Title: Gastrointestinal peptides and small bowel hypomotility are possible causes for fasting and postprandial symptoms in active Crohn's disease.

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SHORT RUNNING HEAD: Motility in Crohn's disease

Abbreviations

Analysis of Variance (ANOVA); Area under the Curve (AUC); au (arbitrary units); Biomedical Research Centre (BRC); Body Mass Index (BMI); Cholecystokinin (CCK); Crohn's disease (CD); C-Reactive protein (CRP); Enteroendocrine cells (EC); Enzyme-linked immunosorbent assay (ELISA); gastrointestinal (GI); Glucagon-like peptide-1 (GLP-1); Healthy volunteer (HV); Interactive data language (IDL); Inflammatory Bowel Disease (IBD); Magnetic Resonance Imaging (MRI); Magnetic Resonance Index of Activity (MaRIA); National Institute of Health Research (NIHR); Polypeptide YY (PYY); Radioimmunoassay (RIA); Region of Interest (ROI); Small Bowel Water Content (SBWC); Sir Peter Mansfield Imaging Centre (SPMIC); Standard error of the Mean (SEM); Visual Analogue Scores (VAS).

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ABSTRACT

Background: Crohn's disease (CD) patients suffer postprandial aversive symptoms which may lead to anorexia and malnutrition. Changes in the regulation of gut hormones and gut dysmotility are believed to play a role.

Objective: This study aims to investigate small bowel motility and gut peptides responses to a standard test meal in CD by using Magnetic Resonance Imaging (MRI).

Design: Fifteen CD patients with active disease (age 36±3 years, BMI 26±1 kg/m^2) and 20 healthy volunteers (HV) (age 31±3 years; body mass index, BMI 24±1 kg/m^2) were studied. They underwent baseline and postprandial MRI scans, symptom questionnaires, and blood sampling following a 400g soup meal (204 kcal). Small bowel motility and other MRI parameters and glucagon-like peptide-1 (GLP-1), polypeptide YY (PYY), and cholecystokinin (CCK) peptides were measured. Data is presented as mean±standard deviation of the mean (SEM).

Results: HV had significantly higher fasting motility index (106±13 a.u.) compared to CD participants (70±8 a.u., $p \leq 0.05$). Postprandial small bowel water content showed a significant time by group interaction ($p<0.05$) with CD showing higher levels from t=210 min postprandially. Fasting levels of GLP-1 and PYY were significantly greater in CD compared to healthy volunteers (GLP-1, CD 50±8 μg/mL versus HV 13±3 μg/mL, $p \leq 0.0001$ and PYY, CD 236±16 pg/mL versus HV 118±12 pg/mL, $p \leq 0.0001$). The meal challenge induced a significant postprandial increase in aversive symptoms scores (fullness, distention, bloating, abdominal pain and sickness) in CD participants compared to healthy volunteers ($p \leq 0.05$).

Conclusions: The decrease in fasting small bowel motility noted in CD may be ascribed to the increased fasting gut peptides. A better understanding of the etiology of aversive symptoms in CD will facilitate identification of better therapeutic targets to improve nutritional status.

KEYWORDS: Gastrointestinal motility; gut peptides; Crohn’s disease; anorexia

INTRODUCTION

Malnutrition is common in patients with Crohn's disease (CD). A recent systematic review reported a reduction in body mass index (BMI) in 37% of CD patients with a reduction in fat-free mass and fat mass in 28% and 31% respectively (1).

Multiple reasons could account for the malnutrition but an abnormal eating behaviour is common in CD, with up to 37% of patients showing abnormal eating patterns (2), with an associated significant reduction in protein intake. Ingestion of food plays a predominant role in symptom generation in Inflammatory Bowel Disease (IBD) patients. In a large prospective multi-centre study, 77% of patients avoided some foods to prevent disease relapse, with 86% of patients avoiding foods when the disease is activity to prevent worsening symptoms (3).

The aetiology for this is still unclear but postprandial dysmotility of the inflamed gut could be an aetiological factor. A delayed gastric empting or attenuated small bowel motility in the fasting or postprandial state could be an important aetiological factor in the patient symptom generation and eventual adoption of a diverse eating behaviour to improve symptom control.

