

Lactose intolerance in irritable bowel syndrome patients with diarrhoea: the roles of anxiety, activation of the innate mucosal immune system and visceral sensitivity

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SUMMARY

Background

Irritable bowel syndrome patients with diarrhoea (IBS-D) often report intolerance to milk; however, the mechanism underlying these symptoms is unknown.

Aim

To assess the role of psychological factors, immune activation and visceral sensitivity on the development of lactose intolerance (LI) in IBS-D patients.

Methods

Fifty-five IBS-D patients and 18 healthy controls (HCs) with lactase deficiency underwent a 20-g lactose hydrogen breath test (LHBT). Patients were categorised as lactose malabsorption (LM; malabsorption only) or LI [malabsorption plus increase in total symptom score (TSS)]. Measurements included (i) psychological status; (ii) enteric biopsies with quantification of mast cells (MCs), T-lymphocytes and enterochromaffin cells; (iii) serum cytokines; (iv) rectal sensitivity before and after lactose ingestion.

Results

LI was more prevalent in IBS-D patients than HCs [25/55 (46%) vs. 3/18 (17%), $P = 0.029$]. IBS-D patients with LI had (i) higher levels of anxiety than those with LM ($P = 0.017$) or HCs ($P = 0.006$); (ii) increased mucosal MCs compared with LM ($P = 0.006$) and HCs ($P < 0.001$); (iii) raised serum TNF- α compared with LM ($P = 0.034$) and HCs ($P < 0.001$) and (iv) increased rectal sensitivity after lactose ingestion compared with LM ($P < 0.001$) or HCs ($P < 0.001$). Severity of abdominal symptoms after lactose ingestion was associated with the increase in visceral sensitivity after lactose intake ($r = 0.629$, $P < 0.001$), MCs ($r = 0.650$, $P < 0.001$) and anxiety ($r = 0.519$, $P < 0.001$).

Conclusions

IBS-D patients with lactose intolerance are characterised by anxiety, mucosal immune activation and increased visceral sensitivity after lactose ingestion. The presence of these biomarkers may indicate an IBS phenotype that responds to dietary therapy and/or mast cell stabilisers (ClinicalTrials.gov Identifier: NCT01286597).

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INTRODUCTION

Lactose Intolerance (LI) is characterised by digestive symptoms, including bloating, abdominal discomfort and diarrhoea, after ingestion of dairy products. In people with lactase deficiency, lactose is not digested and absorbed in the small bowel, but passes into the colon where bacterial fermentation produces gas and short-chain fatty acids and other products that can cause luminal distension and alter digestive function.^{1, 2} The risk of lactose-triggering symptoms in patients with lactase deficiency is associated with the dose ingested, whether lactose is taken with other foods, gastrointestinal (GI) transit time and the intestinal flora.³ The same mechanism is responsible for intolerance to other poorly absorbed, fermentable carbohydrates (e.g. fructose, fructans) and polyols (e.g. sorbitol) that are ubiquitous in the diet.⁴

Patients with irritable bowel syndrome (IBS) are more likely to report intolerance to dairy products and other foods than healthy individuals.⁵ Indeed, the similarity between IBS and food intolerance symptoms is so striking that it has been proposed that malabsorption of easily fermented carbohydrates (such as lactose in patients with lactase deficiency) is the cause of IBS in many patients⁴ and a combined nutrient and lactulose challenge test has been proposed as a diagnostic test in this condition.⁶ The mechanism underlying the link between IBS and food intolerance is uncertain; however, studies suggest a shared aetiology involving both psychological (e.g. anxiety) and GI dysfunction (e.g. altered gut transit, visceral hypersensitivity).^{7, 8} A large body of work in animal models and IBS patients has found links between psychological state and stress with immune activation in the mucosa and the release of various mediators that alter GI motor and sensory function.^{9–12} Moreover, supernatant obtained from the colonic mucosa and jejunal secretions of IBS patients increases the excitability of mesenteric sensory nerves and induces visceral hypersensitivity in animal models.^{13, 14} These findings suggest that brain–gut interactions are mediated by immune factors. Consistent with this view, prospective clinical studies have linked psychological state, life event stress and activation of the immune system to the development of post-infective IBS.¹⁵

We propose that the same ‘neuro-immune’ modulation of visceral function could also be the pathological mechanism underlying food intolerance. This hypothesis was tested using a validated, clinical experimental model of lactose intolerance in a Chinese population with primary lactase deficiency.¹⁶ Recent studies in this popula-

tion have shown that ingestion of 20 g lactose is three times more likely to cause digestive symptoms in IBS patients than in healthy controls¹⁶ and that both high levels of gas production and visceral hypersensitivity increase the likelihood of patients reporting digestive symptoms after ingestion of 20 g lactose.¹⁷ In this study, we assess whether central psychological factors and peripheral immune activation impact visceral sensitivity after ingestion of lactose in this population and whether these neuro-immune effects explain the high prevalence of food intolerance in IBS patients.

