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).

Clinical trial registry: Clinical trials.gov NCT03052465.

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8   **ABSTRACT**

9   **Background**: Crohn's disease (CD) patients suffer postprandial aversive symptoms which

10   may lead to anorexia and malnutrition. Changes in the regulation of gut hormones and gut

11   dysmotility are believed to play a role.

12   **Objective:** This study aims to investigate small bowel motility and gut peptides responses to a

13   standard test meal in CD by using Magnetic Resonance Imaging (MRI).

14   **Design**: Fifteen CD patients with active disease (age 36 ± 3 years, BMI 26 ± 1 kg/m^2) and 20

15   healthy volunteers (HV) (age 31 ± 3 years; body mass index, BMI 24 ± 1 kg/m^2) were studied.

16   They underwent baseline and postprandial MRI scans, symptom questionnaires, and blood

17   sampling following a 400g soup meal (204 kcal). Small bowel motility and other MRI

18   parameters and glucagon-like peptide-1 (GLP-1), polypeptide YY (PYY), and cholecystokinin

19   (CCK) peptides were measured. Data is presented as mean±standard deviation of the mean

20   (SEM).

21   **Results:** HV had significantly higher fasting motility index (106 ± 13 a.u.) compared to CD

22   participants (70 ± 8 a.u., $p\leq0.05$). Postprandial small bowel water content showed a significant

23   time by group interaction ($p<0.05$) with CD showing higher levels from t=210 min

24   postprandially. Fasting levels of GLP-1 and PYY were significantly greater in CD compared

25   to healthy volunteers (GLP-1, CD 50 ± 8 μg/mL versus HV 13 ± 3 μg/mL, $p\leq0.0001$ and PYY,

26   CD 236 ± 16 pg/mL versus HV 118 ± 12 pg/mL, $p\leq0.0001$). The meal challenge induced a

27   significant postprandial increase in aversive symptoms scores (fullness, distention, bloating,

28   abdominal pain and sickness) in CD participants compared to healthy volunteers ($p\leq0.05$).

29   **Conclusions:** The decrease in fasting small bowel motility noted in CD may be ascribed to the

30   increased fasting gut peptides. A better understanding of the etiology of aversive symptoms in

31   CD will facilitate identification of better therapeutic targets to improve nutritional status.

33 **INTRODUCTION**

34 Malnutrition is common in patients with Crohn's disease (CD). A recent systematic review

35 reported a reduction in body mass index (BMI) in 37% of CD patients with a reduction in fat-

36 free mass and fat mass in 28% and 31% respectively (1).

37 Multiple reasons could account for the malnutrition but an abnormal eating behaviour is

38 common in CD, with up to 37% of patients showing abnormal eating patterns (2), with an

39 associated significant reduction in protein intake. Ingestion of food plays a predominant role in

40 symptom generation in Inflammatory Bowel Disease (IBD) patients. In a large prospective

41 multi-centre study, 77% of patients avoided some foods to prevent disease relapse, with 86%

42 of patients avoiding foods when the disease is activity to prevent worsening symptoms (3).

43 The aetiology for this is still unclear but postprandial dysmotility of the inflamed gut could be

44 an aetiological factor. A delayed gastric empting or attenuated small bowel motility in the

45 fasting or postprandial state could be an important aetiological factor in the patient symptom

46 generation and eventual adoption of a diverse eating behaviour to improve symptom control.

47 Recent technological advances in image analysis have allowed us to use magnetic resonance

48 imaging (MRI) to measure CD activity through intestinal motility (4). A significant negative

49 correlation is observed between terminal ileal motility and histological (4), biochemical (5)

50 and radiological measures of disease activity (4). The exact aetiology of this

51 pathophysiological finding is still unclear.