Recent technological advances in image analysis have allowed us to use magnetic resonance imaging (MRI) to measure CD activity through intestinal motility (4). A significant negative correlation is observed between terminal ileal motility and histological (4), biochemical (5) and radiological measures of disease activity (4). The exact aetiology of this pathophysiological finding is still unclear.

Enteroendocrine cells (EC) are intraluminal nutrient sensors. They play a pivotal role in orchestrating physiological functions in the gastrointestinal tract. Exposure of the ileum to fatty acids decreases jejunal (6) and duodenal motility (7) through polypeptide YY (PYY)-mediated mechanisms. Glucagon-like peptide-1 (GLP-1) has been shown to decrease small bowel motility (8, 9). Moreover, cholecystokinin (CCK) has been shown to delay gastric emptying by increasing pyloric motility (10, 11), with CCK modulation (12) affecting food intake underlying its anorectic role in eating behaviour. CD patients with active small bowel inflammation have significant up-regulation of EC cells with an increase in ileal expression of GLP-1 (13), PYY (2-fold) (14) and (CCK) (3-fold) (15) levels. This increase in plasma peptide levels is associated with the symptoms of nausea and anorexia, with both symptoms, and tissue and plasma EC-peptide expression decreasing to normality in remission (14). We hypothesise that an increase in CCK, GLP-1 or PYY-EC activity could potentially lead to symptoms of nausea through delayed gastric emptying and attenuated post-prandial small bowel motility. To our knowledge, this mechanistic link has never been described.

We have shown that a soup test meal challenge is effective at inducing a change in multiple physiological quantified end-points and in monitoring markers of gastrointestinal motility in a single MRI study acceptable to participants (16, 17). Our developed technique for the assessment of small bowel motility showed excellent inter and intra observer agreement (17). We now aim to investigate fasting and postprandial changes in gut motility, intestinal physiology and related symptoms in a cohort of CD patients with active disease and healthy volunteers (HV) to investigate the aetiology of patient symptoms.

SUBJECTS AND METHODS

Study population

This is a single-centre open-label clinical trial conducted between November 2015 and February 2017 at the National Institute of Health Research Nottingham Biomedical Research Centre (NIHR Nottingham BRC). CD patients (18-75 years) with active disease were recruited from the Inflammatory Bowel Disease clinic at Nottingham University Hospitals

Trust. Active disease was defined as ulceration seen at ileocolonoscopy, intestinal inflammation or deep ulceration seen on computer tomography or MR enterography, with the disease activity quantified via the Magnetic Resonance Index of Activity (MaRIA) score of >7 (18) or faecal calprotectin of >250µg/g (19, 20) or C-Reactive protein (CRP) >5mg/dl. These measures of disease activity were to be quantified within four weeks of recruitment. Stable doses of immunosuppressive and biological agents were permitted. No changes in medication were allowed at inclusion until data collection of the outcome measures has been completed. All participants had a good command of the English language and had the capacity to give informed written consent.

Age-, body mass index- and gender-matched HV participants were recruited from an existing participant database in the NIHR Nottingham BRC and from the local healthy populations of Nottingham University Hospitals and the University of Nottingham. Potential participants with a history of inflammatory bowel disease, smokers (21), a history of bowel resections or any gastrointestinal surgery, history of pancreatic insufficiency, thyroid disease, diabetes, protein-pump inhibitor usage or any medication that affects gastric emptying or small bowel transit and any potential participants scoring very highly on the depression scale questionnaire were excluded. Standard MRI exclusion criteria were applied.

This study was approved by the Research Ethics Committee (NRES approval 15/EM/0003) of the Health Research Authority. This study is registered on clinical trials.gov with identifier NCT03052465. All participants gave informed written consent.

Outcome measures

The primary outcome of this study was to compare fasting and postprandial small bowel motility between CD and HV participants as measured through MRI. Secondary outcomes were gall bladder contraction, gastric volumes, small bowel water content, plasma GLP-1, PYY and CCK, symptoms scores and MRI disease activity scores as quantified by the MaRIA score (18).