METHODS

Subjects

The study was performed in an adult Chinese population with primary lactase deficiency on genetic testing and previous positive 40 g LHBT. Consecutive patients aged 16–75 years with IBS-D based on the Rome III criteria were recruited between September, 2010 and April 2011 from the out-patient clinic at the Sir Run Run Shaw Hospital in Hangzhou, China (~95% self-referred from community). Healthy controls (HCs) with no history of GI disease or digestive symptoms were recruited by advertisement. No participant had undergone abdominal surgery, had active medical disease or allergic disorders. Subjects taking anti-inflammatory drugs were excluded (e.g. aspirin, steroids and antihistamines).

Study design

As presented in Figure 1, screening, psychosocial status and digestive symptoms were assessed by validated questionnaires and colonoscopy with two biopsies each from the sigmoid colon, ascending colon and terminal ileum. Two weeks after colonoscopy, the 20 g LHBT was performed with barostat measurements of rectal sensitivity obtained before and after the procedure to assess the effect of lactose malabsorption and fermentation on rectal sensitivity. Additionally, serum cytokines were measured after the 20 g LHBT.

The 20 g LHBT was performed as part of a larger series of studies in which three doses of lactose (10 g, 20 g, 40 g) were tested.¹⁶ Thus, although placebo was not used, participants and investigators were blinded to the dose of lactose administered. Similarly, investigators performing measurement of rectal sensitivity and those measuring mucosal immune cells and serum cytokines were unaware of clinical data, questionnaire scores and LHBT results until the end of this study. This study was

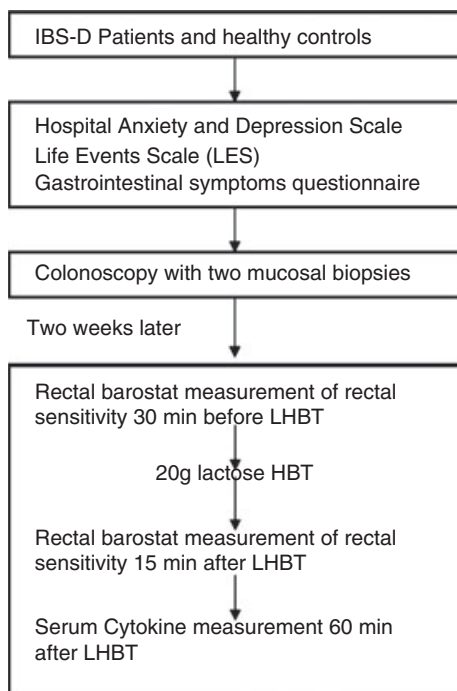


Figure 1 | Study design.

approved by the ethical committee of Sir Run Run Shaw Hospital, conducted in accordance with the Declaration of Helsinki and written informed consent was obtained from all participants. Registration: ClinicalTrials.gov Identifier: NCT01286597.

Assessment of psychosocial status and GI symptoms

Psychological state was assessed by Hospital Anxiety and Depression Score (HADS) and psychosocial stress by The Life Events Scale (LES) of Miller and Rahe modified and validated for use in Chinese populations (Table S1). The Bowel Disease Questionnaire (BDQ) measured frequency and severity of digestive symptoms during the previous 3 months.

