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Randomised clinical trial: gluten may cause depression in subjects with non-coeliac gluten sensitivity – an exploratory clinical study

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SUMMARY

Background
Current evidence suggests that many patients with self-reported non-coeliac gluten sensitivity (NCGS) retain gastrointestinal symptoms on a gluten-free diet (GFD) but continue to restrict gluten as they report 'feeling better'.

Aim
To investigate the notion that a major effect of gluten in those with NCGS is on mental state and not necessarily on gastrointestinal symptoms.

Methods
Twenty-two subjects (24–62 years, five male) with irritable bowel syndrome who had coeliac disease excluded but were symptomatically controlled on a GFD, undertook a double-blind cross-over study. Participants randomly received one of three dietary challenges for 3 days, followed by a minimum 3-day washout before crossing over to the next diet. Challenge gluten-free food was supplemented with gluten (16 g/day), whey (16 g/day) or not supplemented (placebo). End-points included mental state as assessed by the Spielberger State Trait Personality Inventory (STPI), cortisol secretion and gastrointestinal symptoms.

Results
Gluten ingestion was associated with higher overall STPI state depression scores compared to placebo [$M = 2.03$, 95% CI (0.55–3.51), $P = 0.010$] but not whey [$M = 1.48$, 95% CI (−0.14 to 3.10), $P = 0.07$]. No differences were found for other STPI state indices or for any STPI trait measures. No difference in cortisol secretion was identified between challenges. Gastrointestinal symptoms were induced similarly across all dietary challenges.

Conclusions
Short-term exposure to gluten specifically induced current feelings of depression with no effect on other indices or on emotional disposition. Gluten-specific induction of gastrointestinal symptoms was not identified. Such findings might explain why patients with non-coeliac gluten sensitivity feel better on a gluten-free diet despite the continuation of gastrointestinal symptoms.

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INTRODUCTION

Gluten, the major protein of wheat, has been established as the causative agent in the development of coeliac disease, characterised by small intestinal injury and immunological activation.[1] Gluten has also been implicated as a causal factor in the development of chronic functional gastrointestinal symptoms similar to those classified as irritable bowel syndrome (IBS).[2] In fact, non-coeliac gluten sensitivity (NCGS) has been proposed as a defined entity in which IBS-like symptoms markedly improve on a gluten-free diet (GFD), but coeliac disease has been excluded.[3, 4] However, understanding of this putative entity remains poor and controversial. Several descriptions of it have included patients with intraepithelial lymphocytosis in the duodenum and evidence of immunological activation that potentially might be part of the spectrum of coeliac disease.[5–8] Furthermore, descriptions of the entity often do not take into account the potential for symptomatic improvement by reduction in other symptom-inducing components of wheat, especially fructans, one of the short-chain poorly absorbed carbohydrates (FODMAPs).[9]

Two recent studies have challenged NCGS patients on a GFD, who had normal duodenal biopsies and/or were HLA-DQ2/8 negative, with carbohydrate-deplete gluten in a blinded fashion.[9, 10] The first, a parallel group study found that patients were significantly worse with gluten for overall symptoms, pain, bloating, satisfaction with stool consistency and tiredness. No clues to the mechanisms were elucidated.[10] The second (comprising two back-to-back challenges) used a cross-over design on a low FODMAP dietary background and could find no evidence of gluten-specific triggering of symptoms in such patients.[9] Interestingly, participants opted to continue following a GFD upon study completion as they subjectively described "feeling better" (unpublished observations).

Psychological health has been extensively explored within the coeliac population, where several neurological and psychiatric illnesses are common.[11, 12] Among them, a high prevalence of anxiety and depression has been reported in treated patients.[13–17] In the majority of cases, this anxiety and depression is reported particularly as a personality trait whereby the behaviours and feelings are consistent and relatively enduring.[13–17] However, a high prevalence of transitory mood state has been reported in untreated coeliac disease patients.[13] Interestingly, reversal of this effect was observed after 1 year on a GFD.[13] This observed change in temporary predisposition in coeliac patients following the removal of gluten may be similar in patients with NCGS.

