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**Corticotrophin releasing factor increases ascending colon volume after a fructose test meal in healthy humans: a randomised control trial<sup>1-5</sup>**

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<sup>3</sup> Abbreviations used: CI, confidence interval; CRF, corticotrophin releasing factor; FODMAPS, fermentable oligo-di-mono-saccharides and polyhydric alcohols; IBS, irritable bowel syndrome; LUBT, lactose [<sup>13</sup>C] ureide breath test; MRI, magnetic

resonance imaging; OCTT, oro-caecal transit time; SBWC, small bowel water content; VAS, visual analogue scores

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This trial was registered at [ClinicalTrials.gov](https://clinicaltrials.gov) as NCT01763281

**RUNNING HEAD: EFFECTS OF CRF ON FRUCTOSE MALABSORPTION**

## 1 ABSTRACT

2 **Background:** Poorly absorbed, fermentable carbohydrates can provoke irritable  
3 bowel syndrome (IBS) symptoms by escaping absorption in the small bowel and  
4 being rapidly fermented in the colon in some susceptible subjects. IBS patients are  
5 often anxious and stressed and stress accelerates small bowel transit which may  
6 exacerbate malabsorption.

7 **Objective:** In this study we investigated the effect of intravenous injection of  
8 corticotrophin releasing factor (CRF) on fructose malabsorption and the resulting  
9 volume of water in the small bowel.

10 **Design:** We performed a randomised, placebo controlled, cross-over study of CRF  
11 versus saline injection in 11 male and 10 female healthy subjects, examining the  
12 effect on the malabsorption of a 40 g fructose test meal and its transit through the  
13 gut which was assessed by serial Magnetic Resonance imaging (MRI) and breath  
14 hydrogen measurement. Orocaecal transit was assessed using the lactose-ureide  
15 C<sup>13</sup> breath test and the adrenal response to CRF assessed by serial salivary cortisol  
16 measurements.

17 **Results:** (Mean  $\pm$  SD) CRF injection caused a significant rise in salivary cortisol  
18 which lasted 135 minutes. Small bowel water content (SBWC) rose from baseline,  
19 peaking at 45 minutes after fructose ingestion while breath hydrogen peaked later at  
20 75 minutes. The area under the curve (AUC) for SBWC from -15 - 135 minutes was  
21 significantly lower after CRF versus saline (mean difference [95% CI] 7433 [275,  
22 14591] mL.min,  $P = 0.04$ ). Ascending colon volume rose after CRF, significantly  
23 more for male volunteers than female ( $P = 0.025$ ).

24 **Conclusions:** CRF constricts the small bowel and increases fructose malabsorption  
25 as shown by increased ascending colon volumes. This mechanism may help to

- 26 explain the increased sensitivity of some stressed individuals to fructose
- 27 malabsorption.
- 28 This trial was registered at [ClinicalTrials.gov](https://clinicaltrials.gov) as NCT01763281.

## 29 INTRODUCTION

30 IBS is characterised by abdominal pain and erratic bowel habits, and food  
31 undoubtedly plays a role in causing symptoms. Poorly absorbed fermentable oligo-  
32 di-mono-saccharides and polyhydric alcohols (FODMAPs) have been shown in a  
33 randomised, placebo controlled trial to provoke symptoms of pain, bloating and  
34 flatulence in IBS patients (1, 2). A recent randomized control trial (RCT) showed a  
35 low FODMAPs diet reduced symptoms in IBS patients (3). However malabsorption  
36 per se is not enough to provoke symptoms as clearly shown in a study of lactose  
37 malabsorption in China (4). It affected 90% of the Chinese population, however only  
38 a minority experienced symptoms. Anxiety was a strong predictor of developing  
39 symptoms during a lactose challenge (4) suggesting an interaction between  
40 FODMAP malabsorption and psychological state.

41 One of the most consistent features in IBS patients is the association with anxiety,  
42 depression and somatisation (5). Patients often report that the onset of the condition  
43 was associated with stress (6). However the link of symptoms to stressful events is  
44 not straightforward and when stress and bowel symptoms are recorded over  
45 prolonged periods the correlation of symptoms and stress is only modest ( $r = 0.27$ )  
46 (7). Others have shown a chronic activation of the hypothalamic- pituitary adrenal  
47 axis in IBS-D patients who have elevated basal and stimulated cortisol levels which  
48 correlate with anxiety symptoms (8). Previous studies have shown that psychological  
49 stress (9) and clinical anxiety are both associated with accelerated small bowel  
50 transit (10). We have previously investigated IBS-D patients using MRI and shown  
51 that they have constricted small intestines and accelerated mouth to caecum transit  
52 time which correlated with anxiety (11). We also recently demonstrated that IBS-D  
53 patients show a failure of the ascending colon to relax postprandially (12) which

54 could lead to increased wall tension and hence increased symptoms when the colon  
55 is distended by the arrival of FODMAPs such as fructose or lactose. Previous  
56 animal studies showed an acceleration of whole gut transit with stress and suggest  
57 that CRF is a key element, since CRF antagonist can block this acceleration (13,  
58 14). Recently we have shown that CRF injections constrict the small bowel in  
59 healthy volunteers to levels seen in IBS-D patients, suggesting that a similar  
60 mechanism might be operating in humans (15). Our previous MRI study showed that  
61 40 g of fructose distended the small bowel, increasing its volume 4 fold. In some  
62 individuals, a portion escaped absorption, entered the colon, leading to a rise in  
63 breath hydrogen (16).