52 Enteroendocrine cells (EC) are intraluminal nutrient sensors. They play a pivotal role in

53 orchestrating physiological functions in the gastrointestinal tract. Exposure of the ileum to

54 fatty acids decreases jejunal (6) and duodenal motility (7) through polypeptide YY (PYY)-

55 mediated mechanisms. Glucagon-like peptide-1 (GLP-1) has been shown to decrease small

56   bowel motility (8, 9). Moreover, cholecystokinin (CCK) has been shown to delay gastric

57   emptying by increasing pyloric motility (10, 11), with CCK modulation (12) affecting food

58   intake underlying its anorectic role in eating behaviour. CD patients with active small bowel

59   inflammation have significant up-regulation of EC cells with an increase in ileal expression of

60   GLP-1 (13), PYY (2-fold) (14) and (CCK) (3-fold) (15) levels. This increase in plasma

61   peptide levels is associated with the symptoms of nausea and anorexia, with both symptoms,

62   and tissue and plasma EC-peptide expression decreasing to normality in remission (14). We

63   hypothesise that an increase in CCK, GLP-1 or PYY-EC activity could potentially lead to

64   symptoms of nausea through delayed gastric emptying and attenuated post-prandial small

65   bowel motility. To our knowledge, this mechanistic link has never been described.

66   We have shown that a soup test meal challenge is effective at inducing a change in multiple

67   physiological quantified end-points and in monitoring markers of gastrointestinal motility in a

68   single MRI study acceptable to participants (16, 17). Our developed technique for the

69   assessment of small bowel motility showed excellent inter and intra observer agreement (17).

70   We now aim to investigate fasting and postprandial changes in gut motility, intestinal

71   physiology and related symptoms in a cohort of CD patients with active disease and healthy

72   volunteers (HV) to investigate the aetiology of patient symptoms.

73   **SUBJECTS AND METHODS**

74   **Study population**

75   This is a single-centre open-label clinical trial conducted between November 2015 and

76   February 2017 at the National Institute of Health Research Nottingham Biomedical Research

77   Centre (NIHR Nottingham BRC). CD patients (18-75 years) with active disease were

78   recruited from the Inflammatory Bowel Disease clinic at Nottingham University Hospitals

79   Trust. Active disease was defined as ulceration seen at ileocolonoscopy, intestinal

80   inflammation or deep ulceration seen on computer tomography or MR enterography, with the

81   disease activity quantified via the Magnetic Resonance Index of Activity (MaRIA) score of

82   >7 (18) or faecal calprotectin of >250µg/g (19, 20) or C-Reactive protein (CRP) >5mg/dl.

83   These measures of disease activity were to be quantified within four weeks of recruitment.

84   Stable doses of immunosuppressive and biological agents were permitted. No changes in

85   medication were allowed at inclusion until data collection of the outcome measures has been

86   completed. All participants had a good command of the English language and had the

87   capacity to give informed written consent.

88   Age-, body mass index- and gender-matched HV participants were recruited from an existing

89   participant database in the NIHR Nottingham BRC and from the local healthy populations of

90   Nottingham University Hospitals and the University of Nottingham. Potential participants

91   with a history of inflammatory bowel disease, smokers (21), a history of bowel resections or

92   any gastrointestinal surgery, history of pancreatic insufficiency, thyroid disease, diabetes,

93   protein-pump inhibitor usage or any medication that affects gastric emptying or small bowel

94   transit and any potential participants scoring very highly on the depression scale questionnaire

95   were excluded. Standard MRI exclusion criteria were applied.

96   This study was approved by the Research Ethics Committee (NRES approval 15/EM/0003) of

97   the Health Research Authority. This study is registered on clinical trials.gov with identifier

98   NCT03052465. All participants gave informed written consent.

99   **Outcome measures**

100   The primary outcome of this study was to compare fasting and postprandial small bowel

101   motility between CD and HV participants as measured through MRI. Secondary outcomes

102   were gall bladder contraction, gastric volumes, small bowel water content, plasma GLP-1,

103   PYY and CCK, symptoms scores and MRI disease activity scores as quantified by the MaRIA

104   score (18).

