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Corticotrophin releasing factor increases ascending colon volume after a fructose test meal in healthy humans: a randomised control trial[1-5]

Kathryn A. Murray, Ching Lam, Sumra Rehman, Luca Marciani, Carolyn Costigan, Caroline L. Hoad, Melanie R. Lingaya, Rawinder Banwait, Stephen J. Bawden , Penny A. Gowland and Robin C. Spiller

[1] From the Sir Peter Mansfield Imaging Centre, School of Physics and Astronomy, University of Nottingham, Nottingham UK (KAM, CC, CLH, SJB and PAG); NIHR Nottingham Digestive Diseases Biomedical Research Unit, Nottingham University Hospitals, University of Nottingham, Nottingham UK (CL, ML, RB, LM, RCS) and Nottingham Digestive Diseases Centre, School of Medicine, University of Nottingham, Nottingham UK (CL, SR, LM and RCS)

AUTHOR LIST FOR INDEXING: Murray, Lam, Rehman, Marciani, Costigan, Hoad, Lingaya, Banwait, Bawden, Gowland and Spiller.

[2] Address correspondence to: Professor Robin Spiller, Nottingham Digestive Diseases Centre and NIHR Biomedical Research Unit, Queen's Medical Centre, E Floor, West Block, Nottingham University Hospitals, University of Nottingham, Nottingham NG7 2UH, UK. Tel: +44 (0) 115 8231090. Fax: +44 (0) 1158231409. E-mail: Robin.Spiller@nottingham.ac.uk.

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

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

[4] Supplemental Figure 1 is available in the Online Supplemental Material

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This trial was registered at ClinicalTrials.gov as NCT01763281

RUNNING HEAD: EFFECTS OF CRF ON FRUCTOSE MALABSORPTION

ABSTRACT

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

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

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

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

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

explain the increased sensitivity of some stressed individuals to fructose malabsorption.

This trial was registered at ClinicalTrials.gov as NCT01763281.

INTRODUCTION

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

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

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

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

SUBJECTS AND METHODS

Study participants

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

Study design

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

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

Barrett, Nuneaton, UK) with 5ml of pure lemon juice (PLJ) (Healthy Food Brands, West Sussex, UK) added to improve palatability. This dose of 40 g was selected as our previous study (16) showed that with a 40g dose, good distension of the small bowel is obtained and easily seen on the MR images. They received serial scans after this at time 15, 45, 75, 105, 135, 195, 255 and 315 minutes postprandially, with samples of saliva for cortisol measurement, end expiratory breath for hydrogen (H_2) measurement (Gastro$^+$ Gastrolyzer, Bedfont Scientific, Kent, UK) and symptom questionnaires, all being collected after each scan. Pulse and blood pressure measurements were taken after each scan and a State-Trait Anxiety Inventory (STAI) questionnaire was administered on a single occasion halfway through the scan day.

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

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

participation and the trial ended after the final volunteer had completed both arms. The study was registered on clinicaltrials.gov identifier NCT01763281.

MRI protocol

Images were collected using a whole-body, research-dedicated, 1.5T MR scanner (Achieva, Philips Medical System, Best, The Netherlands). Each imaging period lasted for 10 minutes and volunteers were positioned supine with a 16-element coil wrapped around the abdomen. The volunteers were allowed to sit upright away from the scanner between scans. The volume of freely mobile water in the small bowel (SBWC) was measured as described previously (18), using a coronal single-shot turbo spin-echo sequence. This acquired 24 slices in a single 24 second expiration breath hold (TR/TE = 8000/320 ms, 512x512 reconstructed matrix, voxel size 0.78x0.78x7 mm^3). A coronal dual-echo gradient echo sequence was used to determine the volume of the ascending colon (12) as well as the volume of gas. This sequence allowed simultaneous 24 slice collection of both in-phase and out-of-phase images in a single 15 second expiration breath hold (TR/TE1/TE2 = 157/2.30/4.60 ms, 256x256 reconstructed matrix, voxel size1.76x1.76x7 mm^3). Gastric volumes were measured with a balanced gradient echo sequence (TR/TE = 2.98 / 1.49 ms, flip angle 80^o, 256 x 256 reconstructed matrix, reconstructed in-plane resolution 1.56 x 1.56 x 5 mm^3, SENSE 2.0) (19), acquiring 50 transverse slices in a 16.5 second breath hold.

Lactose Ureide Breath Test (LUBT)

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

baseline breath sample before receiving their test drink (details above). The drink was mixed with 500 mg of labelled ^{13}C lactose ureide (Euriso-top®, Saint-Aubin Cedix, France). Breath samples were taken every 10 minutes for an hour, then every 15 minutes for an additional 4 hours. Analysis of breath samples was carried out on an IRIS®-Lab analyser (Wagner Analysen Technik, Bremen, and Germany) and the result was expressed as delta over baseline: the difference between the $^{13}CO_2/^{12}CO_2$ ratio in the post meal breath sample and the corresponding ratio in the baseline sample. The OCTT was manually determined by two experienced operators looking at plots of delta over baseline as a function of time and was taken as the time at which there was a rise of more than 2 ppm in ^{13}C above the baseline after consumption of the drink.

Data analysis, statistics and sample size

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

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

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

RESULTS

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

study, and as a result the data for males and females are presented separately. Normally distributed data are presented in tables as mean ± SD, while non-normally distributed data are shown as median [IQR]. Data in the figures are presented as the average at each time point across the study day and the error bars are the standard error of the mean (SEM).