64 We hypothesised that accelerating small bowel transit using CRF intravenous  
65 injections would exacerbate fructose malabsorption as assessed by breath hydrogen  
66 and colonic volumes after a fructose challenge. We therefore carried out a RCT of  
67 CRF versus a saline placebo in healthy volunteers who ingested a 40 g fructose  
68 meal.

## 69 **SUBJECTS AND METHODS**

### 70 **Study participants**

71 A total of 21 healthy volunteers (11 male and 10 female) were recruited. Of these, 1  
72 male withdrew consent, and 20 (age  $23 \pm 3$  years, BMI  $24.4 \pm 3.4$  kgm<sup>-2</sup>) were  
73 randomised to take part. Participants were considered eligible if they were non-  
74 smokers, aged between 18 and 60 years old, BMI between 18 and 30 kg m<sup>-2</sup>, and  
75 without any history of serious acute or chronic illness, particularly gastrointestinal  
76 disease. Pregnant or breast feeding females were excluded, and pregnancy tests  
77 were available to verify this. Any participants on antibiotics, probiotics, or medication  
78 that interferes with gastrointestinal motility were excluded. Subjects were not allowed  
79 to have taken part in a clinical study within the 3 months prior to the present study.  
80 All volunteers completed the Patient Health Questionnaire 15 (PHQ 15) and the  
81 Hospital Anxiety and Depression Scale (HADS), and were screened for MRI  
82 contraindications with a safety screening questionnaire prior to randomisation. The  
83 participants were recruited and enrolled by KAM, SR and CL. CL also created the  
84 computer-generated randomisation code for the participants, allocated and  
85 administered their treatments and was the only person involved who was not blinded  
86 on the study day. All participant data were given a special identifier and therefore,  
87 during data analysis, KAM, SR and CL remained blinded to allocated treatment to  
88 avoid any possible bias.

89

### 90 **Study design**

91 The study was a single-centre, randomised, two-way, double-blind, crossover study,  
92 consisting of a screening visit and two MRI scan days which were approximately 7  
93 days apart. Data were collected at the 1.5T MRI scanning unit of the Sir Peter

94 Mansfield Imaging Centre, located at the University Park campus of the University of  
95 Nottingham. The participants were asked to fast from 20:00 h on the day before  
96 scanning and refrain from alcohol, caffeine and strenuous activity for 18 hours prior.  
97 They were also asked to refrain from eating foods such as bran, wheat, rye, fruit and  
98 vegetables high in FODMAPs (fermentable, oligo-,di- mono-saccharides and  
99 polyhydric alcohols ) and excessively spicy foods on the day before the study, as  
100 these could all alter intestinal volumes. On arrival, they were asked to rinse their  
101 mouth with mouthwash (Corsodyl Daily, GlaxoSmithKline Consumer Healthcare,  
102 Brentford, UK) to reduce the number of oral bacteria which could ferment oral  
103 carbohydrate to give a misleading early breath hydrogen rise. A sustained rise in  
104 breath hydrogen of more than 20 ppm was considered to be a sign of malabsorption.  
105 Volunteers underwent a baseline scan before having an intravenous cannula  
106 positioned (0.8 mm cannula, Biovalve, E.C Laboratories, VYGON, France). A local  
107 anaesthetic cream (EMLA, AstraZeneca, Luton) was applied to the arm to minimise  
108 discomfort during the process. Following cannulation, the volunteers had a second  
109 scan before receiving an intravenous dose of either a saline solution (0.9% NaCl) or  
110 100 µg human Corticotrophin releasing factor (CRF [Corticotrophin Trifluoroacetate,  
111 FERRING GmbH, Kiel]). Due to the short half-life of CRF, the bolus injection lasted  
112 for only 1 second and was followed by a 5mL saline flush. The short bolus injection  
113 time followed by the saline flush was to allow the peptide to reach the peripheral  
114 system quickly. The dosage was prepared before the participants entered the clinical  
115 area and they only saw a colourless liquid in the syringe on both arms of the trial. As  
116 a result, both arms of the study were sufficiently similar to prevent participants and  
117 researchers ever knowing which treatment was received. Volunteers were then given  
118 a test drink consisting of 500 mL of water containing 40 g of fructose (Holland &

119 Barrett, Nuneaton, UK) with 5ml of pure lemon juice (PLJ) (Healthy Food Brands,  
120 West Sussex, UK) added to improve palatability. This dose of 40 g was selected as  
121 our previous study (16) showed that with a 40g dose, good distension of the small  
122 bowel is obtained and easily seen on the MR images. They received serial scans  
123 after this at time 15, 45, 75, 105, 135, 195, 255 and 315 minutes postprandially, with  
124 samples of saliva for cortisol measurement, end expiratory breath for hydrogen (H<sub>2</sub>)  
125 measurement (Gastro+ Gastrolyzer, Bedford Scientific, Kent, UK) and symptom  
126 questionnaires, all being collected after each scan. Pulse and blood pressure  
127 measurements were taken after each scan and a State-Trait Anxiety Inventory  
128 (STAI) questionnaire was administered on a single occasion halfway through the  
129 scan day.

