array coil. The actual scan duration was approximately 15 minutes. After the localising scan, three different imaging sequences were used. A coronal dual echo fast field echo (FFE) to visualise the abdominal anatomy and colonic volumes, secondly the MRCP sequence was used to assess the small bowel water content and lastly the sequence used to localise the transit capsules in the gut. All of these sequences were scanned using an expiration breath hold to prevent respiratory motion artefact.

4.6.2 Imaging parameters

The sequence used to define the colon anatomy: Coronal dual echo Breath Hold Asset, (see section 2.13) flip angle of 80°, 7mm contiguous interleaved slices. Field of view 45 cm, with a phase FOV of 80%. ASSET factor 2, TR of 175 milliseconds and two echoes, TE 2 milliseconds and TE 4 milliseconds.

The coronal sequences used to identify the transit capsules: Coronal LAVA breath hold: 3D fat saturated sequence with a flip angle of 120°, TE 2 milliseconds, TR of 4 milliseconds. Phase FOV 80%. Slice thickness of 2.6mm with 64 locations (or slices).

The water-sensitive sequences used to suppress background tissue and measure small bowel water content: Coronal small bowel water sequence used a coronal single shot fast spin echo sequence with fat saturation. A 90° flip angle, TE of 324 milliseconds and a TR of 1.2 seconds ensured a heavily T2 weighted sequence. This gave high-intensity signal from areas with freely mobile fluid and little signal from body tissues. A FOV of 400 cm (phase FOV 80%), 32 slices with a slice thickness of 7 mm.

MRCP new

This optimised coronal small bowel water sequence used a coronal single shot fast spin echo sequence with fat saturation. A 90° flip angle, TE of 327 milliseconds and a TR of 1.4 seconds ensured a heavily T2 weighted sequence. This gave high-intensity signal from areas with

freely mobile fluid and little signal from body tissues. An FOV of 400 cm, 32 slices with a slice thickness of 7 mm.

Objective 1

For our cohort of patients with newly diagnosed coeliac disease, the mean difference of their fasting small bowel water content (the primary outcome) before and after the initiation of GFD was calculated using Paired Test at 5% level of significance.

Objectives 2 and 3

This analysis was repeated for the two secondary outcomes, namely the volume of the fasting colon and the gastrointestinal transit time.

Co-variate analyses in the coeliac disease group

The potential association between co-variables (gastrointestinal symptoms, Marsh grading etc.) and the three MRI outcomes were assessed. The healthy control cohort is included to provide pilot reference data to inform the design of future studies. The small bowel water content, transit time and segmented colonic volumes among the coeliac disease cases was compared to the controls, and also the mean difference pre- and post-measurement between cases and controls.

4.6.3 Screening blood tests

One blood sample was collected from the healthy controls at their first study visit only. This was sent immediately for standard serology for coeliac disease markers and no blood was stored. A positive coeliac serology result was considered an exclusion factor for the study for healthy controls. None of the healthy controls followed a gluten free diet.

4.6.4 Hydrogen breath tests

Fasting breath hydrogen (H_2) was measured at both study day visits, Visit 2 and Visit 4, 12 months apart. Participants were asked to have their last meal before 8pm the day before and then abstain from food or drink, except for water. They were also asked not to smoke on the day of the test and the day before.

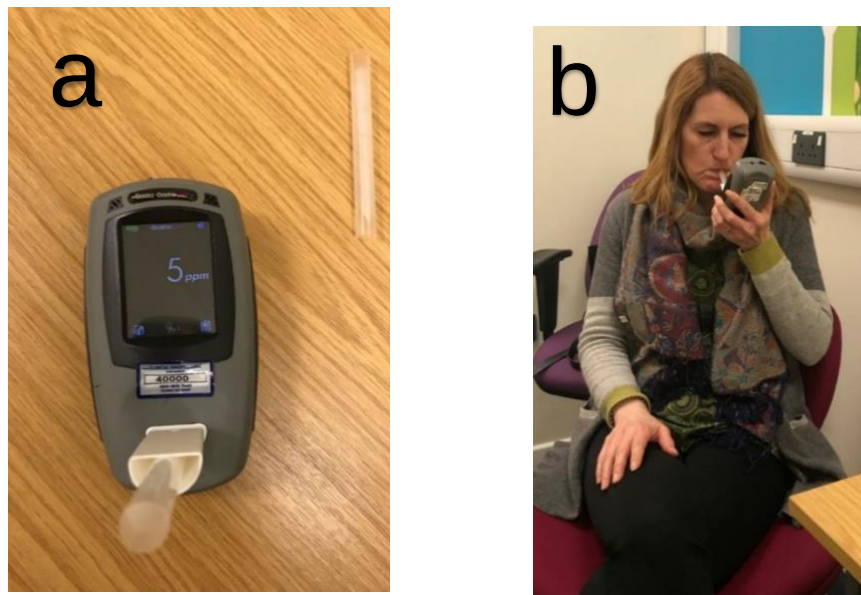


Figure 4-12 (a) Portable hand-held breath H_2 meter (b) demonstrating the use of the portable hand-held breath H_2 meter (permission given)

On the study day itself they were instructed not to chew gum, and to brush their teeth and rinse their mouth with water and not use mouthwash, and to not wear perfume, sprays or aftershave containing alcohol. H₂ tests were performed using a portable hand-held breath H₂ meter (Gastro+ Gastrolzyer, Bedfont Scientific, Kent, UK) Figure 4-12

4.6.5 Stool diaries

Participants recorded the time and consistency (on the Bristol scale) of every bowel movement for the seven days prior to both study days (see **Appendix 4 Stool diary**). Stool form is classified on a scale from 1-7, with Type 1 being Separate hard lumps, like nuts (difficult to pass and can be black) through to type 7 being 'watery and entirely liquid'.

4.6.6 Stool samples

On each study day a baseline stool sample was also collected from both the patients and the healthy controls. Participants were given a stool collection kit including gloves, card tray, sample pot, plastic box and freezer "ice packs" to keep the sample cool until it was brought along to the study day. No patient travelled for >30 miles to reach the hospital and the expectation was that samples were usually >2 hours old on receipt in the department.

This is in accordance with infection control advice from Nottingham University Hospitals Microbiology service. On both study days samples were brought to the SPMIC and transferred to an interim dedicated

refrigeration unit on site. They were then transferred to the -80°C freezers at the NIHR NDD BRC for later evaluation.

The intent is to use the stool sample collected during the MARCO study to look at the gut microbiome and SCFAs in incident coeliacs and post GFD.

As well as the DNA being analysed at the Teagasc Next Generation DNA Sequencing Facility in the Alimentary Pharmabiotic Centre at University College Cork (UCC) Ireland, it has been agreed to use the HUMI - Human Microbiome Research, Research Programs Unit at the University of Helsinki, Finland to analyse the SCFA's and gut metagenomes in stool samples collected in 24 of the CD patients to provide a longitudinal functional profile of the change in the Gut microbiome in this cohort, along with a control set from the healthy controls.

Such analyses are costly and it has only recently been possible to source funding for this work and to have organised all the necessary legal material transfer agreements. It is estimated that it will take up to a year to extract and analyse the data. This is beyond the timelines for this thesis and therefore the microbiota and SCFA analysis does not form part of this thesis.

4.6.7 Symptom questionnaires

Symptom intensity on each study day was measured prior to the MRI scan using a composite of visual analogue scales (VAS, range, 0–100

mm) for each symptom (i.e. abdominal bloating, flatulence, nausea and abdominal pain) (**Appendix 5 Study day symptom questionnaire**).

4.6.8 Anxiety, Depression and somatisation

Psychometric assessment was performed at both screening visits using the following questionnaires:

Hospital Anxiety and Depression Scale (HADS) is fourteen item ordinal scale that is commonly used in hospital and outpatient settings to assess the level of anxiety and depression that an individual is experiencing (**Appendix 5 Hospital Anxiety and Depression Scale (HADS)** [138].

There are seven questions related to anxiety and seven relating to depression giving a maximum score of 21 for both depression and anxiety, 8-10 is associated with low level anxiety/depression, 11 and above shows significant depression/anxiety. It has been in use for over thirty years and is widely used in the evaluation of the impact on the patient of GI disorders [138] .

The Personal Health Questionnaire (PHQ15) (**Appendix 7 PHQ 15**) [139] is a widely used self-administered screening instrument to assess somatization, the description of multiple symptoms that cannot be accounted for by medical or organic disease. It uses criteria from the Diagnostic and Statistical Manual of Mental disorders, version IV, from the American Psychiatric Association and has 15 somatic symptoms, each symptom scored on a Likert scale from 0 (“not bothered at all”) to 2 (“bothered a lot”) giving a minimum score of 0 and a maximum score of

30. PHQ-15 scores of 5, 10, 15, represent cut-off points for low, medium, and high somatic symptom severity, respectively [140].

The results from these questionnaires were then separately scored for the three GI symptoms on the scale - abdominal pain, nausea and disordered bowel function from the PHQ-15 giving a range of 0-6 to give further information on the symptoms specifically associated with coeliac disease.

4.6.9 Patient and public involvement

With the support of Andrew Wragg, an experienced Patient and Public Involvement & Engagement facilitator at the NIHR Nottingham Digestive Disease Biomedical Research Unit, QMC Hospital, Nottingham. and with the advice of Coeliac UK, a Coeliac Patient Focus Group was set up to inform our initial research protocol. Members of the group were recruited using a local coeliac support group affiliated to Coeliac UK, and the NDDC BRU Facebook page (**Figure 4-13**).



Figure 4-13 Facebook advertisement for participants in Coeliac focus group to inform the MARCO protocol and patient literature.

At the initial meeting the PPI facilitator gave a presentation on patient involvement and engagement in research and I then presented our research idea and the background to the project.

All aspects of the trial were discussed with members of this group. Their opinion has also sought on the form and content of all patient-facing documentation such as invitation letter, information sheets and recruitment posters. Their feedback was incorporated into the design of that documentation, with some simplification of the language used and more clarity to the aims of the study, and also consideration of the patient journey during the trial.

This group has been briefed via email on the progress of the trial and they are keen to help in the dissemination of the results of this study and the direction of future coeliac research in Nottingham.

4.7 ETHICAL APPROVALS AND GOOD CLINICAL PRACTICE (GCP)

STANDARDS

Once the protocol was developed and the accompanying Patient Information Sheet and documentation was reviewed by our PPI group, University of Nottingham sponsorship was obtained and ethical approval for the study was sought from the National Research Ethics Committee and was assigned to the South East Coast – Surrey for proportionate review. After some minor changes to the information sheets were made and the documentation resubmitted. The study was finally approved by the United Kingdom National Research Ethics Service (14MP002) on the 12/2/2015.

The study was carried out according to Good Clinical Practice as defined by the Declaration of Helsinki. Nottingham University Hospitals NHS Trust Research and Development departmental approval was granted (local study number 15/LO/0253) and the study was registered on ClinicalTrials.gov (Identifier: NCT02551289).

4.8 CONFIDENTIALITY AND DATA PROTECTION

Individual participant medical information obtained as a result of this study was considered confidential and disclosure to third parties prohibited with the exceptions noted below. Participant confidentiality was further ensured by utilising identification code numbers to correspond to treatment data in the computer files. Such medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare. If information is disclosed during the study that could pose a risk of harm to the participant or others, the researcher would discuss this with the CI and where appropriate report accordingly.

