World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i3.225

World J Gastroenterol 2012 January 21; 18(3): 225-236
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Stress-induced visceral analgesia assessed non-invasively in rats is enhanced by prebiotic diet

Muriel Larauche, Agata Mulak, Pu-Qing Yuan, Osamu Kanauchi, Yvette Taché

Muriel Larauche, Pu-Qing Yuan, Yvette Taché, CURE: Digestive Diseases Research Center and Oppenheimer Family Center for Neurobiology of Stress, Department of Medicine, Digestive Diseases Division at the University of California Los Angeles, VA Greater Los Angeles Healthcare System, Los Angeles, CA 90073, United States

Agata Mulak, Department of Gastroenterology and Hepatology, Wroclaw Medical University, Borowska 213, 50-556 Wroclaw, Poland

Osamu Kanauchi, Shiga University of Medical Science, Shiga and Central Laboratories for Frontier Technology, Kirin Holdings Co. Ltd., 1-13-5, Fukuura, Kanazawa-ku, Yokohama, 236-0004, Japan

Author contributions: Larauche M designed experiments, carried out research, analyzed data, and prepared the manuscript; Mulak A carried out research, analyzed data, and prepared the manuscript; Yuan PQ analyzed data; Kanauchi O designed experiments, and analyzed data; Taché Y designed experiments, evaluated data and prepared the manuscript; all authors have read and approved the final manuscript.

Supported by Central Labs for Frontier Technology Kirin Holdings Co., Ltd Japan and the National Institute of Health grants, P50 DK-64539 (to Taché Y and Larauche M), Center Grant DK-41301 (Animal Core, to Yvette Taché), R01 DK-33061 (to Yvette Taché); and Veterans Administration Research Career Scientist Award

Correspondence to: Dr. Muriel Larauche, VA Greater Los Angeles Healthcare System, 11301 Wilshire Boulevard, CURE Building 115, Room 111, Los Angeles, CA 90073, United States. mlarauche@mednet.ucla.edu
Telephone: +1-310-4783711-41827 Fax: +1-310-2684963

Received: May 15, 2011 Revised: July 6, 2011
Accepted: October 27, 2011
Published online: January 21, 2012

Abstract

AIM: To investigate the influence of repeated water avoidance stress (rWAS) on the visceromotor response (VMR) to colorectal distension (CRD) and the modulation of the response by a prebiotic diet in rats using a novel surgery-free method of solid-state manometry.

METHODS: Male Wistar rats fed a standard diet with or without 4% enzyme-treated rice fiber (ERF) for 5 wk were subjected to rWAS (1 h daily x 10 d) or no stress. The VMR to graded phasic CRD was assessed by intraluminal colonic pressure recording on days 0 (baseline), 1 and 10 (45 min) and 11 (24 h) after rWAS and expressed as percentage change from baseline. Cecal content of short chain fatty acids and distal colonic histology were assessed on day 11.

RESULTS: WAS on day 1 reduced the VMR to CRD at 40 and 60 mmHg similarly by 28.9% ± 6.6% in both diet groups. On day 10, rWAS-induced reduction of VMR occurred only at 40 mmHg in the standard diet group (36.2% ± 17.8%) while in the ERF group VMR was lowered at 20, 40 and 60 mmHg by 64.9% ± 20.9%, 49.3% ± 11.6% and 38.9% ± 7.3% respectively. The visceral analgesia was still observed on day 11 in ERF- but not in standard diet-fed rats. By contrast the non-stressed groups (standard or ERF diet) exhibited no changes in VMR to CRD. In standard diet-fed rats, rWAS induced mild colonic histological changes that were absent in ERF-fed rats exposed to stress compared to non-stressed rats. The reduction of cecal content of isobutyrate and total butyrate, but not butyrate alone, was correlated with lower visceral pain response. Additionally, ERF diet increased rWAS-induced defecation by 26% and 75% during the first 0-15 min and last 15-60 min, respectively, compared to standard diet, and reduced rats' body weight gain by 1.3 fold independently of their stress status.

CONCLUSION: These data provide the first evidence of psychological stress-related visceral analgesia in rats that was enhanced by chronic intake of ERF prebiotic.

Key words: Stress-related visceral analgesia; Water avoidance stress; Colorectal distension; Enzyme-treated rice fiber prebiotic; Short chain fatty acids; Defecation; Rat; Solid-state manometry

Peer reviewer: Damian Casadesus Rodriguez, MD, PhD, Calixto Garcia University Hospital, J and University, Vedado, Havana City, Cuba

Larauche M, Mulak A, Yuan PQ, Kanauchi O, Taché Y. Stress-induced visceral analgesia assessed non-invasively in rats is enhanced by prebiotic diet. *World J Gastroenterol* 2012; 18(3): 225-236 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i3/225.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i3.225

INTRODUCTION

Irritable bowel syndrome (IBS) is a common functional bowel disorder of unclear etiology, characterized by recurrent abdominal pain and altered bowel habits without structural abnormalities affecting 10%-15% of the US[1,2] and 5%-10% of the Asian adult population[3,4]. Stress is an important factor in the onset, maintenance and exacerbation of IBS symptoms[5-7]. Recently, interactions between intestinal microbiota, mucosal barrier function, and immune system have been identified to play a role in IBS pathogenesis[8,9]. An imbalance in the gastrointestinal microbial population induced by infection, dietary changes or antibiotics can produce low grade inflammation as observed in a subset of IBS patients[10]. Alterations in gut transit, which can be related to dietary factors, stress or antibiotics, may also contribute to the abnormalities observed in enteric microbiota metabolic activity including fermentation processes[11]. Prebiotics as indigestible food constituents provide specific substrates ready to be metabolized by the beneficial gut microbiota thereby stimulating their growth or activity[8,12]. Prebiotics are also a source of short chain fatty acids (SCFAs) that provide energy to the epithelium and exhibit anti-inflammatory properties[13].

Visceral hypersensitivity, a key feature of IBS[14], can be associated with a low grade colonic mucosal inflammation in a subset of patients[15]. Therefore, the concept that anti-inflammatory action of probiotics and prebiotics could be beneficial in visceral pain has emerged[16]. In fact, there are reports that *Escherichia coli Nissle 1917* inhibits the visceral hypersensitivity associated with trinitrobenzene sulphonic acid colitis[17]. Similarly, *Lactobacillus paracasei* prevents the visceral hypersensitivity associated with inflammation in mice with gut microbiota altered by antibiotics[18]. Recently, a new prebiotic, enzyme-treated rice fiber (ERF) made from rice bran containing dietary fiber and fat soluble fraction has been shown to reduce inflammation and clinical symptoms in the murine dextran sodium sulfate colitis model by modulating the colonic microbiota environment and regulating immune cell differentiation[12] and to prevent 3-d intermittent restraint stress-induced IBS-like symptoms in rats[19].

Existing evidence from several groups including ours indicates that stress induces alterations of colonic functions (increased permeability, mucus secretion, motility, myenteric nerves activation and serotonin release) and the development of visceral hypersensitivity in rats[20-22]. In particular, we previously developed a psychological stress model of intermittent water avoidance stress (WAS)[23] which upon repeated exposure (rWAS) in rats induces sustained visceral hyperalgesia when monitored by electromyography (EMG)[24,25]. In rats, rWAS also induces colonic epithelial alterations associated with cytokines increase, mast cell activation and antigen sensitization[24,26-28], which may all contribute to greater visceral sensitivity[29]. Administration of probiotics chronically throughout the 10 d of rWAS prevents the stress-related colonic epithelial alterations and antigen load in the mucosa[30]. By contrast, we have recently shown in mice that exposure to rWAS can induce stress-related visceral analgesia in response to colorectal distension (CRD) when monitored non-invasively using a novel method of solid-state manometry, which avoids prior surgery to implant recording electrodes and subsequent single housing which are required in commonly used EMG monitoring of visceral pain[31].

Therefore in the present study, we first examined whether exposure to rWAS induced visceral analgesia in rats using the non-invasive monitoring of the visceromotor response (VMR) to graded phasic CRD. Second, we tested whether the chronic feeding of 4% ERF prebiotic would affect rWAS-related alterations of visceral sensitivity and colonic motor functions and potential underlying mechanisms by monitoring histological changes in the colonic mucosa and cecal content of SCFAs.

MATERIALS AND METHODS

Animals

Male Wistar rats (Harlan Laboratories, Indianapolis, IN) were fed *ad libitum* a standard diet (composition detailed in Table 1) with or without 4% ERF (formulated in pellets for 4 wk, then in powder form) (Kirin Holdings Company, Laboratory, Japan) starting from weaning (~ 21 d old, weight ~ 50-74 g) and throughout the 48 experimental days. Rats were housed in pairs, under standard conditions of temperature and humidity, on bedding with enrichment. At the end of the experiment, animals were euthanized by an intraperitoneal overdose of anesthesia (urethane 25%) and thoracotomy. Experimental protocols were performed in accordance with animal protocol No. 12049-09 approved by the Greater Los Angeles Institutional Animal Care and Use Committee.

Preparation and chemical composition of enzyme-treated rice fiber

The ERF diet was prepared as detailed previously[12] and composed roughly of 70% dietary fiber by weight[19,32] formulated to have 4% of ERF in standard food (Table 1). In the standard diet, 4% ERF was replaced by cellulose (30 g/kg) (Table 1).

