Increased fasting small bowel water content in untreated coeliac disease and scleroderma as assessed by MRI

Ching Lam[1], David S. Sanders[2], Peter Lanyon[3], Klara Garsed[4], Stephen Foley[5], Susan Pritchard[6], Luca Marciani[1], Caroline L Hoad[1,6], Carolyn Costigan[1], Penny Gowland[6], Robin Spiller[1]

1. Nottingham Digestive Diseases Centre, University of Nottingham and National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, Nottingham University Hospitals NHS Trust and University of Nottingham
2. Sheffield Teaching Hospitals NHS Foundation Trust and University of Sheffield, Sheffield, UK
3. Nottingham University Hospitals Trust, Nottingham, UK
4. Derby Teaching Hospitals NHS Foundation Trust, Derby, UK
5. Sherwood Forest Hospitals NHS Foundation Trust, Mansfield, UK
6. Sir Peter Mansfield Magnetic Resonance Centre, University of Nottingham, UK

Abstract

Background and aims: The regular overnight migrating motor complex (MMC) ensures that the normal fasting small bowel water content (SBWC) is minimised. We have applied our recently validated non-invasive magnetic resonance technique to assess SBWC in newly diagnosed coeliac disease (CD), scleroderma (SCD) and irritable bowel syndrome (IBS) conditions, possibly associated with small intestinal bacterial overgrowth (SIBO).

Methods: 20 CD and 15 SCD patients with gastrointestinal symptoms were compared to 20 healthy volunteers (HV) and 26 IBS with diarrhoea (IBS-D) patients as previously reported. All underwent a fasting, magnetic resonance imaging (MRI) scan on a 1.5 T Philips Achieva MRI scanner to assess fasting SBWC and colonic volumes. Stool and symptom diaries were completed for 1 week.

Results: Median (Interquartile range, IQR)

Compared to healthy volunteers, all the patients had significantly increased stool frequency and Bristol stool form score. SBWC was significantly increased in CD 109(53-224) vs. 53(31-98) mL in HV, $p<0.01$ and 42 (28-67) in IBS-D, $p<0.01$. Variable increase in SBWC was also found in SCD, median 77(39-158) but this was not significant, $p=0.2$. Colonic volumes were similar for all

groups being 547 (442-786) for CD, 511 (453-789) for SCD, 612 (445-746) for HV and 521 (428-757) mL for IBS-D. When CD patients were subdivided according to the Marsh classification, the higher grades had larger colonic volumes.

Conclusion: Fasting SBWC as assessed by MRI is significantly increased in newly diagnosed CD and SCD but decreased in IBS-D. Future studies should test whether increased resting fluid predisposes to SIBO.

Key words:

Small bowel, magnetic resonance imaging, irritable bowel syndrome, coeliac disease, scleroderma

Key Summary

1. Summarise the established knowledge on this subject

    - There is limited published data on the normal motor patterns of the small bowel and resting small bowel water content in the human gastrointestinal tract
    - The novel MRI technique is a non-invasive tool that has helped to shed some light on the human gastrointestinal physiology
    - The MRI technique is able to assess fasting small bowel water
    - The MRI technique is able to assess colonic volumes

2. What are the significant and/or new findings of this study?

    - The fasting small bowel water content in patients with newly diagnosed coeliac disease and scleroderma is increase
    - A small subgroup of patients with coeliac disease who has more severe mucosal damage based on the Marsh grading was associated with large colonic volumes.
    - The increased water secretion in the small bowel may predispose these groups of patients to small intestinal bacterial overgrowth.
    - Further larger studies would be needed to affirm this hypothesis.

Introduction

The normal fasting small bowel contains little resting secretions since the interdigestive migrating motor complex, which passes down the bowel every 1-2 hours, clears these into the colon [1]. This is important as it prevents excessive bacterial proliferation which would otherwise damage the small intestinal mucosa leading in severe cases to malabsorption. Diseases such as

scleroderma which impair MMCs[2] can be complicated by small intestinal bacterial overgrowth (SIBO) which can be prevented by stimulating MMCs with octreotide [3]. Assessment of the motor patterns which lead to increased resting secretions and hence provide fertile conditions for bacterial growth, has until recently required intestinal intubation so there is little published data. Early radiological studies in coeliac disease noted the increase in resting fluids which caused a characteristic dilution and flocculation of barium solutions but they were not able to quantify this [4]. We have validated a novel MRI technique for assessing small bowel water content (SBWC) [5] . We have also shown that Ondansetron, a 5HT-3 receptor antagonist (5-HT3RA) shown to be effective in IBS-D, normalized fasting SBWC while reducing antroduodenal motility [6] supporting the idea that reduced fasting SBWC could be an indicator of excessive small bowel motility. We planned to exploit this new , non-invasive, highly patient acceptable technique to assess small and large bowel water in conditions known to be associated with SIBO such as scleroderma [7] and coeliac disease [8] and to compare this with our data in IBS-D, where there has been much interest in the possibility of SIBO as a cause of symptoms.