Interestingly, the relationship between psychological health and NCGS has seldom been studied. One recent publication explored trait anxiety and depression in patients with NCGS where patients consumed four slices of gluten-containing white bread per day for 3 days.[17] Results revealed that patients had higher trait anxiety and depression scores at baseline compared to healthy controls but that these scores did not differ significantly following the consumption of gluten. Mood state was not explored, although mood change and other extra-intestinal symptoms including forgetfulness were common symptoms related to gluten intake reported by recently surveyed NCGS participants.[18] It may be that the reversal of mood state among this entity, not personality trait, contributes to why such patients feel better when following a GFD despite the continuation of gastrointestinal symptoms.

This concept was investigated in the current exploratory study of participants with IBS in whom coeliac disease had been excluded and a GFD had led to self-reported improvement in gastrointestinal symptoms. It was hypothesised that the ingestion of gluten by participants with NCGS would have a significant effect on mental state and not necessarily on gastrointestinal symptoms. The effects of gluten on gastrointestinal symptoms were reported in part together with those of a preceding study.[9]

MATERIALS AND METHODS

Participants

Participants were recruited from a preceding study in which subjects with self-reported NCGS were challenged with diets containing varying amounts of gluten, as recently reported.[9] They were all invited to participate in the current exploratory study aimed in part to determine the effect of gluten on mental state. As the time between participation in the two studies varied from 8 to 17 months, inclusion/exclusion criteria were re-confirmed. Participants were included if they were aged >16 years of age, met Rome III criteria for IBS prior to implementation of a GFD, had currently reported well controlled gastrointestinal symptoms and had been adherent to a GFD for at least the preceding 6 weeks. Coeliac disease was excluded by either a normal duodenal biopsy (Marsh 0) performed at endoscopy while on a gluten-containing diet and/or by the absence of the HLA-DQ2 and HLA-DQ8 haplotype. Exclusion criteria included other significant gastrointestinal disease (such as cirrhosis or inflammatory bowel disease); other clinically significant co-morbidity; intake of nonsteroidal

anti-inflammatory agents; use of systemic immunosuppressant medication; reported psychiatric disorder; excessive alcohol intake; pregnancy or inability to give written informed consent.

Study protocol

The exploratory study was a randomised, placebo-controlled, double-blind, cross-over dietary rechallenge study. SLP and JRB recruited, enrolled and assigned participants to a computer-generated randomisation sequence, held by an independent observer. Upon enrolment, participants were educated on a diet low in FODMAPs and it was asked that they continue a GFD low in FODMAPs for the duration of the study. After a 3-day baseline period, participants then received one of the three dietary challenges consecutively for 3 days, followed by a minimum 3-day and maximum 14-day washout period between each diet. Participants were required to report symptom resolution before crossing over to the next diet. Challenge food was supplemented with gluten, whey or not supplemented (placebo). Whey protein isolate was used as a protein control and has a supposed rapid digestibility in the gut.[19, 20] All meals and snacks were supplied to participants (labelled 'Diet A', 'Diet B' and 'Diet C') during dietary challenges. Measurements included mental state, cortisol secretion and gastrointestinal symptoms. Mental state and cortisol secretion were assessed prior to (baseline) and on day 3 of each dietary challenge. Gastrointestinal symptoms were assessed daily for the study duration. Participants unable to continue a treatment due to intolerable gastrointestinal symptoms were permitted to cease the study food of that particular arm, but continue to collect data as per day 3 and collect symptom and food diaries when not on the study diet. Participants then resumed any remaining treatment arms following the allocated washout period. The study was approved by Eastern Health Research and Ethics Committee and was registered with the Australian Clinical Trials Registry (ACTRN12613000768796).