130 The primary outcome was the effect of CRF on the area under curve volume versus  
131 time curve for water in the small bowel (in mL.min). Secondary outcomes were  
132 gastric volumes (in mL), breath hydrogen (in ppm), ascending colon volumes (in mL),  
133 ascending colon gas volumes (in mL), oro-caecal transit time (min) and symptom  
134 VAS questionnaires on the study days (in mm). Ascending colon volumes were  
135 reported as the % change from baseline. While volumes were expected to increase  
136 on both arms of the study in response to fructose (17), assessing the % change from  
137 immediately before intravenous injection (t = -45 min) until the point where CRF no  
138 longer had an effect was done to determine if the increase was significantly greater  
139 as a result of acute experimental stress.

140 The study was carried out following Good Clinical Practice (GCP) protocols and the  
141 Declaration of Helsinki with approval by the University of Nottingham Medical School  
142 Ethics Committee. Volunteers gave written informed consent prior to their

143 participation and the trial ended after the final volunteer had completed both arms.

144 The study was registered on clinicaltrials.gov identifier NCT01763281.

### 145 **MRI protocol**

146 Images were collected using a whole-body, research-dedicated, 1.5T MR scanner  
147 (Achieva, Philips Medical System, Best, The Netherlands). Each imaging period  
148 lasted for 10 minutes and volunteers were positioned supine with a 16-element coil  
149 wrapped around the abdomen. The volunteers were allowed to sit upright away from  
150 the scanner between scans. The volume of freely mobile water in the small bowel  
151 (SBWC) was measured as described previously (18), using a coronal single-shot  
152 turbo spin-echo sequence. This acquired 24 slices in a single 24 second expiration  
153 breath hold (TR/TE = 8000/320 ms, 512x512 reconstructed matrix, voxel size  
154 0.78x0.78x7 mm<sup>3</sup>). A coronal dual-echo gradient echo sequence was used to  
155 determine the volume of the ascending colon (12) as well as the volume of gas. This  
156 sequence allowed simultaneous 24 slice collection of both in-phase and out-of-phase  
157 images in a single 15 second expiration breath hold (TR/TE1/TE2 = 157/2.30/4.60  
158 ms, 256x256 reconstructed matrix, voxel size 1.76x1.76x7 mm<sup>3</sup>). Gastric volumes  
159 were measured with a balanced gradient echo sequence (TR/TE = 2.98 / 1.49 ms,  
160 flip angle 80°, 256 x 256 reconstructed matrix, reconstructed in-plane resolution 1.56  
161 x 1.56 x 5 mm<sup>3</sup>, SENSE 2.0) (19), acquiring 50 transverse slices in a 16.5 second  
162 breath hold.

### 163 **Lactose Ureide Breath Test (LUBT)**

164 A previously validated LUBT protocol was used (20). Participants ingested 1 g of  
165 unlabelled lactose ureide (Euriso-top®, Saint-Aubin Cedix, France) 3 times a day  
166 with meals on the day before each study day, to stimulate glucose ureide hydrolase  
167 enzyme activity in the colonic bacteria. On the study day, participants provided a

168 baseline breath sample before receiving their test drink (details above). The drink  
169 was mixed with 500 mg of labelled  $^{13}\text{C}$  lactose ureide (Euriso-top®, Saint-Aubin  
170 Cedix, France). Breath samples were taken every 10 minutes for an hour, then every  
171 15 minutes for an additional 4 hours. Analysis of breath samples was carried out on  
172 an IRIS®-Lab analyser (Wagner Analysen Technik, Bremen, and Germany) and the  
173 result was expressed as delta over baseline: the difference between the  $^{13}\text{CO}_2/^{12}\text{CO}_2$   
174 ratio in the post meal breath sample and the corresponding ratio in the baseline  
175 sample. The OCTT was manually determined by two experienced operators looking  
176 at plots of delta over baseline as a function of time and was taken as the time at  
177 which there was a rise of more than 2 ppm in  $^{13}\text{C}$  above the baseline after  
178 consumption of the drink.

#### 179 **Data analysis, statistics and sample size**

180 SBWC was measured using a previously described and validated method (18).  
181 Ascending colon volumes were measured using Analyze® 9.0 (Biomedical Imaging  
182 Resource, Mayo Clinic, Rochester, MN, USA) (12) and the volume of gas in the  
183 ascending colon was assessed from Analyze-generated object maps using a  
184 programme written in-house (IDL®, Research Systems Inc, Boulder, Colorado,  
185 USA). This programme first summed the in phase and out of phase coronal images  
186 of the colon. Colonic gas was operator-defined as a region of interest where the sum  
187 of the two images appeared completely black. These regions were then  
188 automatically summed along the entire ascending colon, giving a total gas volume.  
189 Gastric volumes; consisting of liquid and gas in the stomach, were defined using an  
190 intensity based region growing algorithm developed in IDL® (Research Systems Inc,  
191 Boulder, Colorado, USA) (19). All symptom scores were assessed using a 100 mm  
192 visual analogue score (VAS), and the STAI questionnaire was scored as described