Repeated water avoidance stress

The procedure of rWAS was performed as described before[23]. Each adult Wistar rat (232-311 g) was placed on a pedestal (10 cm × 8 cm × 8 cm) affixed to the center of a Plexiglas® standard rat cage floor (45 cm length × 25 cm width × 25 cm height) for 1 h daily for 10 consecutive days between 8 and 10 am. The Plexiglas® rat cage

Table 1 Chemical composition of standard and enzyme-treated rice fiber diets

Content (g/kg)	Standard	ERF[1]
Casein[2]	146.0	140.4
Vitamin mix	10.0	10.0
Mineral mix	35.0	35.0
Choline chloride	2.0	2.0
Cellulose[2]	30.0	-
ERF	-	40.0
Corn oil	50.0	50.0
Corn starch	727.0	722.6

[1]Enzyme-treated rice fiber (protein, 14.9%; dietary fiber, 74.5%); [2]According to AIN 93G formula, protein and dietary fiber contents in two diets were adjusted to the same value using casein and cellulose, respectively. ERF: Enzyme-treated rice fiber.

was filled with room temperature water (25 ℃) up to 1 cm from the top of the pedestal. Non-stressed rats were kept in their home cage and handled daily (5 min).

Measurement of colonic motor function and visceral pain

Fecal pellet output: In rats subjected to rWAS protocol, fecal output was monitored as the total number of pellets expelled for the 1-h period of rWAS exposure on each of the 10 d with a time course response monitored every 15 min.

Assessment of visceral pain response to CRD: Visceral sensitivity to CRD was assessed using the non-invasive manometric method that we recently developed and validated for use in mice and rats[31,33]. Briefly, a PE50 catheter was taped 3.5 cm below the pressure sensor of a miniaturized pressure transducer catheter (SPR-524 Mikro-Tip catheter; Millar Instruments, Houston, TX). A custom-made balloon (2 cm wide × 5 cm long)[33,34], prepared from an infinitely compliant high-density polyethylene 16 micron gauge plastic bag (STOUT, C4348N16, Wichita, KS), was tied over the catheter at 1 cm below the pressure sensor with silk 4.0 (Henry Schein Inc., Melville, NY).

On experimental day, rats were briefly anesthetized with isoflurane (3% in O_2) and the lubricated "balloon-pressure sensor" catheter was introduced into the rectum and distal colon such that the distal end of the balloon was positioned at 1 cm from the anus and the catheter secured to the tail with tape. Each animal was placed in a Bollman cage covered with a light tissue blanket and left to rest for 30 min before the CRD procedure. Each balloon was connected to the barostat and the miniaturized pressure transducer to a preamplifier (model 600; Millar Instruments, Houston, TX). The intracolonic pressure (ICP) signal was acquired using CED Micro1401/SPIKE2 program. The CRD protocol consisted of two CRDs at 60 mmHg to unfold the balloon immediately followed by two series of graded phasic distensions to constant pressures of 10, 20, 40 and 60 mmHg. Each CRD lasted 20 s and was applied at a 4 min inter-stimulus interval (Figure 1). A similar CRD paradigm has been used previously to assess visceral pain-related responses in rats[35].

Data analysis

The phasic component of the intracolonic pressure (pICP) was extracted from the ICP signal recorded[31,33]. The VMR was defined as the increase in area under the curve (AUC) of pICP during CRD over the mean value of pre- and post-distension 20 s periods. To examine the pressure-response relationship and adjust for inter-individual variations of the signal[35], ICP amplitudes were normalized for each rat to the highest pressure (60 mmHg) in the 1st set of CRD. This value served as 100% response (control) in the baseline period of data collection before exposure to WAS (day 0) and represented the baseline VMR. The VMR to the subsequent CRDs was expressed either as % from their baseline values or mean change from the baseline response (Δ VMR in %) at different pressures of distension as validated in our previous studies[25,31,33]. In some cases, to determine correlations between visceral pain responses and inflammatory scores or cecal organic acid content, the non-normalized cumulative AUC response during the CRD procedure on day 11 (for all pressures of distensions) was used[36]. For subgrouping analysis, each rat exhibiting VMR to CRD at 60 mmHg with a value higher than that obtained at baseline (> 10%) was categorized as hyperalgesic, while each rat presenting value lower than that obtained at baseline (< 10%) was categorized as analgesic.

Experimental protocol

Rats were fed 4% ERF diet (2 groups) or standard control diet (2 groups) starting at the weaning period for 37 d until they reached the age of 6-6.5 wk (weight ~ 232-311 g) and handled every 3-4 d (petting, 5 min). All experimental protocols were performed in the morning and conducted in conscious non-fasted rats. All groups were habituated to the experimental conditions for VMR monitoring (training to Bollman cages, 90 min per day for 3 consecutive days before day 0), then tested for baseline CRD response (day 0). Thereafter rats were exposed daily to 1 h WAS for 10 consecutive days or no stress (days 1-10), while still maintained under standard or 4% ERF diet. Non-stressed animals were kept in their home cages and handled daily. Body weight was monitored in the morning, every 2-4 d for 37 d and daily during the stress period before each stress session as well as in non-stressed groups. Body weight gain was expressed as % of initial body weight. Fecal pellet output (FPO) was monitored during each rWAS session. On days 1 (acute WAS) and 10 (rWAS), 45-50 min after the end of the stress session, and on day 11, 24 h after the last rWAS session, rats were subjected to CRD protocol and VMR was monitored in all groups (Figure 1). Immediately after the last CRD, animals were euthanized and cecum and distal colon, at a level caudal to the balloon placement, were collected.

Colonic histological assessment

The distal colonic segment was rinsed with cold phosphate buffer saline, fixed in 4% paraformaldehyde and kept at 4 ℃ until processed. After fixation, paraffin-embedded specimens were sectioned at 5 μm (whole thickness) and stained with HE. Inflammatory scores were

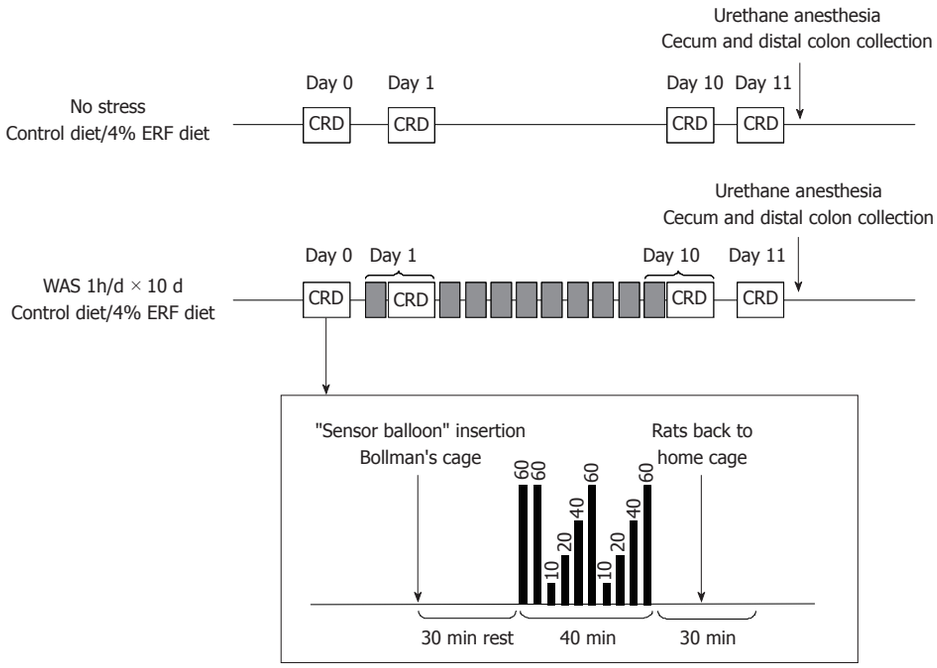

Figure 1 Treatment and colorectal distension (black column with number in mmHg) protocol for the assessment of visceral pain in Wistar rats exposed to daily intermittent water avoidance stress. Grey boxes represent the daily sessions of water avoidance stress (1 h). ERF: Enzyme-treated rice fiber; CRD: Colorectal distension.

Table 2 Baseline visceromotor response to colorectal distension at day 0

CRD (mmHg)	Standard diet[1]		ERF diet[1]	
	No stress (n = 7) VMR (%)[2]	WAS (n = 7) VMR (%)[2]	No stress (n = 9) VMR (%)[2]	WAS (n = 8) VMR (%)[2]
10	-0.3 ± 1.2	0.9 ± 1.9	2.8 ± 2.8	0.89 ± 1.0
20	5.1 ± 1.8[a]	26.8 ± 12.2	30.6 ± 12.2	30.7 ± 7.0
40	71.4 ± 6.2	77.3 ± 11.8	71.1 ± 11.2	88.7 ± 7.8
60	100 ± 0	100 ± 0	100 ± 0	100 ± 0

[1]Rats were fed standard or 4% enzyme-treated rice fiber (ERF) diet (2 groups each before to be assigned to no stress or water avoidance stress, WAS) starting at the weaning period for 37 d before performing the baseline visceromotor response (VMR) to colorectal distension (CRD); [2]Each value represents the mean ± SE of n as indicated in parenthesis; [a]$P < 0.05$ *vs* all other groups at 20 mmHg - 2-way ANOVA, Bonferroni post test.

established following a scale of 0 to 3: 0: normal, 1: mild, 2: moderate, 3: severe based on the occurrence of inflammatory cell infiltration and mucosa microscopic aspect (submucosal edema, vascular dilation, mucosal erosion, atrophy).

Cecal content of organic acids

The cecum was cut open and its content flash frozen less than 15 min post collection and kept at -80 ℃ until assayed for SCFAs levels as previously described[12]. Briefly, 0.3 g of the cecal content was weighed and 1 mL of Milli-Q water added. The mixture was incubated at 4 ℃ for 60 min. After centrifugation at 12 000 r/min at 4 ℃ for 10 min, the obtained supernatant was filtered using a 0.22 μm filter. Organic acids were separated with the Shimpack SPR-H 250 L (Shimadzu Co. Ltd., Kyoto, Japan). The mobile phase was 4 mmol/L of p-toluene sulfonic acid, and the detector was electric conductivity (Shimadzu CDD-6A, Kyoto Japan).