Experimental protocol

This study was open label. Participants were asked to fast from 2000 h the previous evening and to avoid alcohol, caffeine, strenuous exercise and any medication that could affect gut function for 18 h before the experiment. On the day of the scan, participants attended the 1.5T Philips Achieva MRI scanner (Philips Healthcare, Best, the Netherlands) at the Sir Peter Mansfield Imaging Centre (SPMIC) at the University Park Campus, University of Nottingham at 0800.

Participants underwent a baseline fasting scan (defined at t = -20 min time point), together with a fasting baseline blood sample. They were then asked to consume all their test meal within a maximum time of 20 min. The participants then underwent a first immediate postprandial scan (defined as t = 0 min).

This was followed with data collection (MRI, questionnaire data and 10 ml blood samples) time points every 15 min for the first 60 min and then every 30 min up to 270 min. At each time point, participants filled a 100mm Visual analogue scale (VAS) symptoms questionnaire scoring their feeling of fullness, bloating, distension, abdominal pain/discomfort and nausea (14). At the end of the 270 min participants were given a volume (750mls-1250mls) of oral contrast agent (2.5% Mannitol,0.2 % locust bean gum) to drink (within 60 minutes) and a further MRI scan (within 30 minutes) was undertaken to quantify disease activity. See **supplementary figure 1**.

Test meal

The test meal consisted of: cream of chicken soup (400g) (or mushroom for vegetarians) (Heinz, Wigan, UK) (14, 22), The nutrient content of this meal/100g was: energy (kcal) 51, protein 1.5 g (1.5%), carbohydrate 4.7 g (4.5%), fat 2.9 g (2.9%). For a complete list of contents please see https://www.heinz.co.uk/products/soup/ranges/classics/cream-of-chicken.

Magnetic Resonance Imaging

Participants were scanned using a range of sequences (22). At each time point scans were acquired to assess gastric volume (23), gall bladder volume (24), small bowel water content (25) and small bowel motility (26).

Gastric emptying was assessed using a balanced gradient echo sequence to yield a good contrast between the stomach contents and other abdominal organs (23).

The content of apparent freely mobile water in the small bowel was assessed as previously described (25). This sequence yields high-intensity signals from areas with freely mobile fluid and dark signals from poorly mobile or bound water and all other body tissues.

Small bowel motility was assessed using a single slice cine-MRI acquisition set at six contiguous parallel coronal planes through the small bowel (22).

Gall bladder volume was measured pre- and post-prandial, at every acquisition time point using the same images as for the gastric volumes as previously shown (24). These functional MRI measures were acquired prior to the use of any anti-spasmodic agents administered during serial image acquisition.

Plasma collection and peptides assays analysis

Fasting 10 ml blood sample was drawn and collected. After the test meal, data were acquired every 15 min for the first 60 min and every 30 min thereafter to 270 min. Plasma peptides (total GLP-1, total PYY) were analysed through enzyme-linked immunosorbent assay (ELISA) techniques (Millipore, UK) as previously shown (14). The concentrations of serum CCK were measured by radioimmunoassay (RIA) (Euro Diagnostic Products, Sweden) as previously shown (27).

MRI measures of disease activity

Small bowel was scanned before and 10 minutes after 40mg of hyoscine butyl bromide was injected intravenously to reduce small bowel motility. Participants were scanned within 30 minutes. Initially a true fast imaging with a steady sequence was acquired in the coronal plane. Axial T1 sequences were acquired before and 70 s after intravenous administration of 0.2 ml/kg body weight of gadolinium chelate (gadodiamide 0.5 mmol/l) at a rate of 2 ml/s.

Data analysis

Motility assessment

All dynamic data was processed with Dual Registration of Abdominal Motion (Motilent, London, UK). Registration results were further analysed using a customised graphical user interface written in MATLAB (MathWorks, Natick, MA, USA) (17).