Study food preparation

Study food was prepared according to Australian and New Zealand Food Standards. Study food was supplied and other potential inducers of symptoms were minimised by the food being gluten-free, dairy-free, low FODMAP (fermentable, poorly absorbed short-chain carbohydrates) and low in food chemicals. During dietary challenges, food was supplemented with 16 g/day whole-wheat gluten, 16 g/day whey protein isolate or no additional protein (placebo). All meals and snacks were provided but participants were asked to provide perishable items themselves. Guidance was given as to which perishable foodstuffs were appropriate. The meal plan was adequate in macronutrients, micronutrients and provided 8 MJ energy daily. Meals and snacks were similar in taste, texture and appearance across the three treatment conditions, confirmed with preliminary testing in five healthy people where the food containing the gluten could not be differentiated from those that did not.

The gluten used was commercially available, carbohydrate-depleted wheat gluten (Vital Wheat Gluten; Penford Australia Ltd, Tamworth, NSW, Australia) and contained 75% protein, 1.8% crude fibre, 6.9% lipid, 15.6% starch and 0.6% ash, as shown on reversed-phase high-performance liquid chromatography (HPLC). On the basis of size-exclusion HPLC, the protein content had a distribution of 6.6% nongluten protein (albumin/globulin), 53.4% glutenin and 40.0% gliadin. The whey protein isolate (Resource Beneprotein Instant Protein Powder; Nestle Healthcare Nutrition, Inc., Minneapolis, MN, USA) was lactose-free and low FODMAP, as measured by methodologies described previously.[21, 22]

Measurements

Adherence to the GFD was assessed by specific questioning and using a flow chart to give a numerical score.[23] This was cross-checked with the assessment of participants' baseline 3-day food diary. Adherence to dietary challenges was assessed by entries into a tick-box diary and unused food was counted at the end of each dietary challenge. Participants were also asked to document any additional foods consumed. These were evaluated by an experienced dietician for gluten and FODMAP content using the Monash University low FODMAP database.

Mental state was assessed using the State Trait Personality Inventory (STPI). The STPI was selected based on simplicity, validity and reliability.[24] It is an 80-item self-report questionnaire, with eight 10-item scales for measuring state and trait anxiety, depression, anger and curiosity. State items are used to assess current emotional state and are rated on a four-point intensity scale, where 1 = not at all; and 4 = very much so. Trait items assess emotional disposition and are rated on a four-point intensity scale, where 1 = almost never; and 4 = almost always. The range of possible scores for each subscale can vary from a minimum of 10 to a maximum of 40. The STPI was completed during the baseline period and on day 3 of each dietary challenge.

Salivary cortisol secretion was used as a biomarker of stress. Collection instructions were provided to participants to ensure influential factors were controlled, including sample collection taken at standardised times. The Salimetrics Oral Swab (SOS; Salimetrics, State College, PA, USA) was used to collect saliva samples and stored inside a Swab Storage Tube (clear sterile plastic tube; Salimetrics). Saliva samples were obtained during the baseline period and on day 3 of each dietary challenge. All saliva samples were transported on ice and frozen at −20 °C until being assayed externally (Stratech Scientific APAC Pty Ltd, Sydney, NSW, Australia) by competitive immunoassay using commercially available kits (Salimetrics). The results were expressed as micrograms per decilitre (μg/dL).

Gastrointestinal symptoms were assessed using a 100 mm visual analogue scale (VAS) as previously applied.[9, 10] The VAS is part of the validated IBS-SSS questionnaire.[25] Items on the VAS include overall abdominal symptoms, abdominal pain, bloating, wind, stool consistency, tiredness and lethargy and nausea. The VAS was completed daily for the duration of the study. Daily VAS scores were combined to obtain an average over the baseline period and each of the three dietary challenges (gluten, whey and placebo).