105   **Experimental protocol**

106   This study was open label. Participants were asked to fast from 2000 h the previous evening

107   and to avoid alcohol, caffeine, strenuous exercise and any medication that could affect gut

108   function for 18 h before the experiment. On the day of the scan, participants attended the 1.5T

109   Philips Achieva MRI scanner (Philips Healthcare, Best, the Netherlands) at the Sir Peter

110   Mansfield Imaging Centre (SPMIC) at the University Park Campus, University of

111   Nottingham at 0800.

112   Participants underwent a baseline fasting scan (defined at t = -20 min time point), together

113   with a fasting baseline blood sample. They were then asked to consume all their test meal

114   within a maximum time of 20 min. The participants then underwent a first immediate

115   postprandial scan (defined as t = 0 min).

116   This was followed with data collection (MRI, questionnaire data and 10 ml blood samples)

117   time points every 15 min for the first 60 min and then every 30 min up to 270 min. At each

118   time point, participants filled a 100mm Visual analogue scale (VAS) symptoms questionnaire

119   scoring their feeling of fullness, bloating, distension, abdominal pain/discomfort and nausea

120   (14). At the end of the 270 min participants were given a volume (750mls-1250mls) of oral

121   contrast agent (2.5% Mannitol,0.2 % locust bean gum) to drink (within 60 minutes) and a

122   further MRI scan (within 30 minutes) was undertaken to quantify disease activity. See

123   **supplementary figure 1**.

Test meal

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The test meal consisted of: cream of chicken soup (400g) (or mushroom for vegetarians)

125

(Heinz, Wigan, UK) (14, 22), The nutrient content of this meal/100g was: energy (kcal) 51,

126

protein 1.5 g (1.5%), carbohydrate 4.7 g (4.5%), fat 2.9 g (2.9%). For a complete list of

127

contents please see https://www.heinz.co.uk/products/soup/ranges/classics/cream-of-chicken.

128

Magnetic Resonance Imaging

129

Participants were scanned using a range of sequences (22). At each time point scans were

130

acquired to assess gastric volume (23), gall bladder volume (24), small bowel water content

131

(25) and small bowel motility (26).

132

Gastric emptying was assessed using a balanced gradient echo sequence to yield a good

133

contrast between the stomach contents and other abdominal organs (23).

134

The content of apparent freely mobile water in the small bowel was assessed as previously

135

described (25). This sequence yields high-intensity signals from areas with freely mobile fluid

136

and dark signals from poorly mobile or bound water and all other body tissues.

137

Small bowel motility was assessed using a single slice cine-MRI acquisition set at six

138

contiguous parallel coronal planes through the small bowel (22).

139

Gall bladder volume was measured pre- and post-prandial, at every acquisition time point

140

using the same images as for the gastric volumes as previously shown (24). These functional

141

MRI measures were acquired prior to the use of any anti-spasmodic agents administered

142

during serial image acquisition.

143

Plasma collection and peptides assays analysis

144

Fasting 10 ml blood sample was drawn and collected. After the test meal, data were acquired

145

146   every 15 min for the first 60 min and every 30 min thereafter to 270 min. Plasma peptides

147   (total GLP-1, total PYY) were analysed through enzyme-linked immunosorbent assay

148   (ELISA) techniques (Millipore, UK) as previously shown (14). The concentrations of serum

149   CCK were measured by radioimmunoassay (RIA) (Euro Diagnostic Products, Sweden) as

150   previously shown (27).

151   **MRI measures of disease activity**

152   Small bowel was scanned before and 10 minutes after 40mg of hyoscine butyl bromide was

153   injected intravenously to reduce small bowel motility. Participants were scanned within 30

154   minutes. Initially a true fast imaging with a steady sequence was acquired in the coronal

155   plane. Axial T1 sequences were acquired before and 70 s after intravenous administration of

156   0.2 ml/kg body weight of gadolinium chelate (gadodiamide 0.5 mmol/l) at a rate of 2 ml/s.

157   **Data analysis**

158   *Motility assessment*

159   All dynamic data was processed with Dual Registration of Abdominal Motion (Motilent,

160   London, UK). Registration results were further analysed using a customised graphical user

161   interface written in MATLAB (MathWorks, Natick, MA, USA) (17).