The technique used in this study quantified the motility of the bowel using the pixel signal changes through the time series, within a defined region of interest (ROI) placed over all visible small bowel loops (17) as previously described. This method utilizes changes in signal intensities that occur when the small bowel contents move between segments in regions showing bolus movement of contents as well as those showing more oscillatory motion, rather than looking for continuous motion throughout the time series. Motility measures are presented as arbitrary units (a.u.) and are calculated as the mean across the total small bowel ROI.

Small bowel water content (SBWC), Gall bladder and gastric volumes

These parameters were quantified as previously validated (25), using in house software written in interactive data language (IDL) (Research Systems Inc. Boulder, Colorado, USA).

Visual analogue scale (VAS)

Symptoms regarding appetite, satiety and abdominal pain were scored at each time point using a previously validated questionnaire (14).

MARIA score

MRE variables were evaluated by a Nottingham University Hospitals clinical gastrointestinal (GI) MRI radiologist with > 10 years' experience (Dr. Khalid Latief) in each segment including: bowel wall thickening, enhancement of the bowel wall after administration of intravenous contrast with gadolinium (relative contrast enhancement), presence of ulcers, mural oedema, regional enlarged lymph nodes (>10 mm), peri-enteric vascularization (comb sign), peri-enteric fluid, fat stranding, and fibro-fatty proliferation. The MaRIA score in each segment was calculated according to a formula, as previously defined (18).

Sample size and Statistical analyses

Previous literature showed 2-fold higher plasma PYY in CD (area under the curve, AUC 22990 ± 5585) vs HV (10700 ± 1886) and a higher plasma GLP-1 in CD (AUC 1027 ± 220) vs HV (1347 ± 350) (28). Assuming α of 0.05, power of 80%, a maximum sample of 15 participants in each group was needed to show a difference. Similar comparisons for CCK showed significant differences between CD and HV in 10 participants/group with a strong correlation (r=0.6) between mean CCK plasma concentration and gastric emptying half-life (15). Assuming α of 0.05, power of 80% a total sample size of 19 participants was needed to show a difference. To allow for missing data (~10%), we aimed to recruit a maximum of 20 participants in each of the CD and HV cohorts.

The primary analysis was an across-group analysis with further sub-analyses undertaken if the primary comparison is significant. Normality of the data was assessed using Shapiro–Wilk's test. Parametric data was presented as mean ± standard error of the mean (SEM) or median ± interquartile range if non-parametric. All statistical analyses were performed using GraphPad Prism 7.01 (La Jolla, USA). A p-value less than 0.05 was considered statistically significant. Analyses of variance (ANOVA) was used to assess the significance of differences between and within each group with different time points. When the analysis of variance was significant, post hoc test assessments of the individual time points were performed using the Dunnett's (for parametric data) or Dunn's test (for nonparametric data) for within group comparison and Sidak's test was used for between groups to account for multiple comparisons. The Pearson correlation coefficient was used to measure the strength of correlation between MaRIA scores and the different variables (measured as AUC).

RESULTS

Participants

Nineteen CD participants with active disease (age 36±3 years, BMI 26±1 kg/m^2) as well as 20 HV participants (age 31±3 years, BMI 24±1 kg/m^2) were recruited. One CD participant was lost to follow-up and the data from two CD participants were excluded from the final analyses: one because of a low MaRIA score of 3.39 and another because of an unanalysable MRI data set. One other participant was excluded because of a high score on the hospital anxiety and depression questionnaire. See supplementary figure 2, table 1 and supplementary table 1. CD participants had a mean disease duration of 12.3±1.7 years, mean HBI of 6±1, mean CRP of 18.2±3.4mg/dl and mean fecal calprotectin of 787.2±146.8μg/g. All patients had ileal involvement. Four patients were being prescribed anti-tumor necrosis factor therapy, one patient vedolizumab, three patients immunomodulators, with the rest on no medication at the time of recruitment. Eight patients were surgically naïve.

All HV participants and CD participants completed the study and tolerated the experimental procedures well without any adverse event.

Small bowel motility

The HV participants started with a significantly higher (106±13 a.u., $p<0.05$) fasting motility index compared to CD participants (70±8 a.u.) (**Figure 1**). No significant difference in the postprandial time by group interaction was observed in small bowel motility between the CD and HV groups.