Lactose hydrogen breath test (LHBT)

The concentration of hydrogen in end-alveolar breath samples was detected using a Handhold Micro H₂ Meter (Micro Medical Ltd, Basingstoke, UK). Breath hydrogen and symptoms were recorded at 15 min intervals for 3 h after ingestion of 20 g lactose. A positive result of LHBT was defined by a ≥ 20 ppm breath H₂ increase on at least two consecutive readings. The number and the severity of individual symptoms (nausea, bloating, abdominal pain, borborygmi and diarrhoea) during the test were assessed by a Likert scale.¹⁶ Total

symptom score (TSS) was calculated as the sum of the highest intensity value for each symptom. Consistent with recommendations of the National Institutes of Health,² lactose intolerance (LI) was diagnosed if an increase in breath hydrogen during LHBT was accompanied by an increase in symptoms (TSS ≥ 1) on at least two consecutive measurements; lactose malabsorption (LM) was diagnosed if the increase in breath hydrogen was not accompanied by patient reports of symptoms.¹⁶

Histology and immunohistochemistry

Microscopic colitis, eosinophilic infiltration and epithelial abnormalities including microorganisms were excluded by standard microscopy. For immunohistochemistry, 4- μ m sections were incubated in complete medium for 1 h at room temperature with the following monoclonal antibodies: mast cell tryptase (mast cell marker, Abcam, Cambridge, MA, USA, 1:100); chromogranin A (enterochromaffin cells marker, Abcam, 1:10); and CD4, CD8 (T cell markers, Abcam, 1:50). Quantification of mucosal immune cells was performed at $\times 400$ magnifications, 400 μ m \times 400 μ m in lamina propria by three experimenters who were unaware of clinical data and LHBT results.

Rectal barostat

A double-lumen polyvinyl catheter (CTD-Synectics LTD, Stockholm, Sweden) with an adherent, infinitely compliant barostat bag (800 mL capacity, 10 cm long, 20 cm maximal diameter), finely folded, was inserted into the rectum and secured with the proximal border 5 cm from the anal verge. The bag was unfolded by 200 mL of air for 2 min and then deflated. A conditioning ramp distension at 1 mL/s continued to an intra-bag pressure of 40 mmHg at which point rectal capacity was recorded.^{17, 18} The bag was deflated and then measurements of rectal sensations were obtained during a second distension. Volume thresholds for first sensation, urge to defecate, discomfort/pain were recorded.^{17, 18}

Serum cytokine

Serum TNF- α , INF- γ , IL-4 and IL-10 level were quantified using enzyme-linked immunosorbent assay kits (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions (Excell bio, Shanghai, China). Optical density was measured at a wavelength of 450 nm. Density values were correlated linearly with the concentrations of cytokine standards.

Statistical analysis

Continuous, normally distributed data are reported as mean \pm s.d. and nonnormally distributed data as median and interquartile range. Comparisons of continuous data between groups were made by unpaired *t*-tests. Comparison of rectal sensation thresholds before and after LHBT used paired *t*-tests. Categorical data were calculated as percentage in each group of subjects, and proportional differences between groups were calculated using χ^2 analysis. Relationships between pairs of variables were evaluated using the Pearson rank test.

Our aim was to assess whether immune factors are involved in food intolerance. In particular, mast cells have been implicated in GI dysfunction in both Western and Far Eastern populations.^{19–21} The primary analysis was a comparison of the number of mast cells in the enteric mucosa in IBS patients with and without LI after 20 g LHBT. Power calculations based on published studies of mast cells in enteric biopsies from IBS patients (9.7 ± 2.4 /hpf) and HCs (4.5 ± 2.3 /hpf)²¹ indicate that 18 participants are required to identify a 20% difference in mast cell numbers between groups with 90% power ($P < 0.05$).

One-way analysis of variance (ANOVA) and L-S-D's testing that provides results corrected for multiple comparisons were used to compare mean values between IBS-D patients with LI, IBS-D patients with LM, and HCs. All analyses were conducted using the SPSS version 16.0 statistical package.

RESULTS

Demographic characteristics

Sixty-three IBS-D patients and 20 HCs were recruited. Three patients with ulcerative colitis at colonoscopy were excluded and five patients declined the barostat studies. Two controls with digestive symptoms were excluded. Thus, 55 patients and 18 HCs were included in the final analysis (Table 1). No participant had the C/T-13910 or related single nucleotide polymorphism (SNP) associated with lactase persistence and all participants had a positive 40 g LHBT documented during a previous study.¹⁶ Lactose intake in IBS patients and HCs in this Chinese population is low (<12 g/day).¹⁶

Hydrogen breath test

The number of participants with lactose malabsorption (>20 ppm rise in breath hydrogen) was similar in IBS-D patients and HCs [48/55 (87%) vs. 16/18 (89%), $P = 0.856$]; however, the proportion with LI was greater

