Gluten Causes Gastrointestinal Symptoms in Subjects Without Celiac Disease: A Double-Blind Randomized Placebo-Controlled Trial

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OBJECTIVES: **Despite increased prescription of a gluten-free diet for gastrointestinal symptoms in individuals who do not have celiac disease, there is minimal evidence that suggests that gluten is a trigger. The aims of this study were to determine whether gluten ingestion can induce symptoms in non-celiac individuals and to examine the mechanism.**

METHODS: **A double-blind, randomized, placebo-controlled rechallenge trial was undertaken in patients with irritable bowel syndrome in whom celiac disease was excluded and who were symptomatically controlled on a gluten-free diet. Participants received either gluten or placebo in the form of two bread slices plus one muffin per day with a gluten-free diet for up to 6 weeks. Symptoms were evaluated using a visual analog scale and markers of intestinal inflammation, injury, and immune activation were monitored.**

RESULTS: **A total of 34 patients (aged 29–59 years, 4 men) completed the study as per protocol. Overall, 56% had human leukocyte antigen (HLA)-DQ2 and/or HLA-DQ8. Adherence to diet and supplements was very high. Of 19 patients (68%) in the gluten group, 13 reported that symptoms were not adequately controlled compared with 6 of 15 (40%) on placebo ($P=0.0001$; generalized estimating equation). On a visual analog scale, patients were significantly worse with gluten within 1 week for overall symptoms ($P=0.047$), pain ($P=0.016$), bloating ($P=0.031$), satisfaction with stool consistency ($P=0.024$), and tiredness ($P=0.001$). Anti-gliadin antibodies were not induced. There were no significant changes in fecal lactoferrin, levels of celiac antibodies, highly sensitive C-reactive protein, or intestinal permeability. There were no differences in any end point in individuals with or without DQ2/DQ8.**

CONCLUSIONS: **"Non-celiac gluten intolerance" may exist, but no clues to the mechanism were elucidated.**

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INTRODUCTION

In clinical practice, some patients have symptoms of irritable bowel syndrome (IBS) that respond well to a gluten-free diet but they have no markers of celiac disease. The published scientific literature is largely devoid of the so-called "non-celiac gluten intolerance" and "wheat intolerance," yet they are widely believed to be very common (1–3). In the evaluation of exclusion diets, wheat has been found to be one of the most common factors inducing gastrointestinal symptoms (4), but it is not known whether gluten is the responsible agent, as wheat, the major cereal removed from the gluten-free diet, contains other components that include other proteins, lipids, and carbohydrates. Of particular importance are fructans, which are poorly absorbed carbohydrates and can induce symptoms by themselves (5,6).

The role of gluten in celiac disease is clear. The toxic peptide sequences have been defined (7,8), the genetic susceptibility loci identified, and the pathological processes comparatively well known. Deamidation of these gliadin epitopes by tissue transglutaminase enables them to be presented with a high affinity to major histocompatibility complex class II T cells in genetically

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susceptible individuals (human leukocyte antigen (HLA)-DQ2 or HLA-DQ8 being expressed in 99.4% of patients with celiac disease) (9). This process initiates a cascade of events, resulting in mucosal inflammation, small intestinal villous atrophy (10), increased intestinal permeability (11), malabsorption of macronutrients and micronutrients (12), and resultant complications of celiac disease. To date, the literature regarding the effect of gluten outside celiac disease has been limited to experiments in cancer cell lines and to uncontrolled clinical studies (1,2,13–17). Whether gluten itself can contribute to gastrointestinal symptoms and/or induce injury to the proximal small intestine in non-celiac patients has never been directly assessed.

The aims of this study were to examine the hypotheses that gluten can cause gastrointestinal symptoms in patients without celiac disease and to preliminary screen for potential mechanisms of whether gluten does so by causing intestinal injury and/or inflammation in such subjects. To do this, a randomized, double-blind, placebo-controlled, dietary rechallenge trial was conducted in subjects with IBS who had celiac disease excluded by best practice methods and who reported a symptom response to a gluten-free diet.

METHODS

Patients

Patients were recruited between July 2007 and December 2008 through advertisements in e-newsletters and community/state newspapers in metropolitan Melbourne, and by referral in private dietetic practice. The inclusion criteria were age >16 years, symptoms of IBS fulfilling Rome III criteria that have improved on a gluten-free diet, and adherence to the diet for at least 6 weeks immediately before screening. Celiac disease was excluded by either (i) absence of the HLA-DQ2 and HLA-DQ8 haplotype or (ii) a normal duodenal biopsy (Marsh 0) performed at endoscopy while on a gluten-containing diet in individuals expressing the HLA-DQ2 or HLA-DQ8 haplotype. Patients with significant gastrointestinal disease (such as cirrhosis or inflammatory bowel disease), excessive alcohol intake, intake of non-steroidal anti-inflammatory agents, and unable to give written informed consent were excluded.