Gallbladder volumes

Two CD patients had a cholecystectomy prior to recruitment. No difference in the fasting gall bladder volumes were observed between the two groups. No significant difference in the postprandial time by group interaction was observed in gall bladder volumes between the CD and HV groups. The difference in gallbladder volumes between HV and CD participants from fasting to 150 min are shown in **Figure 2**.

Gastric volumes

The baseline gastric volumes showed a small amount of fasting gastric secretions (**Figure 3**, HV: 29±5 mL, CD: 25±4 mL) with no differences observed between the groups. No significant difference in the postprandial time by group interaction was observed in gastric volumes between the CD and HV groups. Gastric volume increased upon feeding (HV: 388±18 mL, CD: 324±26 mL). The average time to empty half of the stomach contents ($T_{1/2}$) in HV and CD participants was 43±4 min, 63±7.5 min respectively, with no significant difference observed between the groups.

Small bowel water content

The data in **Figure 4** shows a small amount of fasting small bowel water content in both groups (HV: 44±6 mL, CD: 36±9 mL). No difference in the fasting small bowel water content was visualised between groups. A significant increase ($p<0.05$) was seen in CD participants compared to HV (measured as area under the curve (AUC) CD: 19778±2119 mL/min, HV: 14197±1249 mL/min). A significant difference in the time by group interaction was observed in small bowel water content between the CD and HV groups ($p=0.0352$). An increase in postprandial water volume in CD when compared to healthy volunteers was observed at t=210 min ($p=0.0388$), t=240 min ($p=0.0168$) and 270 min ($p=0.0048$).

Total GLP-1

Figure 5 show higher ($p<0.0001$) fasting GLP-1 levels in CD participants compared to HV (CD: 50±8 µg/mL, HV: 13±3 µg/mL). The test meal did not induce a significant postprandial response in either group. No significant difference in the time by group interaction was observed between HV and CD groups. Significantly higher ($p<0.0001$) postprandial GLP-1 levels were reported in CD participants compared to HV (AUC CD: 12293±1586 µg/mL, HV: 3317±762 µg/mL).

Total PYY

The CD participants showed higher (236±16 pg/mL, $p<0.0001$) fasting PYY plasma levels when compared to HV (118±12 pg/mL) (**Figure 6**). The test meal did not induce a significant postprandial response in either group. No significant difference in the time by group interaction was observed between HV and CD groups. CD participants exhibited a significantly higher postprandial PYY response compared to the HV (AUC CD: 62782±4313 pg /mL, HV: 34744±3169 pg/mL, $p<0.0001$).

CCK

No significant difference was seen in fasting and postprandial levels of CCK between the two groups (**Figure 7**). No significant difference in the time by group interaction was observed in postprandial CCK between the CD and HV groups. ~~In CD group, a significant difference (p<0.0001) was seen across the different time points with a significant increase (p<0.05) from time 0 to 90 min compared to fasting plasma levels. Within HV group, plasma CCK levels showed a significant increase (p<0.05) immediately after feeding to 60 min in comparison to fasting concentrations.~~

Symptom VAS data

Fasting and postprandial VAS scores recorded from CD participants and HV are shown in **Figure 8**. CD participants showed a significantly higher ($p<0.01$) fasting fullness and abdominal pain scores compared to HV (CD: 21±6 mm, 18±5 mm HV: 5±3 mm, 0.5±0.3 mm). CD participants also showed a significantly higher ($p<0.05$) fasting distention scores compared to HV (CD: 14±5 mm HV: 2±1 mm).

No significant difference in the time by group interaction was observed in VAS between the

CD and HV groups. The meal induced a significantly ($p<0.05$) higher postprandial fullness scores in CD participants compared to HV (AUC CD: 6795±1440 mm/min HV: 2907±703 mm/min). A significantly higher postprandial ($p<0.0001$) VAS scores of bloating, distention and abdominal pain were noted in CD participants compared to HV (AUC CD: 5558±1293 mm/min, 5071±1253 mm/min, 3187±873 mm/min HV: 565±257 mm/min, 303±191 mm/min, 7±5 mm/min). The CD participants showed a significantly ($p<0.01$) higher sickness scores compared to HV healthy volunteers (AUC, CD: 2024±927 mm/min HV: 75±75 mm/min).