Statistical analyses

A linear mixed model analysis for cross-over designs was undertaken for each of the mental health indices separately, with dietary condition and order of testing treated as fixed factors and participants as the random factor. A number of models and covariance structures were fitted to the data. The comparison of salivary cortisol across dietary challenges was assessed by repeated measures ANOVA. The gastrointestinal symptom data were not normally distributed across dietary challenges and so were analysed using the Friedman test. Where required, pairwise comparisons between each of the challenge conditions were undertaken and Type 1 error was controlled by the use of Hochberg False Discovery Rate (FDR) test.[26, 27] Statistical analyses were performed using GraphPad Prism Version 6.01 (GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistics Version 21 (SPSS Inc., Chicago, IL, USA).

RESULTS

Study population

Twenty-two participants agreed to participate in the study. The subjects who participated in the preceding study, but were not able to return did so due to pregnancy/breast feeding, travel, time constraints or being unwilling to eat gluten. Detailed descriptions of the 22 participants have been recently reported.[9] Briefly, they were aged 24–62 years and five were male. Predominant bowel habits were diarrhoea in eight, constipation in 10 and alternating in four. Twelve participants were HLA-DQ2 and/or HLA-DQ8 positive. Twenty-one participants reported symptom resolution during the allocated washout period. One participant had an extended washout period (11.5 weeks) between her second and third diet treatment, but was included as her data did not influence the result of any analysis. Baseline participant characteristics are shown in Table 1.

Dietary compliance

All participants undertook the three dietary challenges. One patient ceased the whey challenge (treatment first received) prematurely because of intolerable symptoms after lunch on day 2. Data continued to be collected as per day 3.

Nearly all meals (99%, 96% and 99%) were consumed in the gluten, whey and placebo challenges respectively. All patients adhered to the gluten-free, low FODMAP diet. Seven participants consumed snacks high in natural food chemicals (e.g. one banana), but this did not differ across the dietary challenges within participants.

Effect on mental state

Two participants were considered outliers at baseline (>2 s.d. from the mean) and their responses were removed from analysis. A linear mixed model for cross-over designs was applied to the remaining 20 participants with fixed effects of condition (challenge) and order (sequence), as well as the interaction between these two factors, and participants entered as random effects. The model of best fit to the data for each of the STPI state and trait variables, determined by the lowest -2

Table 1 | Participant characteristics at baseline

Number of participants	22
Gender	5 male
Median age (range)	48 (24–62) years
Median body mass index (range)	23 (17–32) kg/m^2
Predominant bowel habit	
Diarrhoea	36%
Constipation	46%
Mixed/Alternating	18%
HLA type	
DQ2 or DQ8 positive	55%

restricted log likelihood value, was that with repeated measures on condition and unstructured covariance matrix.

The tests of fixed effects revealed that condition ($F = 5.994$, $P = 0.011$) had a significant effect on STPI state depression score, but order ($F = 3.036$, $P = 0.06$) did not. Further, no significant interaction between condition and order was observed ($F = 1.623$, $P = 0.20$). Exploration of the main effect of challenge condition revealed that state depression was significantly higher in the gluten condition than the placebo condition ($P = 0.010$) (Table 2; Figure 1). Figure 2 shows paired participant STPI state depression scores across the three conditions. This increase in STPI state depression score following gluten ingestion compared to placebo met criteria for statistical significance after controlling the FDR ($P = 0.017$). Effect size between gluten and placebo ($d = 0.64$) was moderate. Although state depression was higher in the gluten condition than in the whey condition, this difference failed to reach significance ($P = 0.07$, $d = 0.43$) (Table 2; Figure 1). There was no difference between whey and placebo conditions ($P = 0.61$, $d = 0.12$) (Table 2; Figure 1). Eighteen participants (90%) had equal ($n = 4$) or higher ($n = 14$) STPI state depression scores on gluten compared to placebo. Condition had no effect on the other STPI state or trait indices (Table 2).

Effect on salivary cortisol concentrations

One participant produced insufficient saliva for analysis and two participants failed to provide salivary samples on one or more dietary challenges. Their results were removed from analysis. No differences were found in salivary cortisol levels between or during the dietary challenges, $F(2,36)=1.17$, $P = 0.31$ (Figure 3).