We hypothesised that resting SBWC would be increased in coeliac and scleroderma patients. The logic being that increased SBWC in untreated CD patients would reflect both the net effect of impaired absorption associated with villous atrophy and increased secretion associated crypt hyperplasia and also the impaired motility reported in untreated coeliac disease [9, 10] while increased SBWC in SCD would reflect the associated impaired motility and reduced absorption. We planned to compare these with a group of previously studied IBS-D patients who acted as a control for the effect of diarrhoea.

Materials and methods:

Subjects:

20 patients who were newly diagnosed with CD and on a gluten diet were recruited from gastroenterology clinics in secondary care (Nottingham University Hospital Trusts, Nottingham and Royal Hallamshire Hospital and University of Sheffield, Sheffield). Diagnosis of coeliac

disease was confirmed by duodenal (D2) biopsy. 15 patients with SCD who had gastrointestinal symptoms such as oesophageal reflux, abdominal pain, diarrhoea and constipation were recruited from a secondary care hospital (Nottingham Treatment Centre and Nottingham University Hospitals Trusts, Nottingham). The results from these 2 groups were compared with previous groups of 20 healthy volunteers (HV) and 26 untreated irritable bowel syndrome with diarrhoea (IBS-D) patients who underwent similar MRI fasting scans using the same criteria and restrictions as previously reported[11]. The IBS-D patients satisfied the Rome III criteria for IBS-D [12] and had been evaluated to exclude other causes of diarrhoeal disorders including coeliac disease, microscopic colitis and bile salt diarrhoea.

This study was approved by the local ethics committees (06/Q2404/74, 10/H0906/50, L/07/2011). All subjects had given written consent and the study was carried out according to the Good Clinical Practice Principles and Declaration of Helsinki. All subjects were asked to abstain from alcohol, caffeine and strenuous exercise from the night before. If they were on any medication that might have affected gastrointestinal motility, this was stopped a week before the study day. Subjects were asked to fast overnight before the study day. All subjects had completed a MRI safety questionnaire to ensure safety and exclude any MRI incompatible metal implants. All subjects were asked to fill in a 7-day stool diary based on the Bristol Stool Form Scale, Hospital Anxiety and Depression Questionnaire and the Patient Health Questionnaire 15 (PHQ-15).

MRI scanning protocol:

All subjects were scanned in the 1.5 T Philips Achieva MRI scanner (Philips, Best, The Netherlands) in the morning. Subjects were in a supine position with a SENSE-4 element body coil wrapped around the abdomen during the scanning phase. Subjects were in the scanner for approximately 15 minutes. 3 different imaging sequences were used for this study. Firstly, a coronal dual echo fast field echo (FFE) sequence was used to visualise the abdominal anatomy. This acquisition has 24 coronal planes and 45 continuous transverse images with a resolution of 1.76 mm x 1.76 mm and a slice thickness of 7mm. Secondly, small bowel water was assessed

using similar protocol to our previous studies [5, 6, 11]. This is a single breath-hold scan using a single shot, fast spin echo sequence which gives a high intensity signals from areas with free fluid and little signal from other body tissues. The third imaging sequence was to assess colonic volumes. This method was recently described and it involves a coronal dual echo FFE sequence with 24 continuous slices with an in-plane resolution of 1.76 x 1.76 mm and 7 mm slice thickness [13]. All these sequences were done in an expiration breath holds of between 15 to 24 s.

Data analysis and statistics:

Data was compared with a cohort of 20 HV who previously had a baseline fasting MRI scan as part of other research studies. These subjects were age and sex matched to the cohort of CD patients.

SBWC measurement was analysed with an in-house software which was previously described and validated [5] against naso-duodenal infusion of mannitol/ saline into the small bowel. The colonic volumes were manually segmented from the coronal images using Analyze9^{TM} software (Mayo Foundation, Rochester, MN, USA). The total colonic volume is a total sum of the segmented colon measured from each image slices [13]. The volumes of small bowel water content and colonic volumes are expressed in millilitres (mL).

Power and statistical analysis:

Power calculation was not performed as these MRI assessments have never been performed before in such subjects.