193 by the Spielberger State-Trait Anxiety Inventory (21). Salivary cortisol was  
194 determined using enzyme-linked immunosorbent assay (Salimetrics, Suffolk UK).  
195 Statistical analyses were carried out using Prism 6 (GraphPad Software Inc., San  
196 Diego, CA, USA). Data were **first tested** for normality using the Shapiro-Wilk's test of  
197 normality, after which paired, two-tailed t-tests were used to determine the  
198 significance of the differences for normally distributed data and Wilcoxon signed rank  
199 tests were used to test the significance of differences of non-normally distributed  
200 data. The varied responses of males and females to the treatments were  
201 investigated and two way analysis of variance (ANOVA) was used to determine the  
202 effect of treatment and gender on the outcomes. Differences were considered  
203 significant at  $P < 0.05$ .

204 Previous work in healthy volunteers using 40 g fructose in 500 mL (16), showed a  
205 postprandial SBWC volume at 75 minutes of  $413 \pm 123$  mL (mean  $\pm$  SD). This  
206 indicates that using 15 participants we should be able to detect a 27% change in  
207 SBWC with 90% power with  $\alpha < 0.05$ . Another study previously using **CRF** showed a  
208 reduction of SBWC by 36% in 15 healthy subjects when CRF was given  
209 intravenously (15). To allow for dropouts, 20 participants were enrolled in the study.

210

## 211 **RESULTS**

212 Study procedures were well tolerated by the volunteers. All 20 successfully  
213 completed the study (see Consort diagram in **Supplemental Figure 1**) and were  
214 included in the analyses. There were no adverse reactions to cannulation or injection  
215 and only a few reported feeling flushed after injection. Pulse and blood pressure  
216 measurements did not change. There were differences noted between the response  
217 to an injection followed by a fructose meal for males and females on both arms of the

218 **study**, and as a result the data for males and females are presented separately.

219 Normally distributed data are presented in tables as mean  $\pm$  SD, while non-normally  
220 distributed data are shown as median [IQR]. Data in the figures are presented as the  
221 average at each time point across the study day and the error bars are the standard  
222 error of the mean (SEM).

223

### 224 **Stress response**

225 The salivary cortisol concentrations throughout the study day are shown in **Figure 1**.

226 Cortisol levels were initially higher on both arms of the study, but fell at the point of  
227 cannulation. After CRF injection, salivary cortisol concentrations rose steadily and  
228 peaked after 30 minutes at  $0.49 \pm 0.27 \mu\text{g dL}^{-1}$ . In comparison, cortisol levels after  
229 injection with saline rose to a maximum of  $0.18 \pm 0.23 \mu\text{g dL}^{-1}$ . The cortisol response  
230 lasted until 135 minutes after drinking fructose, and the time period from 15 minutes  
231 before to 135 minutes after ( $t = -15 - 135$  minutes) was selected as being  
232 physiologically relevant for comparisons. The  $t = -15 - 135$  min AUC (**Table 1**) for  
233 salivary cortisol on the CRF arm of the study was significantly greater than saline  
234 (**mean difference [95% CI] 22.4 [12.3, 32.5]  $\mu\text{g dL}^{-1} \cdot \text{min}$ ,  $P = 0.0002$ ). After CRF**  
235 injection, female participants had a numerically higher salivary cortisol concentration  
236 than males (Table 1) but this difference was not statistically significant (mean  
237 difference  **$15.3 \pm 8.5 \mu\text{g dL}^{-1} \cdot \text{min}$ ,  $P = 0.09$ ; *Student's t test*).**

238

### 239 **Breath H<sub>2</sub>**

240 The breath H<sub>2</sub> concentration of the 20 volunteers across the study day for both  
241 treatment arms is shown in **Figure 2**. Consumption of fructose led to an immediate  
242 increase in H<sub>2</sub> concentration, which peaked at 75 minutes postprandial ( $54 \pm 20$  ppm

243 CRF arm,  $44 \pm 12$  ppm saline arm) and then returned to baseline levels. This trend  
244 was seen for both arms of the study, and there was no significant difference between  
245 the CRF and saline arm. Table 1 shows the differences between the breath H<sub>2</sub>  
246 responses for males and females. There were no significant differences between the  
247 CRF and saline arms for either group, although CRF injection in males produced a  
248 numerically larger volume of breath H<sub>2</sub> than saline median difference [95% CI] 2400  
249 [-3675, 7193] ppm.min,  $P = 0.38$ . Breath H<sub>2</sub> was significantly larger for males after  
250 both CRF and saline injection (Table 1); 6 males showed a rise in breath H<sub>2</sub> of more  
251 than 20 ppm after CRF compared to 2 females, while 7 males showed an increase  
252 after saline injection, compared to only 3 females. The gender effect on the  
253 measured breath H<sub>2</sub> was significant ( $P = 0.035$ ; two way ANOVA), there was also a  
254 significant time effect ( $P = 0.0001$ ; two way ANOVA), with a positive time x gender  
255 interaction ( $P = 0.0001$ ; two way ANOVA).