162   The technique used in this study quantified the motility of the bowel using the pixel signal

163   changes through the time series, within a defined region of interest (ROI) placed over all

164   visible small bowel loops (17) as previously described. This method utilizes changes in signal

165   intensities that occur when the small bowel contents move between segments in regions

166   showing bolus movement of contents as well as those showing more oscillatory motion, rather

167   than looking for continuous motion throughout the time series. Motility measures are

168   presented as arbitrary units (a.u.) and are calculated as the mean across the total small bowel

169   ROI.

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

171   These parameters were quantified as previously validated (25), using in house software

172   written in interactive data language (IDL) (Research Systems Inc. Boulder, Colorado, USA).

173   *Visual analogue scale (VAS)*

174   Symptoms regarding appetite, satiety and abdominal pain were scored at each time point

175   using a previously validated questionnaire (14).

176   **MARIA score**

177   MRE variables were evaluated by a Nottingham University Hospitals clinical gastrointestinal

178   (GI) MRI radiologist with > 10 years' experience (Dr. Khalid Latief) in each segment

179   including: bowel wall thickening, enhancement of the bowel wall after administration of

180   intravenous contrast with gadolinium (relative contrast enhancement), presence of ulcers,

181   mural oedema, regional enlarged lymph nodes (>10 mm), peri-enteric vascularization (comb

182   sign), peri-enteric fluid, fat stranding, and fibro-fatty proliferation. The MaRIA score in each

183   segment was calculated according to a formula, as previously defined (18).

184   **Sample size and Statistical analyses**

185   Previous literature showed 2-fold higher plasma PYY in CD (area under the curve, AUC 22990

186    $\pm$ 5585) vs HV (10700 $\pm$ 1886) and a higher plasma GLP-1 in CD (AUC 1027 $\pm$ 220) vs HV

187   (1347 $\pm$ 350) (28). Assuming α of 0.05, power of 80%, a maximum sample of 15 participants

188   in each group was needed to show a difference. Similar comparisons for CCK showed

189   significant differences between CD and HV in 10 participants/group with a strong correlation

190   (r=0.6) between mean CCK plasma concentration and gastric emptying half-life (15). Assuming

191 α of 0.05, power of 80% a total sample size of 19 participants was needed to show a difference.

192 To allow for missing data (~10%), we aimed to recruit a maximum of 20 participants in each

193 of the CD and HV cohorts.

194 The primary analysis was an across-group analysis with further sub-analyses undertaken if the

195 primary comparison is significant. Normality of the data was assessed using Shapiro–Wilk's

196 test. Parametric data was presented as mean ± standard error of the mean (SEM) or median ±

197 interquartile range if non-parametric. All statistical analyses were performed using GraphPad

198 Prism 7.01 (La Jolla, USA). A p-value less than 0.05 was considered statistically significant.

199 Analyses of variance (ANOVA) was used to assess the significance of differences between

200 and within each group with different time points. When the analysis of variance was

201 significant, post hoc test assessments of the individual time points were performed using the

202 Dunnett's (for parametric data) or Dunn's test (for nonparametric data) for within group

203 comparison and Sidak's test was used for between groups to account for multiple

204 comparisons. The Pearson correlation coefficient was used to measure the strength of

205 correlation between MaRIA scores and the different variables (measured as AUC).

206 **RESULTS**

207 **Participants**

208 Nineteen CD participants with active disease (age 36±3 years, BMI 26±1 kg/m^2) as well as 20

209 HV participants (age 31±3 years, BMI 24±1 kg/m^2) were recruited. One CD participant was

210 lost to follow-up and the data from two CD participants were excluded from the final

211 analyses: one because of a low MaRIA score of 3.39 and another because of an unanalysable

212 MRI data set. One other participant was excluded because of a high score on the hospital

213 anxiety and depression questionnaire. See supplementary figure 2, table 1 and supplementary

214 table 1. CD participants had a mean disease duration of 12.3±1.7 years, mean HBI of 6±1,

215 mean CRP of 18.2±3.4mg/dl and mean fecal calprotectin of 787.2±146.8μg/g. All patients

216 had ileal involvement. Four patients were being prescribed anti-tumor necrosis factor therapy,

217 one patient vedolizumab, three patients immunomodulators, with the rest on no medication at

218 the time of recruitment. Eight patients were surgically naïve.