MaRIA scores

The mean value of the MaRIA score was 20.3±1.9 (see the supplementary table for individual scores). There was moderate non-significant correlation between fasting small bowel motility and disease activity as measured through MaRIA scores (r=0.52 (95% confidence interval -0.050, 0.8303, p=0.07). There was no significant correlation between MaRIA scores and postprandial small bowel motility, small water content and GLP-1, PYY or CCK levels.

DISCUSSION

The primary aim of this study was to understand the altered intestinal physiology and aversive patient symptoms observed in active CD in the fasted and the postprandial state. Any alterations might help better understand symptom generation that may be leading to altered eating behaviour and malnutrition.

Participants with CD had a lower fasting small bowel intestinal motility, with otherwise no difference observed in the postprandial phase when compared to HV. We have shown significantly higher fasting and postprandial levels for both GLP-1 and PYY with associated aversive symptoms being reported to a significantly greater extent in CD than in HV.

Although small bowel hypomotility, elevated plasma GI peptide levels and aversive patient symptoms could be interlinked in the fasting state; our data suggests that this hypothesis may not hold true in the postprandial phase. Possibly, symptom generation in the postprandial phase could be related to GI-peptide mediated alterations in the gut-brain axis rather than altered intestinal physiology. A decrease in MR motility in CD, in the prepared bowel, was previously described, with motility correlated to histological and biochemical measures of disease activity and patient symptoms (29). Global small bowel hypomotility involving normal-looking bowel has been described in CD, with motility variance negatively correlating to key patient symptoms like diarrhoea, pain and clinical symptom scores (29, 30).

The fasting and postprandial plasma levels of GLP-1 were significantly higher in CD. This was confirmed in previous CD studies (31). Similarly, plasma fasting and postprandial PYY levels were significantly elevated in CD group as we previously described (14, 31). The higher fasting GLP-1 and PYY observed in CD could be the cause of the fasting small bowel hypomotility observed in CD. We observed no difference in the fasting and postprandial plasma CCK levels, although the observed postprandial CCK response was only significant in the CD rather than the healthy group despite the absolute levels not being significant in between groups. The CD location in this recruited cohort was predominantly ileal or ileocolonic rather than proximal in the duodenum where the CCK-secreting I cells are located. Although earlier data in IBD murine models suggested that EC upregulation occurs irrespective of the anatomical location of intestinal inflammation (32), this observation was later refuted in CD (13). This might explain the lack of difference in CCK concentration between both groups.

No significant difference was seen in the fasting and postprandial gall bladder volumes in CD compared to healthy volunteers. The lack of difference in CCK levels between the two groups explains the lack of difference in the gall bladder volumes as CCK is integral for the gall bladder contraction in response to a fat stimulus. Additionally, previous data showed a similar non-significant fasting gall bladder volumes in both healthy volunteers and patients with small bowel CD (33, 34).

Similarly to postprandial small bowel motility, a delay in gastric emptying was expected in the CD cohort with no difference in gastric emptying observed between CD and HV in this study. Both PYY and GLP-1 may delay gastric emptying (35). In a previous study, delayed gastric emptying was observed in an inflammatory bowel disease cohort with similar plasma CCK and elevated plasma GLP-1 to healthy participants (31). In that study, gastric emptying was measured through a breath test so the findings are not comparable to our study.