256

### 257 Gastric emptying

258 The volume of liquid and air in the stomach was easily visualised and quantified. The  
259 AUC for gastric volume from  $t = -15$  min –  $t = 135$  min is shown in Table 1 for both  
260 arms of the study. The maximum gastric volume was no different after CRF ( $484 \pm$   
261  $67$  mL) than after saline injection ( $469 \pm 88$  mL), when all subjects were considered  
262 together ( $P = 0.40$ ). There were however differences in gastric volumes between the  
263 male (**Figure 3A**) and female participants (**Figure 3B**) across the study day. CRF  
264 significantly delayed gastric emptying in female participants relative to the saline  
265 (mean difference  $\pm$  SD in AUC ( $t = -15 - t = 135$  min)  $5067 \pm 6062$  mL.min,  $P =$   
266  $0.027$ , *Student's t test*), but this delay was not observed for the male participants,  
267 where the gastric volume was greater for saline than that for CRF (mean difference  $\pm$

268 **SD** 1959 ± 8463 mL.min,  $P = 0.48$ , *Student's t test*). The difference between male  
269 and female gastric emptying **was not significant** on the CRF arm of the study ( $P =$   
270 0.085, two way ANOVA), but there was a significant time effect ( $P = 0.0001$ , two way  
271 ANOVA) and time x gender interaction ( $P = 0.0001$ , two way ANOVA). Differences  
272 between males and females were also not significant on the **saline** arm of the study  
273 ( $P = 0.72$ , two way ANOVA), and while there was a significant time effect ( $P =$   
274 0.0001, two way ANOVA) there was no interaction.

275

### 276 **Small bowel water content (SBWC)**

277 After the fructose drink, the volume of free water in the small bowel increased from  
278 (mean ± SD) 74 ± 50 mL at  $t = -15$  minutes and peaked at 416 ± 133 mL after CRF  
279 and 75 ± 43 mL peaking at 489 ± 144 mL after saline. The time to peak was 45  
280 minutes postprandial, and volumes returned to baseline by the end of the study day  
281 (**Figure 4**). There was a reduction in SBWC in the CRF treatment arm relative to the  
282 saline arm and this could be seen on the MR images. **Figure 5** shows a  
283 representative example of the differences seen 45 minutes postprandial. **Over the**  
284 **entire study day there was no significant difference, mean difference ± SD 5291 ±**  
285 **18987mL.min,  $n = 20$   $P = 0.1$ , *Student's t test***). The CRF injection did however  
286 decrease small bowel water immediately after the fructose drink but this effect only  
287 lasted for 135 minutes postprandially, paralleling the cortisol response. The AUC for  
288 these time points (Table 1) was significantly lower after CRF than observed after  
289 saline, **mean difference [95% CI] 7433 [275, 14591] mL.min ( $n = 20$ ,  $P = 0.04$ , paired**  
290 ***Student's t test*)** . There were significant differences between male and female  
291 **SBWC on both arms of the study (Table 1). The effect of time was significant on both**

292 arms of the study as obtained with two way ANOVA, with a positive time x gender  
293 interaction on the CRF arm (Table 1).

294

### 295 **Ascending colon volume**

296 The percentage change in the volume of the ascending colon from immediately  
297 before the injection (t – 45 min) was assessed for both the CRF and saline arms of  
298 the study. **Figure 6** shows the trend across the study day after both CRF and saline  
299 injection. The volume increased from baseline (t – 45) of  $210 \pm 77$  to  $270 \pm 109$  mL  
300 (29%) 45 minutes after the fructose drink for the CRF arm of the study, significantly  
301 greater than the increase from baseline of  $226 \pm 74$  to  $252 \pm 83$  mL (12%) observed  
302 after the saline injection, (data not shown,  $P = 0.048$ ; *Student's t test*). Male  
303 volunteers had a significantly larger colon on their CRF arm of the study, but there  
304 were no significant treatment differences recorded for female volunteers (Table 1).  
305 Male volunteers also had significantly larger colons than females after CRF (mean  
306 difference [95% CI] 7729 [1096, 14362] mL.min,  $P = 0.025$ ; *Student's t test*) but not  
307 saline (mean difference [95% CI] 2991 [-492.6, 6474] mL.min,  $P = 0.09$ ; *Student's t*  
308 *test*). Ascending colon gas volumes were also determined but the change on the  
309 CRF arm of the study (507 [232, 1449] mL.min v 350 [198, 934] mL.min for saline),  
310 was not significantly different from the change observed with saline ( $P = 0.45$ ).

311

### 312 **Orocaecal transit time (OCTT)**

313 OCTT was manually assessed by 2 operators, and defined as the first sustained rise  
314 of 2ppm in  $^{13}\text{C}$  concentration after the drink. Data were inconsistent and did not show  
315 the smooth rise that is characteristic of LUBT curves, data from only 18 volunteers  
316 could be reliably analysed. Transit time with saline (mean  $\pm$  SD)  $49 \pm 20$  min was

317 significantly shorter than after injection with CRF (mean  $\pm$  SD)  $59 \pm 23$  min, mean  
318 difference [95% CI]  $10.6 [2.1,19.0]$  min,  $P = 0.02$ . The median oro-caecal transit time  
319 for male volunteers was numerically shorter than for females but these differences  
320 were not statistically significant.