Effect on gastrointestinal symptoms

Comprehensive descriptions of gastrointestinal symptom results have been recently published.[7] No differences were identified across the dietary challenges for overall gastrointestinal symptoms (Figure 4) and for individual symptoms (data not shown). The order of the dietary challenges was associated with degree of symptomatic response, with the first intervention being associated with greater symptomatic changes (mean 15.5 mm) than the second (mean 5.3 mm) or third (mean 4.0 mm) challenges, regardless of whether it contained gluten, whey or placebo.[9]

DISCUSSION

The term NCGS has been defined as one or more of a variety of immunological, morphological or symptomatic manifestations that are precipitated by the ingestion of gluten in people in whom coeliac disease has been excluded.[28] Despite the supposedly sound definition, the NCGS entity is complex. While initial work was suggestive of a gluten-specific effect on gastrointestinal symptoms among this entity,[10] the subsequent double-blind, placebo-controlled, randomised, cross-over studies, including the present study, have failed to observe this interaction.[7] We have hypothesised that the reason why patients might feel better on the GFD is that gluten is having a detrimental effect on their mental state and the cessation of gluten improves their well-being rather than the gastrointestinal symptoms *per se*. Indeed, short-term exposure to gluten appeared to specifically induce current feelings of depression in the present study.

The observed change in current feelings of depression is appreciable. According to Spielberger's norms for state depression scores,[24] the mean scores of participants in

Table 2 | Comparison of STPI state and trait indices for placebo, whey and gluten dietary challenges. Gluten was associated with a significantly higher state STPI depression score across the three groups. Data shown as mean, standard deviation (s.d.) and effect size (η_p^2)

STPI		Mean (s.d.)				
		Placebo	Whey	Gluten	*P*-value	η_p^2
State indices	Depression	19.20 (3.82)	19.75 (4.98)	21.45 (4.86)	**0.011**	**0.38**
	anxiety	17.55 (4.36)	17.05 (3.97)	18.05 (5.09)	0.65	0.05
	Curiosity	22.65 (7.86)	23.35 (6.48)	21.40 (6.31)	0.25	0.06
	Anger	10.80 (1.28)	10.70 (1.53)	11.25 (2.02)	0.56	0.04
Trait indices	Depression	18.45 (4.65)	19.85 (5.40)	18.80 (6.18)	0.54	0.02
	Anxiety	18.30 (4.24)	18.80 (3.44)	18.45 (3.95)	0.21	0.04
	Curiosity	27.95 (6.71)	27.05 (7.22)	27.75 (7.45)	0.70	0.01
	Anger	15.90 (5.43)	14.80 (4.19)	15.35 (4.77)	0.11	0.06

Bold values were used to highlight significance.

Aliment Pharmacol Ther 2014; 39: 1104-1112

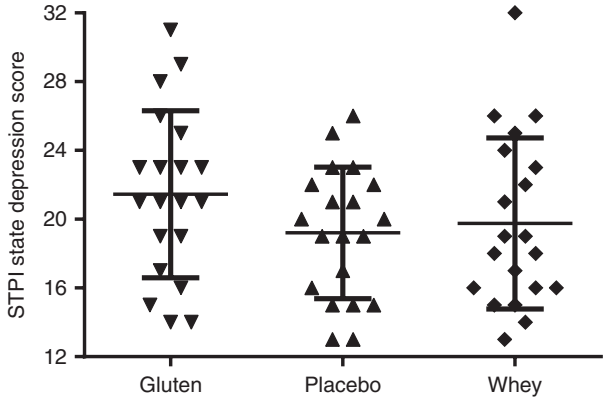

Figure 1 | STPI state depression scores during the gluten, whey and placebo dietary challenges. A linear mixed model of fixed effects revealed that condition had a significant effect on STPI state depression score. Pairwise sub-analyses revealed that state depression was significantly higher in the gluten condition than placebo. No differences were found between gluten and placebo or placebo and whey.