219 All HV participants and CD participants completed the study and tolerated the experimental

220 procedures well without any adverse event.

221 **Small bowel motility**

222 The HV participants started with a significantly higher (106±13 a.u., $p < 0.05$) fasting motility

223 index compared to CD participants (70±8 a.u.) (**Figure 1**). No significant difference in the

224 postprandial time by group interaction was observed in small bowel motility between the CD

225 and HV groups.

226 **Gallbladder volumes**

227 Two CD patients had a cholecystectomy prior to recruitment. No difference in the fasting gall

228 bladder volumes were observed between the two groups. No significant difference in the

229 postprandial time by group interaction was observed in gall bladder volumes between the CD

230 and HV groups. The difference in gallbladder volumes between HV and CD participants from

231 fasting to 150 min are shown in **Figure 2**.

232 **Gastric volumes**

233 The baseline gastric volumes showed a small amount of fasting gastric secretions (**Figure 3**,

234 HV: 29±5 mL, CD: 25±4 mL) with no differences observed between the groups. No

235 significant difference in the postprandial time by group interaction was observed in gastric

236 volumes between the CD and HV groups. Gastric volume increased upon feeding (HV:

237 388±18 mL, CD: 324±26 mL). The average time to empty half of the stomach contents ($T_{1/2}$)

238 in HV and CD participants was 43±4 min, 63±7.5 min respectively, with no significant

239 difference observed between the groups.

240 **Small bowel water content**

241 The data in **Figure 4** shows a small amount of fasting small bowel water content in both

242 groups (HV: 44±6 mL, CD: 36±9 mL). No difference in the fasting small bowel water content

243 was visualised between groups. A significant increase ($p < 0.05$) was seen in CD participants

244 compared to HV (measured as area under the curve (AUC) CD: 19778±2119 mL/min, HV:

245 14197±1249 mL/min). A significant difference in the time by group interaction was observed

246 in small bowel water content between the CD and HV groups ($p = 0.0352$). An increase in

247 postprandial water volume in CD when compared to healthy volunteers was observed at

248 t=210 min ($p = 0.0388$), t=240 min ($p = 0.0168$) and 270 min ($p = 0.0048$).

249 **Total GLP-1**

250 **Figure 5** show higher ($p < 0.0001$) fasting GLP-1 levels in CD participants compared to HV

251 (CD: 50±8 µg/mL, HV: 13±3 µg/mL). The test meal did not induce a significant postprandial

252 response in either group. No significant difference in the time by group interaction was

253 observed between HV and CD groups. Significantly higher ($p < 0.0001$) postprandial GLP-1

254 levels were reported in CD participants compared to HV (AUC CD: 12293±1586 µg/mL, HV:

255 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

278 CD and HV groups. The meal induced a significantly (p<0.05) higher postprandial fullness

279 scores in CD participants compared to HV (AUC CD: 6795±1440 mm/min HV: 2907±703

280 mm/min). A significantly higher postprandial (p<0.0001) VAS scores of bloating, distention

281 and abdominal pain were noted in CD participants compared to HV (AUC CD: 5558±1293

282 mm/min, 5071±1253 mm/min, 3187±873 mm/min HV: 565±257 mm/min, 303±191 mm/min,

283 7±5 mm/min). The CD participants showed a significantly (p<0.01) higher sickness scores

284 compared to HV healthy volunteers (AUC, CD: 2024±927 mm/min HV: 75±75 mm/min).

285 **MaRIA scores**

286 The mean value of the MaRIA score was 20.3±1.9 (see the supplementary table for individual

287 scores). There was moderate non-significant correlation between fasting small bowel motility

288 and disease activity as measured through MaRIA scores (r=0.52 (95% confidence interval -

289 0.050, 0.8303, p=0.07). There was no significant correlation between MaRIA scores and

290 postprandial small bowel motility, small water content and GLP-1, PYY or CCK levels.