### 321 Questionnaires

322 One volunteer did not return a STAI questionnaire on the CRF arm of the study, and  
323 STAI analyses are therefore performed on data from 19 volunteers. The average  
324 State anxiety score after CRF injection was  $32.7 \pm 7$ , significantly greater than the  
325 average score after saline injection,  $28.8 \pm 7$  ( $P = 0.047$ ), while there were no  
326 significant differences between the two treatments for the Trait anxiety score. Using  
327 Spearman rank correlation coefficient, there was a significant correlation between  
328 cortisol concentration and State-anxiety scores ( $r = 0.53$ ,  $P = 0.02$ ) for the CRF arm  
329 but not the saline. There were no correlations between cortisol concentration and T-  
330 anxiety scores for either treatment. STAI scores also did not correlate with SBWC,  
331 ascending colon volume or breath  $H_2$ . There were no significant differences between  
332 the two treatment arms for measures of bloating, distension, fullness or nausea  
333 (**Table 2**). All volunteers were within the normal range of the HADS (anxiety 3 (1.3 –  
334 5.8), depression 0.5 (0 – 2.5) and PHQ-15 (2 (0.25 – 3) questionnaires.

335

### 336 DISCUSSION

337 This study sought to simulate experimentally the psychological and physiological  
338 changes that are seen in anxious patients with IBS whom we have previously shown  
339 to have constricted small bowels, accelerated small bowel transit and incompliant  
340 ascending colons (11, 12). We hypothesised that accelerated transit, by reducing the  
341 time for absorption, would exacerbate fructose malabsorption and increase colonic

342 volumes. Our study confirmed earlier studies using the same MRI technique which  
343 showed that CRF reduced small bowel water content (15). It should be noted  
344 however that since we used a very different meal, the shape of the small bowel  
345 water content looked rather different. The previous study (15) used a mixed solid/  
346 liquid phase meal in which the liquid phase was orange juice which contained  
347 glucose in approximately equal amounts (3 g) as fructose together with sucrose  
348 which are all rapidly absorbed. This leads to an initial rapid fall in SBWC which then  
349 rises as pancreatic secretions are stimulated by the later emptying, solid phase. Our  
350 current study used a liquid only test meal containing a much large dose (40g) of  
351 fructose which, in the absence of glucose, is poorly absorbed. This increased small  
352 bowel water content and caused increased colonic gas and fluid with a concomitant  
353 rise in breath hydrogen as we have previously shown (16). In keeping with other  
354 studies we showed that intravenously administered CRF inhibits gastric emptying in  
355 females and delays small intestinal transit in both genders (22). The new finding  
356 was that CRF increased ascending colonic volumes after fructose ingestion,  
357 suggesting that acute stress could worsen symptoms due to ingestion of FODMAPs.  
358 The CRF effect on the hypothalamic-adrenal axis as shown by salivary cortisol was  
359 only significant for 135 minutes, in keeping with its known short half-life (23). This is  
360 also in keeping with binding of CRF with CRF-binding protein, which increases after  
361 injection and neutralises the biological activity of CRF. Levels of bound and free CRF  
362 are undetectable after 2 hours (24). Similarly its effect on the stomach, small bowel  
363 and colon were only apparent for the first 135 minutes suggesting the end organ  
364 effects are short lived after a single injection. The CRF effect on males and females  
365 differed, with females showing a higher though not significant salivary cortisol  
366 concentration. This is in keeping with previous studies, where cortisol levels were

367 found to be, depending on the stressor, either comparable between men and women  
368 or higher in women (25).

369 Gastric emptying has been shown to be inhibited by acute stress in dogs (26), rats  
370 (27) and humans (28), also by the action of intravenous or intraperitoneal  
371 administration of CRF (22). The results for the complete cohort of volunteers showed  
372 a greater AUC after CRF, but this was not significantly different after saline. The  
373 effect on gastric emptying of females was more pronounced however, and they  
374 showed a significant delay in emptying on the CRF arm relative to the saline arm. A  
375 similar effect has been recorded with male and female mice; the females showed  
376 significantly slower upper gastrointestinal transit relative to males after an acute  
377 stressor (29). It should be noted that all the gender comparisons were unplanned  
378 post hoc analyses. A larger sample size would have been necessary if any of these  
379 differences had been the primary endpoint.