Data generated as a result of this trial is available for inspection on request by the participating physicians, the University of Nottingham representatives, the REC, local R&D Departments and the regulatory authorities.

4.9 RESOURCES

Funding

This work was supported by a College of Radiographers Doctoral Fellowship grant. This was inaugurated in 2015 for eligible members of the College registered with Health and Care Professions Council to undertake doctoral studies aligned to the College of Radiographers' research priorities. After a detailed submission and evidence of support

from the author's employer (Medical Physics Department at NUH) and the supervisors at the University of Nottingham an award was granted under the 'effectiveness of technical approaches' theme.

The scanning and other research costs were met by internal Nottingham Digestive Diseases Centre research funds.

Participants were not paid to participate in the trial. However, each participant was offered an inconvenience allowance of up to £20 per visit to cover costs of travel and the burden of involvement.

5 MARCO RESULTS

In this pilot study all study participants tolerated the MRI scanning protocol. As described in the Consort diagram in Chapter 4, out of the initial 36 patients and 36 healthy controls who started the study, 30 patients and 33 healthy controls returned for at least part of their follow up assessments.

The table below (Table 3) summarises the demographics and clinical parameters of the coeliac patients in the study. As expected, the coeliac patients recruited were predominately female, and mean age at recruitment was 40 years, with male patients mean age considerably older at 59 years

Table 3 Demographics and key clinical variables of the MARCO Coeliac patients.

Sex	Age	BMI	Presenting symptoms	Baseline TTG	12 month TTG	Biopsy grading	12 month Biagi score
F	39	27.4	Fatigue	+	—	1	3
F	28	20.5	GI	+	—	1	1
F	55	35.7	Anaemia	+	N/A	1	N/A
F	61	19.2	GI + Anaemia	+	N/A	1	3
M	62	27.5	GI + Fatigue	+	—	1	3
M	69	22.1	Lower GI	+	—	1	3
F	54	20.2	Osteoporosis	+	↓	1	3
F	32	20.1	Diarrhoea	+	—	3a	3
F	45	29.3	Diarrhoea	+	—	3a	3
F	20	22.8	Constipation Anaemia	+ +	↓	3a	3

F	24	37.7	Fatigue	+	—	3a	N/A
M	67	33.0	Dyspepsia	+	—	3a	2
M	44	21.2	Fatigue	+	↓	3a	3
F	55	39.7	Anaemia + diarrhoea	+	—	3a	2
M	60	39.8	Diarrhoea	+	—	3a	3
M	66	22.7	Incidental finding	+	—	3a	3
M	70	27.7	GI	+	—	3a	3
F	47	20.8	+ve TTG	+		3a	N/A
F	38	17.7	Incidental finding	+	—	3a	3
M	59	37.4	GI + Diarrhoea	+	—	3a	3
F	34	31.8	Anaemia +Osteoporosis	+	—	3a	3
F	31	20.8	Mouth ulcers	+	—	3a	3
F	61	18.0	Anaemia	+	↓	3a	2
F	27	23.2	Anaemia +F	+	N/A	3a	N/A
F	21	20.0	GI	+	—	3b	N/A
F	58	22.1	Diarrhoea	+	—	3b	3
F	25	23.9	Diarrhoea + Anaemia	+	—	3b	3
F	33	23.3	Unavailable	+	↓	3b	2
M	40	20.5	Anaemia Diarrhoea	+ +	—	3b	3
M	53	33.5	Mouth ulcers	+	—	36b	3
F	43	22.3	Anaemia	+	—	3b	3
F	48	23.0	Anaemia	+	↓	3b	2
F	53	27.5	GI	+	—	3c	3
M	59	30.4	Incidental finding	+	↓	N/A	3
F	38	17.9	GI +Constipation	+	—	N/A	3
F	31	20.9	GI + fatigue	+	—	N/A	N/A

Key: Positive TTG = + Negative TTG = -

TTG trending towards normal values = ↓ Not available = N/A

5.1 PRIMARY OUTCOMES

The three primary outcomes were Small Bowel Water Content, Colon volumes and Whole Gut Transit Time.

5.2 SMALL BOWEL WATER CONTENT

The primary outcome was the difference in SBWC in coeliac patients (n=29) before and after treatment initiation with a GFD. No significant difference ($P=0.26$) in SBWC was seen for the coeliac patients between baseline (164 ± 17) ml and 12 month follow up (147 ± 17) ml.

No significant difference ($P=0.41$) in SBWC was seen for the healthy controls (n=31) at baseline (107 ± 13) ml and 12 month follow up (112 ± 14) ml.

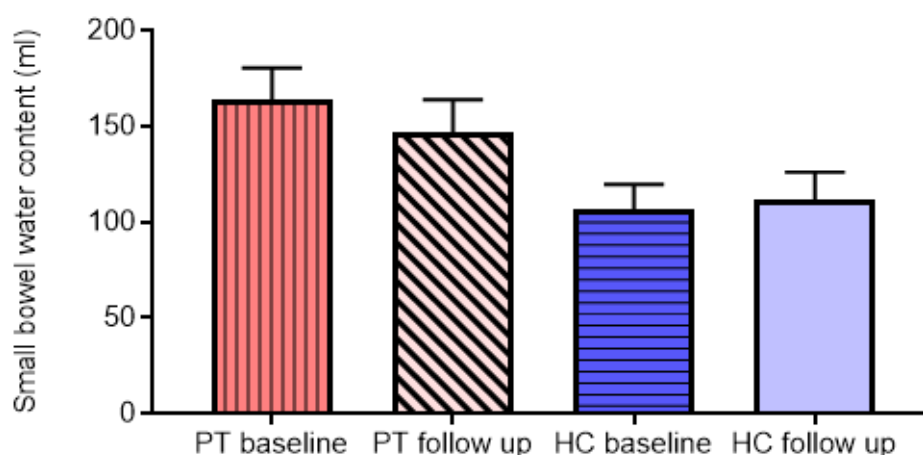


Figure 5-1 Small bowel water content in ml at baseline and 12 month follow up for healthy controls (HC) and coeliac patients (PT).

A significant difference ($P=0.003$) was seen in SBWC between coeliac patients (151 ± 15) ml and healthy controls at baseline (99 ± 12) ml. No

significant difference ($P= 0.09$) in SBWC in coeliac patients (147 ± 17) ml and healthy controls (112 ± 15) ml at 12 month follow up.

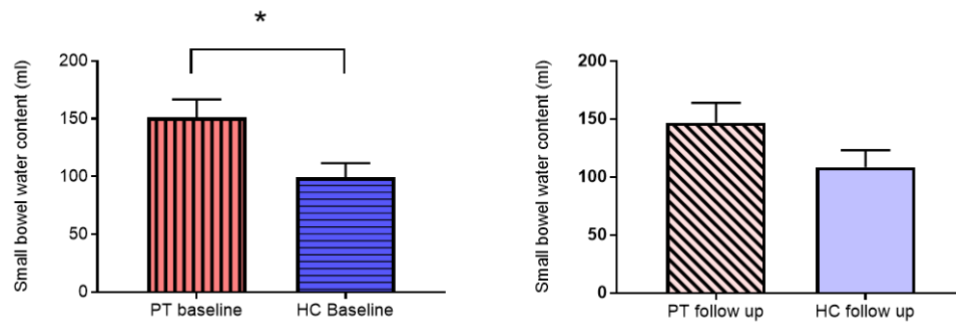


Figure 5-2 SBWC in ml for coeliac patients (PT) and healthy controls (HC) at baseline (* $P=0.003$) and 12 month follow up.

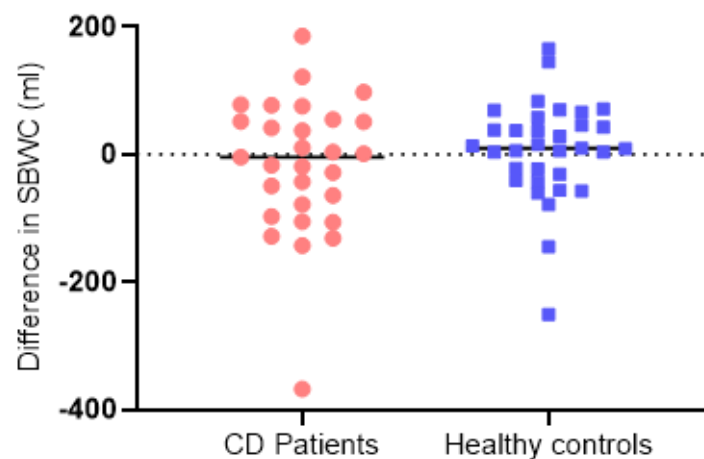


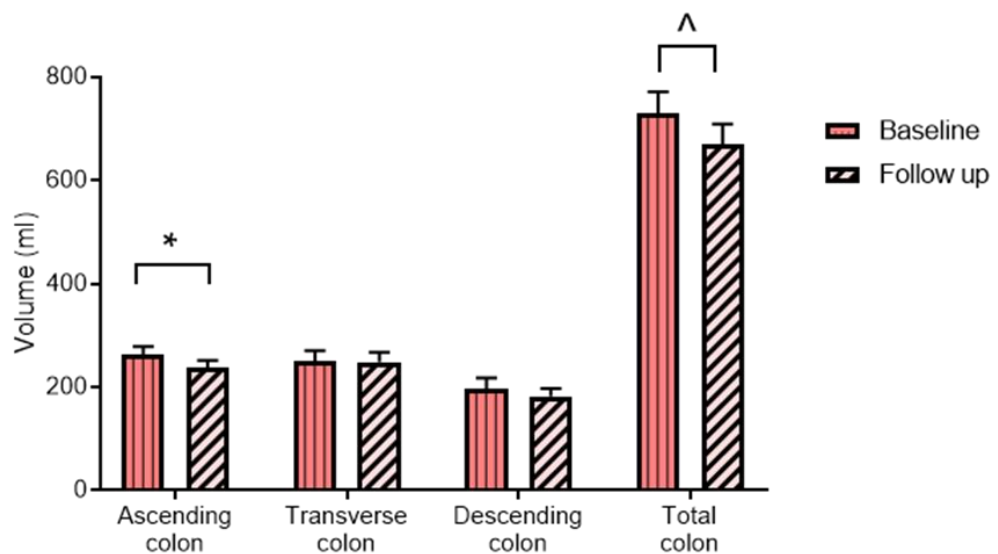
Figure 5-3 Changes in small bowel water content (ml) for coeliac patients and healthy controls.

5.3 COLON VOLUMES

Suitable images were obtained in most participants with only two coeliac patient data sets unusable due to patient's inability to hold their breath

sufficiently for the required scan time. Regional and total colon volumes are described. (All data are in Table 4)

The primary outcome was the difference in colon volumes in coeliac patients before and after (n=27) treatment initiation with a GFD. There was a significant difference in ascending colon volumes (P=0.02, and overall colon volume P=0.04).



There were no significant differences in regional or total colon volumes

Figure 5-4 Coeliac patients colon volumes in ml (n=27) at baseline and follow up. * P=0.02, ^ P=0.04.
at baseline and follow up for the healthy controls.