Postprandial small bowel water content typically showed two peaks. The first peak is the gastric phase and represents emptying of the gastric contents in the proximal small bowel. The second peak is called the intestinal phase (36) which represents the increase in small bowel water content due pancreatico-biliary and enterocyte secretion. A bimodal postprandial peak was also seen in the small bowel water content in CD group, with a significant difference in time by group interaction in postprandial small bowel water content between CD and HV. The noted increase could be due to the significant postprandial CCK response in the CD group which leads to an increase in bile acid production and pancreatic secretion. Additionally, the meal may have acted as an osmotic stimulus causing the increase in the small bowel water content seen in the intestinal phase in CD cohort (37). Moreover, the observed increase in small bowel water content in CD group might have induced an increase in small bowel motility by stimulating smooth muscle contraction due to the increase in small bowel distention thus nullifying any postprandial differences between groups. Additionally, the increase in small bowel distention might have made small bowel motility more easily quantifiable in this unprepared MRI visit hence decreasing any possible difference to healthy participants. None of the CD participants had predominant stricture which may have caused a delay in intestinal transit and might explain the increase in small bowel water content. Finally, the increase in measureable small bowel water content in CD might merely represent a delay in the distal small bowel emptying its contents in the ascending colon.

The CD group demonstrated significantly higher fasting and postprandial symptom VAS scores, with more predominant symptoms of fullness, distention and abdominal pain scores compared to HV. The additional small bowel distention from the increase in water content in CD might explain the significant difference in the measured symptoms. However, the actual difference in small bowel water content is small and accounts to only 40 mL which might not been enough to account for such a drastic difference in symptoms. Another possible mechanism for these exaggerated symptoms might be from the upregulated gut-brain axis which we have not investigated in this study.

There were possible limitations to this work. The test meal used was small in volume and nutrients and did not induce a significant change in postprandial GLP-1, PYY and CCK. However, as we had previously shown (14), it was well tolerated by all the patients and acted as a good stimulus to gastrointestinal physiological responses. Another limitation is the relatively small participant population, but such detailed phenotyping made recruiting a larger cohort for this exploratory study unfeasible. We as well assayed total PYY and GLP-1 rather than the active peptides. In vivo, active GLP-1 is rapidly degraded to GLP-1 (9-36). Similarly, PYY (1-36) is metabolised to the active PYY (3-36). Both of these activities are undertaken through DPP-4 (38, 39).We have previously shown that DPP-4 expression is decreased in CD (40). In this present study, we used a protease inhibitor to further minimise DPP4 activity and peptide degradation. In previous work we also have shown no significant difference between active and total GLP-1 in small bowel CD and HV (28). Similarly, total PYY, has been shown to have a temporal pattern similar to that of PYY (3–36) after meals (41). For these reasons, we assayed total rather than the active peptides.

In this work we successfully quantified fasting and postprandial small bowel motility and possibly ascribed a putative role for EC peptides in the aetiology of disordered intestinal motility and anorectic symptoms in CD. A better understanding of the role EC peptides in the altered eating behaviour and malnutrition has pharmacological relevance. EC peptide modulators (Exendin 9-39 (42) and dexloxiglumide (43)) are now available. Further work is now needed to deconstruct the gut-brain axis and possibly open a new therapeutic pathway in CD therapy, thus improving nutritional status, disease outcomes and quality of life.

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CONFLICT OF INTEREST

None

AUTHORS’ CONTRIBUTION

AK, CLH, LM, RCS, PAG, SAT and GWM designed research; AK, CLH, LM, AN, SR, ML, YF, GS, GWM conducted research; AK, AM, CLH, ML, YF, GS and KL analyzed data or performed statistical analysis; AK, CLH, PAG, RCS, SAT, AM, LM, and GWM wrote the paper; GWM had primary responsibility for final content.

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Tables 1: Patient demographics

	HV participants	CD participants
Number	20	15
Age	31±3 years	36±3 years
Gender	10F:10M	8F:8M
BMI	24±1 kg/m^2	26±1 kg/m^2
Smoking Status	Non-smokers	Non-smokers
Mean CRP (SEM)	N/A	18.2±3.4mg/dl
Mean Faecal Calprotectin (SEM)	N/A	787.2±146.8µg/g

Figure legends:

Figure 1: Time courses of the small bowel motility in Crohn's disease (CD) and in healthy volunteers. A. Significantly lower fasting small bowel motility in CD when compared to healthy volunteers. B. Time courses of the small bowel motility for CD and healthy volunteer. No significant difference in the time by group interaction was observed between CD and healthy volunteer groups. Data presented as mean ± SEM (healthy volunteer: n=20, CD: n=15). ANOVA was used to assess the significance of differences between and within each group for different time points.