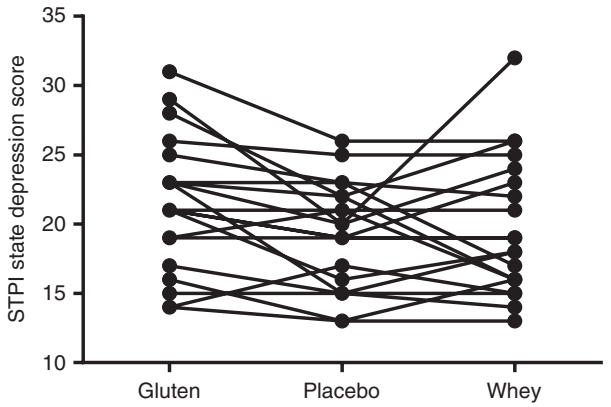

Figure 2 | Paired STPI state depression scores across the gluten, whey and placebo dietary challenges. State depression scores were significantly higher in the gluten condition compared to placebo. No significant differences were found between gluten and whey or placebo and whey.

Figure 3 | Salivary cortisol concentrations during the gluten, whey and placebo dietary challenges. The comparison of salivary cortisol across dietary challenges was assessed by repeated measures ANOVA. No differences were seen across the gluten, whey or placebo dietary challenges. Data shown as box and whisker plots (bar = median, box = interquartile range, whiskers = 10–90 percentile).

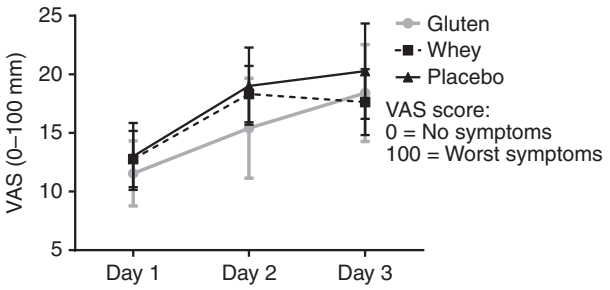

Figure 4 | Overall gastrointestinal symptoms over the 3-day study period during the gluten, whey and placebo dietary challenges. Gastrointestinal symptom data were analysed using the Friedman test. There were no significant differences in overall gastrointestinal symptoms during the gluten, whey or placebo dietary challenges. Data shown represent the mean ± SEM.

this study went from being largely 'neutral depressive' in the placebo condition to being 'mild depressive' following the consumption of gluten.[24] However, Spielberger's norms are based on clinical populations whereas patients in the current study had not been diagnosed as clinically depressed. That said, this observed change over a 3-day intervention is plausible. Personality and mood states are widely accepted as being transitory and rapidly changing, often from moment to moment.[29] If such a change is indeed a gluten-specific effect, the mechanisms involved require elucidation.

One explanation might be alterations in cortisol secretion as circulating concentrations of cortisol are greater with negative affect (i.e. aversive moods such as anxiety, hostility and depression). However, the degree to which this association is due to stable individual differences (i.e. traits) or transient differences in affect (i.e. states) remains unclear.[30] There is currently no evidence that gluten ingestion can stimulate cortisol secretion, but this link has been seldom studied.[31] In the current study,

cortisol concentrations during each dietary period were measured in saliva, a technique that has been shown to provide a feasible, accurate and practical alternative to blood determinations.[32, 33] These were similar across all dietary treatments, indicating that state depression may not be as closely associated with cortisol secretion compared to trait depression, as previously described.[30]

A second potential mechanism is via alteration of brain serotonin (5-hydroxy-tryptophan, 5-HT). Decreased brain 5-HT concentration has been long suggested as a cause of depression.[34] The synthesis of 5-HT in the brain is dependent on the availability of its amino acid precursor, tryptophan. Interestingly, recent work has identified a link between protein ingestion, tryptophan production and concentrations of 5-HT in the brain.[35] In this study, rats consuming food supplemented with food-grade wheat for 2 h had modest reductions in concentration of tryptophan in the brain suggesting that 5-HT pathways are remarkably sensitive to various proteins present in food.[35] Whether carbohydrate-depleted gluten results in reductions of tryptophan concentration in the human brain requires further exploration. Nonetheless, serotonergic dysfunction due to impaired availability of tryptophan has been shown to play a role in various psychological conditions including depression.[36–39]