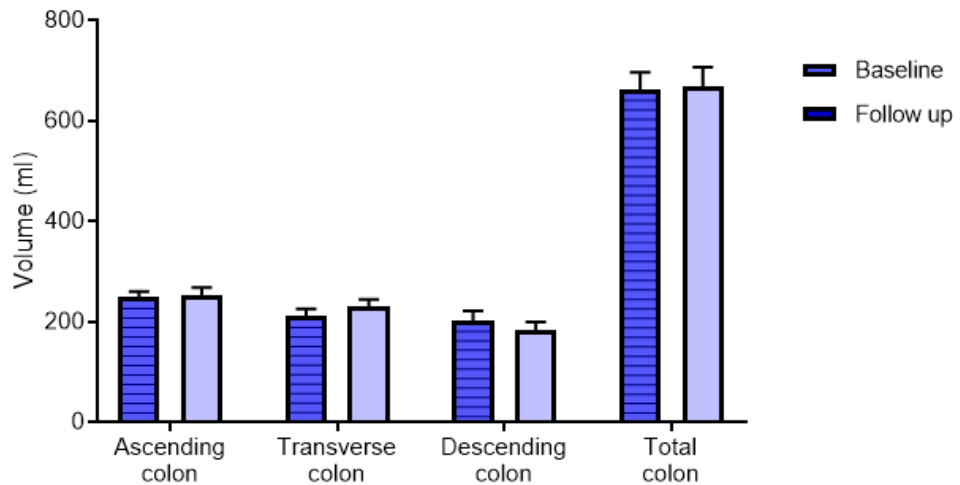


Figure 5-5 Healthy controls colon volumes in ml (n= 32) at baseline and follow up.

The overall regional and total colon volumes for both cohorts are given in

Table 4 Colon volumes. **Figure 5-6** shows the changes in total colon volume for the two cohorts, with a greater reduction in colon volume seen

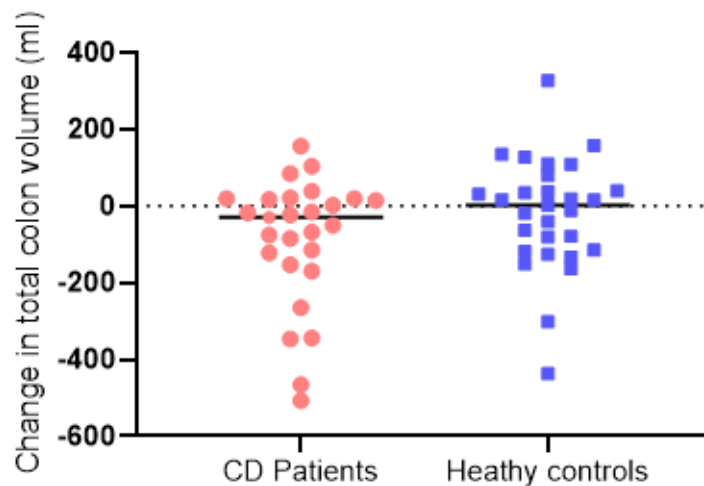


Figure 5-6 Change in total colon volume (ml) for CD patients and healthy controls

in the coeliac patients.

Table 4 Colon volumes

Baseline PT and HC all data (mean $\pm$ SEM)	Patient	Healthy controls	P
Ascending colon volume (ml)	267 $\pm$ 11	248 $\pm$ 12	P=0.24
Transverse colon volume (ml)	246 $\pm$ 16	211 $\pm$ 14	P=0.06
Descending colon volume (ml)	189 $\pm$ 18	209 $\pm$ 21	P=0.21
Total colon volume (ml)	701 $\pm$ 38	663 $\pm$ 34	P=0.20

Follow up Patient and healthy controls (mean $\pm$ SEM)	Patient (n=27)	Healthy Controls (n=33)	P
Ascending colon volume (ml)	238 $\pm$ 14	254 $\pm$ 15	P=0.21
Transverse colon volume (ml)	248 $\pm$ 19	231 $\pm$ 14	P= 0.25
Descending colon volume (ml)	183 $\pm$ 15	183 $\pm$ 1	P= 0.39
Total colon volume (ml)	669 $\pm$ 41	668 $\pm$ 39	P=0.36

CD patients (n=27)	Baseline (mean $\pm$ SEM)	Follow up (mean $\pm$ SEM)	P
Ascending colon volume (ml)	263 $\pm$ 15	238 $\pm$ 14	P=0.02
Transverse colon volume (ml)	251 $\pm$ 21	248 $\pm$ 19	P=0.41
Descending colon volume (ml)	197 $\pm$ 21	183 $\pm$ 15	P=0.34
Total colon volume (ml)	731 $\pm$ 41	669 $\pm$ 41	P=0.04

Healthy controls (n=33)	Baseline (mean $\pm$ SEM)	Follow up (mean $\pm$ SEM)	P
Ascending colon volume (ml)	251 $\pm$ 13	254 $\pm$ 15	P=0.41
Transverse colon volume (ml)	220 $\pm$ 15	231 $\pm$ 14	P= 0.15
Descending colon volume (ml)	200 $\pm$ 19	183 $\pm$ 1	P= 0.11
Total colon volume (ml)	665 $\pm$ 35	668 $\pm$ 39	P=0.44

5.4 TRANSIT

Quantifying transit: each transit capsule was allocated a score based on its position in the colon and whole gut transit time (WGTT) was assessed using the weighted average position score (WAPS), (in arbitrary position score units) of the five capsules on the coronal sequence as described previously [133].

The primary outcome was the difference in transit time in coeliac patients before and after treatment initiation with a GFD.

A significant difference ($P= 0.04$) was seen in coeliac patients in transit time WAPS between baseline (2.2 ± 0.3) and 12 month follow up (1.7 ± 0.2). No significant difference in transit at baseline (1.3 ± 0.2) and 12

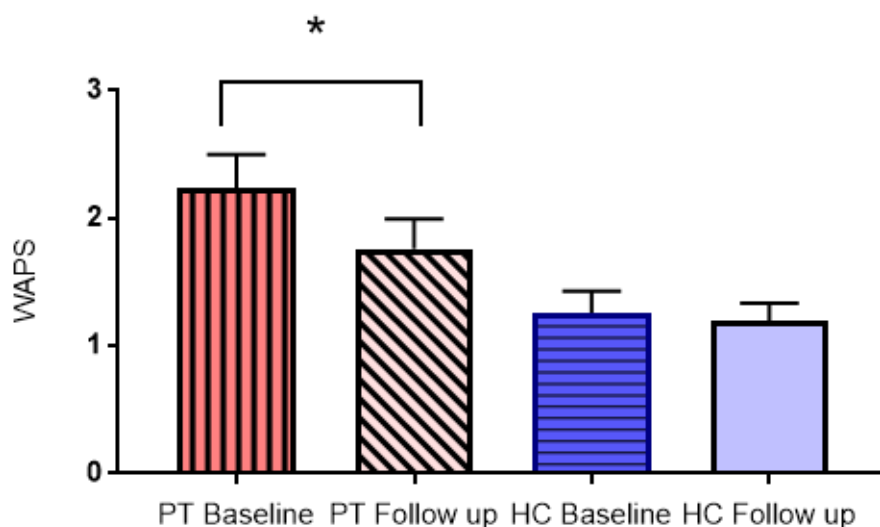


Figure 5-7 Baseline and follow up Transit time in WAPS shown for coeliac patients * ($P= 0.04$) and healthy controls.

month follow up (1.3 ± 0.1) was seen in the healthy controls. As described in methods, the WAPS can be extrapolated (with some assumption) to transit time in hours which is presented in the tables below for completeness.

Transit	Median WAPS Baseline	Median WAPS 12 months	Transit hours Baseline	Transit hours Follow up	P value (WAPS)
Patients (n=29)	2.2 ± 0.3	1.7 ± 0.2	63	52	0.04
Healthy controls (n=32)	1.3 ± 0.2	1.3 ± 0.1	44	39	0.44

Table 5 A significant difference in transit time between baseline and follow up was seen for the coeliac patients (P=0.04). No significant difference was observed for the healthy controls.

Median WAPS	HV Median WAPS	Patients Median WAPS	HV (hours)	PT (hours)	P value
Baseline (n=36)	1.3 ± 0.2	2.2 ± 0.3	44	67	0.01
12 months (n=29)	1.3 ± 0.1	1.8 ± 0.3	39	52	0.04

Table 6 Significant differences in transit time between coeliac patients and healthy controls was seen at baseline (P=0.01) and at 12 month follow up (P=0.04).

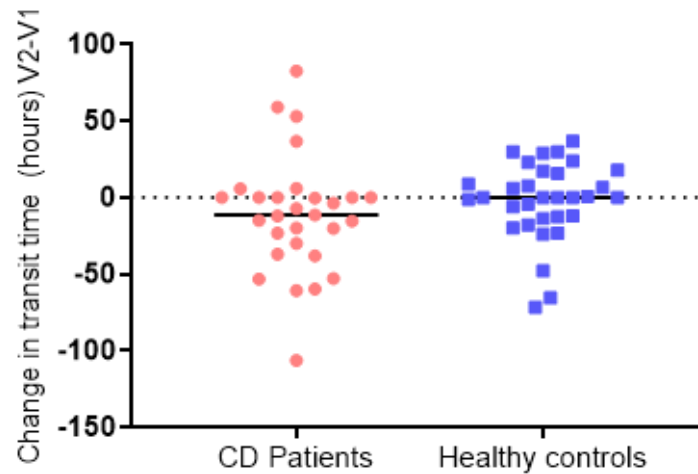


Figure 5-8 Change in transit time (hours) for coeliac patients and healthy controls.

5.5 SECONDARY OUTCOMES

The outcome measure was the difference in BMI in coeliac patients at baseline and 12 month follow up. A small but significant ($p=0.05$) increase was seen in the BMI of the coeliac patients at follow up (baseline mean 25.4 ± 1.2 kg/m², follow up mean 25.7 ± 1.7 kg/m²) (**Figure 5-9**).

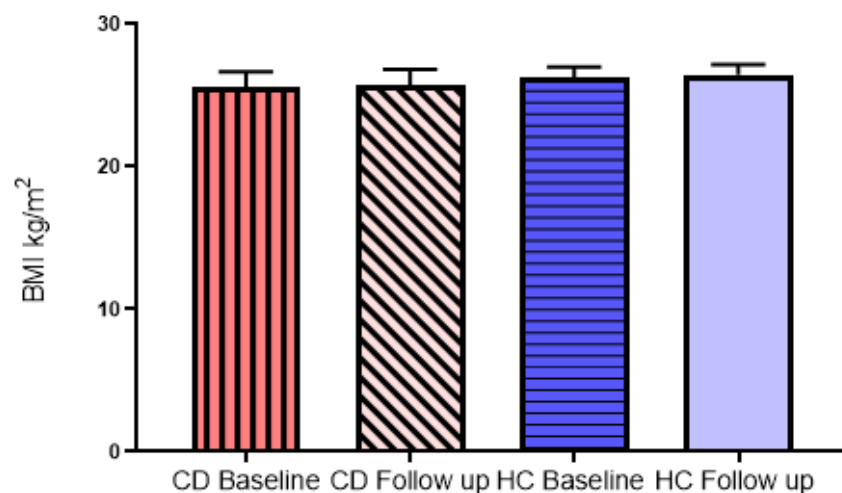


Figure 5-9 Body Mass Index (BMI) at baseline and follow up for coeliac patients and healthy controls.