Table 1 | Demographic, psychological and social characteristics of IBS-D patients and Healthy Controls (HCs) with results of 20 g Lactose Hydrogen Breath Test. IBS-D patients had higher levels of anxiety and Life Event Stress than HCs. The patient group also had increased prevalence of LI and higher total symptom score after lactose ingestion

	IBS-D (<i>n</i> = 55)	Healthy controls (<i>n</i> = 18)	<i>P</i>
Age (years), mean \pm s.d.	43.4 \pm 11.2	43.6 \pm 11.4	0.950
Gender: male/female	39/16	12/6	0.925
BMI (kg/m ²), mean \pm s.d.	23.5 \pm 2.49	22.7 \pm 3.04	0.291
Married, N (%)	47(85.5%)	16(88.9%)	0.713
Anxiety	5.19 \pm 3.04	3.05 \pm 1.55	0.006
Depression	4.96 \pm 3.28	4.33 \pm 1.45	0.436
Life Event Stress (Inter-Quartile Range)	70.5 (43.5–113.75)	8.0 (0–36.5)	<0.001
LI prevalence (20 g lactose HBT)	25 (45.5%)	3 (16.7%)	0.020
Total Symptom Score (TSS mean \pm s.d.)	5.12 \pm 2.96	0.67 \pm 1.57	<0.001

in the IBS than in the HC group [25/55 (46%) vs. 3/18 (16%), $P = 0.020$] and mean TSS was higher (5.12 ± 2.96 vs. 0.67 ± 1.57 , $P < 0.001$) (Table 1). Thus, 25 IBS-D patients were categorised as LI and 23 as LM only. IBS patients with LI and LM on LHBT and also those participants with negative 20 g LHBT (7 IBS patients, 2 controls) had similar demographic characteristics, severity of IBS symptoms (Table 2). Per-protocol, IBS patients with a negative 20 g LHBT were excluded from the primary analysis; however, the significance of results were unchanged if these individuals were included in the LM group.

Psychological state (HADS) and Life Event Stress (LES)

Anxiety (5.19 ± 3.04 vs. 3.05 ± 1.55 , $P = 0.006$) and LES [70.5 (43.5–113.75) vs. 8.0 (0–36.5), $P < 0.001$] were both higher in IBS-D patients than in HCs (Table 1). Furthermore, anxiety was higher in patients with LI than in those with LM (5.78 ± 3.27 vs. 3.52 ± 2.23 , $P = 0.017$) and HCs (3.05 ± 1.55 , $P = 0.006$). Similar scores for depression were present in all groups (Table 2).

Rectal sensitivity

There were no differences in volume thresholds for rectal sensations between groups before lactose intake. After

Table 2 | Demographic, psychological and psychosocial characteristics of IBS-D patients with lactose intolerance (LI) and with lactose malabsorption (LM) as assessed by 20 g Lactose Hydrogen Breath Test. IBS-D patients with LI had higher levels of anxiety than those with LM. The severity of malabsorption (H₂ excretion) and GI symptoms were similar in both IBS groups

	IBS-D with LI (n = 25)	IBS-D with LM (n = 23)	P	IBS-D without LI (N = 30)*	P
Age (years), mean ± s.d.	41.5 ± 11.9	44.1 ± 9.98	0.97	43.4 ± 10.8	0.97
Gender: Male:female	17:8	15:8	0.84	18:12	0.54
Body mass index (kg/m ²), mean ± s.d.	23.4 ± 2.5	23.6 ± 2.37	0.42	23.6 ± 2.5	0.78
Married, N (%)	21 (84.0%)	19 (82.6%)	0.89	24 (80%)	0.70
Severity of GI symptoms†	3.8 ± 1.6	3.5 ± 2.2	0.36	3.6 ± 2.1	0.57
Frequency of GI symptom†	4.6 ± 0.9	4.9 ± 1.1	0.25	4.7 ± 0.9	0.46
HADs anxiety	5.8 ± 3.3	3.5 ± 2.2	0.02	3.8 ± 2.3	0.01
Depression	4.9 ± 3.5	5.8 ± 3.0	0.46	5.0 ± 3.2	0.87
Life event scale (stress)	105.50 (58–166.5)	60 (15–104)	0.20	65 (40–123)	0.36
Peak H ₂ value (ppm)	54 (37.5–71.5)	56 (26.5–70)	0.69	58 (29.6–75)	0.46
Total H ₂ excretion (ppm × min)‡	2385 (1278–5182)	3195 (1185–5508)	0.80	3045 (1076–5467)	0.74

* Includes 7 IBS-D patients with no increase in breath hydrogen on 20 g LHBT.