Statistical analysis

Each experimental group included 5-10 rats. Data were analyzed using one-way ANOVA or 2-way ANOVA followed by Bonferroni *post hoc* test or by paired or unpaired Student *t* test as specified to assess the difference between treatment groups. Contingency statistical analyses for correlation between visceral sensitivity and cecal organic acid content were performed using two-tailed Fisher's exact test. Based on the Grubb's test results, three out of the total number of 34 rats on two different days of CRD were found to be outliers and excluded from VMR data analysis. Due to technical issues (i.e., expulsion of the balloon before CRD or probe failure during CRD), 5 rats were excluded from some parts of the analysis immediately after stress (day 1: 2 rats) or 24 h later (day 11: 3 rats). A P value < 0.05 was considered significant.

RESULTS

Visceral pain

Rats were fed a standard diet or 4% ERF diet for 48 d and CRD was performed on days 37 (baseline, day 0), 38 (day 1), 47 (day 10) and 48 (day 11) with or without (no stress) 1-h daily WAS from days 38 to 47. At day 0, the VMR to baseline CRD in each of the four groups of rats was similar at all pressures of distensions, except at 20 mmHg in rats fed standard diet to be assigned to no stress which presented a lower VMR [$F(3.108)=184.7$, $P < 0.001$] compared to the groups fed ERF diet ($P < 0.05$) (Table 2).

Table 3 Visceromotor response after water avoidance stress or no stress in rats fed standard or enzyme-treated rice fiber diet

Time[1] Post WAS	CRD pressure (mmHg)	Standard diet[1]				ERF diet1			
		No stress		WAS		No stress		WAS	
		VMR (%)[2]	*n*	VMR (%)[2]	*n*	VMR (%)[2]	*n*	VMR (%)[2]	*n*
45 min day 1	40	15.8 ± 17.6	7	- 32.4 ± 15.2[a]	7	26.0 ± 23	8	- 29.5 ± 12.3[b]	8
	60	1.0 ± 9.7		- 30.0 ± 10.8[a]		12.9 ± 13.8		- 28.7 ± 6.0[b,c]	
45 min day 10	40	-26.4 ± 11.7	7	- 36.2 ± 17.8[b]	7	3.7 ± 21.3	8	- 49.3 ± 11.6[b,c]	9
	60	-23.7 ± 18.6		- 20.6 ± 12.2		21.6 ± 23.5		- 38.9 ± 7.3[b]	
24 h day 11	40	7.5 ± 38.7	5	- 28.2 ± 15.0	7	- 4.1 ± 21.5	6	- 49.8 ± 12.6[b]	9
	60	30.4 ± 15.4		- 24.3 ± 9.6[c]		5.8 ± 15.1		- 34.3 ± 10.8[b]	

[1]Rats were fed 4% enzyme-treated rice fiber (ERF) diet or standard control diet starting at the weaning period for 37 d, then tested for baseline visceromotor response (VMR) to colorectal distention (CRD) response (day 0) and thereafter exposed daily to 1 h water avoidance stress (WAS) for 10 consecutive d or no stress (days 1-10), while still maintained under standard or 4% ERF diet. Non-stressed animals were kept in their home cages. On days 1 (acute WAS) and 10 [repeated water avoidance stress (rWAS)], 45-50 min after the end of the stress session, and on day 11, 24 h after the last rWAS session, rats were subjected to CRD protocol and VMR was monitored in all groups; [2]Each value represents the mean ± SE of % VMR changes from baseline (day 0) in number of animal indicated by n in columns). Minus values represent analgesia. [a]$P < 0.05$, [b]$P < 0.01$ *vs* baseline; [c]$P < 0.05$ *vs* respective diet non stressed group, 2-way ANOVA, Bonferroni post hoc test.

Repeated water avoidance stress induces an immediate analgesia compared to baseline in standard diet fed rats

Non-stressed rats on standard diet exhibited no statistically significant changes in VMR compared to baseline at all pressures of CRD when tested on days 1 ($n = 7$), 10 ($n = 7$) and 11 ($n = 5$) after baseline recording [$F(3.99) = 1.248$, $P = 0.28$] (Table 3 and Figure 2). By contrast, acute WAS (day 1) decreased the VMR to CRD (analgesia) at 40 mmHg and 60 mmHg by 32.4% ± 15.2% and 30.0% ± 10.8% respectively compared to baseline ($n = 7$, $P < 0.05$, Bonferroni post hoc test) (Table 3 and Figure 2A). After 10 consecutive days of rWAS, rats tested within 45-50 min after the last session of stress exhibited a decreased VMR to CRD (analgesia) at 40 mmHg by 36.2% ± 17.8% compared to baseline ($n = 7$, $P < 0.01$) but not at 60 mmHg ($n = 7$, $P > 0.05$) (Table 3 and Figure 2B). The VMR to CRD was not significantly different from baseline values on day 11, 24 h after the last stress session ($n = 7$, $P > 0.05$) (Table 3 and Figure 2C). Two-way ANOVA showed a significant influence of rWAS treatment [$F(3.88) = 2.761$, $P = 0.0468$] and time [$F(3.88) = 77.56$, $P < 0.0001$]. However, there was no interaction between treatment and time. In rats fed standard diet, 85.7%, 66.7% and 85.7% of animals developed visceral analgesia in response to rWAS when tested at day 1, 10 or 11, respectively, while 100% exhibited visceral analgesia in rats fed ERF diet consistently over the 3 d of testing.

ERF diet potentiates the immediate and induces delayed visceral analgesia to rWAS compared to baseline in rats

In non-stressed rats fed 4% ERF diet tested on days 1 ($n = 8$), 10 ($n = 8$) and 11 ($n = 6$) after baseline recording, the VMR was not significantly different to the baseline at all pressures of CRD [$F(3.104) = 0.6390$, $P = 0.59$]; $P > 0.05$) (Table 3 and Figure 2). By contrast, one session of WAS decreased the VMR to CRD compared to baseline at 40 and 60 mmHg by 29.5% ± 12.3% and 28.7% ± 6.0% respectively ($n = 8$, $P < 0.01$ each) (Table 3 and Figure 2A). Likewise when rats were exposed daily to rWAS and tested on day 10 (Figure 2B) the VMR to CRD was decreased significantly at 20, 40 and 60 mmHg by 25% ± 7.8%, 49.3% ± 11.6% and 38.9% ± 7.3%, respectively compared to baseline ($n = 9$, $P < 0.05$, $P < 0.001$ and $P < 0.001$, respectively) and the decreased VMR was maintained on day 11 (Table 3 and Figure 2C).

ERF diet compared with control diet decreases the percentage of rats developing hyperalgesia in response to rWAS at 60 mmHg CRD over the 10 d of stress

We further performed subgrouping analysis of VMR in animals developing hyperalgesia vs analgesia compared to their basal VMR at 60 mmHg and combined this number over the 10 d of stress (three testing days) for each group. In the non-stressed rats fed either standard or ERF diet, there was no statistical difference ($P > 0.05$, Fisher's exact test) in the percentage of rats that exhibited hyperalgesia throughout the whole 10 d of stress (40%, 28.6% and 80% of rats fed the standard diet, $n = 7$/group *vs* 57%, 57% and 60% fed ERF diet, $n = 7$/group, at days 1, 10 and 11, respectively). By contrast in rWAS groups, while 14.3%, 33.3% and 14.3% fed standard diet developed hyperalgesia at day 1, 10 and 11 ($n = 6$-8/group) respectively, none did in the ERF diet fed group ($n = 8$-9/group) ($P = 0.0478$, Fisher's exact test).

Cecal organic acids analysis

Cecum content was collected on day 48 immediately after the last CRD. In non-stressed rats, 4% ERF diet significantly increased cecal butyrate content compared with standard diet (9.2 ± 0.8 mmol/L per g *vs* 6.1 ± 0.8 mmol/L per g, $P < 0.05$; Figure 3A). In rWAS exposed rats fed ERF diet, cecal butyrate content values were still maintained at similar levels (9.5 ± 1.1 mmol/L per g), however the values did not reach significance when compared with rWAS + standard diet group (7.3 ± 1.4 mmol/L per g) due to a trend to increased butyrate in the rWAS + standard diet group. No statistical differences were detected for the other cecal organic acids even though there was a tendency for isobutyrate to be lower in rats fed ERF diet and exposed or not to rWAS compared to standard diet