A small but not significant ($P=0.08$) gain was seen in the mean BMI in the healthy control cohort at 12 month follow up (baseline 26.2 ± 0.7 kg/m²), follow up (26.4 ± 0.7 kg/m²).

5.6 STUDY DAY SYMPTOMS

The outcome measure was the difference in study day combined GI symptom scores in coeliac patients between baseline and follow up visits. There was a significant difference between study day symptoms at baseline and follow up for coeliac patients ($P=0.03$). No significant difference between study day symptoms at baseline and follow up for healthy controls ($P=0.4$). A significant difference was found between baseline study day symptoms between the patient and healthy control cohort ($P=0.0001$) and at follow up the study day symptoms were also significantly different ($P=0.01$) (**Figure 5-10**).

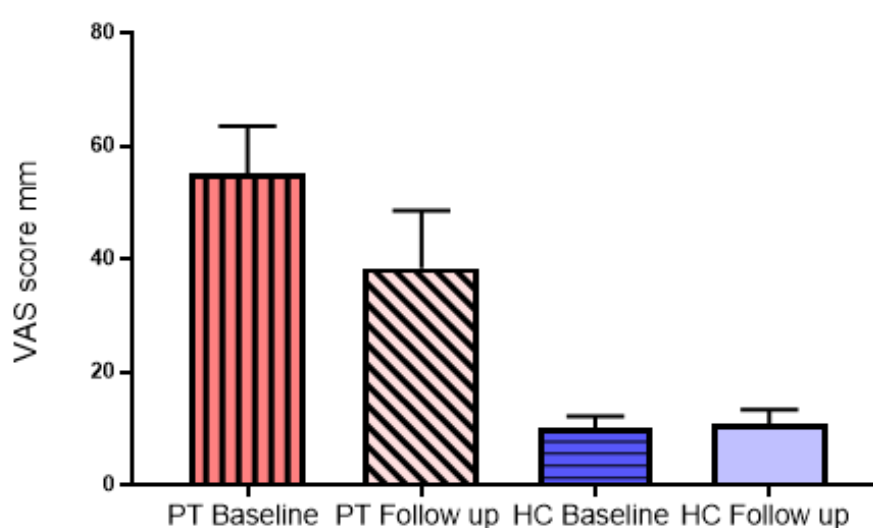


Figure 5-10 Study day symptoms at baseline and follow up for coeliac patients and healthy controls.

5.7 ANXIETY, DEPRESSION AND SOMATISATION

The outcome measure was the difference in PHQ_15 scores in coeliac patients before and after treatment initiation with a GFD. The PHQ_15 score was significantly higher ($P= 0.0003$) at baseline than at follow up for the coeliac patients. There was no significant difference in the PHQ_15 score ($P= 0.26$) at baseline and at follow up for the healthy controls (**Figure 5-11**).

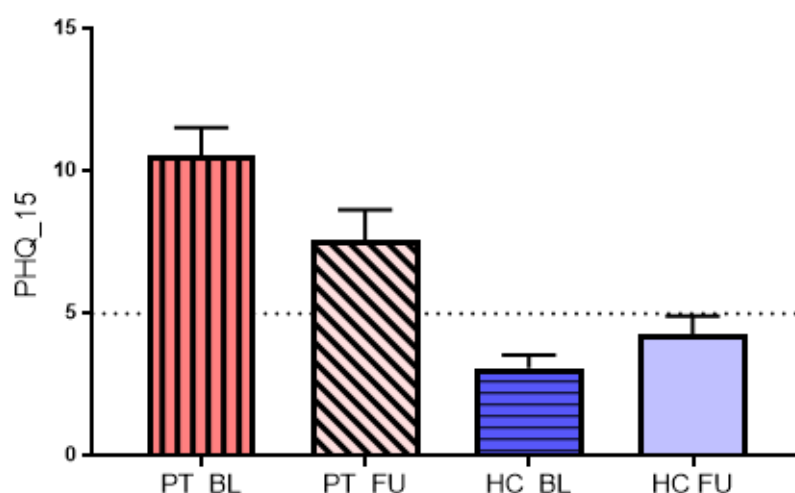


Figure 5-11 PHQ_15 values at baseline (BL) and follow up (FU) for coeliac patients (PT) and healthy controls (HC). The dotted line shows cut – off point for low somatic symptom severity.

The PHQ_15 score also higher for healthy controls at follow up, but both scores were below the lower cut off point of 5 on the scale for severity of symptoms.

PHQ_3 – the three questions relating to GI symptoms only

There was a significant improvement $P=0.001$) in PHQ-3 score for patients between baseline and follow up. At 12 month follow up GI symptoms as measured using the PHQ_3 subset of questions in patients were reduced and the GI symptom score was raised in healthy controls.

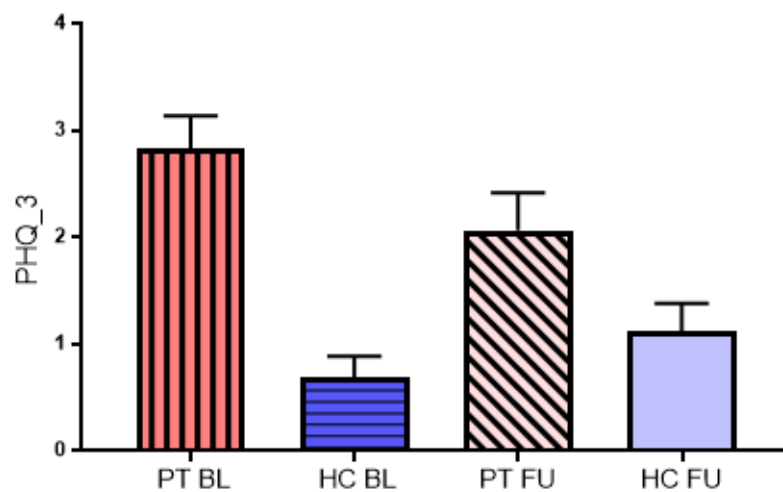


Figure 5-12 PHQ_3 values at baseline (n=36 for both cohorts) and follow up (coeliac patients n= 30, healthy controls n=33).

Hospital Anxiety and Depression Scale (HADS)

The main outcome measure was the difference in anxiety, depression and combined HADs scores in coeliac patients before and after treatment initiation with a GFD. The results are summarised in (**Figure 5-13**)

Coeliac patients (mean±SEM)	Baseline	Follow up	p
Anxiety	8.4 ± 0.8	6.9 ± 0.8	0.10
Depression	5.6 ± 0.6	3.3 ± 0.5	0.004
HADS	13.8 ± 1.4	10.5 ± 1.2	0.04

Healthy Controls	Baseline	Follow up	p
Anxiety	4.9 ± 0.5	5.3 ± 0.5	0.3
Depression	2.3 ± 0.4	3.5 ± 0.6	0.55
HADS	7.2 ± 0.9	8.4 ± 0.9	0.14

Table 7 Summary of the participants Hospital Anxiety and Depression Scale scores at baseline and follow -up.

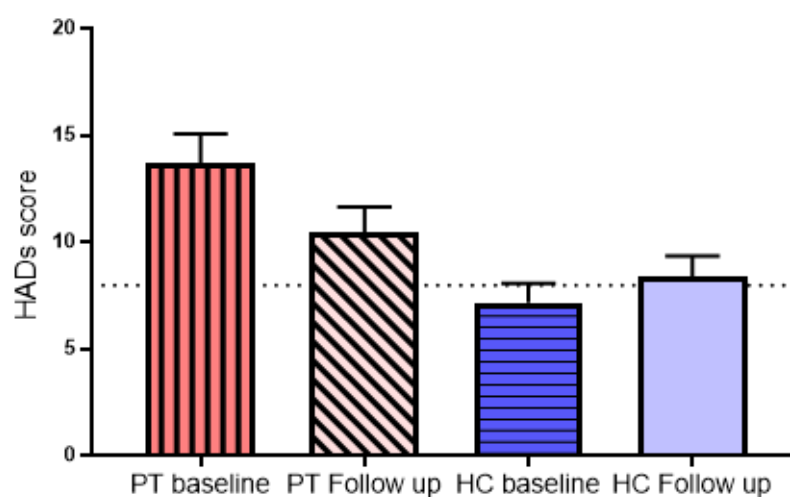


Figure 5-13 Hospital Anxiety and Depression Scale scores (combined) at baseline and follow up for coeliac patients and healthy controls. The dotted line shows cut off point of 8 for anxiety /depression.

Values for anxiety and depression at baseline were significantly higher in the patient population than in healthy controls (**Figure 5-13**).

5.8 HYDROGEN BREATH TESTS (HBT)

The outcome measure was the difference between fasting breath H₂ values in coeliac patients before and after gluten free diet treatment. Fasting breath H₂ concentrations were measured in parts per million (p.p.m.) No significant differences were found between the baseline and follow up HBT values for either cohort.

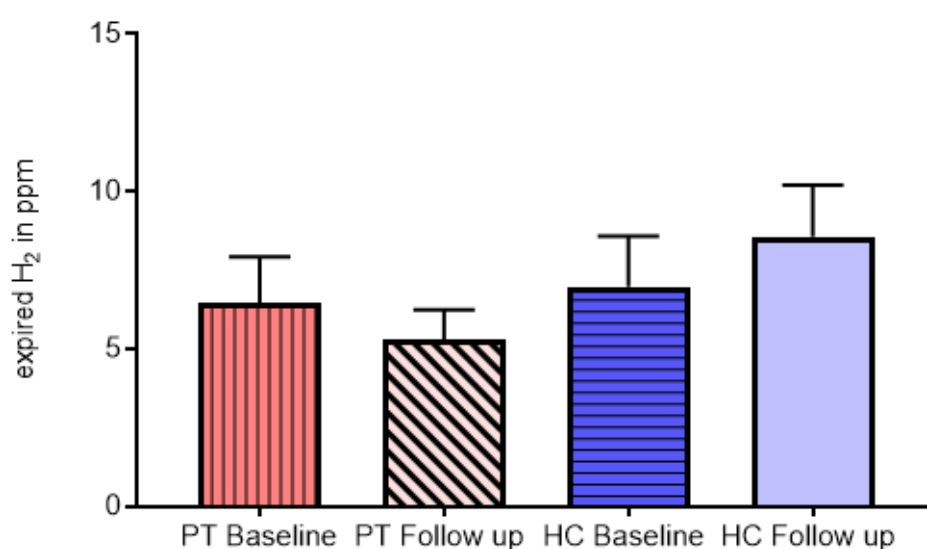


Figure 5-14 Hydrogen breath tests results (in parts per million) at baseline and follow up for coeliac patients and healthy controls.

The coeliac patients (n=29) who reported adherence with their gluten-free diet treatment (Biagi score level 2 or above (**Figure 5-15**) had a small

but insignificant decrease in fasting breath H₂ (baseline mean 7.0 ±1.8, follow up mean 5.4 ± 1.0).