Statistical analysis was carried out using GraphPad Prism 6 for Windows (GraphPad Software, La Jolla California, USA). Normality of the data was tested by using the D'Agostino and Pearson omnibus normality test. Comparisons between 2 groups were performed using a 2-tailed unpaired t test for variables with a normal distribution or the Mann-Whitney's test for those with a non-normal distribution. For multiple comparisons, 1-way ANOVA was used for normally distributed variables and Kruskal-Wallis test for non-normally distributed variables. The data are expressed as mean (±standard deviation; SD) when normally distributed and as median

(interquartile range; IQ) when non-normally distributed. Results of comparison test is significant if $p \leq 0.05$. Post-hoc assessments were performed by using Bonferroni's multiple comparison test for normally distributed variables and Dunn's multiple comparison test for non-normally distributed variables. In the post-hoc assessments, result was considered significant if $p \leq 0.03$, thus accounting for the effect of multiple comparisons.

Results:

General demographics

All subjects tolerated the MRI scanning protocol well. There were no adverse events during this study period. 20 CD patients (12 female and 8 male; age 45.6±14.1), 15 SCD (13 female and 2 male; age 62.9±12.9), were recruited and compared with previously scanned 26 IBS-D patients (17 female and 9 male; 48.5±11.0) and 20 HV (12 female and 8 male; age 42.9±15.3). 5 of 20 CD had iron deficiency anaemia, low folate and low B12 levels were each noted in 1 of 20 but none had hypocalcaemia or hypoalbuminaemia. This group was further subtyped according to the D2 biopsy results based on the modified Marsh-Oberhuber grading system for CD [14]. 8 CD had Marsh 3c, 8CD had Marsh 3b, 2 CD had Marsh 3a and 2 CD had Marsh 1.

Bowel patterns

As Figure 1 and Table 1 show there was a significantly increased daily stool frequency in all 3 patient groups compared to HV. Average Bristol Stool Form score was also increased in all 3 patient groups but only for IBS-D did this reach statistical significance.

Psychological assessments

Both CD and SCD showed similar PHQ-15 scores to IBS-D patients and significantly higher than the HV. Similarly, patients' anxiety and depression scores were all increased compared with HV. (See Table 1).

Small bowel water content (SBWC)

The MRI scans of the fasting small bowel showed significantly increased SBWC in the CD group compared with HV and IBS-D groups being 109 (53-224) versus 53 (31-98) mL and 42 (28-67) mL respectively; p<0.01(Kruskal-Wallis). There was no significant difference in fasting SBWC between the CD and SCD groups, 109 (53-224) and 77 (39-158) respectively; adjusted p >0.9. There was increased in fasting SBWC in SCD compared to the IBS-D (adjusted p=0.13) but not the HV group; adjusted p >0.9 (Figure 2). In the CD group, there was no difference in the fasting SBWC when comparing within the group who had anaemia (n=5) vs normal haemoglobin level (n=15). The values were 178 (63-870) and 107 (50-148) ml respectively, p=0.31.

Figure 3 shows some examples of coronal image of the abdomen from a (A) HV, (B) patient with CD and (C) patient with SCD. The bright signal represents free water. Image B shows a CD patient with an extremely large volume of water in the small bowel compared to a HV. MRI analysis of the small bowel water content showed 1334 mL of free fluid in the small bowel. Image C shows dilated small bowel and free water in the small bowel of a SCD patient. Most of the water appears to be in the proximal small bowel.

Total colonic volume

Overall there were no significant difference between CD (547 [442-786]) versus HV (612 [445-746]) mL; p=0.89, IBS-D (521 [428-757]) mL versus HV; p=0.67 and SCD (511 [453-789]) mL versus HV; p=0.65. (Figure 4).

However, in the CD group, patients were further subtyped according to the severity of the D2 biopsy based on the modified Marsh grading (Figure 5). There was a significant difference in the total colonic volumes as the severity of the Marsh 3 subtype grading increased. The total colonic volumes for the Marsh 3a, 3b and 3c subtypes were 395 (355-436), 519 (423-757) and 696 (528-806) mL respectively; p=0.05. The stool frequency in Marsh 3c at 1.2 (1.0-1.3) was reduced compared to grade 3a and 3b which were 1.4 (1.0-3.0), 1.9 (1.6-2.1) and respectively; p= 0.02 (Kruskal-Wallis). See figure 6.

Correlation between MR parameters and bowel symptoms

There were no correlations between fasting SBWC and psychological distress symptoms such as anxiety or depression. There were no significant correlations between fasting SBWC and stool frequency and consistency (Table 2).

Discussion

This is the first attempt to relate fasting SBWC to clinical features and as such provides new insights into disease but also raises many new questions. We were not able to perform a power calculation prior to the start of the study as MRI has never been performed in these groups of patients. However with our normal fasting small bowel water content of 53(33) ml from a recently performed study in normal volunteers[15] we can calculate that using our technique in 25 healthy volunteers we would be able to detect a 27 ml difference with an 80% power. Whether such an increase would be clinically significant remains to be determined however the striking variability in fasting small bowel motor activity which occurs during the passage of the migrating motor complex suggests that any change would need to be substantial to be able to be detected by a single measurement which is not synchronized to the motor patterns. Plainly the effects we observed in our patients were substantial since the statistical test performed showed a p value of <0.01 is very robust and the difference seen would not be due to chance.