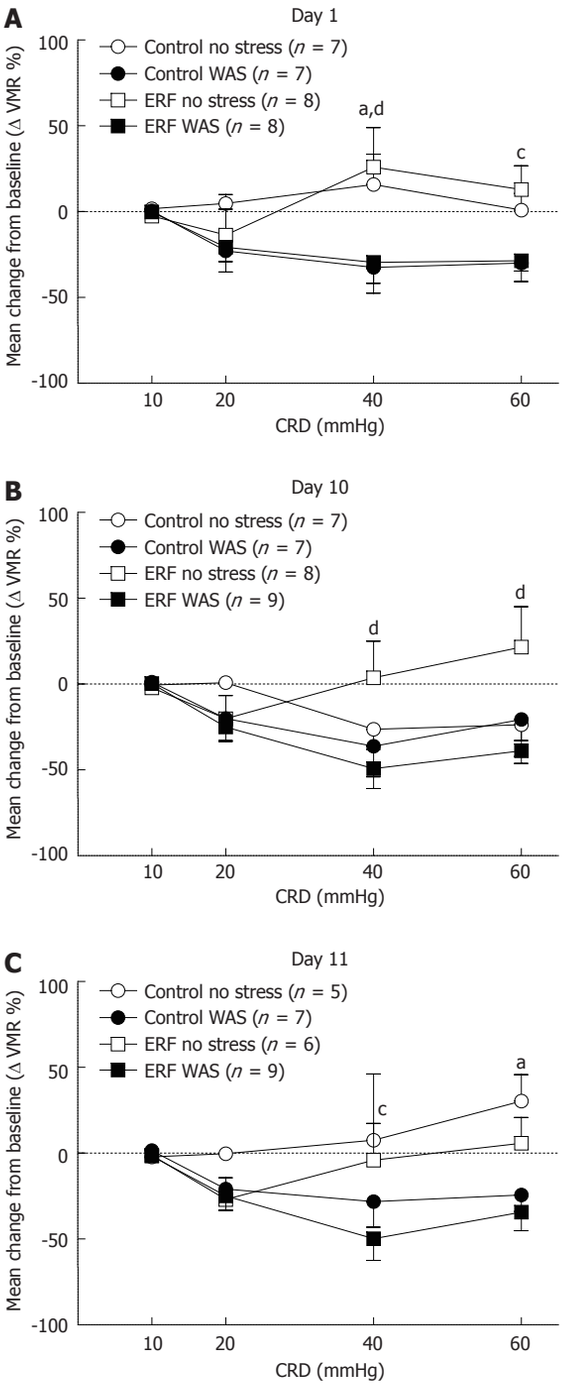

Figure 2 Influence of water avoidance stress or no stress on visceral sensitivity to colorectal distension in rats fed standard or prebiotic (4% enzyme-treated rice fiber) diet ad libitum. A: Immediate visceral motor response (VMR) at day 1; B: Immediate VMR at day 10; C: VMR 24 h at day 11. Data are expressed as mean ± SE of number of animals indicated in parenthesis for each group. [a]$P < 0.05$ standard diet no stress *vs* standard diet water avoidance stress (WAS); [c]$P < 0.05$, [d]$P < 0.01$ enzyme-treated rice fiber (ERF) no stress *vs* ERF WAS; 2-way ANOVA followed by Bonferroni post hoc test. CRD: Colorectal distention.

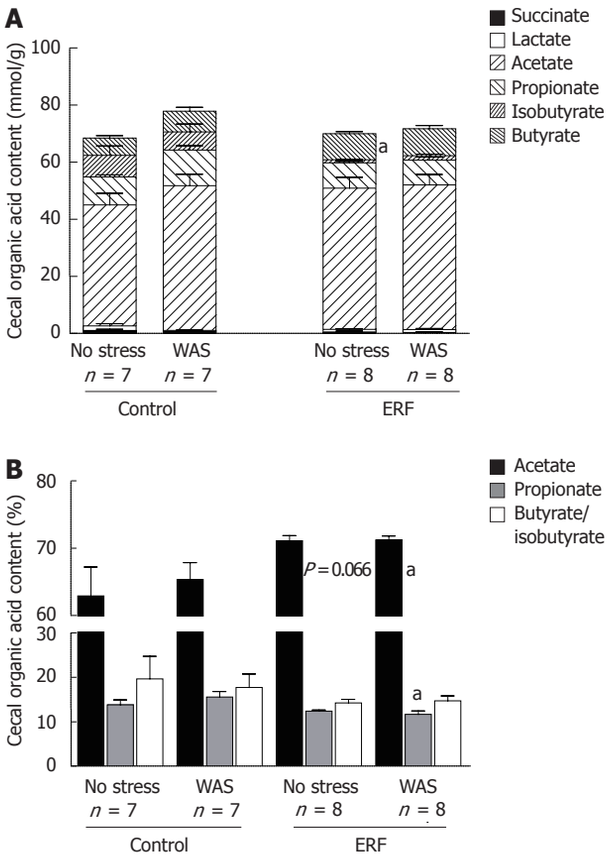

Figure 3 Cecal organic acid content in mmol/L per g (A) or % (B) in rats fed either standard or 4% enzyme-treated rice fiber prebiotic diet and exposed or not to repeated water avoidance stress in rats. Data are expressed as mean ± SE, of number of rats indicated as *n* below columns. [a]$P < 0.05$ *vs* control diet same stress status group; unpaired student *t* test. WAS: Water avoidance stress; CRD: Colorectal distention.

fed rats (1.51 ± 0.64 mmol/L per g *vs* 6.30 ± 2.79 mmol/L per g, $P = 0.10$; 1.04 ± 0.11 mmol/L per g *vs* 7.52 ± 3.37 mmol/L per g, respectively, $P = 0.06$).

The ratio of SCFAs, acetate:propionate:butyrate/isobutyrate, in the cecal content of rats fed ERF diet compared to standard diet, stressed or not, showed that ERF diet increased the percentage of acetate in stressed rats (71.2% ± 0.6% *vs* 65.4% ± 2.5%, $P < 0.05$) as well as in non-stressed rats, although not significantly (71.2% ± 0.7% *vs* 63.0% ± 4.3%, $P = 0.07$) (Figure 3B). ERF diet also decreased the percentage of propionate in stressed animals (11.7% ± 0.8% *vs* 15.6% ± 1.2%, $P < 0.05$; Figure 3B).

Interestingly, lower concentrations in cecal content of isobutyrate and total butyrate (isobutyrate + butyrate), but not butyrate alone, were correlated with lower visceral pain response (overall AUC during CRD on day 11) ($R_p = 0.41$, $P = 0.0368$ and $R_p = 0.42$, $P = 0.0335$, respectively) in stressed and non-stressed animals fed either ERF or standard diet.

Colonic histological assessment

In animals fed standard diet, rWAS evoked slight colonic inflammation with mild invasion of inflammatory cells and submucosal edema that was rarely observed in non-stressed animals. The inflammatory score was statistically different between these groups (1.3 ± 0.3 *vs* 0.3 ± 0.2 arbitrary units, $P < 0.05$, unpaired *t* test) (Figure 4A, B and E) and statistical analysis confirmed that positive inflammatory scores occurred less frequently than expected by chance in stressed animals ($P < 0.05$, two-tailed Fisher's exact test). The inflammatory score of 4% in ERF-fed

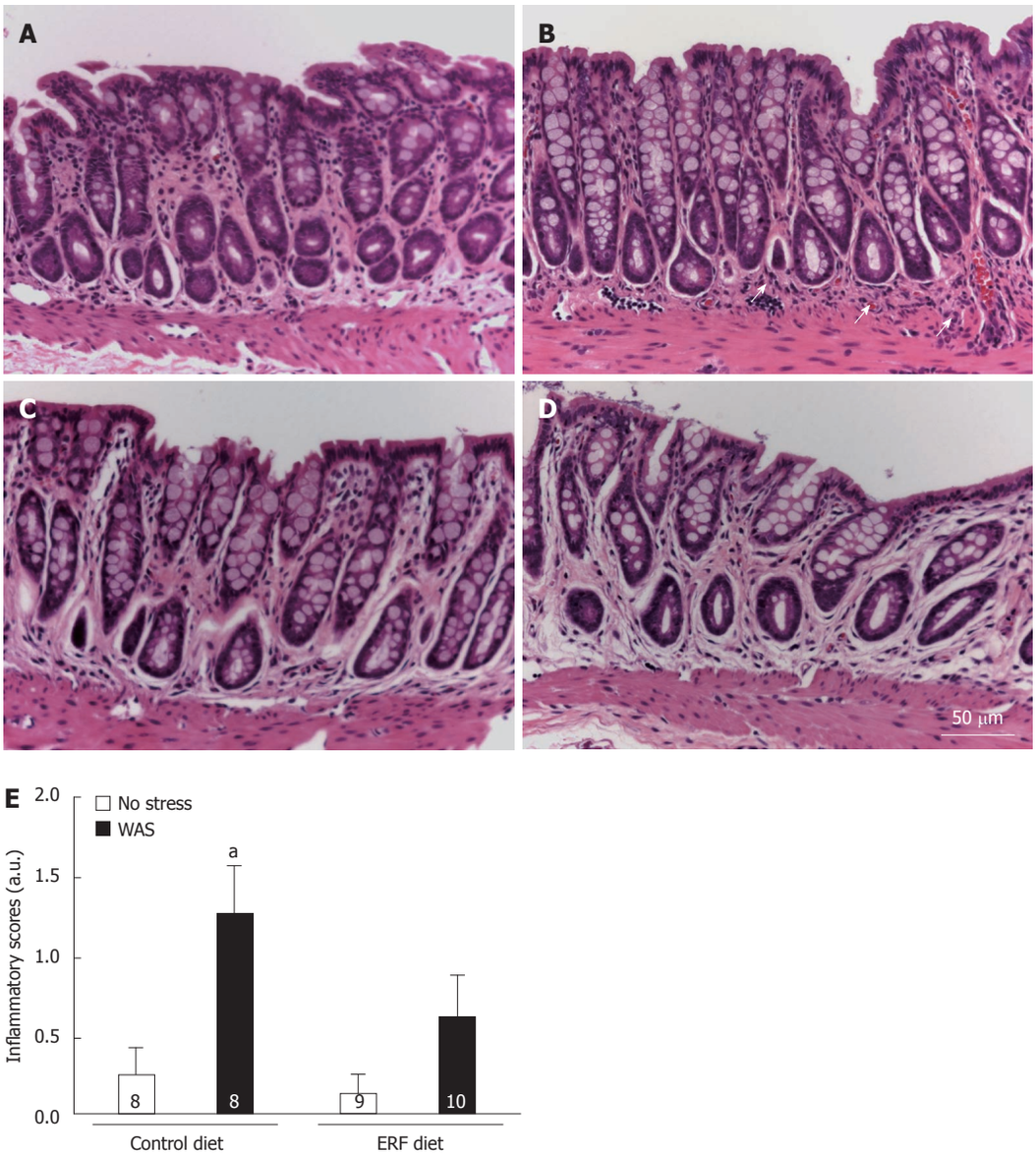

Figure 4 Inflammatory scores in distal colon of rats fed either standard or 4% enzyme-treated rice fiber diet and exposed or not (no stress) to repeated water avoidance stress. A-D: Representative histological sections of the rat distal colon stained with hematoxylin and eosin. The arrows point to an area in the lamina propria with inflammatory cell infiltration in stressed rats fed with standard diet (B) that was not observed in non-stressed rats fed with standard (A) or 4% enzyme-treated rice fiber (ERF) (C) diet and stressed rats fed with 4% ERF diet (D). E: Inflammatory scores. Data are expressed as mean ± SE, of number of rats indicated as n in each column. [a]$P < 0.05$ *vs* opposite diet same stress status group; unpaired student *t* test. WAS: Water avoidance stress; a.u.: Arbitrary unit.

animals exposed to stress was similar to that of non-stressed animals (fed ERF or standard diet, $P > 0.05$ paired and unpaired *t* test, respectively) (Figure 4C-E). Of the animals exposed to rWAS, 4 out of 10 in the ERF-fed group (40%) and 7 out of 8 in the standard diet-fed group (87.5%) exhibited positive inflammatory scores ($P = 0.07$, two-tailed Fisher's exact test).