† During the last 3 months before interview.

‡ Amount of H₂ excretion (ppm × min): expressed as area under the concentration–time curves (AUC, ppm, 3 h). Data are expressed as median and 25–75% quartile values.

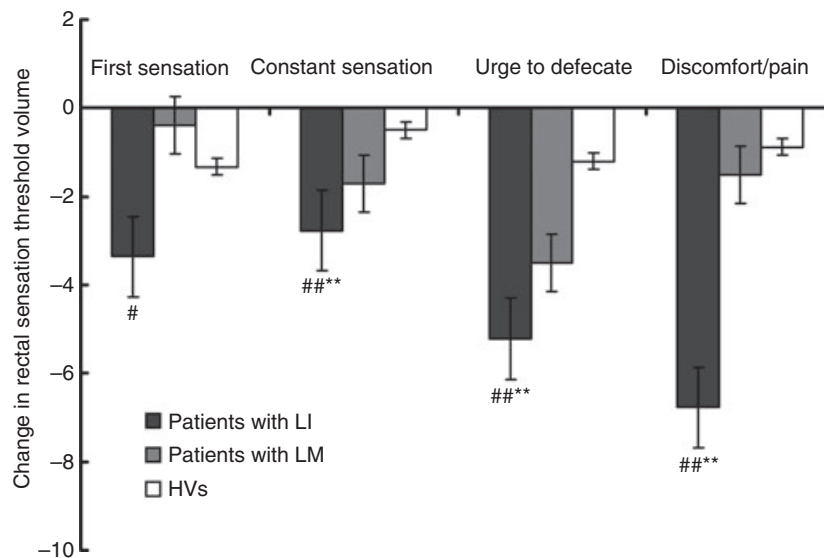


Figure 2 | Effect of lactose ingestion on rectal sensation thresholds in study participants. Negative values (reduction in volume threshold) indicate an increase in visceral sensitivity. The effect of lactose ingestion on sensation threshold was more pronounced in lactose intolerance (LI) compared with lactose malabsorption (LM) patients and healthy controls (HCs) for all sensations (comparison with LM: **P* < 0.05, ***P* < 0.01; HCs: #*P* < 0.05, ###*P* < 0.01).

LHBT, the threshold of rectal discomfort/pain in IBS-D patients fell and was lower than HCs (*P* = 0.002) (Table S2). Further analysis revealed that this increase in visceral sensitivity was seen in IBS-D patients with LI, but not in IBS-D patients with LM (Figure 2).

Mucosal immune cells

Histology showed no microscopic colitis, eosinophilia or parasites in any participant. Immunohistochemistry revealed increased MCs (primary outcome) in the terminal ileum, ascending and sigmoid colon, increased

ECCs in terminal ileum and sigmoid colon and increased CD4+ (not CD8+) T cells in the sigmoid colon in IBS-D patients compared with HCs (Figure 3). MC counts in the terminal ileum were higher in IBS patients with LI compared with patients with LM [11.25 (9.8–19.5) vs. 7.75 (6.47–11.7, $P < 0.05$)] and HCs [7.14 (6.05–9.29), $P < 0.05$ compared to LI group] (Table 3).

Serum cytokines

TNF- α release was increased (8.51 ± 2.32 vs. 5.75 ± 2.12 pg/mL, $P = 0.001$), and IL-10 was decreased (10.33 ± 4.96 vs. 13.67 ± 5.32 pg/mL, $P = 0.034$) after LHBT in IBS-D patients compared with HCs. No differences were observed between these two groups for IL-4 and IFN- γ ($P > 0.05$). Similarly, TNF- α levels were higher in IBS-D patients with LI compared with those