The coeliac patients who reported complete adherence (n=24) (Biagi score level 3) with their gluten free diet treatment had a small but insignificant decrease fasting breath H₂ (baseline mean 6.8 ±2.1 ppm, follow up mean 4.9 ± 1.1 ppm)

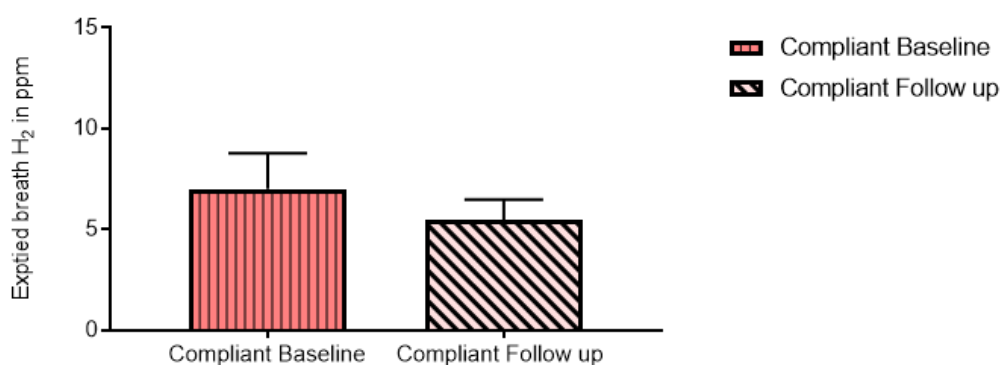


Figure 5-15 Hydrogen breath tests results (in parts per million) at baseline and follow up for coeliac patients reporting Biagi level 2 or above GFD adherence (n=29).

5.9 STOOL DIARIES

The outcome measure was the difference in stool form and frequency in coeliac patients before and after treatment initiation with a GFD. Not all participants supplied stool diaries at baseline (n=33 for both cohorts) and at follow up (coeliac patients n=27, and controls n=31).

No significant difference between stool frequency at baseline and follow up for coeliac patients ($P=0.31$). A change in stool form was seen at baseline and follow up for coeliac patients but this was not significant ($P=0.058$) (**Figure 5-16**).

No significant difference was found between stool frequency at baseline and follow up for healthy controls ($P=0.34$). No significant difference between stool form at baseline and follow up for healthy controls was seen ($P=0.30$) (**Figure 5-17**).

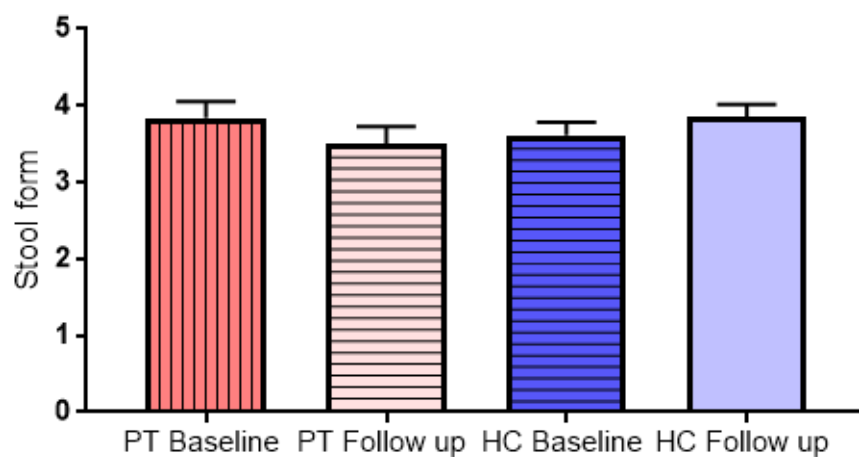


Figure 5-16 Stool form at baseline and follow up for coeliac patients and healthy controls.

No significant differences between the two groups were found with stool data, including form and frequency at baseline or follow up.

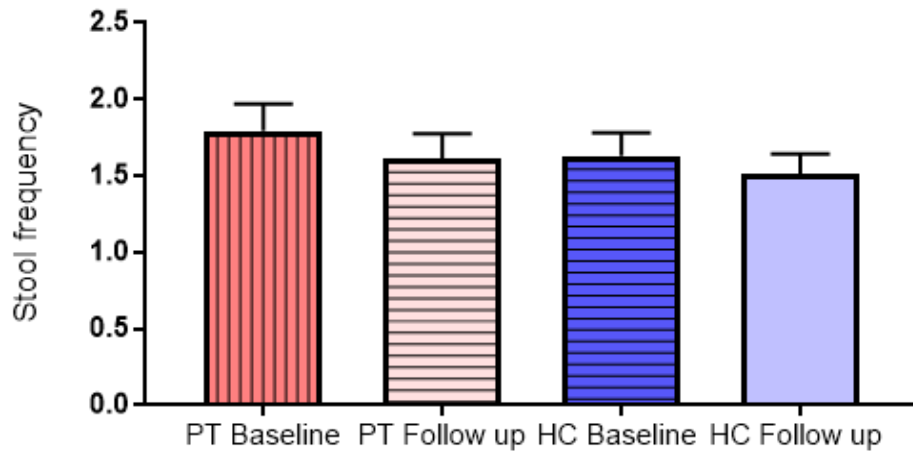


Figure 5-17 Stool frequency at baseline and follow up for coeliac patients and healthy controls.

5.10 BIAGI SCORES

All the healthy controls had a Biagi score of 0, i.e. level one at both baseline and follow up. All patients were also level one at baseline, i.e. did not follow a GFD. At follow up (n=30), although all patients had followed a gluten free diet to some extent, one patient, due to their personal circumstances was not compliant with a GFD and remained in level one. A further five followed a GFD with occasional errors of

adherence, i.e. level two, and the remaining 24 were level three and reported excellent adherence with a score of 3 or 4.

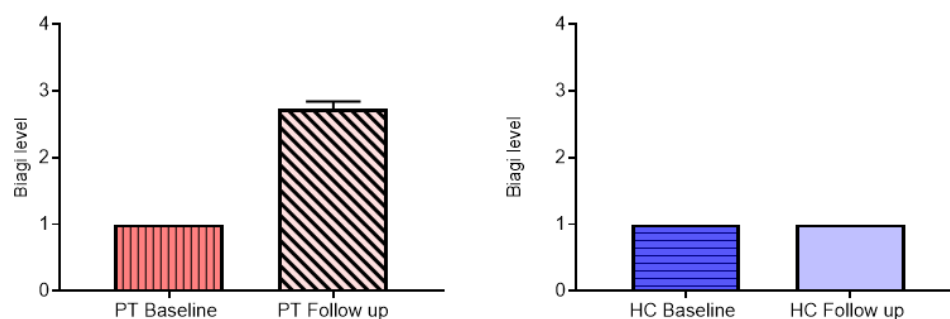


Figure 5-18 Biagi levels at baseline (both cohorts n=36) and follow up (CD patients n= 31 and controls n=32).

5.11 SEROLOGY

Sixteen months after recruitment commenced for the MARCO study, NUH laboratories changed the methodology and ranges for TTG (IgA) testing making direct comparison impossible for these values at baseline and follow up for the majority of patients in the study. All serology for CD patients was positive at baseline, follow-up results have been given as:

- Within normal limits or
- TTG reducing but not yet within normal limits (6 month results were available for most patients) or
- Positive

5.12 CORRELATIONS

Multiple linear regression (least squares) analysis found no correlation between SBWC volume difference (i.e. Scan 2 – Scan 1 volumes) and Marsh Grading. However changes in total colon volume between Scan 1 and Scan 2 were related to their modified Marsh grading, with a greater decrease in colon size seen in patients with a Marsh grading of 3 (a, b or c) (mean -71.3 ml) compared to Marsh grading of 1 (mean 52.8 ml) although there was a wide variation in 95% confidence intervals (CI)

Figure 5-19. There was also a greater decrease in whole gut transit time difference in patients with a Marsh grading of 3 (mean -12) compared to Marsh grading of 1 (mean 7.2) Again the wide range in 95% CI makes this comparison limited in value.

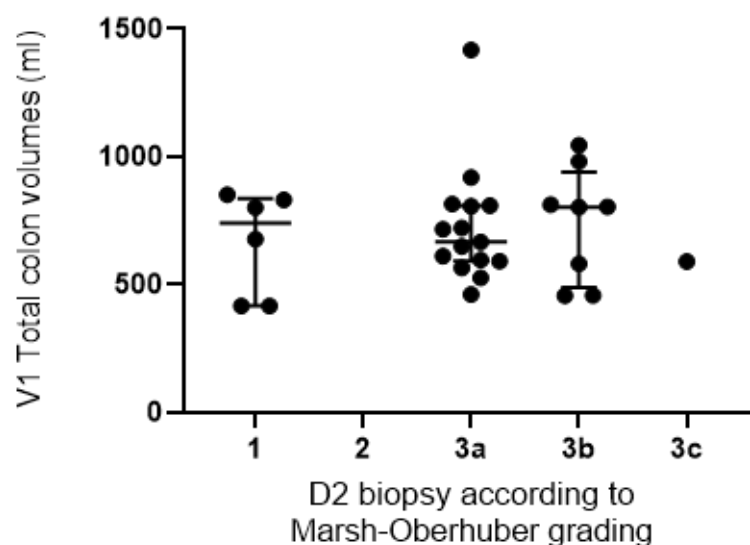


Figure 5-19 Total colonic volume versus severity of modified Marsh grading for D2 biopsy.

There were no correlations between the primary outcomes at baseline SBWC, colon volumes and transit and psychological distress symptoms such as anxiety or depression or general health such as PHQ_15.

There was however, correlation between higher PHQ_15 scores, more severe study day symptoms and higher HADS scores both at baseline and follow up.

6 DISCUSSION

Coeliac disease is one of most common genetic disorders, affecting approximately 1% of individuals worldwide and the only current treatment is a strict gluten-free diet for life [141]. As well as the social and economic burden of this disease, patients find the time to diagnosis, the invasive endoscopic biopsies required for an adult diagnosis, and the necessity for a gluten containing diet for accurate antibody testing onerous.

In 2018 Coeliac UK, a charity for people living with coeliac disease and other gluten related conditions, set key research priorities around coeliac disease [104, 142]. Priority 4 was 'Finding less invasive tests to recognise coeliac disease', including an alternative to endoscopic biopsies and the gluten challenge for diagnosis; this was a higher priority than priority 8, 'Finding a cure for the disease'.

The idea for the MARCO study originated from previous work in our group and fits well with these research priorities. This observational study evaluated the effect of a gluten free diet treatment on newly diagnosed coeliac patients using MRI techniques. The overall aim underpinning this work was to use non-invasive MRI techniques to provide novel insights into coeliac disease, the effect of GFD, and the relationship of gastrointestinal function and symptoms.

The study tested the hypotheses that in adults patients newly diagnosed with coeliac disease the treatment with gluten-free diet (GFD) will reduce the water content of the fasting small bowel, the volume of the fasting colon and whole gut transit time.

Several secondary outcomes were also evaluated, including clinical measurements and patient reported outcomes.