291 **DISCUSSION**

292 The primary aim of this study was to understand the altered intestinal physiology and aversive

293 patient symptoms observed in active CD in the fasted and the postprandial state. Any

294 alterations might help better understand symptom generation that may be leading to altered

295 eating behaviour and malnutrition.

296 Participants with CD had a lower fasting small bowel intestinal motility, with otherwise no

297 difference observed in the postprandial phase when compared to HV. We have shown

298 significantly higher fasting and postprandial levels for both GLP-1 and PYY with associated

299 aversive symptoms being reported to a significantly greater extent in CD than in HV.

300 Although small bowel hypomotility, elevated plasma GI peptide levels and aversive patient

301 symptoms could be interlinked in the fasting state; our data suggests that this hypothesis may

302 not hold true in the postprandial phase. Possibly, symptom generation in the postprandial

303 phase could be related to GI-peptide mediated alterations in the gut-brain axis rather than

304 altered intestinal physiology. A decrease in MR motility in CD, in the prepared bowel, was

305 previously described, with motility correlated to histological and biochemical measures of

306 disease activity and patient symptoms (29). Global small bowel hypomotility involving

307 normal-looking bowel has been described in CD, with motility variance negatively correlating

308 to key patient symptoms like diarrhoea, pain and clinical symptom scores (29, 30).

309 The fasting and postprandial plasma levels of GLP-1 were significantly higher in CD. This

310 was confirmed in previous CD studies (31). Similarly, plasma fasting and postprandial PYY

311 levels were significantly elevated in CD group as we previously described (14, 31). The

312 higher fasting GLP-1 and PYY observed in CD could be the cause of the fasting small bowel

313 hypomotility observed in CD. We observed no difference in the fasting and postprandial

314 plasma CCK levels, although the observed postprandial CCK response was only significant in

315 the CD rather than the healthy group despite the absolute levels not being significant in

316 between groups. The CD location in this recruited cohort was predominantly ileal or

317 ileocolonic rather than proximal in the duodenum where the CCK-secreting I cells are located.

318 Although earlier data in IBD murine models suggested that EC upregulation occurs

319 irrespective of the anatomical location of intestinal inflammation (32), this observation was

320 later refuted in CD (13). This might explain the lack of difference in CCK concentration

321 between both groups.

322 No significant difference was seen in the fasting and postprandial gall bladder volumes in CD

323 compared to healthy volunteers. The lack of difference in CCK levels between the two groups

324 explains the lack of difference in the gall bladder volumes as CCK is integral for the gall

325 bladder contraction in response to a fat stimulus. Additionally, previous data showed a similar

326 non-significant fasting gall bladder volumes in both healthy volunteers and patients with

327 small bowel CD (33, 34).

328 Similarly to postprandial small bowel motility, a delay in gastric emptying was expected in

329 the CD cohort with no difference in gastric emptying observed between CD and HV in this

330 study. Both PYY and GLP-1 may delay gastric emptying (35). In a previous study, delayed

331 gastric emptying was observed in an inflammatory bowel disease cohort with similar plasma

332 CCK and elevated plasma GLP-1 to healthy participants (31). In that study, gastric emptying

333 was measured through a breath test so the findings are not comparable to our study.

334 Postprandial small bowel water content typically showed two peaks. The first peak is the

335 gastric phase and represents emptying of the gastric contents in the proximal small bowel. The

336 second peak is called the intestinal phase (36) which represents the increase in small bowel

337 water content due pancreatico-biliary and enterocyte secretion. A bimodal postprandial peak

338 was also seen in the small bowel water content in CD group, with a significant difference in

339 time by group interaction in postprandial small bowel water content between CD and HV. The

340 noted increase could be due to the significant postprandial CCK response in the CD group

341 which leads to an increase in bile acid production and pancreatic secretion. Additionally, the

342 meal may have acted as an osmotic stimulus causing the increase in the small bowel water

343 content seen in the intestinal phase in CD cohort (37). Moreover, the observed increase in

344 small bowel water content in CD group might have induced an increase in small bowel

345 motility by stimulating smooth muscle contraction due to the increase in small bowel

346 distention thus nullifying any postprandial differences between groups. Additionally, the

347 increase in small bowel distention might have made small bowel motility more easily

348 quantifiable in this unprepared MRI visit hence decreasing any possible difference to healthy

349 participants. None of the CD participants had predominant stricture which may have caused a

350 delay in intestinal transit and might explain the increase in small bowel water content. Finally,

351 the increase in measureable small bowel water content in CD might merely represent a delay

352 in the distal small bowel emptying its contents in the ascending colon.