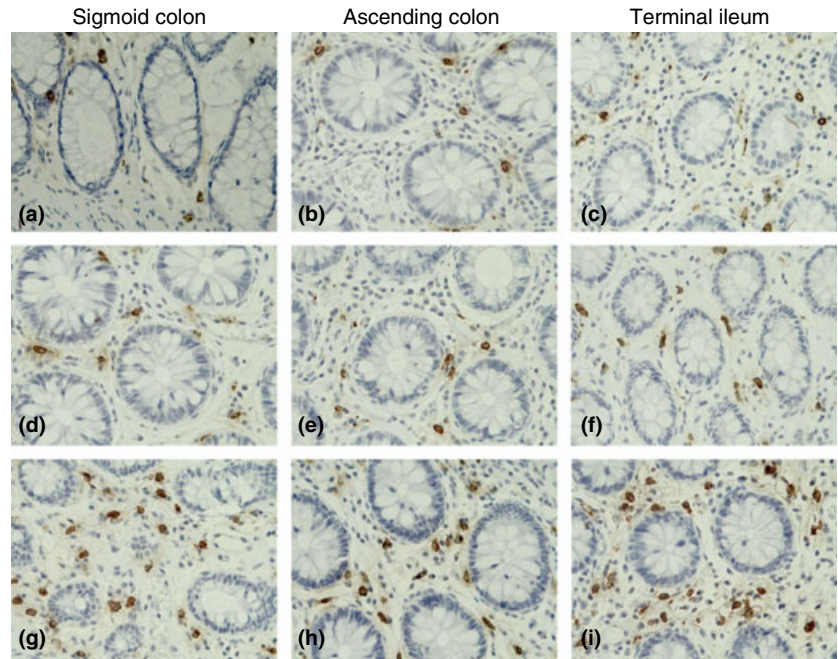


Figure 3 | Representative photomicrographs showing tryptase positive mast cells (MCs) in the colonic mucosa of a healthy control (HCs) (a–c), an IBS-D patient with lactose malabsorption (LM) (d–f) and an IBS-D patient with lactose intolerance (LI) (g–i). IBS-D patients with LI had increased mucosal MCs compared with LM and HCs.

Table 3 | Mucosal immune cells in IBS-D patients with lactose intolerance (LI), IBS-D patients with lactose malabsorption (LM) and Healthy Controls (HCs). IBS-D patients with LI had increased mucosal mast cells (MCs) and other cells involved in the innate mucosal immune system compared with LM and HCs

	IBS-D patients with LI (n = 25)	IBS-D patients with LM (n = 23)§	HCs (n = 18)
MCs sigmoid	9.4 (7.3–10.8)†	7.3 (6.2–9.3)	6.6 (6.0–7.4)
MCs ascending	10.2(7.7–15.2)†	7.0 (6.2–9.7)	7.0 (6.3–8.0)
MCs terminal ileum	11.2 (9.8–19.5)**‡	7.8 (6.4–11.7)	7.1 (6.0–9.2)
ECCs sigmoid	6.7 (4.2–9.6)‡	4.8 (3.2–6.3)‡	2.8 (1.8–3.6)
ECCs ascending	3.6 (2.6–6.5)	4.4 (3.4–5.7)	3.5 (2.3–4.6)
ECCs terminal ileum	5.3 (4.3–8.9)‡	4.5(3.0–7.0)‡	3.5 (2.6–3.8)
CD8+T sigmoid	38.5 ± 10.9	32.1 ± 13.9	32.6 ± 17.6
CD8+T ascending	46.1 ± 11.3	49.1 ± 11.3	43.9 ± 15.8
CD8+T terminal ileum	70.1 ± 12.3†	55.1 ± 12.3	44.9 ± 12.8
CD4+ sigmoid	58.5 ± 9.9‡	40.8 ± 5.4	35.6 ± 12.4
CD4+ ascending colon	68.5 ± 9.90	55.8 ± 17.5	55.6 ± 15.4
CD4+ terminal ileum	88.5 ± 10.9	75.8 ± 18.5	65.8 ± 16.3

Compared with patients with LM * $P < 0.05$, ** $P < 0.01$; compared to HCs † $P < 0.05$, ‡ $P < 0.01$.

§ Excludes 7 IBS-D patients with no increase in breath hydrogen on 20 g LHBT. Inclusion of these patients in this group did not alter results.

with LM (8.98 ± 2.7 vs. 7.0 ± 1.58 pg/mL, $P = 0.016$) and HCs (6.21 ± 1.72 pg/mL, $P < 0.001$ compared to LI group) (Table S3).