To test these hypotheses 36 incident coeliacs were recruited, over one third of all newly diagnosed coeliacs referred to the specialist dietetic service at Nottingham University Hospitals during the study recruitment window, and more than half of those who responded to the initial contact about the study were recruited. 83% of patients recruited to MARCO and 92% of the healthy controls returned for at least part of their follow –up assessments at 12 months. Although formal power calculations were not made as this was a pilot study it was estimated that data from 24 patients would be adequate to see a difference in our primary outcome, SBWC between the 2 scan time points.

All patients in the study were reasonably well characterised against the inclusion and exclusion criteria. They had access to the local standard of care dietetic consultations for newly diagnosed coeliac patients, which comprised a face to face session with the specialist coeliac dietician, either as part of a group of newly diagnosed adult patients, or as an individual. Local dietetic services for coeliac disease are well established and the study benefitted from this. In the clinical standard care pathway they were followed up with a telephone review at three months and a further face to face visit at 12 months. They underwent coeliac screening blood tests, (serology, full blood count, ferritin, serum folate, vitamin B12, calcium, alkaline phosphatase), associated autoimmune conditions (thyroid-stimulating hormone and thyroid hormone(s), and serum glucose), liver disease (aspartate aminotransferase/alanine

aminotransferase) [34], weight / height measurements, symptom review and nutritional assessment. Additionally, participants in the MARCO study had their gluten free dietary adherence measured at baseline and follow up study visits using the Biagi questionnaire as described in [119]. Repeat endoscopic biopsies were not performed as they are not part of the clinical pathway.

The patients in the study comprised a mix of mucosal damage severity as reflected from the Marsh grading. All patients in the study were on a gluten containing diet at the baseline assessments, and all attempted a gluten –free diet with specialist dietetic support during the study. One patient reported poor adherence although their TTG levels has returned to normal at follow up. Another five reported some errors of adherence, (Biagi level 2) and three of these had serology levels above normal values, though all were much lower that their baseline values. Of those that reported excellent adherence four also had TTG levels above the normal threshold, although again their values were tending towards normal compared to baseline.

This may indicate partial or delayed mucosal recovery due to gluten exposure. It must be noted that although positive coeliac antibody tests may correspond to gluten intake, it is also known that low TTG titres do not necessarily correlate with mucosal recovery [34]. This heterogeneity of Biagi scores post treatment and varying TTG recovery discussed above suggested that breaking down the results according to perceived adherence would not be beneficial. [30].

Silvester et al [30] described the limitations of using TTG levels to measure mucosal recovery in a meta-analysis in 2027 – reporting a low sensitivity (below 50%) in detection of persistent villous atrophy. A study comparing surrogate markers such as a point of care tests (POCT), serum EMA and TTG and adherence scores with re-biopsy and combining the adherence score with other markers only gave a sensitivity of 70.6% (59.7-80.0%) compared to duodenal histology follow up [143]. A convincing case for repeat duodenal biopsies is presented by Sharkey et al [144] quoting the sensitivity of TTG antibody testing as only 43.6% for detecting ongoing villous atrophy for 595 patients in his study. However as repeat biopsies were not standard of care for patients in our Trust, dietary adherence and serum TTG were the indicators used for mucosal recovery in this MARCO observational study.

The MRI techniques used in this study to measure SBWC, transit and colonic volumes are all possible to use on most MRI scanners in clinical use in the UK. Other, more specialised techniques such as relaxometry to look at the bowel wall signal and motility studies of the small bowel would provide further useful information in colic disease evaluation and research.

6.1 PRIMARY OUTCOMES

The non-invasive method of imaging coeliac patients was reproducible, easily interpreted, involved only one imaging session at a given time

point, was easily tolerated by the participants (both patients and healthy controls) and used no ionising radiation. The findings are discussed below.

6.1.1 Small bowel water content

SBWC was increased in the coeliac patients at baseline compared to the healthy controls, and did not decrease significantly at follow up. SBWC was not significantly changed for the healthy controls between base line and follow up scans.

Increased small bowel fluid has been observed in patients with coeliac disease radiologically by Rubesin *et al* [145] as far back as 1989. Lam *et al* discussed that increased SBWC in patients with untreated coeliac disease may reflect both the net effect of impaired absorption associated with villous atrophy and increased secretion associated with crypt hyperplasia and also the impaired motility reported in untreated disease [110]. This would be expected to reverse in treated patients but the follow-up time of 12 months in this study may not have been enough for sufficient mucosal healing to have occurred. Biopsy results at 12 months were not available for this study.

Rubio-Tapia *et al* at the Mayo Clinic observed mucosal recovery in about 35% of 381 adults with coeliac disease 2 years after starting a GFD and in about 66% of those patients after 5 years; however, 82% of them had improved clinically after 2 years [146]. This suggests that the sustained high volume of SBWC in these patients, despite the improvement on

reported symptoms, PHQ-15 and PHQ-2, and HADs scores may be due to persistent mucosal damage despite serological and symptomatic recovery.

This data gives an overall measurement of the SBWC of the entire small bowel. Accurate anatomical segmenting of the small bowel into its regions from the SBWC sequence can be difficult as there is so much inter subject variability in anatomy and the resolution of the sequence is low. Although SBWC was not analysed for the different small bowel segments to see if there were regional differences in the ileum vs the jejunum for example, this may be possible to analyse at a later date.

6.1.2 Colon volumes

Colon morphology can be assessed on the fasted state using MRI, which provides colonic volumes in the natural, undisturbed (unprepared) colon [132]. Colon volumes have been shown to be altered in irritable bowel syndrome and constipation [131]. In this study regional and overall colon volumes were measured at both scanning time points, baseline and 12 month follow up. Baseline and follow-up data was available for 27 coeliac patients with only 2 scan sets being unsuitable due to patient movement. The study has shown that overall colon volume greater in coeliac patients at baseline compared to follow up, and the volume of the ascending colon was also significantly increased in these patients with no change seen in the healthy controls. Although fasting transverse and

descending colon segmental volumes were all numerically larger in coeliac patients compared with controls the differences were not statistically significant. There was a wide variation of volumes in both groups, so the changes in volumes seen in each participant was also examined.

The reason for the increased colon volumes in the patient group remains to be fully understood. Previous work related a change in coeliac patients' colon volumes to their modified Marsh grading, with a greater relative decrease in colon volume seen those with a higher Marsh grading (i.e. more damaged mucosa) [129]. Lam *et al* also found a significant increase in the total colonic volumes in untreated coeliac patients as the severity of the Marsh 3 subtype grading increased and suggested that the elevated peptide YY and glucagon-like peptide-2 (GLP-2) seen in adults with untreated coeliac disease [147] could impede intestinal motility, leading to an enlargement of both the small bowel and the colon [130]. The decrease in GLP-2 seen in adults after GFD treatment may be due to the mucosal healing [148].

In the MARCO study hormone peptides were not measured so this link remains to be tested. The reduction in the ascending colon in patients may be due to change in the output from the small bowel as the malabsorption improves and the possible microbiota changes as there is less substrate to ferment and produce bacteria. Changes in the microbiota will be quantified when the MARCO stool data from the study is available, these data are currently being analysed.

6.1.3 Transit

Whole Gut Transit Time was significantly delayed in the coeliac patient cohort at baseline and although this improved at follow-up it was still significantly slower than in the controls. As expected no significant change in transit time at follow-up was observed on the healthy controls, which reinforces the reproducibility and worth of the technique. Whole gut transit time was also significantly slower in the coeliac patients compared to the healthy controls both at baseline and follow-up, although there was smaller difference at follow-up suggesting again that the patients' transit was normalising.

Other reports in the literature point to alteration of transit in the gastrointestinal tract of coeliac patients. Delayed oro-coecal and oesophageal transit has been noted previously in coeliac patients [47-49] and this is consistent with the dyspeptic symptoms often seen in this disease such as early satiety and epigastric discomfort [70]. In contrast, colonic transit measured by radiopaque markers (Metcalf method) showed somewhat faster transit times in untreated coeliac disease by Bai *et al* [149].

Delayed whole gut transit time in untreated coeliac patients may be due to a number of inter-related factors such as mucosal damage and inflammation disturbing gut motility, malabsorption of food constituents and gut hormone derangement. Although this global transit delay can improve after treatment with a GFD [48], the delayed gastric emptying

in particular may not normalise [150]. Interestingly, the coeliac patients in this study who had greater mucosal damage shown on duodenal biopsy (modified Marsh grading 3) at diagnosis showed a greater decrease in transit time compared to those patients with Marsh 1 grading. This suggests that a recovery in mucosal damage could be associated with normalising of transit times.

Malabsorption of food leading to the steatorrhea experienced by some of these patients may also lead to transit delay as described by Spiller *et al* in the 1980s. Mouth-caecum transit time in the coeliac patients in Spillers' study was linearly related to the 24-hour faecal fat excretion. Interestingly, although these patients had impaired insulin release they had normal post- prandial peptide profiles. No relationship between stool form or frequency and transit was observed in the MARCO study. Short chain fatty acid analysis for the MARCO stool samples is planned and may help further characterising the effect of GFD and the transit findings. There are therapeutic implications of delayed transit, particularly delayed gastric emptying as coeliac disease is frequently associated with thyroid dysfunction and other autoimmune disorders and for these gut dysmotility, particular this delayed gastric emptying could affect drug absorption [58].

The MRI WGTT measurement technique has some limitations. Some patients may find the capsules difficult to swallow although this was not the case for any of the MARCO participants. The MRI method has been validated against radiopaque markers using a 24 hours imaging point. Whilst this is common for scintigraphic methods to assess transit [151,

152] the extrapolation of WAPs scores to transit hours beyond this validation window is only made on an assumption of linearity, which remains to be tested. Therefore in this thesis the more robust weighted average position scores (WAPS) were presented first and a transit time in hours calculated later for completeness.

The repeated measurements in the healthy controls showed little change and offered an opportunity to look at reproducibility of the measurement. The mean WGTT in hours for both visits for the healthy controls was 41.6 hours. This is a little slower than the scintigraphy study transit data for healthy males collected by Krevsky *et al* [153] but similar to the mean WGTT of 43.4 hours from a larger scintigraphy and radiopaque marker transit study of 23 healthy controls by Horikawa *et al* [154] in 1999.

6.1.4 Secondary outcomes

A number of secondary outcomes were also measured. Weight change is common after adoption of a GFD with coeliac patients, with different studies showing either weight gain [155] or normalisation of BMI [156] following treatment. In this study there was a small but significant increase in the mean BMI of the coeliac patients at follow-up. Given the change in their diet and the expected improvement in small bowel absorptive function this minor increase in BMI is not unexpected and did not correlate with their overall levels of anxiety, depression or somatisation.

Health related quality of life is considered a critical endpoint in the assessment of treatment efficacy for coeliac patients [157]. Quality of life reported by the incident coeliacs, as measured by PHQ_15 and HADs questionnaires, was poorer than that in the healthy controls. Although these measures improved after diagnosis and treatment their wellbeing and psychological distress was still poorer overall than the controls at follow up. The study day symptom scores as measured on a GI symptoms VAS questionnaire (gas, bloating, abdominal pain and diarrhoea) were significantly greater at baseline than at follow-up for the coeliac patients and although had improved following the GFD were still significantly higher than the controls at both time points.