380 The results showed a significantly increased postprandial rise in ascending colon  
381 volume as the fructose entered the colon on the CRF arm of the study, as well as an  
382 increased (though not significantly so) ascending colon gas volume, suggesting CRF  
383 possibly increased fructose malabsorption. Post prandial breath hydrogen was not  
384 significantly increased by CRF but this depends on the colonic bacteria and as our  
385 study shows does not reliably reflect malabsorption. Although the increase in  
386 ascending colon gas was not significant this may have been due to our study being  
387 underpowered for this more variable endpoint. It has previously been hypothesized  
388 that FODMAPs trigger gastrointestinal symptoms by distension of the colonic lumen,  
389 mainly through the production of gas (2). Our results show that the colon volume was  
390 increased by fructose ingestion, an effect further increased by CRF from 0-135  
391 minutes post injection. Male volunteers had a significantly larger increase in their

392 ascending colon volume than females on the CRF arm of the study, but this gender  
393 difference was not seen on **the saline arm**. This observation did not correlate with  
394 symptoms for bloating, distension, fullness or nausea and was also somewhat  
395 surprising considering that abdominal bloating is reported more frequently by  
396 females, although this may be a result of them describing the symptom in a different  
397 way (30).

398 Most healthy volunteers seem able to tolerate changes in gas loads, unlike patients  
399 with functional disorders such as IBS who show visceral hypersensitivity (5). The  
400 colonic responses to stress are also more pronounced in IBS patients (31, 32); the  
401 reasons for this are still unknown.

402 Previous studies have shown that CRF increases small bowel motor activity in IBS-D  
403 patients more than controls but whether or not this accelerated transit was not  
404 assessed (33, 34), while other studies have indicated a delay in small bowel transit  
405 due to CRF injection (35). The present study using the C13-ureide breath test  
406 showed a delay in oro-caecal transit. Stengel and Taché (36) have highlighted that  
407 injection of CRF inhibits duodenal transit, although they reported that results on  
408 stress-induced changes of small intestinal motility are conflicting. It may well be that  
409 the constriction of the small bowel which reduces SBWC does not always lead to  
410 faster transit if the CRF induced motor pattern is non-propulsive. It is worth noting  
411 that this recently validated OCTT test (37) was standardised for use with a solid  
412 meal, and may not be optimal for assessment of transit with an osmotically active  
413 liquid meal such as we used.

414 All participants in the study received a standard dose of CRF; it is likely that a  
415 dosage based on individual weight would have been more appropriate. Another  
416 limitation of the study was that no gender-based hormonal fluctuations were

417 considered when assessing the response to CRF. It has been recorded that women  
418 are more vulnerable to stress-related illnesses (38), and the degree of  
419 gastrointestinal motor responsiveness to acute stress in experimental animals at  
420 least, varies depending on gender, oestrus cycles and prior exposure to stress (39).  
421 The reasons why the male and female gastrointestinal responses to acute stress are  
422 so varied require further exploration.

423 MRI has allowed the non-invasive assessment of the small bowel and colon after  
424 intravenous CRF injection followed by a fructose meal, and has demonstrated for the  
425 first time that CRF combined with a FODMAP challenge increases ascending colon  
426 volume, possibly due to increased fructose malabsorption. This may explain why  
427 food intolerances can be inconsistent from day to day, perhaps depending on the  
428 psychological state of the subject. Future studies should focus on the effects of acute  
429 stress stimuli in sufferers of functional gastrointestinal disorders such as IBS in  
430 whom this effect may be even more pronounced.

431

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438 data, KAM had primary responsibility for writing the paper; RCS had primary  
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## TABLES

**Table 1: Comparison of study outcomes after intravenous dosing of CRF or saline in healthy volunteers**

	CRF <sup>1,2</sup>	Saline	P-value <sup>3</sup>
Salivary cortisol <sup>4</sup> ( $\mu\text{g dL}^{-1} \cdot \text{min}$ ) (N = 20)	43.6 $\pm$ 20.1	21.2 $\pm$ 11.3	0.0002
Females (N = 10)	51.3 $\pm$ 22.6	22.0 $\pm$ 11.2	0.004
Males (N = 10)	36.0 $\pm$ 14.6	20.4 $\pm$ 11.9	0.005
Breath H <sub>2</sub> <sup>5</sup> (ppm.min) (N = 20)	1500 (743 – 7868)	3420 (1043 – 6739)	0.99
Females (N = 10)	818 (679 – 1635)	1208 (758 – 3735)	0.2
Males (N = 10)	9210 $\pm$ 9750	6311 $\pm$ 4031	0.38
Comparison of males versus females P	0.035	0.077	
Gastric volume <sup>6</sup> (mL.min) (N = 20)	31776 $\pm$ 9560	30222 $\pm$ 7571	0.4
Females (N = 10)	36601 $\pm$ 7388	31534 $\pm$ 6645	0.03
Males (N = 10)	26952 $\pm$ 9307	28910 $\pm$ 8547	0.48
Comparison of males versus females P	0.085	0.72	
SBWC (mL.min) (N = 20) <sup>7</sup>	48515 $\pm$ 15719	55948 $\pm$ 19169	0.04
Females (N = 10)	52902 $\pm$ 19704	65501 $\pm$ 20890	0.04
Males (N = 10)	44129 $\pm$ 9521	46396 $\pm$ 11687	0.57
Comparison of males versus females P	0.067	0.009	
AUC of % change from baseline against time in ACV <sup>8,9</sup> (N = 20) expressed as %.min	1983 (-2246 – 6941)	-603.5 (-1610 – 2895)	0.048
Females (N = 10)	-1358 (-2494 – 2175)	-1248 (-1935 – 569.9)	0.66
Males (N = 10)	6921 (1788 – 9995)	1153 (-1112 – 4978)	0.037
Comparison of males versus females P	0.026	0.09	
OCTT <sup>10</sup> (min) (N = 18)	60 (40 – 75)	40 (40 – 52.5)	0.02
Females (N = 9)	75 (45 – 75)	50 (40 – 62.5)	0.22
Males (N = 9)	40 (30 – 75)	40 (30 – 50)	0.077