Effects of neuro-immune factors on rectal sensitivity and lactose tolerance

Anxiety scores were associated with MC count in sigmoid colon ($r = 0.451$, $P < 0.001$), ascending colon ($r = 0.351$, $P = 0.005$) and terminal ileum ($r = 0.489$, $P < 0.001$). Applying established criteria, patients with anxiety (≥ 8 scores on HADs, $N = 11$) had higher MC counts compared with those without anxiety [14 (9–16) vs. 8(7–9), $P < 0.05$]. Categorical analysis revealed that patients with a high mast cell count in the terminal ileum [>11 (mean), $N = 20$] had a significant increase in visceral sensitivity (i.e. decrease in urgency, discomfort/pain threshold) compared with those with less mast cell [≤ 11 (mean), $N = 30$]. Anxiety was associated also with the increase in rectal sensitivity after lactose ingestion [first sensation ($r = 0.246$, $P = 0.045$), urgency ($r = 0.435$, $P < 0.001$), discomfort/pain ($r = 0.519$, $P < 0.001$)] and the severity of abdominal symptoms (TSS) ($r = 0.519$, $P < 0.001$). TSS was also associated with the increase in rectal sensitivity [first sensation ($r = 0.310$, $P = 0.008$), urgency ($r = 0.511$, $P < 0.001$), discomfort/pain ($r = 0.594$, $P < 0.001$), MCs in terminal ileum ($r = 0.650$, $P < 0.001$) and serum TNF- α release ($r = 0.291$, $P = 0.050$). (Table S4).

DISCUSSION

Digestive symptoms are often attributed to intolerance of dairy products and other foods by members of the community and, in particular, by patients with functional gastrointestinal (GI) diseases such as the irritable bowel syndrome (IBS)^{5, 22} However, studies have been confounded by discrepancies between self-reports and objective evidence of food intolerance, and a lack of standardised tests able to discriminate between health and disease states^{2, 23} This study applied a validated, clinical experimental model of lactose ingestion in IBS patients with lactase deficiency to gain insights into the pathophysiology of food intolerance. The findings show that IBS patients with digestive symptoms after 20 g lactose ingestion are characterised by anxiety, increased numbers of inflammatory cells in the enteric mucosa and the release of pro-inflammatory cytokines. Moreover, in this group of IBS patients, lactose ingestion induced an increase in visceral sensitivity and the magnitude of this effect was associated with the severity of LI symptoms. These observations provide compelling

evidence of brain–gut interaction and describe a novel mechanism by which neuro-immune factors interact with the diet to cause functional GI symptoms.

Anxiety and life event stress are more prevalent in IBS patients than in healthy controls and have effects on mucosal immunity, visceral sensitivity and digestive function in animal models.²⁴ Psychosocial factors are associated with the persistence of low-grade inflammation and GI symptoms in patients who develop IBS-D after enteric infection.¹⁵ In this study, the same findings were present in IBS-D patients with digestive symptoms after lactose ingestion, but not in IBS-D patients who did not report these problems. Compared with healthy controls and IBS-D patients with lactase deficiency but no symptoms after lactose ingestion, patients with LI had increased mucosal mast cells (MC), CD4+ T-lymphocytes and enterochromaffin cells (ECC) with increased release of pro-inflammatory cytokines after lactose ingestion. There was also a positive association between MC count and the severity of LI symptoms. These results suggest the presence of distinct pathophysiology in IBS-D patients with lactase deficiency who report symptoms after ingestion of a modest dose of lactose and IBS-D patients who do not have a clear association between lactose malabsorption and symptoms.

This study also provides novel insights into the mechanism by which lactose ingestion leads to digestive symptoms and how this could be used to direct treatment. Previous *in vitro* studies have documented MC degranulation after application of specific foods or food antigens on mucosal specimens from patients with food intolerance.^{20, 21} This clinical study demonstrates that in IBS patients with LI, this process can be triggered by fermentation products of lactose; an event that is known to release MC proteases and pro-inflammatory cytokines that impact GI motor and sensory function.^{19, 25} This could be the mechanism by which medications that stabilise the MC membrane improve functional GI symptoms. Lunardi *et al.* found that the symptoms of IBS patients with food intolerance improved during an eight-week treatment of sodium cromoglycate, although patients still followed a normal, non-exclusion diet.²⁶ Similarly, Klooker *et al.* reported that Ketotifen decreased discomfort in response to distension in IBS patients with visceral hypersensitivity and that this effect was associated with improved IBS symptoms.²⁷