Stress response

The salivary cortisol concentrations throughout the study day are shown in **Figure 1**. Cortisol levels were initially higher on both arms of the study, but fell at the point of cannulation. After CRF injection, salivary cortisol concentrations rose steadily and peaked after 30 minutes at 0.49 ± 0.27 µg dL^{-1}. In comparison, cortisol levels after injection with saline rose to a maximum of 0.18 ± 0.23 µg dL^{-1}. The cortisol response lasted until 135 minutes after drinking fructose, and the time period from 15 minutes before to 135 minutes after (t = -15 – 135 minutes) was selected as being physiologically relevant for comparisons. The t = -15 – 135 min AUC (**Table 1**) for salivary cortisol on the CRF arm of the study was significantly greater than saline (mean difference [95% CI] 22.4 [12.3, 32.5] µg dL^{-1} .min, *P* = 0.0002). After CRF injection, female participants had a numerically higher salivary cortisol concentration than males (Table 1) but this difference was not statistically significant (mean difference 15.3 ± 8.5 µg dL^{-1} .min, *P* = 0.09; *Student's t test*).

Breath H_2

The breath H_2 concentration of the 20 volunteers across the study day for both treatment arms is shown in **Figure 2**. Consumption of fructose led to an immediate increase in H_2 concentration, which peaked at 75 minutes postprandial (54 ± 20 ppm

CRF arm, 44 ± 12 ppm saline arm) and then returned to baseline levels. This trend was seen for both arms of the study, and there was no significant difference between the CRF and saline arm. Table 1 shows the differences between the breath H_2 responses for males and females. There were no significant differences between the CRF and saline arms for either group, although CRF injection in males produced a numerically larger volume of breath H_2 than saline median difference [95% CI] 2400 [-3675, 7193] ppm.min, *P* = 0.38. Breath H_2 was significantly larger for males after both CRF and saline injection (Table 1); 6 males showed a rise in breath H_2 of more than 20 ppm after CRF compared to 2 females, while 7 males showed an increase after saline injection, compared to only 3 females. The gender effect on the measured breath H_2 was significant (*P* = 0.035; two way ANOVA), there was also a significant time effect (*P* = 0.0001; two way ANOVA), with a positive time x gender interaction (*P* = 0.0001; two way ANOVA).

Gastric emptying

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

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

Small bowel water content (SBWC)

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

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

Ascending colon volume

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

Orocaecal transit time (OCTT)

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

317 significantly shorter than after injection with CRF (mean ± SD) 59 ± 23 min, mean
318 difference [95% CI] 10.6 [2.1,19.0] min, *P* = 0.02. The median orocaecal 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 ± 7, significantly greater than the
325 average score after saline injection, 28.8 ± 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

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

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

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

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

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

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

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

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

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

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

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TABLES

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

	CRF[1,2]	Saline	*P*-value[3]
Salivary cortisol[4] (µg dL^{-1} .min) (N = 20)	43.6 ± 20.1	21.2 ± 11.3	0.0002
Females (N = 10)	51.3 ± 22.6	22.0 ± 11.2	0.004
Males (N = 10)	36.0 ± 14.6	20.4 ± 11.9	0.005
Breath H_2[5] (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 ± 9750	6311 ± 4031	0.38
Comparison of males versus females *P*	0.035	0.077	
Gastric volume[6] (mL.min) (N = 20)	31776 ± 9560	30222 ± 7571	0.4
Females (N = 10)	36601 ± 7388	31534 ± 6645	0.03
Males (N = 10)	26952 ± 9307	28910 ± 8547	0.48
Comparison of males versus females *P*	0.085	0.72	
SBWC (mL.min) (N = 20)[7]	48515 ± 15719	55948 ± 19169	0.04
Females (N = 10)	52902 ± 19704	65501 ± 20890	0.04
Males (N = 10)	44129 ± 9521	46396 ± 11687	0.57
Comparison of males versus females *P*	0.067	0.009	
AUC of % change from baseline against time in ACV[8,9] (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[10] (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

[1]Data are shown as mean ± SD when normally distributed and median (IQR) when non-normal

[2]Unless otherwise stated, data are for area under the curve (AUC) t = -15 min – t = 135 min

[3]*P*-values were calculated using Wilcoxon matched pairs signed rank tests for non-normally distributed data and paired *t*-tests when normally distributed

[4] Not a significant P interaction for sex; for male versus females CRF *P* = 0.083, saline *P* = 0.58

[5] Time x sex interaction: CRF *P* = 0.0001, saline *P* = 0.0051

[6] Time x sex interaction: CRF *P* = 0.0001, saline *P* = 0.52

[7] SBWC: Small bowel water content. Time x sex interaction: CRF *P* = 0.0001, saline *P* = 0.0012

[8] ACV: ascending colon volume, AUC t = -45 – t = 135 min

[9] Time x sex interaction: CRF *P* = 0.0002, saline *P* = 0.02

[10]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[1,2]	Saline	*P*- value[3]
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

[1] Data are presented as AUC median (IQR) mm.min, obtained from VAS

[2] Data are presented for N = 20 volunteers

[3] *P*-values were calculated using Wilcoxon matched pairs signed rank tests

Figure legends

Figure 1: Salivary cortisol concentrations (mean ± 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 ± 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 ± 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 x gender effect (*P* = 0.0001, two way ANOVA).

Figure 4: Small bowel water content (SBWC, mean ± 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*).