There was also a correlation seen between the severity of study day GI symptoms, and poorer PHQ_15 and overall HADS scores. This is not unexpected given the impact of their restrictive diet and continuing symptoms [158].

Elevated breath hydrogen (H_2) concentrations (a measure of carbohydrate malabsorption) have been reported in patients with coeliac disease, IBS and other functional GI diseases [74]. This increase in H_2 in expired air occurs when dietary sugars are not absorbed in the small bowel and are thus fermented in the colon, producing H_2 , methane (CH_4) and volatile fatty acids. The higher fasting H_2 levels seen in untreated coeliac seen in previous studies [75, 159] were not replicated here, and no correlation between fasting H_2 and symptoms were observed in this study. This measurement was also limited by not controlling the participant's diet prior to the study day, although they were fasted from

the previous evening and tested at 8am. A D-xylose challenge H₂ test could have proven more sensitive [160, 161] but time constraints on the study day did not permit this more accurate H₂ measurement.

Stool frequency and Bristol stool form score data were also collected at both study visits. In our patient population the questionnaire did not show any significant difference between the coeliac patients and healthy controls for stool frequency. A decrease in stool form score was observed for the coeliac patients at follow-up but this was just outside the significance threshold ($P=0.058$). No correlation with study-day symptoms or somatisation was seen. This is contrary to what Lam *et al* observed in their coeliac population at baseline with patients showing increased stool frequency and form at time of diagnosis [130]. This may be due to incomplete data in the MARCO dataset (not all participants recorded stool habits) or to the less severe presentation of these patients at baseline than in Lam's study.

It worth noting that coeliac patients may present with a mixture of gut symptoms including severe or occasional diarrhoea, excessive wind and/or constipation. In a study of 770 Italian adults diagnosed with coeliac disease, of those who had GI symptoms, 27% presented with diarrhoea, 13 % constipation and 15% with alternating bowel habit [162].

6.1.5 Validation study

For quantitative research using MRI, moving from one scanner manufacturer to another, or even from one site to another with identical scanner hardware and software, can prove problematic [163]. MRI

sequences on different scanners, or indeed on similar scanners with slightly different versions of software, although using similar parameters and appearing similar to the naked eye, often have very different signal characteristics. This is due to differences in imaging sequence parameters, gradient performance, image reconstruction and filtering methods, and field strength.

Translating the previously validated SBWC measurement to a new scanner platform for the first time for the MARCO study required a validation study. Using published methodology this would have involved a naso-duodenal intubation study with infusion of known volumes of poorly absorbed fluid in the small bowel of a large number of participants. The method developed in this validation study is novel and requires no difficult and invasive intubation procedures. It can now be used to set a new threshold value for SBWC image analysis to ensure reproducibility when moving quantitative experiments between coils, scanners of different field strengths and/or manufacturers and, therefore, between laboratories, therefore making the SBWC analysis more translational.

6.2 LIMITATIONS

There were limitation to this work. Although most participants returned for their follow up assessments, of those who returned not all assessments, particularly stool collection and stool diaries, were completed, although it has to be noted that this was the case only in a small number of participants.

Serologic testing, as well as being used for diagnosis in conjunction with duodenal biopsy, has historically also been used as a marker for dietary adherence but has its limitations [164] and may not be the reliable indicator for mucosal recovery that follow up duodenal biopsies would have provided. This is also echoed by a recent multidisciplinary consensus panel of international experts in coeliac disease concluding that there is no Grade A evidence based recommendations for assessing adherence to a GFD, to date there are no non-invasive biomarkers that indicate mucosal recovery and that there is often some persistent villous damage despite patient reported adherence [34].

Additionally, substantial methodological changes in serological testing in the laboratories at Nottingham University Hospitals NHS Trust during the study meant that TTG levels at follow-up were not directly comparable for patients in the study.

This study did not look at the influence of gut hormones on the outcomes. The analysis of the gut microbiota and SCFAs in stool samples collected in the coeliac patients and healthy controls is not yet available, due to cost and external laboratory timelines so this analysis does not form part of this thesis.

With the colon volume measurements, two data sets from patients who did not hold their breath sufficiently and therefore had the scan degraded by movement artefact were hard to interpret and the data could not be used. This will improve with the newer technological advances in MRI newer scanners as faster scan times are now possible. There is also a wide variability in inter-individual and intra-individual colon volumes

reported in other studies using MRI measurements of morphology. This reflects the variability caused by daily differences in diet, exercise, emotional state and defecation timing, which are not controlled for and would be difficult to standardise.

Participants did not limit their diet before the HBTs and it is thought that any slowly digested foods (such as legumes and pulses, bran and high-fibre cereals) should be restricted for at least 24 hours prior to testing.

Dietary adherence was measured using the Biagi questionnaire, this used in conjunction with the Coeliac Disease Adherence Test (CDAT) devised by Leffler *et al* [165] might have resulted in a more accurate measure of adherence.

6.3 FUTURE WORK

Future work is already planned to look at the gut metagenomes, microbiota and SCFAs in stool samples collected in the coeliac patients and healthy controls and compare these results to their MRI and other outcomes in the MARCO study.

A study with a longer follow up period could give more information on SBWC as a measure of mucosal healing. New relaxometry scans to look at small bowel 'health' are being developed in Nottingham which would allow a closer look at regional the bowel walls.

Also, a more regional investigation of how transit and motility disturbances may be affecting the stomach / small bowel / colon will be

of interest as well as investigating the role of gut hormones on coeliac patient's motor function. The measurement of key hormone peptides in the fasting state and also in response to a meal stimulus was beyond the scope and funding available for this study. These measurements are clearly of interest and could help explain some of the MARCO findings, they will certainly form part of future studies.

6.4 CONCLUSION

This MRI study patients with incident coeliac disease has shown for the first time that treatment with a gluten free diet for a year reduces the small bowel water, significantly reduces the overall colon volume and decreases the whole gut transit time.

These results support the underlying hypotheses of this work and show that MRI can be a useful non-invasive tool in evaluating coeliac patients and this work can add to our understanding of coeliac disease. This work provided also for the first time a normal reference range with repeated seasonal variation test for the MRI endpoints and a new methods to calibrate and standardise such small bowel water volume quantitation between scanners and laboratories.

The MARCO study can help to provide a better understanding of the effects of a GFD diet on the coeliac bowel and non-invasive MRI endpoints may in the future allow evaluation of the effectiveness of new treatments without needing costly and invasive repeat biopsies.

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8 APPENDICES

8.1 APPENDIX 1 ACCEPTED NOMENCLATURE FOR COELIAC DISEASE [166]

Classical coeliac disease presents with signs and symptoms of malabsorption. Diarrhoea, steatorrhoea, weight loss, iron deficiency anaemia or growth failure is required.

Non-classical coeliac disease presents without signs or symptoms of malabsorption (e.g. abdominal pain, constipation, transaminitis etc.)

Symptomatic coeliac disease is characterised by clinically evident gastrointestinal or extra intestinal symptoms.

Asymptomatic coeliac disease is found in persons with positive diagnostic tests for coeliac disease but with no intestinal or systemic symptoms and no overt clinical response to gluten-free diet.

Subclinical coeliac disease occurs without signs or symptoms sufficient to have triggered screening in routine clinical practice. However, previously unrecognised symptoms such as fatigue may improve on a gluten-free diet.

Potential coeliac disease is found in children with positive coeliac serology but with normal small intestinal biopsies (it is important to ensure that adequate biopsies have been taken, including the duodenal bulb). Such persons are at increased risk of progressing to coeliac disease and require long-term follow-up.

Coeliac disease autoimmunity is defined by positive TTGA or EMA serology on two occasions without definitive diagnosis. Such children will require small bowel biopsy to determine whether they have coeliac disease or potential coeliac disease.

Refractory coeliac disease is characterised by persistence or recurrence of malabsorptive symptoms and signs, together with villous atrophy on small intestinal biopsy, despite maintenance of an adequate gluten-free diet for over 12 months.

8.2 APPENDIX 2 SOP FOR TRANSIT MARKER PREPARATION



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Standard Operating Procedure – Gadolinium Transit Markers

1. Manufacture of the capsules in SPMIC workshop

1. The raw material is Polyoxymethylene (commonly referred to as POM and also known as polyacetal or polyformaldehyde), supplied by DuPont under their trade name Delrin.
2. The plastic rod is milled (Figure 1) and lathed (Figure 2), with hemispherical ends until it has these dimensions: 7mm in diameter, and 19.65 mm long with hemispherical ends, with wall thickness of 0.5mm and with a small hole drilled in one end 2mm in diameter. The cuff with the hole is 3 mm thick; the other one is 0.5 mm. This work will be done in the SPMIC workshop by workshop staff.
3. The capsules are then kept in the workshop in a clean bowl until the whole lot has been completed.

4. Screws are also provided, purchased from RS, standard M2 nylon capsules.



Figure 1 CNC control milling machine.

Figure 1 CNC control milling machine.

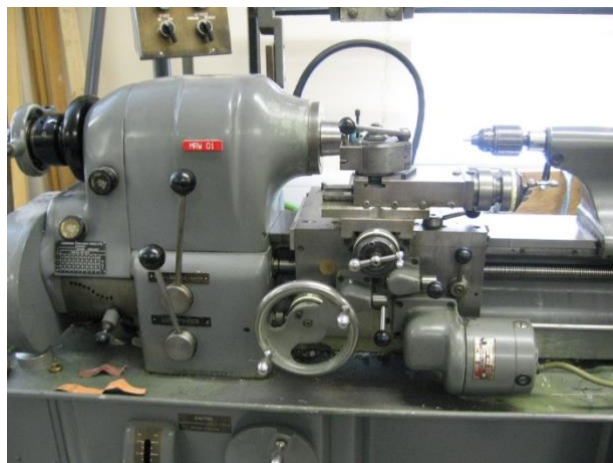


Figure 2 Hardinge lathing machine

Figure 2

Day 1

- Prepare the material: capsules, superglue, gloves, goggles, lab coat, cocktail sticks, polystyrene pad or similar.
- Wear gloves, goggles and a lab coat.
- Clean the work area before starting
- Apply glue to the external surface of the large section where it inserts into the small section. Try to make coverage as uniform as possible using a cocktail stick.
- Promptly join the two sections together. This may require a firm push. Ensure that insertion is complete.
- Clean the capsule with some paper so that no glue is on the external side of the capsule.
- Insert a cocktail stick into the hole on the end of the small section. Insert the other end of the cocktail stick into the polystyrene mount (see figure 5).
- Repeat the same procedure for all the capsules.
- Leave to dry for a day in prep room and label them as do not touch
- Clean up work area

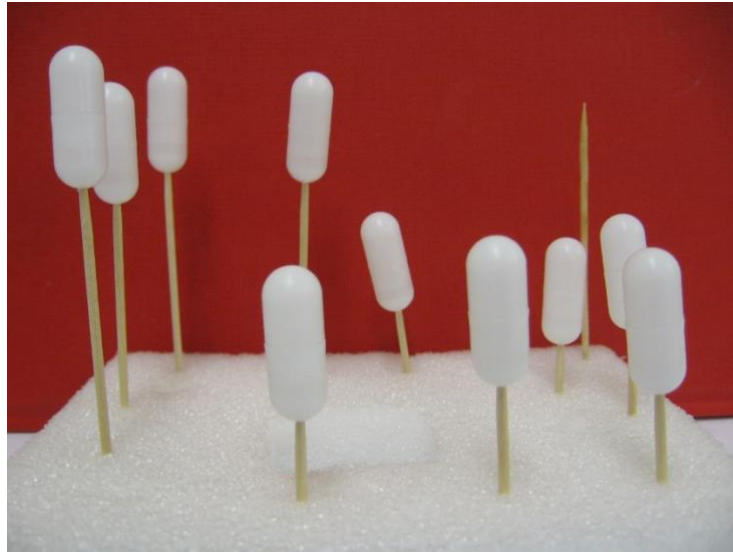


Figure 5
Closed capsules are left on the sticks to dry out.