There was no correlation between inflammatory scores and visceral pain responses ($R_p = 0.17$, $P > 0.05$).

Influence of ERF diet on rWAS-induced defecation

No diarrhea was observed in either group and all the feces expelled during the stress period were formed. Time course study showed that in the standard diet, the average defecation response to WAS took place during the first 0-15 min (1.05 ± 0.12 FPO/15 min per 100 g body weight) followed by little to no defecation during the remaining 15-60 min of exposure (0.22 ± 0.02 FPO/15 min per 100 g body weight). This response was significantly increased in ERF compared with the standard diet group (1.42 ± 0.07 and 0.31 ± 0.03 FPO/15 min per 100 g of body weight, respectively) [$F(3.320) = 85.73$, $P < 0.0001$] (Figure 5A and B). Even though for both diet groups, over the 10 d of WAS, most of the defecation occurred in the first 15 min of exposure to stress, the average defecation in both the 0-15 min and 15-60 min periods were significantly higher in the ERF diet than the standard diet group (1.42 ± 0.07 *vs* 1.05 ± 0.12 and 0.31 ± 0.03 *vs* 0.22 ± 0.02 FPO/15 min per 100 g body weight, respectively; $P < 0.05$; Figure 5A and B).

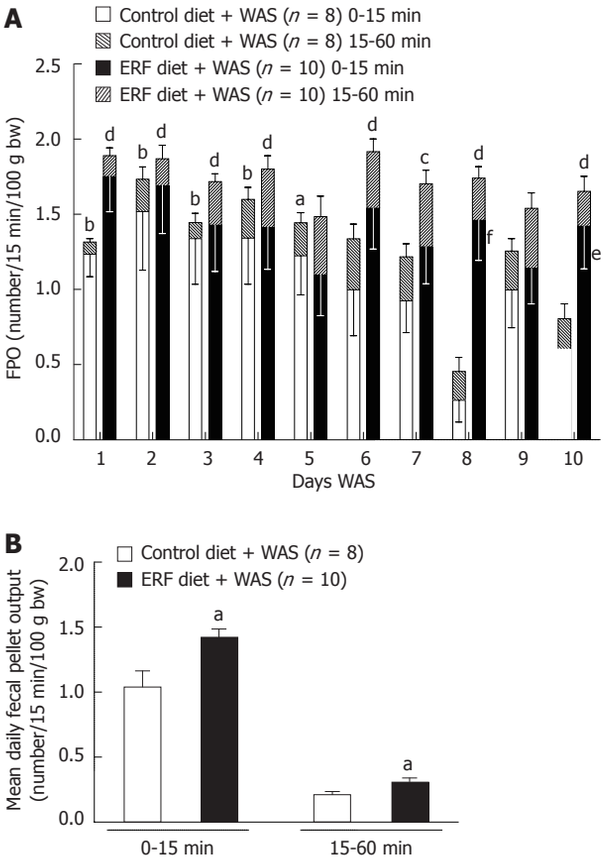

Figure 5 Defecation induced by repeated water avoidance stress in rats fed either standard or 4% enzyme-treated rice fiber prebiotic diet. A: Time-course of hourly defecation expressed as number/15 min per 100 g of body weight for 0-15 min and 15-60 min time periods over the 10 d of 1-h water avoidance stress (WAS). [a]*P* < 0.05, [b]*P* < 0.01 *vs* standard diet interval 0-15 min; [c]*P* < 0.05, [d]*P* < 0.01 *vs* enzyme-treated rice fiber (ERF) diet interval 0-15 min; [e]*P* < 0.05, [f]*P* < 0.01 *vs* standard diet interval 0-15 min; 2-way ANOVA followed by Bonferroni post hoc test; B: Mean daily defecation expressed as number/15 min/100 g of body weight for 0-15 min and 15-60 min intervals. [a]*P* < 0.05 *vs* control diet same time interval; unpaired student *t* test. Data are expressed as mean ± SE, *n* as indicated in parenthesis for each group. FPO: Fecal pellet output.

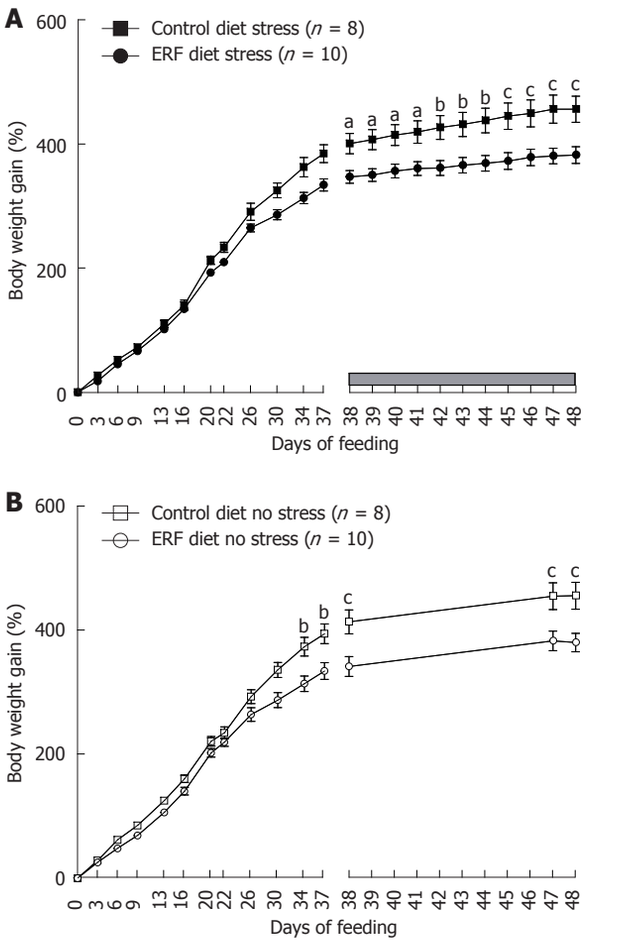

Figure 6 Body weight gain in rats fed either standard or 4% enzyme-treated rice fiber prebiotic diet: influence of repeated water avoidance stress. Rat body weight gain from weaning age (day 21) and during 37 d followed by 10 d of water avoidance stress (WAS) (A) or not (B). Data are expressed as mean ± SE, *n* as indicated in parenthesis for each group. [a]*P* < 0.05, [b]*P* < 0.01, [c]*P* < 0.001 *vs* enzyme-treated rice fiber (ERF) diet group; 2-way ANOVA followed by Bonferroni post hoc test.

ERF diet slows rat body weight gain independently of stress status

The body weights of rats at postnatal day 21 were similar in the four assigned groups before the start of treatments: standard diet + rWAS: 53.4 ± 2.4 g, standard diet + no stress: 58.0 ± 1.2 g, ERF diet + rWAS: 58.2 ± 1.2 g and ERF diet + no stress: 58.3 ± 1.1 g [$F(3.30) = 2.223$, $P = 0.10$]. During the whole experimental period (day 0-48), body weight gain expressed in % from initial body weight showed a significant interaction between diet and time in rats assigned to rWAS [$F(22.368) = 2.455$, $P = 0.0003$] (Figure 6A) or not [$F(14.210) = 2.808$, $P = 0.0007$] (Figure 6B). The percentage of body weight gain started to be significantly higher in standard diet *vs* 4% ERF diet group assigned to rWAS on day 38 (400.9% ± 16.5% *vs* 347.3% ± 10.2%, respectively, $P < 0.05$) or no stress on day 34 (373.3% ± 15.1% *vs* 313.7% ± 12.5%, respectively, $P < 0.01$). Animals on standard diet still maintained a 1.3-fold higher body weight gain than rats on ERF diet over the 10 d of rWAS or no stress (Figure 6A and B). No significant differences were detected in body weight gain between stressed and non-stressed rats in either standard [$F(14.210) = 0.1426$, $P = 0.99$] or ERF [$F(14.240) = 0.1252$, $P = 1.00$] diet groups.

DISCUSSION

In the present study, we demonstrated that exposure to a psychological stressor in the form of WAS 1 h per day once or repeated for 10 d in rats induced stress-related visceral analgesia in as much as 87.3% of the animals tested using a novel non-invasive solid-state manometric method. Additionally, we showed that chronic feeding with a prebiotic diet containing 4% ERF hemi-cellulose-rich dietary fibers, enhanced the expression of stress-induced visceral analgesia at all periods of testing, reduced by 2.2-fold the number of animals displaying stress-induced colonic microscopic alterations, decreased by 76% the isobutyrate cecal content, and slowed rats body weight gain independently of stress exposure and facilitated colonic fecal expulsion.