A third explanation involves the so-called gluten 'exorphins'. These opioid peptides derived from partially digested food proteins including gluten can modulate intestinal function,[31] and can cross the blood–brain barrier and interfere with pain-inhibitory systems, emotionality and memory processes by modulating other hormonal or neurotransmitter systems via the opioid receptors as well as endogenous opioid peptides in the central nervous system (CNS).[40] Such a possibility could be investigated by, for example, the concomitant use of naloxone to block opioid receptors.

A fourth possibility might involve gluten-mediated changes in gut microbiota. Several studies have reported intestinal dysbiosis in patients with coeliac disease.[41] Interestingly, some of the alterations in gut microbiota are restored after adherence to a GFD.[41] This suggests that these changes are secondary consequences of the disease and perhaps directly related to the consumption of gluten. Evidence supporting an important influence of gut microbiota on emotional behaviour and underlying brain mechanisms is well established in adult rodents[42–44] and is emerging in humans. A recent study has provided first evidence that probiotics can modulate the activity of brain regions involved in processing emotion and sensation in adult women.[45] Whether 3 days is sufficient to induce changes in microbiota is uncertain, but this hypothesis requires further investigation in the NCGS population.

It is important to note that several key design issues may have adversely influenced the results. These limitations have been discussed fully with relation to the end-point of gastrointestinal symptoms.[9] With respect to psychological effects, there are four main limitations. First, it suffers from the issues associated with most pilot studies. The number of patients studied is relatively small and the psychological end-points used were restricted to one scale. Secondly, the duration of the dietary challenge might be considered too short to observe the maximum change in psychological states. However, a 3-day gluten challenge has been shown to be long enough to capture the greatest magnitude of change in gastrointestinal symptoms among this entity[17] and psychological states are known to be transitory and rapidly changing.[29] Thirdly, the use of a cross-over design within the IBS population has been criticised mainly on the basis of the possibility of carry-over effects and on the undue influence that dropouts might have on the analysis.[46] While gastrointestinal symptoms had returned to baseline levels before proceeding with the next dietary challenge, an order effect was observed, with significantly more severe symptoms being induced with the first dietary challenge.[9] However, there was no evidence of an order effect on the psychological indices used and the indices were similar in patients after the whey and placebo arms. Furthermore, all participants completed the study. Finally, while adherence to the dietary intervention was ensured using the gold-standard of providing all food, such provision might differ substantially from a participant's usual dietary habits, with consequent increase in the participant's anxiety and negative responses to the intervention.[47] Importantly no difference was observed in anxiety or salivary cortisol levels across the three dietary challenges and depression was only associated with the ingestion of gluten.

In conclusion, the findings of gluten-specific acute changes in current feelings of depression, with no effects on trait indicates, provide a clue that the improvement reported by participants may be in the perception of their general well-being rather than in gastrointestinal symptoms. Such an association requires a larger and more detailed examination.

AUTHORSHIP

Guarantor of the article: Peter R. Gibson.

Author contributions: Study concept and design: SLP, JRB, GWY, JGM, PRG. Recruitment, enrolment and assess-

ment of patients: SLP, JRB. Acquisition of data: SLP, JRB. Analysis and interpretation of data: SLP, JRB, GWY, PRG. Study supervision: JGM, PRG. Drafting of the manuscript: SLP, PRG. All authors approved the final version of the manuscript.

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Declaration of personal interests: Peter R. Gibson has published two books on a diet for irritable bowel syndrome. There were no conflicts of interest to declare for Simone L. Peters, Jessica R. Biesiekierski, Gregory W. Yelland or Jane G. Muir.

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