An important feature of our study was that rectal sensitivity was assessed both before and after LHBT. There

was no difference in rectal threshold volumes between groups at baseline; however, rectal sensitivity increased after lactose ingestion in IBS-D patients with anxiety and high numbers of mucosal mast cells. This effect on visceral sensitivity was associated also with the severity of LI symptoms on multivariate analysis. These findings build on reports that visceral hypersensitivity can be induced in patients with LI after lactose ingestion⁷ and that similar effects are seen also in IBS patients after ingestion of lactulose.²⁸ Together, these observations indicate that this mechanism of disease may not be limited to LI, but shared by a range of poorly absorbed, fermentable carbohydrates (e.g. fructose, fructans, sorbitol) known as “FODMAPs” that are ubiquitous in the diet.⁴

This study has some important strengths: First, a well-defined population of patients and controls with primary lactase deficiency was studied. Second, unselected IBS-D patients attending clinic were recruited and not patients referred for LHBT due to self-reports or clinical suspicion of LI (lactose intake is very low in this population¹⁶). Third, the diagnostic criteria for LM and LI on 20 g LHBT that were applied have been validated locally and in other populations with a high prevalence of lactase deficiency.¹⁶ Indeed, it is likely that the mechanism of disease described here can be generalised because (i) the pathological basis of LM and LI is universal, (ii) prevalence of LI in Chinese individuals with LM lies within the range of reported values in Caucasians²⁹ and (iii) prevalence of IBS-D is similar and the importance of brain–gut interactions in this condition has been demonstrated in many populations.³⁰ Limitations of this study include: First, the relatively small numbers of patients and controls recruited, although, the numbers of subjects in each group were adequate to test the primary hypothesis (see statistics). Second, placebo control was not used and the sensitivity of 20 g LHBT for LM was not assessed; however, in this study, LHBT was not administered for diagnosis as all participants had documented lactase deficiency but, rather, to identify the cause of symptoms in IBS patients with food intolerance. Third, we assessed the number of MCs in the mucosa rather than markers of MC degranulation in blood, tissue or stool because the latter are subject to confounding factors. It should be noted that measurements of MC degranulation are associated with MC count in animal models,³¹ and that our findings demonstrate an association between MC count and LI symptoms. Fourth, rectal sensations were assessed by barostat bag volume rather than pressure. This

approach is validated;^{17, 18} moreover, in this study, we were not primarily comparing rectal sensitivity between groups at baseline, but rather comparing the increase in sensitivity (decreased volume threshold) before and after lactose ingestion.

In conclusion, this study supports the hypothesis that neuro-immune modulation of visceral function is a pathological mechanism underlying food intolerance. Our observations reveal a close relationship between psychological state, increased numbers of mast cells in the enteric mucosa, with the development of visceral hypersensitivity and food intolerance after lactose ingestion in lactase-deficient patients. These observations are not likely to be limited to dairy products, because lactose is just one of many poorly absorbed, fermentable carbohydrates that are ubiquitous in the diet. Future studies will assess whether these findings have clinical relevance in the diagnosis and management of IBS, for example, whether the presence of high numbers of mucosal immune cells on enteric biopsy identifies an IBS-D phenotype that responds to specific dietary therapy (e.g. reduced FODMAP diet) or medical management with H₁ blockers that inhibit MC degranulation.

AUTHORSHIP

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Author contributions: Jianfeng Yang performed the study, analysed the results and drafted the article. Mark Fox contributed to the design of the study, analysed the results and edited the manuscript. Yanqun Cong contributed to the design and performance of the study. Hua Chu, Xia Zheng and Yanqin Long provided essential patient support and performed the lactose HBT tests. Michael Fried contributed to the design of the study and edited the manuscript. Ning Dai contributed to the design of the study, oversaw the conduct of the study and edited the manuscript. All authors had access to the study data and had reviewed and approved the final version of the article.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Life Events Stress Test.

Table S2. Rectal sensation threshold in baseline and after lactose HBT in IBS-D patients and healthy controls (HCs).

Table S3. Serum cytokine level (pg/mL) of D-IBS patients with LI and patients with LM and healthy controls (HCs).

Table S4. Interaction between anxiety, immune activation, rectal sensitivity and lactose tolerance (Pearson rank test).

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