The increase in SBWC in untreated coeliac disease that we observed may represent the imbalance between absorption and secretion created by loss of the absorptive function of villi leaving the crypts, whose main function is secretion [16]. It could also be secondary to the excess serotonin, characteristic of coeliacs [17-19] which has a stimulatory effect on submucosa enteric nerves which drive secretion [20-22]. Equally, given the documented disturbance in gastric emptying and postprandial motility in untreated coeliac disease [23] it may represent impairment of MMCs, something that could be easily tested. This increased fasting secretion would predispose to SIBO which is detected in a proportion of untreated coeliacs, particularly those with more severe disease [8].

We found that overall the changes in small bowel water in coeliac disease were not accompanied by marked changes in colonic volumes. Our previous work has shown that colonic volumes are rather constant [13], possibly because subjects control this by timing defecation to avoid the unpleasant sensation caused by over distension. However, in our study, it is interesting to find that the more severely damaged small bowel mucosa as assessed by the modified Marsh grade was associated with larger colonic volumes. One possible explanation is that the elevated Peptide YY (PYY) [24, 25] and Glucagon-like peptide 1 (GLP2) [26], described in untreated coeliac disease may inhibit intestinal motility leading to an enlargement of both the small bowel and the colon. This was only observed in a small number of patients but it could be tested by repeating our study involving a larger cohort of CD patients before and after a gluten free diet along with measuring postprandial blood levels of these peptides.

Scleroderma deranges small bowel function by the loss of smooth muscle and fibrosis [27] which results in impaired peristalsis in both oesophagus and small intestine. Delayed orocaecal transit is common and 2/3 of patients demonstrate SIBO [7]. The increased fasting contents we have demonstrated are likely due to a combination of reduce small bowel tone and impaired or reduced MMC activity which normally “sweeps” the intestine clear. The slowly moving intestinal contents in these patients would provide ideal conditions for bacteria to proliferate. These increases in SBWC contrast with the decrease seen in IBS-D, where motility is enhanced [28, 29] and as we showed SBWC reduced, possibly driven by anxiety or stress [11, 30, 31].

One limitation of our studies is that we did not prepare our subjects in any way nor attempt to synchronise our scan time to the periodicity of the MMC. We would expect the SBWC to vary in relation to the occurrence of the MMC, being much less just after an MMC had passed distally. This may account for the variability in fasting SBWC seen in all groups. Unfortunately recording small bowel motility to ensure scanning occurred at the same time in the fasting cycle would involve intubation, which we know reduces fasting SBWC [6], possibly because it stimulates

propulsive motility [32]. MRI assessment of motility is coming of age and it may be possible in the future to record both motility and SBWC.

Our findings throw light on the possible causes of SIBO in coeliacs and scleroderma. These ideas should now be tested in a larger study in which the association of SBWC and SIBO is assessed in a group of untreated coeliacs before and after institution of a gluten-free diet. It may also be worthwhile looking at whether prokinetics can reduce fasting SBWC in scleroderma and whether this will reduce the incidence of SIBO in this patient group.

Authorship Statement:

Guarantor of the article: Professor Robin Spiller

Specific author contributions:

CL, KG and SF contributed to the design of the study, performed the research, collected and analysed the data and wrote the paper

DSS and PL recruited participants and wrote the paper

LM, SP, CLH and CC performed the research, collected and analysed data and wrote the paper

RS and PG designed of the study, analysed data and wrote the paper.

All authors approved the final version of the manuscript.

Acknowledgements

We are grateful for the support from the NIHR Nottingham Digestive Diseases Biomedical Research Centre.

Funding

None

Disclosures

The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health. The authors have no competing interests.

References:

1. Deloose, E., et al., *The migrating motor complex: control mechanisms and its role in health and disease.* Nat Rev Gastroenterol Hepatol, 2012. **9**(5): p. 271-85.
2. Vantrappen, G., et al., *The interdigestive motor complex of normal subjects and patients with bacterial overgrowth of the small intestine.* J Clin Invest, 1977. **59**(6): p. 1158-66.
3. Soudah, H.C., W.L. Hasler, and C. Owyang, *Effect of octreotide on intestinal motility and bacterial overgrowth in scleroderma.* N Engl J Med, 1991. **325**(21): p. 1461-7.
4. Bjerkelund, C.J. and O.W. Husebye, *The clinical and roentgenological findings in steatorrhea of varying etiology.* Am J Dig Dis, 1950. **17**(5): p. 139-49.
5. Hoad, C.L., et al., *Non-invasive quantification of small bowel water content by MRI: a validation study.* Phys Med Biol, 2007. **52**(23): p. 6909-22.
6. Marciani, L., et al., *Effects of a 5-HT(3) antagonist, ondansetron, on fasting and postprandial small bowel water content assessed by magnetic resonance imaging.* Aliment Pharmacol Ther, 2010. **32**(5): p. 655-63.
7. Parodi, A., et al., *Small intestinal bacterial overgrowth in patients suffering from scleroderma: clinical effectiveness of its eradication.* Am J Gastroenterol, 2008. **103**(5): p. 1257-62.
8. Rubio-Tapia, A., et al., *Prevalence of small intestine bacterial overgrowth diagnosed by quantitative culture of intestinal aspirate in celiac disease.* J Clin Gastroenterol, 2009. **43**(2): p. 157-61.
9. Bai, J.C., et al., *Abnormal colonic transit time in untreated celiac sprue.* Acta Gastroenterol Latinoam, 1995. **25**(5): p. 277-84.
10. Spiller, R.C., et al., *Delayed mouth-caecum transit of a lactulose labelled liquid test meal in patients with steatorrhoea caused by partially treated coeliac disease.* Gut, 1987. **28**(10): p. 1275-82.
11. Marciani, L., et al., *Postprandial changes in small bowel water content in healthy subjects and patients with irritable bowel syndrome.* Gastroenterology, 2010. **138**(2): p. 469-77, 477 e1.
12. Longstreth, G.F., et al., *Functional bowel disorders.* Gastroenterology, 2006. **130**(5): p. 1480-91.
13. Pritchard, S.E., et al., *Fasting and postprandial volumes of the undisturbed colon: normal values and changes in diarrhea-predominant irritable bowel syndrome measured using serial MRI.* Neurogastroenterol Motil, 2014. **26**(1): p. 124-30.
14. Dickson, B.C., C.J. Streutker, and R. Chetty, *Coeliac disease: an update for pathologists.* J Clin Pathol, 2006. **59**(10): p. 1008-16.
15. Wilkinson-Smith, V.C., et al., *Insights Into the Different Effects of Food on Intestinal Secretion Using Magnetic Resonance Imaging.* JPEN J Parenter Enteral Nutr, 2018. **42**(8): p. 1342-1348.
16. Mellander, A., H. Abrahamsson, and H. Sjovall, *Duodenal secretomotor function in untreated coeliac disease.* Scand J Gastroenterol, 1995. **30**(4): p. 337-43.
17. Sjolund, K. and A. Nobin, *Increased levels of plasma 5-hydroxytryptamine in patients with coeliac disease.* Scand J Gastroenterol, 1985. **20**(3): p. 304-8.
18. Wheeler, E.E. and D.N. Challacombe, *Quantification of enterochromaffin cells with serotonin immunoreactivity in the duodenal mucosa in coeliac disease.* Arch Dis Child, 1984. **59**(6): p. 523-7.
19. Coleman, N.S., et al., *Abnormalities of serotonin metabolism and their relation to symptoms in untreated celiac disease.* Clin Gastroenterol Hepatol, 2006. **4**(7): p. 874-81.
20. Kellum, J.M., et al., *Serotonin induces Cl- secretion in human jejunal mucosa in vitro via a nonneural pathway at a 5-HT4 receptor.* Am J Physiol, 1994. **267**(3 Pt 1): p. G357-63.
21. Munck, L.K., et al., *Failure of tropisetron to inhibit jejunal water and electrolyte secretion induced by 5-hydroxytryptamine in healthy volunteers.* Gut, 1994. **35**(5): p. 637-40.
22. Cooke, H.J., M. Sidhu, and Y.Z. Wang, *5-HT activates neural reflexes regulating secretion in the guinea-pig colon.* Neurogastroenterol Motil, 1997. **9**(3): p. 181-6.
23. Usai, P., et al., *Autonomic dysfunction and upper digestive functional disorders in untreated adult coeliac disease.* Eur J Clin Invest, 1997. **27**(12): p. 1009-15.
24. Sjolund, K. and R. Ekman, *Increased plasma levels of peptide YY in coeliac disease.* Scand J Gastroenterol, 1988. **23**(3): p. 297-300.
25. Wahab, P.J., W.P. Hopman, and J.B. Jansen, *Basal and fat-stimulated plasma peptide YY levels in celiac disease.* Dig Dis Sci, 2001. **46**(11): p. 2504-9.
26. Caddy, G.R., et al., *Plasma concentrations of glucagon-like peptide-2 in adult patients with treated and untreated coeliac disease.* Eur J Gastroenterol Hepatol, 2006. **18**(2): p. 195-202.