353 The CD group demonstrated significantly higher fasting and postprandial symptom VAS

354 scores, with more predominant symptoms of fullness, distention and abdominal pain scores

355 compared to HV. The additional small bowel distention from the increase in water content in

356 CD might explain the significant difference in the measured symptoms. However, the actual

357 difference in small bowel water content is small and accounts to only 40 mL which might not

358 been enough to account for such a drastic difference in symptoms. Another possible mechanism

359 for these exaggerated symptoms might be from the upregulated gut-brain axis which we have

360 not investigated in this study.

361 There were possible limitations to this work. The test meal used was small in volume and

362 nutrients and did not induce a significant change in postprandial GLP-1, PYY and CCK.

363 However, as we had previously shown (14), it was well tolerated by all the patients and acted

364 as a good stimulus to gastrointestinal physiological responses. Another limitation is the

365 relatively small participant population, but such detailed phenotyping made recruiting a larger

366 cohort for this exploratory study unfeasible. We as well assayed total PYY and GLP-1 rather

367 than the active peptides. In vivo, active GLP-1 is rapidly degraded to GLP-1 (9-36). Similarly,

368 PYY (1-36) is metabolised to the active PYY (3-36). Both of these activities are undertaken

369 through DPP-4 (38, 39).We have previously shown that DPP-4 expression is decreased in CD

370 (40). In this present study, we used a protease inhibitor to further minimise DPP4 activity and

371 peptide degradation. In previous work we also have shown no significant difference between

372 active and total GLP-1 in small bowel CD and HV (28). Similarly, total PYY, has been shown

373 to have a temporal pattern similar to that of PYY (3–36) after meals (41). For these reasons, we

374 assayed total rather than the active peptides.

375 In this work we successfully quantified fasting and postprandial small bowel motility and

376 possibly ascribed a putative role for EC peptides in the aetiology of disordered intestinal

377 motility and anorectic symptoms in CD. A better understanding of the role EC peptides in the

378 altered eating behaviour and malnutrition has pharmacological relevance. EC peptide

379 modulators (Exendin 9-39 (42) and dexloxiglumide (43)) are now available. Further work is

380 now needed to deconstruct the gut-brain axis and possibly open a new therapeutic pathway in

381 CD therapy, thus improving nutritional status, disease outcomes and quality of life.

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.