Study protocol

Patients were randomized according to a computer-generated list of random numbers held by an independent observer to either the gluten or the placebo treatment group. Baseline symptom data and a 7-day food diary were collected during a 2-week run-in period. Participants continued on a gluten-free diet throughout the study, but were asked to consume one study muffin and two study slices of bread containing gluten (total intake of 16 g/day) or not (see below) every day for 6 weeks. Both patients and investigators evaluating patients were blinded to the study treatment. At the end of each study week and for 3 weeks after completion of the study intervention, symptoms were evaluated, as previously applied (6). Patients were asked to complete a symptom questionnaire containing the question for the primary outcome detailed below, and a 100-mm visual analog scale, with 0 representing no symptoms, assessing overall symptoms, bloating, abdominal pain, satisfaction with stool consistency, nausea, and tiredness. At weeks 0 and 6, serum, urine, and stool samples were collected for analysis, and intestinal permeability was measured. Food diaries were maintained by patients during the third and sixth study weeks, and unused muffins and bread were returned at the end of weeks 3 and 6 of the treatment period for counting. Patients unable to continue the study because of intolerable symptoms were permitted to cease study food after data were collected as per week 6 (symptom assessment, and blood, urine, and stool samples collected). The protocol was approved by the Eastern Health Research and Ethics Committee.

End points

The primary outcome was the proportion of patients answering "no" on more than half of the symptom assessments to the question, "Over the last week were your symptoms adequately controlled?" This question was asked at the end of each study week or at withdrawal if premature. Secondary outcomes were the change in overall and individual gastrointestinal symptoms as assessed by the visual analog scale, and changes in biomarkers (see below). Compliance with the study treatment was assessed by an unused food count at weeks 3 and 6. Compliance with the gluten-free diet was judged on food diary entries and on specific questioning at the time of review.

Study food preparation

The muffins and bread were prepared and baked commercially in gluten-free ovens and conditions. The base mixes were gluten free. For the gluten group, commercially available, carbohydrate-depleted wheat gluten (Gemtec 1160, Manildra Group, Auburn, NSW, Australia) was added before baking at the amount of 8 g per muffin and 4 g per slice of bread. Analysis of the baked products using a commercially available assay (Biokits Gluten Assay Kit; Tepnel Biosystems, Flintshire, UK; AOAC 991·19 Method) confirmed the preservation of intact gluten and in the amount expected. The gluten used contained 91.7% protein, 1.1% crude fiber, 1.9% lipid, 1.8% starch, and 3.5% ash shown on reversed-phase high-performance liquid chromatography. To assess FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) content, gluten was analyzed as described previously (17) and was shown to be free of short-chain carbohydrates, fructans, fructose, glucose, lactose, sorbitol, mannitol, raffinose, stachyose, nystose, and kestose. On the basis of size-exclusion high-performance liquid chromatography, the protein content had a distribution of 2.3% non-gluten protein (albumin/globulin), 45.7% glutenin, and 52.0% gliadin. Preliminary testing in 10 healthy individuals showed that the muffins and bread containing gluten could not be differentiated from those that did not on the basis of taste or texture.

Measurement of biomarkers

All markers were measured after randomization. Serum was analyzed for antibodies to tissue transglutaminase (i.e., tissue transglutaminase IgA) and whole gliadin (IgA and IgG) by ELISA (enzyme-linked immunosorbent assay) using commercially

available assays (INOVA Diagnostics, San Diego, CA). The manufacturer's reference ranges were used to determine the classification of the serological result. Endomysial antibodies were examined by immunofluorescent staining of distal monkey esophagus (Chemicon Australia, Boronia, VIC, Australia).

Highly sensitive C-reactive protein was measured using an immunoturbidimetric assay (Tina-Quant CRP Roche Diagnostics, Basel, Switzerland). Intestinal permeability was measured using a dual sugar test (18). After an overnight fast, patients emptied their bladder and consumed a solution of lactulose (5 g) and rhamnose (1 g) dissolved in 120 ml of water. All urine over the next 5 h was collected in containers containing boric acid as a preservative, and samples were stored at −80 °C until assayed by high-pressure liquid chromatography as described previously (19). The urinary lactulose-to-rhamnose ratio was calculated. Fecal lactoferrin was measured by ELISA using a commercially available kit (IBD Scan; Techlab, Blacksburg, VA). Two dilutions of each sample were assayed and the results expressed in units of mg/ml feces.