27. Young, M.A., S. Rose, and J.C. Reynolds, *Gastrointestinal manifestations of scleroderma.* Rheum Dis Clin North Am, 1996. **22**(4): p. 797-823.
28. Gorard, D.A., G.W. Libby, and M.J. Farthing, *Ambulatory small intestinal motility in 'diarrhoea' predominant irritable bowel syndrome.* Gut, 1994. **35**(2): p. 203-10.
29. Small, P.K., et al., *Large-scale ambulatory study of postprandial jejunal motility in irritable bowel syndrome.* Scand J Gastroenterol, 1997. **32**(1): p. 39-47.
30. Ditto, B., S.B. Miller, and R.G. Barr, *A one-hour active coping stressor reduces small bowel transit time in healthy young adults.* Psychosom Med, 1998. **60**(1): p. 7-10.
31. Cann, P.A., et al., *Psychological stress and the passage of a standard meal through the stomach and small intestine in man.* Gut, 1983. **24**(3): p. 236-40.
32. Read, N.W., et al., *Effect of gastrointestinal intubation on the passage of a solid meal through the stomach and small intestine in humans.* Gastroenterology, 1983. **84**(6): p. 1568-72.

Tables:

Table 1: Baseline demographics and stool pattern

Mean (SD)	HV	IBS-D	Scleroderma	Coeliac	p (1-way Anova)
N	20	26	15	20	
Female: male	12:8	17:9	13:2	12:8	
Age (years)	42.9±15.3	48.5 ±11.0**	62.9±12.9*,***	45.6 ±14.1	<0.01
PHQ15 (Median, IQR)	1.0 (0-3.0)	11.0 (7.0-13.5)*	10.0 (8.0-11.0)*	10.0 (4.5-14.0)*	<0.01
Anxiety	4.5±2.7	7.7±4.1	6.5±3.7	8.3±3.7*	0.02
Depression	1.4±1.2	4.8±3.3*	4.2±3.4*	4.85±2.9*	<0.01
Stools/ day (Median, IQR)	1.1 (1-1.6)	2.4 (1.8-3.7) *,***	2.1 (1.9-3.7) *	1.4 (1.1-1.9)**	<0.01 (Kruskal-wallis)
Average stool consistency/ day	3.33±0.7	4.5±1.4*	4.2±0.8	4.2±1.2	<0.01

*adjusted p<0.03 versus HV following multiple comparison
** adjusted p<0.03 versus SCD following multiple comparison
*** adjusted p<0.03 versus CD following multiple comparison

Table 2: Correlation between fasting SBWC with psychological distress, stool frequency and consistency

Correlation between fasting SBWC	Spearman, r	P value
Anxiety	0.20	0.10
Depression	0.12	0.32
Stool frequency	-0.07	0.57
Stool consistency	-0.01	0.93

Figures and legends:

Figure 1: Average stool frequency and consistency per day

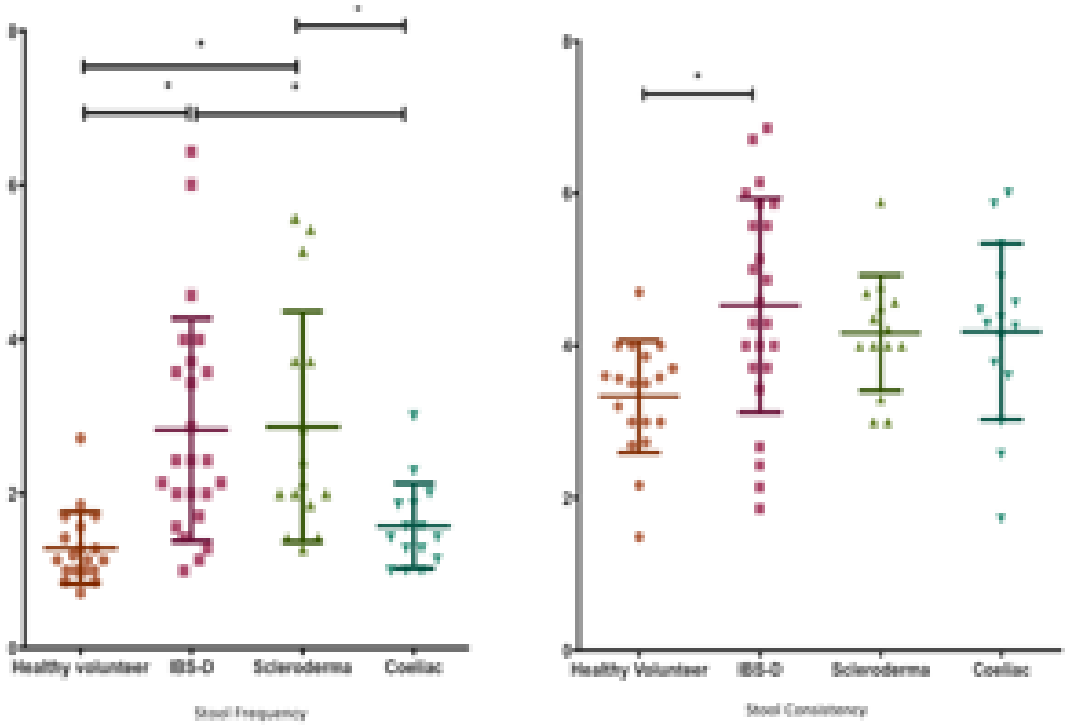

Figure 2: Fasting small bowel water content between HV, IBS-D, SCD and CD

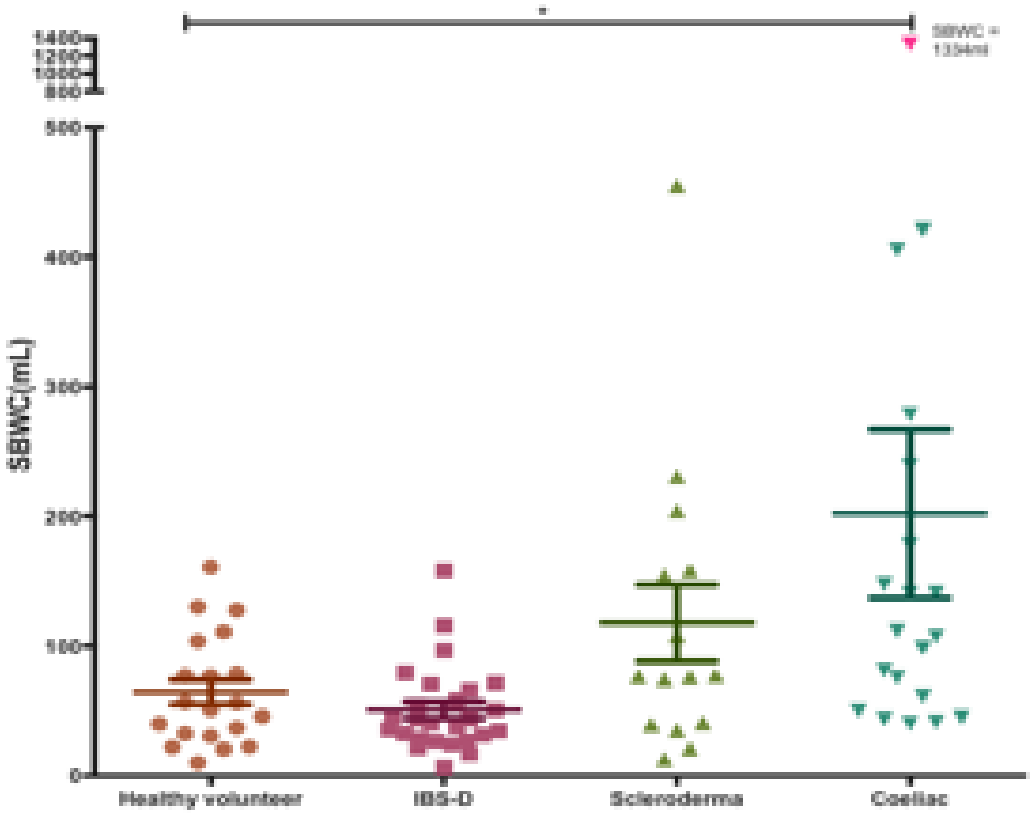

Figure 3: Coronal images of the abdomen showing bright signals which represent free water in the small bowel of a HV (image A), CD (image B) and SCD (image C)