Over the years, a number of acute and chronic stress paradigms have been developed to mimic the alterations of visceral sensitivity seen in IBS patients[37,38]. Acute exposure to WAS is a well-characterized psychological stressor that induces the transcription of *corticotropin-releasing factor* (*CRF*) gene in the paraventricular nucleus of the hypothalamus[23,39] and consequently activates the pituitary-adrenal axis along with inducing a brain CRF receptor subtype 1-mediated stimulation of colonic motor function assessed at the end of the first hour of WAS[23,40]. In animals surgically equipped for EMG monitoring of the VMR to CRD as classically performed[24,35,41], this combined stress paradigm (surgery, post-surgical housing and rWAS 1 h daily for 10 d) induces visceral hypersensitivity in 82%-86% of the animals starting 24 h after the first exposure[41] which is maintained up to 40 d after the last stress session[24,25,42]. In the present experiment however, using a novel non-invasive solid-state manometric method of visceral sensitivity monitoring to CRD that bypassed the surgery and subsequent single housing[31,33], the majority of rats (66.7% to 85.7%) fed a normal diet exposed to rWAS exhibited a consistent visceral analgesic response. This was observed when CRD was performed within 45-50 min after either one or 10 sessions of WAS. Likewise, we recently demonstrated that rWAS for 10 d resulted in the development of visceral analgesia in up to 72.2% of mice tested non-invasively for the VMR to CRD[31]. Taken together these data provide evidence of visceral stress-induced analgesia in rodents as previously established in the somatic pain field[43].

Moreover, previous and present findings point to the possibility that underlying mechanisms of visceral hypersensitivity may involve both disturbances in descending inhibitory pathways along with sensitization of pain pathways in response to stress as demonstrated in somatic pain studies[44]. Indeed, we recently delineated that 69.2% of single housed mice equipped with EMG electrode to record VMR to CRD developed visceral hypersensitivity in response to a similar regimen of rWAS exposure when monitored 24 h after the last stress session[31]. Therefore VMR alterations reflects not only the effect of the stressor *per se* but also the basal state of the animal and conditions associated with visceral pain monitoring, namely the chronic implantation of abdominal wall electrodes, post surgery medications including antibiotics and single housing thereafter[31]. Other studies showed that skin incision correlates with the development of long-lasting visceral hyperalgesia in rats[45], and that stress becomes proalgesic in rats previously exposed to surgery[46]. To date, there is only one report using another minimally-invasive method, the abdominal withdrawal reflex (AWR), which suggests that rWAS in male Wistar rats decreases their threshold to visceral pain[47]. However, AWR monitoring requires observation of abdominal wall musculature contractions and this semi-quantitative score is subjective. Using this non-invasive ICP monitoring, we have previously demonstrated a hyperalgesic response in Wistar rats following a peripheral injection of the selective CRF_1 receptor agonist, cortagine, known to act directly on CRF_1 receptor subtype expressed in the colon[33], further indicating that the analgesia observed in response to an acute psychological stress in the present study is likely to recruit stress-related brain inhibitory mechanisms. Taken together the previous and present data indicate that this new method of VMR monitoring is valuable to unravel both analgesia and hyperalgesia without confounding factors in rats. In the field of somatic pain, the underlying mechanisms of stress-induced analgesia can be mediated by opioid or non-opioid dependent inhibitory systems[43]. Our preliminary results suggest that WAS-induced visceral analgesia in male rats is naloxone-independent[48]. Further studies are warranted to delineate the underlying components of the visceral analgesic response to rWAS in particular, the role of endocannabinoid tone recently reported to be increased by this stressor[42].

Interestingly, chronic feeding of rats with 4% ERF prebiotic diet potentiated the acute or repeated WAS-induced visceral analgesia. This was shown by the prolongation of the analgesic response to 24 h after the last rWAS session and expansion to all CRD pressure levels (20, 40 and 60 mmHg) compared to normal diet in which the visceral analgesia was mainly observed at 40 mmHg within the 45 min post WAS exposure. Additionally, ERF completely prevented the development of hyperalgesia seen in 14.3%-33% of rats fed a standard diet and undergoing CRD at 60 mmHg after WAS on day 1 or rWAS on day 10. These observations support the analgesic potential of ERF prebiotic diet on visceral sensitivity. In a previous report, 4% ERF diet reduced restraint stress-induced visceral hypersensitivity as monitored with AWR[19]. Collectively, these findings indicate that ERF prebiotic diet may be beneficial to strengthen the stress-related visceral analgesia as well as to curtail the development of visceral hypersensitivity.

Prebiotics as dietary carbohydrates are a source of carbon and energy for colonic bacteria which ferment SCFAs primarily to acetate, propionate and butyrate[13,49]. Based on the local site of action of prebiotics, we investigated the potential peripheral colonic mechanisms through which ERF diet enhances the stress-related analgesic response by first assessing changes in organic acid cecal content. The molar ratio among the three major SCFAs (constituting roughly 90% of the SCFAs in the lumen) was ~ 64:15:19 for acetate:propionate:butyrate/isobutyrate in the animals fed standard diet (stressed or non-stressed), which is close to the average ratio of ~ 60:20:20 described in mammals[49] while that of rats fed ERF diet was ~ 71:12:15. The change in butyrate/isobutyrate molar ratio observed in ERF fed rats was linked to an increase in butyrate consistent with our previous study[19] and a concomitant reduction in isobutyrate. Isobutyrate is derived from valine microbial degradation obtained by proteolysis of endogenous and dietary proteins and not from carbohydrates as are propionate and acetate[50]. As both standard and ERF diets contain identical levels of protein (Table 1), differences in diet protein content are unlikely to account for the reduction observed. Despite the observed increase in butyrate in ERF-fed animals, we

found no correlation between the visceral pain response and butyrate cecal concentration which does not support an underlying role in ERF modulatory effect on visceral sensitivity. In addition, while butyrate enemas have been shown to dose-dependently decrease visceral sensitivity in healthy volunteers[51], other reports indicate that they promotes visceral hypersensitivity in rats[52,53] and clinical studies showed that colonic butyrate levels are increased in IBS patients[54]. Interestingly however, we found a positive correlation between isobutyrate and total butyrate (isobutyrate + butyrate) cecal concentrations and visceral pain responses. Reduction in isobutyrate cecal levels associated with a concomitant increase in acetate and decrease in propionate as observed in ERF-fed rats, may have contributed to lowering the pH in the colon of those animals possibly participating to the modulation of the colonic microflora[55,56] and the reduction in visceral pain observed. Indeed, prebiotics alter the composition and balance of microflora, both in the colonic lumen and at the mucosal surface, in which beneficial bacteria such as *Bifidobacteria* and *Lactobacilli* become of greater prominence[57]. Of interest, probiotics (mainly *Lactobacilli*) have been found to exert antinociceptive effects on stress-induced visceral hypersensitivity in rodents, particularly by increasing the expression of μ-opioid and cannabinoid receptors in intestinal epithelial cells[18, 58-60].

Another possible mechanism through which ERF diet may have enhanced visceral analgesia is by preventing rWAS-induced subtle immune and structural histological changes in colonic mucosa as demonstrated by the reduction in the percentage of rats exhibiting histological alterations. Recently, in a murine colitis model, 4% ERF diet has been shown to reduce inflammation by modulating colonic environment and regulating immune cell differentiation[12]. There is also evidence that rWAS in naïve animals increases colonic permeability[27,28,30], which combined with a longer small intestinal transit time may enhance bacterial translocation[61,62], a phenomenon involved in the development of visceral hypersensitivity in rats[29]. Prebiotics and probiotics have a beneficial influence on gastrointestinal motility arising from a combination of increased bacterial mass, increased stool water as well as increased colonic tone, all resulting in accelerated transit[8,63]. Likewise in the present study the well established defecation response to rWAS[23,48] seen in the standard diet group was increased in ERF fed rats either when expressed by rat or per 100 g body weight. WAS is a mild stressor that does not induce diarrhea, the maintenance of a higher defecation in rats fed 4% ERF diet may be beneficial by facilitating fecal evacuation.

Lastly, independently of its effects on visceral sensitivity, colonic epithelial, immune and motor functions, 4% ERF diet compared to standard diet reduced the body weight gain in rats. Numerous reports have described the effect of prebiotics on the food intake regulation, fat mass and weight gain in experimental studies[64]. Although we did not precisely evaluate food intake in our experiment, these data are consistent with our observation. rWAS however, did not alter rats weight gain in the different groups, confirming previous reports[24].

In conclusion, the present findings demonstrate that under conditions of non-invasive solid-state manometry method to monitor visceral pain, a repeated mild psychological homotypic stressor such as WAS induces stress-related visceral analgesia in rats which so far has been mainly established in the somatic pain field. In addition, our data support an enhancing effect of the prebiotic ERF diet on stress-related visceral analgesia together with increased evacuation of colonic content and reduction in body weight gain. When evaluated in the context of research supporting the use of pre- or probiotics as treatment option for IBS[65], these preclinical findings point toward ERF ability to enhance the analgesic response which may be beneficial in IBS patients in whom altered descending inhibitory pathways have been described[66,67].

COMMENTS

Background

Alterations in intestinal microbiota, mucosal barrier function, and immune system have been implicated to contribute to irritable bowel syndrome (IBS) pathogenesis. Visceral hypersensitivity, a key feature of IBS, can be associated with a low grade colonic mucosal inflammation in a subset of patients. Recently, a new prebiotic, enzyme-treated rice fiber (ERF), has been shown to reduce inflammation and major symptoms in the murine dextran sodium sulfate colitis model by modulating the colonic microbiota environment and regulating immune cell differentiation. This study aimed to evaluate in rats the effects of chronic prebiotic diet on stress-related alterations of visceral sensitivity and colonic motor functions when monitored non-invasively, using repeated intermittent exposure to a psychological stressor in the form of water avoidance stress (WAS).