Figure 2: Time courses of the gallbladder volumes in Crohn's disease (CD) and in healthy volunteer groups. A. No significant difference in the fasting gall bladder volumes between CD and healthy volunteer groups. B. Time courses of the gall bladder volumes in CD and healthy volunteer groups. No significant difference in the time by group interaction was observed between CD and healthy volunteer groups. Data presented as mean ± SEM (healthy volunteer: n=20, CD: n=13). ANOVA was used to assess the significance of differences between and within each group for different time points.

Figure 3: Time courses of the stomach content volumes in Crohn's disease (CD) and in healthy volunteers. A. No significant difference in the fasting stomach volumes between CD and healthy volunteers. B. Time courses of the stomach volumes for CD and healthy volunteers. No significant difference in the time by group interaction was observed between CD and healthy volunteer groups. Data presented as mean ± SEM (healthy volunteers: n=20, CD: n=15). ANOVA was used to assess the significance of differences between and within each group for different time points.

Figure 4: Time courses of the small bowel water content in Crohn's disease (CD) and in healthy

volunteers. A. No significant difference in the fasting small bowel water content for CD and healthy volunteers. B. Time courses of the small bowel water content for CD and healthy volunteers showing a significant difference in time by group interaction ($p=0.0352$) with an increase in postprandial water volume in CD when compared to healthy volunteers at t=210 min ($p=0.0388$), t=240 min ($p=0.0168$) and 270 min ($p=0.0048$). Data presented as mean ± SEM (healthy volunteers: n=20, CD: n=15). ANOVA was used to assess the significance of differences between and within each group with different time points. Sidak's test was used for the assessments of the individual time points between groups.

Figure 5: Time courses of the GLP-1 concentrations in Crohn’s disease (CD) and in healthy volunteers. A. Significantly higher fasting GLP-1 concentrations for CD when compared to healthy volunteers. B. Time courses of the GLP-1 concentrations for CD and healthy volunteers. No significant difference in the time by group interaction was observed between CD and healthy volunteer groups. CD participants exhibited a significantly higher postprandial GLP-1 response compared to healthy volunteers (AUC CD: 12293±1586 μg/mL, healthy volunteers: 3317±762 μg/mL, $p<0.0001$). Data presented as mean ± SEM (healthy volunteers: n=20, CD n=15). ANOVA was used to assess the significance of differences between and within each group for different time points.

Figure 6: Time courses of the PYY concentrations in Crohn’s disease (CD) and in healthy volunteers. A. Significantly higher fasting PYY concentrations in CD when compared to healthy volunteers. B. Time courses of the PYY concentrations for CD and healthy volunteers. No significant difference in the time by group interaction was observed between CD and healthy volunteer groups. CD participants exhibited a significantly higher postprandial PYY response compared to healthy volunteers (AUC CD: 62782±4313 pg /mL,

healthy volunteers: 34744±3169 pg/mL, $p<0.0001$). Data presented as mean ± SEM (healthy volunteers: n=20, CD n=15). ANOVA was used to assess the significance of differences between and within each group for different time points.

Figure 7: Time courses of the CCK concentrations in Crohn's disease (CD) and in healthy volunteers. A. No significant difference in fasting CCK concentrations between CD and healthy volunteers. B. Time courses of the CCK concentrations for CD and healthy volunteers. No significant time by group interaction observed. Data are mean ± SEM (healthy volunteers: n=20, CD n=15). ANOVA was used to assess the significance of differences between and within each group for different time points.

Figure 8: Time courses of the fasting and postprandial VAS scores in in Crohn's disease (CD) and in healthy volunteers. CD participants showed a significantly higher fasting fullness ($p<0.01$), abdominal pain ($p<0.01$) and distention scores ($p<0.05$) compared to healthy volunteers. Data presented as mean ± SEM (HV: n=20, CD n=15). ANOVA was used to assess the significance of differences between and within each group for different time points.