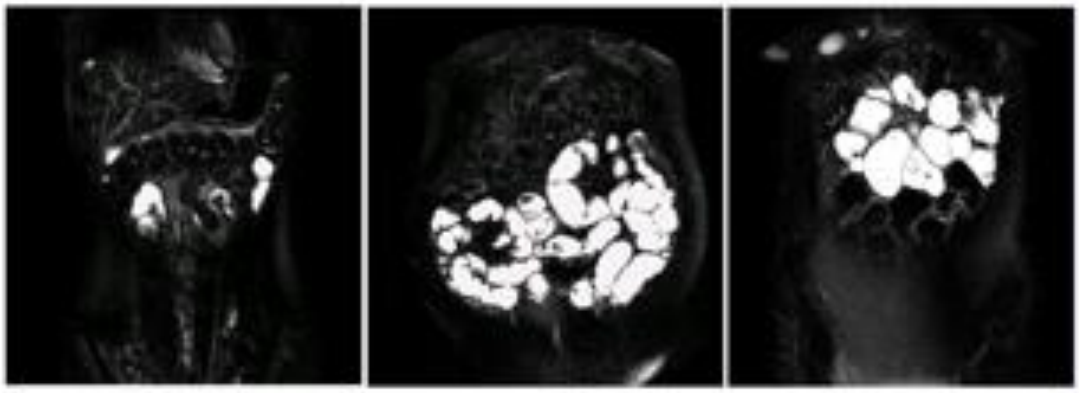

Figure 4: Total colonic volume between each patient group versus HV

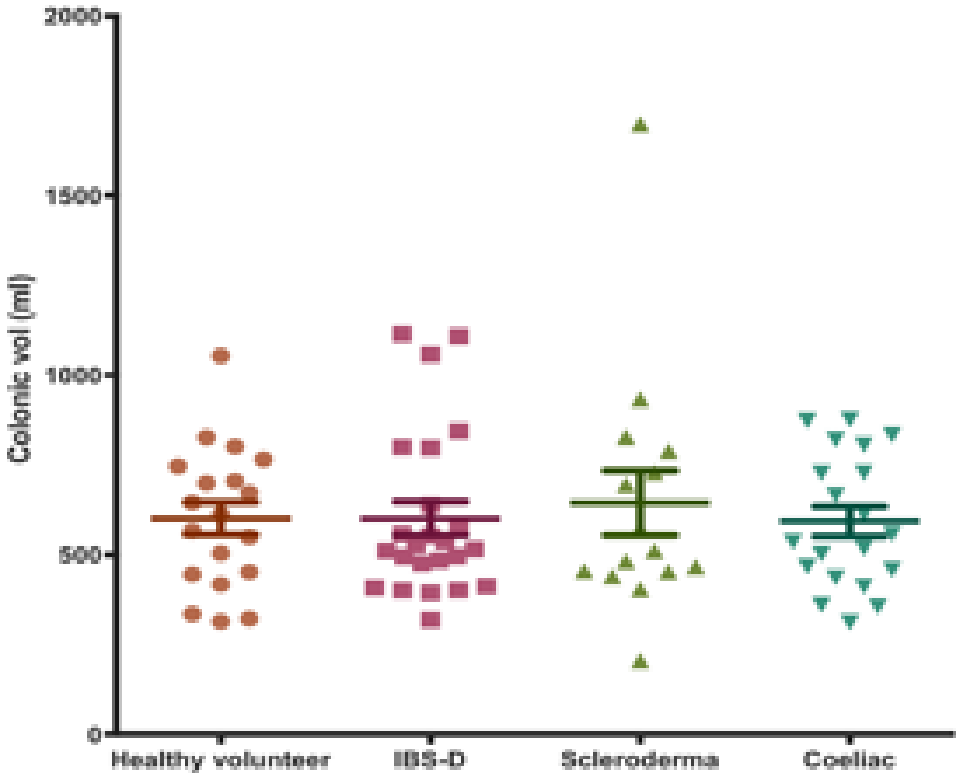

Figure 5: Total colonic volume versus severity of modified Marsh grading for D2 biopsy

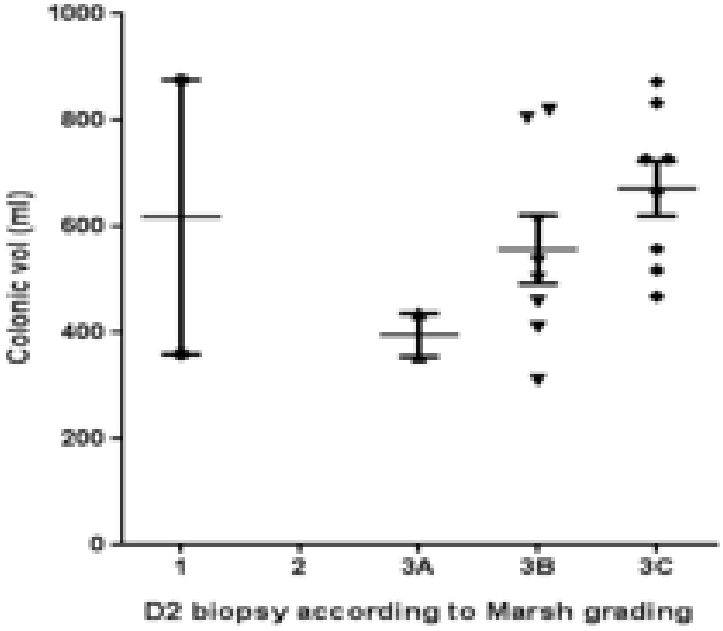

Figure 6: Average stool frequency per day versus modified Marsh 3 subtypes in D2 histology.