Research frontiers

It has been recently suggested that the basal state of the animals largely influences the visceral pain responses to a psychological stressor. Indeed, in mice the impact of repeated intermittent exposure to WAS on the visceral pain responses to colorectal distension varies in function of the basal state of the animal. WAS was reported to induce analgesic response when mice were tested using the novel non-invasive method of solid-state manometry which avoids prior surgery to implant recording electrodes and subsequent single housing. By contrast, mice tested with the traditional method of electromyography with the electrode surgically implanted on the abdominal muscles and post-surgical single housing, developed visceral hyperalgesia following exposure to the same repeated WAS.

Innovations and breakthroughs

The results of the present study demonstrate that under conditions of a non-invasive manometry method to monitor visceral pain, a repeated mild psychological homotypic stressor such as WAS induces stress-related visceral analgesia in rats as well, expanding our previous reports in mice. This opens a new field of investigation on stress-related visceral analgesia in rodents which so far has been little explored compared with the somatic pain field. In addition, the data support an enhancing effect of the prebiotic ERF diet on WAS-related visceral analgesia together with increased evacuation of colonic content and reduction in body weight gain.

Applications

In the context of a critical relationship between the composition and stability of the microbiota and gastrointestinal sensory-motor function as well as neuroimmune interactions within the brain-gut axis, these preclinical findings point toward ERF ability to enhance the analgesic response. In the view of growing reports suggesting that IBS patients present altered descending pain inhibitory pathways, the use of ERF as a prebiotic to enhance visceral analgesia is clinically relevant and could be therapeutically beneficial.

Terminology

Non-invasive method of monitoring visceromotor response (VMR) to colorectal distension (CRD) is a novel surgery-free method of solid-state manometry assessing changes in intraluminal colonic pressure (ICP) induced by abdominal muscles contractions in response to discomfort/painful colorectal distensions.

Enzyme-treated rice fiber is a new prebiotic made from rice bran containing dietary fiber and fat soluble fraction.

Peer review

It is a very well designed investigation, the topic is very interesting for the readers, the article is very well written, the results are applicable, the conclusions are valuable.

REFERENCES

1 **Longstreth GF**, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. *Gastroenterology* 2006; **130**: 1480-1491
2 **Chang L**. Review article: epidemiology and quality of life in functional gastrointestinal disorders. *Aliment Pharmacol Ther* 2004; **20** Suppl 7: 31-39
3 **Chang FY**, Lu CL, Chen TS. The current prevalence of irritable bowel syndrome in Asia. *J Neurogastroenterol Motil* 2010; **16**: 389-400
4 **Liu J**, Hou X. A review of the irritable bowel syndrome investigation on epidemiology, pathogenesis and pathophysiology in China. *J Gastroenterol Hepatol* 2011; **26** Suppl 3: 88-93
5 **Dickhaus B**, Mayer EA, Firooz N, Stains J, Conde F, Olivas TI, Fass R, Chang L, Mayer M, Naliboff BD. Irritable bowel syndrome patients show enhanced modulation of visceral perception by auditory stress. *Am J Gastroenterol* 2003; **98**: 135-143
6 **Posserud I**, Agerforz P, Ekman R, Björnsson ES, Abrahamsson H, Simrén M. Altered visceral perceptual and neuroendocrine response in patients with irritable bowel syndrome during mental stress. *Gut* 2004; **53**: 1102-1108
7 **Musial F**, Häuser W, Langhorst J, Dobos G, Enck P. Psychophysiology of visceral pain in IBS and health. *J Psychosom Res* 2008; **64**: 589-597
8 **Spiller R**. Review article: probiotics and prebiotics in irritable bowel syndrome. *Aliment Pharmacol Ther* 2008; **28**: 385-396
9 **Tana C**, Umesaki Y, Imaoka A, Handa T, Kanazawa M, Fukudo S. Altered profiles of intestinal microbiota and organic acids may be the origin of symptoms in irritable bowel syndrome. *Neurogastroenterol Motil* 2010; **22**: 512-519
10 **Collins SM**, Denou E, Verdu EF, Bercik P. The putative role of the intestinal microbiota in the irritable bowel syndrome. *Dig Liver Dis* 2009; **41**: 850-853
11 **Oufir LE**, Barry JL, Flourié B, Cherbut C, Cloarec D, Bornet F, Galmiche JP. Relationships between transit time in man and in vitro fermentation of dietary fiber by fecal bacteria. *Eur J Clin Nutr* 2000; **54**: 603-609
12 **Komiyama Y**, Andoh A, Fujiwara D, Ohmae H, Araki Y, Fujiyama Y, Mitsuyama K, Kanauchi O. New prebiotics from rice bran ameliorate inflammation in murine colitis models through the modulation of intestinal homeostasis and the mucosal immune system. *Scand J Gastroenterol* 2011; **46**: 40-52
13 **Szilagyi A**. Use of prebiotics for inflammatory bowel disease. *Can J Gastroenterol* 2005; **19**: 505-510
14 **Bouin M**, Plourde V, Boivin M, Riberdy M, Lupien F, Laganière M, Verrier P, Poitras P. Rectal distention testing in patients with irritable bowel syndrome: sensitivity, specificity, and predictive values of pain sensory thresholds. *Gastroenterology* 2002; **122**: 1771-1777
15 **Ohman L**, Simrén M. Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 163-173
16 **Elsenbruch S**. Abdominal pain in Irritable Bowel Syndrome: a review of putative psychological, neural and neuro-immune mechanisms. *Brain Behav Immun* 2011; **25**: 386-394
17 **Liebregts T**, Adam B, Bertel A, Jones S, Schulze J, Enders C, Sonnenborn U, Lackner K, Holtmann G. Effect of E. coli Nissle 1917 on post-inflammatory visceral sensory function in a rat model. *Neurogastroenterol Motil* 2005; **17**: 410-414
18 **Verdú EF**, Bercik P, Verma-Gandhu M, Huang XX, Blennerhassett P, Jackson W, Mao Y, Wang L, Rochat F, Collins SM. Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. *Gut* 2006; **55**: 182-190
19 **Kanauchi O**, Mitsuyama K, Komiyama Y, Yagi M, Andoh A, Sata M. Preventive effects of enzyme-treated rice fiber in a restraint stress-induced irritable bowel syndrome model. *Int J Mol Med* 2010; **25**: 547-555
20 **Larauche M**, Kiank C, Taché Y. Corticotropin releasing factor signaling in colon and ileum: regulation by stress and pathophysiological implications. *J Physiol Pharmacol* 2009; **60** Suppl 7: 33-46
21 **Taché Y**, Kiank C, Stengel A. A role for corticotropin-releasing factor in functional gastrointestinal disorders. *Curr Gastroenterol Rep* 2009; **11**: 270-277
22 **Gareau MG**, Silva MA, Perdue MH. Pathophysiological mechanisms of stress-induced intestinal damage. *Curr Mol Med* 2008; **8**: 274-281
23 **Bonaz B**, Taché Y. Water-avoidance stress-induced c-fos expression in the rat brain and stimulation of fecal output: role of corticotropin-releasing factor. *Brain Res* 1994; **641**: 21-28
24 **Bradesi S**, Schwetz I, Ennes HS, Lamy CM, Ohning G, Fanselow M, Pothoulakis C, McRoberts JA, Mayer EA. Repeated exposure to water avoidance stress in rats: a new model for sustained visceral hyperalgesia. *Am J Physiol Gastrointest Liver Physiol* 2005; **289**: G42-G53
25 **Larauche M**, Bradesi S, Million M, McLean P, Taché Y, Mayer EA, McRoberts JA. Corticotropin-releasing factor type 1 receptors mediate the visceral hyperalgesia induced by repeated psychological stress in rats. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G1033-G1040
26 **Santos J**, Yang PC, Söderholm JD, Benjamin M, Perdue MH. Role of mast cells in chronic stress induced colonic epithelial barrier dysfunction in the rat. *Gut* 2001; **48**: 630-636
27 **Söderholm JD**, Yang PC, Ceponis P, Vohra A, Riddell R, Sherman PM, Perdue MH. Chronic stress induces mast cell-dependent bacterial adherence and initiates mucosal inflammation in rat intestine. *Gastroenterology* 2002; **123**: 1099-1108
28 **Yang PC**, Jury J, Söderholm JD, Sherman PM, McKay DM, Perdue MH. Chronic psychological stress in rats induces intestinal sensitization to luminal antigens. *Am J Pathol* 2006; **168**: 104-114
29 **Ait-Belgnaoui A**, Bradesi S, Fioramonti J, Theodorou V, Bueno L. Acute stress-induced hypersensitivity to colonic distension depends upon increase in paracellular permeability: role of myosin light chain kinase. *Pain* 2005; **113**: 141-147
30 **Zareie M**, Johnson-Henry K, Jury J, Yang PC, Ngan BY, McKay DM, Soderholm JD, Perdue MH, Sherman PM. Probiotics prevent bacterial translocation and improve intestinal barrier function in rats following chronic psychological stress. *Gut* 2006; **55**: 1553-1560
31 **Larauche M**, Gourcerol G, Million M, Adelson DW, Taché Y. Repeated psychological stress-induced alterations of visceral sensitivity and colonic motor functions in mice: influence of surgery and postoperative single housing on visceromotor responses. *Stress* 2010; **13**: 343-354
32 **Tanabe H**, Sugiyama K, Matsuda T, Kiriyama S, Morita T. Small intestinal mucins are secreted in proportion to the settling volume in water of dietary indigestible components in rats. *J Nutr* 2005; **135**: 2431-2437
33 **Larauche M**, Gourcerol G, Wang L, Pambukchian K, Brunnhuber S, Adelson DW, Rivier J, Million M, Taché Y. Cortagine, a CRF1 agonist, induces stresslike alterations of colonic function and visceral hypersensitivity in rodents primarily through peripheral pathways. *Am J Physiol Gastrointest Liver Physiol* 2009; **297**: G215-G227
34 **Tammpere A**, Brusberg M, Axenborg J, Hirsch I, Larsson H, Lindström E. Evaluation of pseudo-affective responses to noxious colorectal distension in rats by manometric recordings. *Pain* 2005; **116**: 220-226
35 **Ness TJ**, Gebhart GF. Colorectal distension as a noxious visceral stimulus: physiologic and pharmacologic characterization of pseudaffective reflexes in the rat. *Brain Res* 1988; **450**: 153-169