Supplementary Material

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

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Supplementary Material
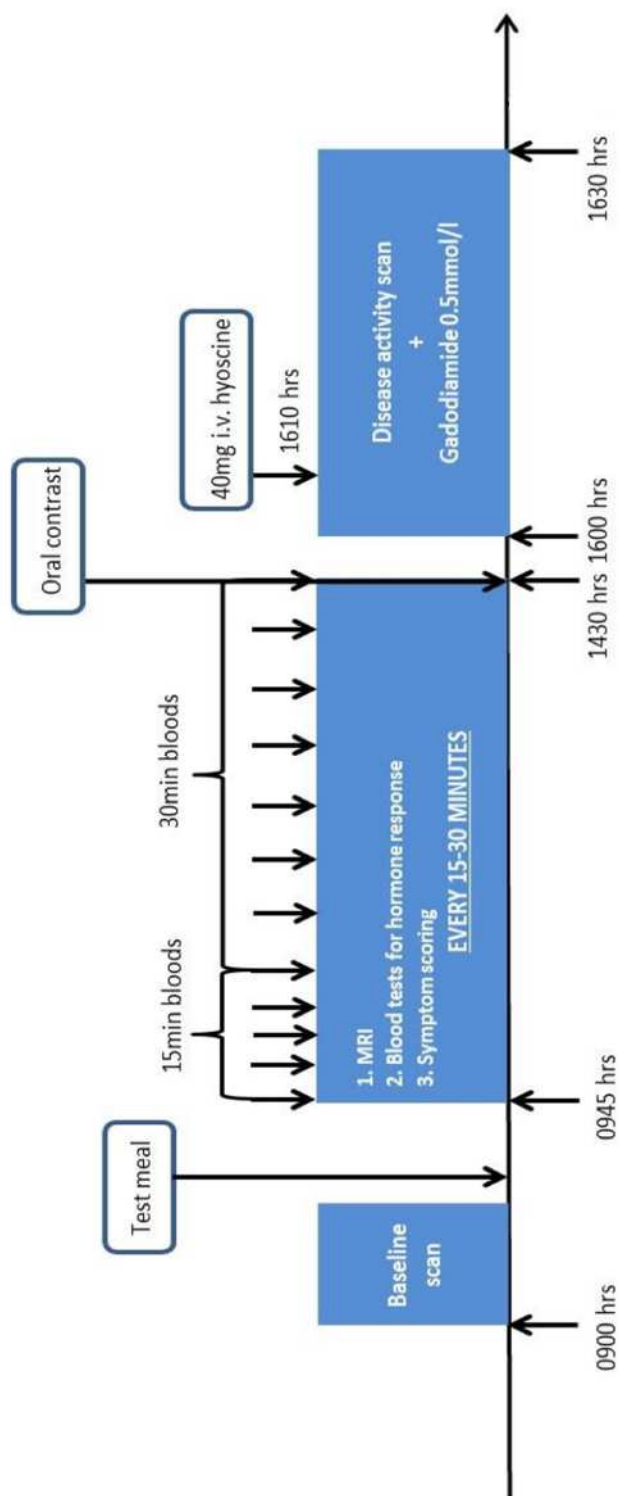
Supplementary figure 1: Diagram of MIC2's study protocol.

Supplementary Material

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Supplementary table 1

	Age years	Sex	Disease Duration years	Montreal Classification	Con Meds	Surgical History	MaRIA	HBI	CRP mg/dl	fC µg/g
1	24	F	12	A1L1B2	Adalimumab	Right hemicolectomy	16.10	2	41	933
2	29	F	4	A2L1B2	Adalimumab	No	11.37	2	7	177
3	39	F	14	A2L1B2	Adalimumab	No	16.72	13	<5	1435
4	57	M	8	A3L3B1	Methotrexate	No	27.48	1	15	393
5	20	F	3	A2L1B1	Nil	No	7.00	6	<5	275
6	52	F	25	A2L1B1p	Nil	Right hemicolectomy	27.07	7	<5	107
7	30	M	8	A2L1B3p	Nil	Right hemicolectomy	24.54	9	32	u/a
8	33	M	3	A2L1B1	Nil	Right hemicolecomy	13.65	2	<5	577
9	46	M	15	A2L1B3	Nil	No	17.50	2	10	u/a
10	33	M	4	A2L1B2	Adalimumab	No	33.75	2	<5	1023
11	51	F	37	A1L1B2	Methotrexate Vedolizumab	3 small bowel resections	17.00	5	9	535
12	30	F	15	A1L3B1	nil	Subtotal colectomy	17.06	6	<5	604
13	53	M	27	A2L1B2	Methotrexate	Right hemicolectomy, small bowel resection, ileocolic resection	23.68	2	<5	1800
14	23	F	3	A2L1B1	Azathioprine	No	24.49	6	13	1587
15	19	M	6	A1L1B1	Nil	No	27.48	18	<5	u/a

Table summarising the demographics and key clinical variables of the CD subjects; Con Meds=concomitant medication at the time of recruitment, MaRIA=Magnetic Resonance Index of Activity, HBI-Harvey-Bradshaw Index, CRP=C-Reactive Protein, fC=Faecal Calprotectin, u/a=unavailable.