Day 2

- Prepare 15 μ M Gadolinium (Trade name: Dotarem®) by diluting 1ml of Dotarem® (279.32mg/ml) in 33ml of distilled water.
- Prepare other materials required: a 1mL syringe with a needle, plastic screws, polystyrene pad or similar material that can support the capsules upright while drying, blue food dye.
- Wear gloves, goggles and a lab coat.
- Check that the capsules are dry.
- Take randomly 10% of the capsules and leave them apart for the leakage test.

Capsules for use

- Using the needle* and syringe, add 0.4ml of the 15 μ M Gadolinium to each capsule.
- This should be slowly to avoid air trapping or overflow.
- Stand the filled capsule upright (hole at the top) in the polystyrene pad until ready to insert screw.
- Put a drop of superglue on the tip of a screw and screw it into the hole for 3 complete rotations. Return to the polystyrene pad so that the capsules stand upright, screw up.
- Leave the capsules to dry for a day in prep room and label them as do not touch
- Clean up work area
- * Practise safe sharps technique while using the needle

Capsules for leakage test

- Follow the same production steps as with 'capsules for use' but replace the Gadolinium solution with 0.4mL blue dye for food use.

Day 3

- Prepare the material: capsules, scalpel blade (stitch cutter blade can be used), surface on which to cut, (can be cardboard tray), file, glue approved for human use (BLUE Histoacryl).
- Wear gloves, goggles and a lab coat.
- Use the scalpel blade to cut the top of the screw off the capsule
- Ensure that the cut surface is smooth and round. If necessary use the file to smooth the cut area and remove any unwanted material from the capsule's surface.
- Add a drop of histoacryl glue to the cut surface of the screw.
- Leave to dry in prep room for a day and label them as do not touch
- Clean up work area.

Day 4

- Check that the capsules are dry and clear the exterior of the capsules of any unwanted material.

Capsules for leakage test

- Prepare the material: capsules, 50 ml plastic test tubes with lid, water bath, thermometer for the water bath, tube rack for the water bath, eppendorfs.
- Fill the water bath with water and set the temperature to be 37°C. Leave it until it reaches the desired temperature (check with the thermometer).
- Fill each test tube with 45 ml tap water and put one capsule in.
- Close the test tube and put it in the test rack in the water bath.
- Leave the test tube in the water bath for 48 hours
- After 48 hours remove the test tube from the water bath
- Visually inspect the water in the test tube for evidence of blue discolouration. Any discolouration implies leakage and the batch should be discarded.

Capsules for experiment

- Keep the capsule in a covered plastic dish, and label the dish with content of capsules, date of assembly, confirmation of leakage test.

3. Preparation for use

Before giving the capsules to a participant:

- Prepare a plastic container with 2.5 litre of tap water
- Add a ½ capful of Milton disinfectant
- Add the markers to the container, ensuring that any floating capsules have their entire surface exposed to the cleaning solution.
- Leave the capsules in the bowl for 10 - 20 minutes.

- Drain the container, using a clean sieve to collect the markers.
- Rinse the markers using drinkable tap water.
- Allow to air dry, then blot with a paper towel if required.
- Ensure that the participant knows to swallow the markers whole, without biting or chewing,
- Participants should be offered a drink to assist swallowing. The exact drink may vary according to the study.

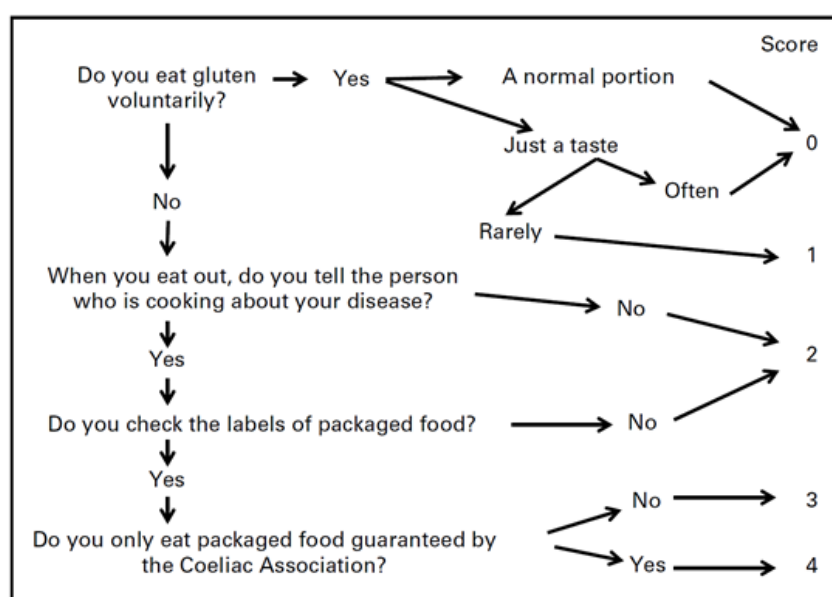
8.3 APPENDIX 3 BIAGI SCORE



Title of Study: MRI assessment in newly diagnosed coeliac disease and following gluten-free diet treatment

Participant number				Initials				DoB	D	D	M	M	Y	Y
								Today's Date	D	D	M	M	Y	Y

Biagi Score



8.4 APPENDIX 4 STOOL DIARY

MRI assessment in newly diagnosed coeliac disease and following gluten-free diet treatment

Stool Diary

Participant Number	Date	Hours of Pain?	Score each 0-3 using the following scale				Initials	DoB	M	M	M	M	M	M	M	M	M	M
Day	(e.g. Monday 05/Jun/10)	(If no pain enter '0')	0 = none	1 = mild (distinct but negligible)	2 = moderate (annoying)	3 = severe (disabling)												
			Gas/flatulence	Bloating	Abdominal Pain		STOOL FORM AND TIME (Form = score 1-7 from 'Stool Form' list below; Time = time of stool)											
							1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	Continue on back if > 7 bowel movements for 1 particular day				
1							Form											
2							Time											
3							Form											
4							Time											
5							Form											
6							Time											
7							Form											
							Time											

Stool Form

- 1: Separate hard lumps, like nuts 
- 2: sausage shaped but lumpy 
- 3: like a sausage or snake but with cracks on the surface 
- 4: like a sausage or snake, smooth and soft 

- 5: soft blobs with clear cut edges 
- 6: fluffy pieces with ragged edges, a mushy stool 
- 7: watery, no solid pieces. Entirely liquid 

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[illegible]

Symptom category:	Visual Analogue Scale: mark the point on the line that best describes how bad each symptom has been 0 = 'not at all' ; 100 = 'as bad as it could be'
Gas/ Flatulence:	
Bloating:	
Abdominal Pain:	
Diarrhoea:	

8.6 APPENDIX 5 HOSPITAL ANXIETY AND DEPRESSION SCALE

(HADS)

Please complete each of the following questions, checking the one response that comes closest to how you have been feeling in the past week.

1. I feel tense or 'wound up':

- 1 ☐ Most of the time
- 2 ☐ A lot of the time
- 3 ☐ Sometimes
- 4 ☐ Never

2. I still enjoy the things I used to enjoy:

- 1 ☐ Definitely as much
- 2 ☐ Not quite as much
- 3 ☐ Only a little
- 4 ☐ Hardly at all

3. I get a sort of frightened feeling as if something awful is about to happen:

- 1 ☐ Definitely and quite badly
- 2 ☐ Yes, but not too badly
- 3 ☐ A little, but it doesn't worry me
- 4 ☐ Never

4. I can laugh and see the funny side of things:

- 1 ☐ As much as I always could
- 2 ☐ Not quite as much now
- 3 ☐ Definitely not as much now
- 4 ☐ Never

5. Worrying thoughts go through my mind:

- 1 ☐ All of the time
- 2 ☐ A lot of the time
- 3 ☐ Sometimes, but not too often
- 4 ☐ Rarely

6. I feel cheerful:

- 1 ☐ Never
- 2 ☐ Not often
- 3 ☐ Sometimes
- 4 ☐ Most of the time

7. I can sit at ease and feel relaxed:

- 1 ☐ Definitely
- 2 ☐ Usually
- 3 ☐ Not often
- 4 ☐ Never

8. I feel as if I am slowed down:

- 1 ☐ Nearly all the time
- 2 ☐ Very often
- 3 ☐ Sometimes
- 4 ☐ Never

9. I get a sort of frightened feeling like 'butterflies' in the stomach:

- 1 ☐ Never
- 2 ☐ Occasionally
- 3 ☐ Quite often
- 4 ☐ Very often

10. I have lost interest in my appearance:

- 1 ☐ Definitely
- 2 ☐ I don't take as much care as I should
- 3 ☐ I may not take quite as much care
- 4 ☐ I take just as much care as ever

11. I feel restless, as if I have to be on the move:

- 1 ☐ Very much
- 2 ☐ Quite a lot
- 3 ☐ Not Very much
- 4 ☐ Never

12. I look forward with enjoyment to things:

- 1 ☐ As much as I ever did
- 2 ☐ Somewhat less than I used to
- 3 ☐ Definitely less than I used to
- 4 ☐ Hardly at all

13. I get sudden feelings of panic:

- 1 ☐ Very often
- 2 ☐ Quite often
- 3 ☐ Not very often
- 4 ☐ Never

14. I can enjoy a good book or TV program:

- 1 ☐ Often
- 2 ☐ Sometimes
- 3 ☐ Not often
- 4 ☐ Rarely

Thank you very much for filling in this questionnaire

Please check that you have answered all the questions

8.7 APPENDIX 7 PHQ 15

During the past 4 weeks, how much have you been bothered by any of the following problems?	Not bothered at all	Bothered a little	Bothered a lot
a. Stomach pain	☐	☐	☐
b. Back pain	☐	☐	☐
c. Pain in your arms, legs, or joints (knees, hips, etc)	☐	☐	☐
d. Menstrual cramps or other problems with your periods [Women only]	☐	☐	☐
e. Headaches	☐	☐	☐
f. Chest pain	☐	☐	☐
g. Dizziness	☐	☐	☐
h. Fainting spells	☐	☐	☐
i. Feeling your heart pound or race	☐	☐	☐
j. Shortness of breath	☐	☐	☐
k. Pain or problems during sexual intercourse	☐	☐	☐
l. Constipation, loose bowels, or diarrhoea	☐	☐	☐
m. Nausea, gas, or indigestion	☐	☐	☐
n. Feeling tired or having low energy	☐	☐	☐
o. Trouble sleeping	☐	☐	☐

Thank you very much for filling in this questionnaire
Please check that you have answered all the questions