36 **Brusberg M**, Arvidsson S, Kang D, Larsson H, Lindström E, Martinez V. CB1 receptors mediate the analgesic effects of cannabinoids on colorectal distension-induced visceral pain in rodents. *J Neurosci* 2009; **29**: 1554-1564

37 **Larauche M**, Mulak A, Taché Y. Stress and visceral pain: From animal models to clinical therapies. *Exp Neurol* 2011; **17:** 213–234

38 **Larauche M**, Mulak A, Taché Y. Stress-related alterations of visceral sensation: animal models for IBS study. *J Neurogastroenterol Motil* 2011; **17**: 213-234

39 **Kresse AE**, Million M, Saperas E, Taché Y. Colitis induces CRF expression in hypothalamic magnocellular neurons and blunts CRF gene response to stress in rats. *Am J Physiol Gastrointest Liver Physiol* 2001; **281**: G1203-G1213

40 **Martinez V**, Taché Y. CRF1 receptors as a therapeutic target for irritable bowel syndrome. *Curr Pharm Des* 2006; **12**: 4071-4088

41 **Schwetz I**, Bradesi S, McRoberts JA, Sablad M, Miller JC, Zhou H, Ohning G, Mayer EA. Delayed stress-induced colonic hypersensitivity in male Wistar rats: role of neurokinin-1 and corticotropin-releasing factor-1 receptors. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G683-G691

42 **Hong S**, Fan J, Kemmerer ES, Evans S, Li Y, Wiley JW. Reciprocal changes in vanilloid (TRPV1) and endocannabinoid (CB1) receptors contribute to visceral hyperalgesia in the water avoidance stressed rat. *Gut* 2009; **58**: 202-210

43 **Butler RK**, Finn DP. Stress-induced analgesia. *Prog Neurobiol* 2009; **88**: 184-202

44 **Villanueva L**, Le Bars D. The activation of bulbo-spinal controls by peripheral nociceptive inputs: diffuse noxious inhibitory controls. *Biol Res* 1995; **28**: 113-125

45 **Cameron DM**, Brennan TJ, Gebhart GF. **Hind paw incision** in the rat produces long-lasting colon hypersensitivity. *J Pain* 2008; **9**: 246-253

46 **Rivat C**, Laboureyras E, Laulin JP, Le Roy C, Richebé P, Simonnet G. Non-nociceptive environmental stress induces hyperalgesia, not analgesia, in pain and opioid-experienced rats. *Neuropsychopharmacology* 2007; **32**: 2217-2228

47 **Yu YB**, Yang J, Zuo XL, Gao LJ, Wang P, Li YQ. Transient receptor potential vanilloid-1 (TRPV1) and ankyrin-1 (TRPA1) participate in visceral hyperalgesia in chronic water avoidance stress rat model. *Neurochem Res* 2010; **35**: 797-803

48 **Larauche M**, Mulak A, Kim YS, Million M, Taché Y. Repeated psychological stress induces an opiate independent immediate visceral analgesia but no sustained visceral hyperalgesia as assessed by a new non-invasive manometry method. *Neurogastroenterol Motil* 2010; **b**87

49 **Hamer HM**, Jonkers D, Venema K, Vanhoutvin S, Troost FJ, Brummer RJ. Review article: the role of butyrate on colonic function. *Aliment Pharmacol Ther* 2008; **27**: 104-119

50 **Rasmussen HS**, Holtug K, Mortensen PB. **Degradation of** amino acids to short-chain fatty acids in humans. An in vitro study. *Scand J Gastroenterol* 1988; **23**: 178-182

51 **Vanhoutvin SA**, Troost FJ, Kilkens TO, Lindsey PJ, Hamer HM, Jonkers DM, Venema K, Brummer RJ. The effects of butyrate enemas on visceral perception in healthy volunteers. *Neurogastroenterol Motil* 2009; **21**: 952-e76

52 **Tarrerias AL**, Millecamps M, Alloui A, Beaughard C, Kemeny JL, Bourdu S, Bommelaer G, Eschalier A, Dapoigny M, Ardid D. Short-chain fatty acid enemas fail to decrease colonic hypersensitivity and inflammation in TNBS-induced colonic inflammation in rats. *Pain* 2002; **100**: 91-97

53 **Bourdu S**, Dapoigny M, Chapuy E, Artigue F, Vasson MP, Dechelotte P, Bommelaer G, Eschalier A, Ardid D. Rectal instillation of butyrate provides a novel clinically relevant model of noninflammatory colonic hypersensitivity in rats. *Gastroenterology* 2005; **128**: 1996-2008

54 **Treem WR**, Ahsan N, Kastoff G, Hyams JS. Fecal short-chain fatty acids in patients with diarrhea-predominant irritable bowel syndrome: in vitro studies of carbohydrate fermentation. *J Pediatr Gastroenterol Nutr* 1996; **23**: 280-286

55 **Ito Y**, Moriwaki H, Muto Y, Kato N, Watanabe K, Ueno K. Effect of lactulose on short-chain fatty acids and lactate production and on the growth of faecal flora, with special reference to Clostridium difficile. *J Med Microbiol* 1997; **46**: 80-84

56 **Walker AW**, Duncan SH, McWilliam Leitch EC, Child MW, Flint HJ. pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. *Appl Environ Microbiol* 2005; **71**: 3692-3700

57 **Stoidis CN**, Misiakos EP, Patapis P, Fotiadis CI, Spyropoulos BG. Potential benefits of pro- and prebiotics on intestinal mucosal immunity and intestinal barrier in short bowel syndrome. *Nutr Res Rev* 2010; **21**: 1-9

58 **Ait-Belgnaoui A**, Han W, Lamine F, Eutamène H, Fioramonti J, Bueno L, Theodorou V. Lactobacillus farciminis treatment suppresses stress induced visceral hypersensitivity: a possible action through interaction with epithelial cell cytoskeleton contraction. *Gut* 2006; **55**: 1090-1094

59 **Kamiya T**, Wang L, Forsythe P, Goettsche G, Mao Y, Wang Y, Tougas G, Bienenstock J. Inhibitory effects of Lactobacillus reuteri on visceral pain induced by colorectal distension in Sprague-Dawley rats. *Gut* 2006; **55**: 191-196

60 **Rousseaux C**, Thuru X, Gelot A, Barnich N, Neut C, Dubuquoy L, Dubuquoy C, Merour E, Geboes K, Chamaillard M, Ouwehand A, Leyer G, Carcano D, Colombel JF, Ardid D, Desreumaux P. Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. *Nat Med* 2007; **13**: 35-37

61 **Khalif IL**, Quigley EM, Konovitch EA, Maximova ID. Alterations in the colonic flora and intestinal permeability and evidence of immune activation in chronic constipation. *Dig Liver Dis* 2005; **37**: 838-849

62 **Porras M**, Martín MT, Yang PC, Jury J, Perdue MH, Vergara P. Correlation between cyclical epithelial barrier dysfunction and bacterial translocation in the relapses of intestinal inflammation. *Inflamm Bowel Dis* 2006; **12**: 843-852

63 **Jouët P**, Sabate JM, Flourie B, Cuillerier E, Gambini D, Lemann M, Jian R, Coffin B. Effects of therapeutic doses of lactulose vs. polyethylene glycol on isotopic colonic transit. *Aliment Pharmacol Ther* 2008; **27**: 988-993

64 **Roberfroid M**, Gibson GR, Hoyles L, McCartney AL, Rastall R, Rowland I, Wolvers D, Watzl B, Szajewska H, Stahl B, Guarner F, Respondek F, Whelan K, Coxam V, Davicco MJ, Léotoing L, Wittrant Y, Delzenne NM, Cani PD, Neyrinck AM, Meheust A. Prebiotic effects: metabolic and health benefits. *Br J Nutr* 2010; **104** Suppl 2: S1-63

65 **Moayyedi P**, Ford AC, Talley NJ, Cremonini F, Foxx-Orenstein AE, Brandt LJ, Quigley EM. The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. *Gut* 2010; **59**: 325-332

66 **Berman SM**, Naliboff BD, Suyenobu B, Labus JS, Stains J, Ohning G, Kilpatrick L, Bueller JA, Ruby K, Jarcho J, Mayer EA. Reduced brainstem inhibition during anticipated pelvic visceral pain correlates with enhanced brain response to the visceral stimulus in women with irritable bowel syndrome. *J Neurosci* 2008; **28**: 349-359

67 **Wilder-Smith CH**, Schindler D, Lovblad K, Redmond SM, Nirkko A. Brain functional magnetic resonance imaging of rectal pain and activation of endogenous inhibitory mechanisms in irritable bowel syndrome patient subgroups and healthy controls. *Gut* 2004; **53**: 1595-1601

S- Editor Tian L **L- Editor** O'Neill M **E- Editor** Xiong L