<sup>1</sup>Data are shown as mean  $\pm$  SD when normally distributed and median (IQR) when non-normal

<sup>2</sup>Unless otherwise stated, data are for **area under the curve (AUC)** t = -15 min – t = 135 min

<sup>3</sup>P-values were calculated using Wilcoxon matched pairs signed rank tests for non-normally distributed data and paired *t*-tests when normally distributed

<sup>4</sup> **Not a significant P interaction for sex; for male versus females CRF  $P = 0.083$ , saline  $P = 0.58$**

<sup>5</sup> **Time x sex interaction: CRF  $P = 0.0001$ , saline  $P = 0.0051$**

<sup>6</sup> **Time x sex interaction: CRF  $P = 0.0001$ , saline  $P = 0.52$**

<sup>7</sup> **SBWC: Small bowel water content. Time x sex interaction: CRF  $P = 0.0001$ , saline  $P = 0.0012$**

<sup>8</sup> **ACV: ascending colon volume, AUC t = -45 – t = 135 min**

<sup>9</sup> **Time x sex interaction: CRF  $P = 0.0002$ , saline  $P = 0.02$**

<sup>10</sup>**OCTT: Orocaecal transit time. This is not an AUC, no 2-way ANOVA performed on the data**

**Table 2: Effect of CRF versus saline on abdominal symptoms**

		CRF <sup>1,2</sup>	Saline	P- value <sup>3</sup>
Symptoms	Fullness	488 (151 – 703)	362 (205 – 561)	0.25
	Bloating	153 (33 – 393)	101 (29 – 301)	0.32
	Distension	102 (17 – 171)	113 (3.4 – 323)	0.99
	Nausea	41 (5 – 89)	8 (0 – 93)	0.60
	Abdominal pain	60 (14 – 166)	68 (3 – 284)	0.29

<sup>1</sup> Data are presented as AUC median (IQR) mm.min, obtained from VAS

<sup>2</sup> Data are presented for N = 20 volunteers

<sup>3</sup> P-values were calculated using Wilcoxon matched pairs signed rank tests

## Figure legends

Figure 1: Salivary cortisol concentrations (mean  $\pm$  SEM) throughout the study day for the 20 volunteers for the CRF (●) and saline (▪) arms of the study. The time of injection just before  $t = -45$  min is indicated with the solid arrow, while the time at which the fructose drink is taken at  $t = 0$  min is shown with the dashed arrow. Salivary cortisol concentrations were significantly larger ( $P = 0.0005$ , *Student's t test*) after injection with CRF.

Figure 2: Mean  $\pm$  SEM breath  $H_2$  concentration of the 20 volunteers throughout the study day for the CRF (●) and saline (▪) arms of the study. The time of injection just before  $t = -45$  min is indicated with the solid arrow, while the time at which the fructose drink is taken at  $t = 0$  min is shown with the dashed arrow. There was no significant difference in breath  $H_2$  concentration for the two arms of the study ( $P = 0.99$ , *Student's t test*).

Figure 3: Mean  $\pm$  SEM gastric volumes for (A) 10 male and (B) 10 female volunteers after intravenous injection of CRF (●, solid connecting line) or saline (▪, dashed connecting line), followed by a **fructose** drink. The time of injection just before  $t = -45$  min is indicated with the solid arrow, while the time at which the fructose drink is taken at  $t = 0$  min is shown with the dashed arrow. Only female volunteers showed a significantly different gastric emptying between CRF and **saline** and there was a significant time  $\times$  gender effect ( $P = 0.0001$ , two way ANOVA).

Figure 4: Small bowel water content (SBWC, mean  $\pm$  SEM) for 20 volunteers after intravenous injection of CRF (●) or saline (▪), followed by a **fructose** drink. The time

of injection just before  $t = -45$  min is indicated with the solid arrow, while the time at which the fructose drink is taken at  $t = 0$  min is shown with the dashed arrow. SBWC was significantly larger on the saline arm of the study from  $t = -15 - t = 135$  min ( $P = 0.04$ , *Student's t test*).

Figure 5: An example of heavily T2-weighted coronal MR images from the abdominal region of a single volunteer 45 minutes after a **fructose** drink. On these images, freely mobile water is shown as bright white and tissues are dark. The volume of water in the small bowel (SBWC) after intravenous CRF (left) and saline (right) are compared.

Figure 6: The percentage change in ascending colon volume (ACV) for 20 volunteers from immediately before injection of CRF (●) or saline (▪) followed by a **fructose** drink. The time of injection just before  $t = -45$  min is indicated with the solid arrow, while the time at which the fructose drink is taken at  $t = 0$  min is shown with the dashed arrow. The % change was significantly greater on the CRF arm of the study ( $P = 0.048$ , *Student's t test*).