Statistical analyses

Power calculations were based on a placebo effect using a similar end point and rechallenge methodology of ~20% (6) and an estimate of 60% response to gluten as there were no previous data on which this could be judged. This indicated that 30 patients were required in each group to achieve a power of 80% and a P value 0.05. The study was terminated early because of difficulty with recruitment of patients in whom celiac disease had been definitely excluded (see above).

To determine the relationship between tolerable symptoms ("yes"/"no") over the 6 weeks, a generalized estimating equation, was used (primary outcome). The change in symptom severity was calculated as the scored difference between commencement and 1 week and was tested by the independent samples t-test for within-group comparisons and ANCOVA (analysis of covariance) between groups. A linear mixed effects model assessed symptom severity scores across the treatment period (longitudinal data). The correlation between measured symptoms and their model residuals was assessed using Pearson's correlation coefficient. Changes in biomarker levels after therapy within each dietary group were assessed by paired t-test using log-transformed data. Comparison of change in biomarker levels between each group was assessed using ANCOVA and that of change in the indices using an independent samples t-test. Blinding was assessed by using the κ-agreement statistic, in which a value of 1 indicated complete agreement and 0 indicated no agreement. All statistical analyses were conducted using the R Statistical Software Package (R Development Core Team, R: A Language and Environment for Statistical Computing, Vienna, Austria). Two-tailed P values at or below 0.05 were considered statistically significant.

RESULTS

Less than one-third of respondents to the advertisements were deemed suitable for screening. Of those 103 subjects, only 39 met the inclusion criteria and were enrolled. Subject flow is shown in

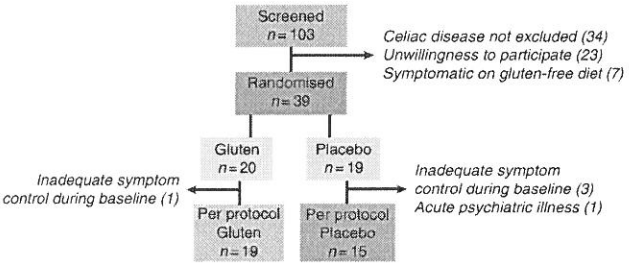

Figure 1. Recruitment pathway and reasons for screen failure and withdrawals.

Figure 1. After randomization, five patients had to be withdrawn. Thus, 34 patients completed the study as per protocol: 19 received gluten and 15 received placebo.

The details of those patients are shown in **Table 1**. All patients were negative for tissue transglutaminase and endomysial antibodies and there were no differences for whole gliadin antibodies between the gluten or placebo groups, including those within the DQ2/8-positive group.

All patients adhered to the gluten-free diet during the study. Alcohol intake did not differ during the treatment period, and did not differ between groups. Nearly all food supplements (95 and 96%) were consumed in the placebo and gluten groups, respectively. The blinding technique was successful, supported by a κ score of 0.24 (low agreement between actual treatment and participant guessing).

Nine patients ceased the study diet prematurely because of intolerable symptoms. Six patients were in the gluten arm and they withdrew after a median of 7 (range 2–18) days, whereas three in the placebo arm withdrew after 16 (range 11–21) days. There were no statistical differences between the groups in frequency and timing of withdrawal. Serum, urine, and stool samples were collected from all of these patients upon cessation of the diet as per week 6.

Significantly more patients in the gluten group (68%; $n = 13/19$) reported the answer, "no" (the primary outcome question) compared with those on placebo (40%; $n = 6/15$) for more than half of the study therapy duration ($P = 0.001$; generalized estimating equation). As shown in **Figure 2**, changes in symptoms from baseline to end of week 1 as scored on the visual analog scale after 1 week's therapy were significantly greater in those patients who consumed the gluten diet for overall symptoms, pain, bloating, satisfaction with stool consistency, and tiredness, but not for wind or nausea. Over the entire study period, the severity scores of pain, satisfaction with stool consistency, and tiredness were significantly higher for those consuming the gluten diet (**Figure 2**). The correlation between model residuals to estimate symptom score redundancy was assessed. Correlation coefficients ranged between 0.3 and 0.9, with the highest correlation between overall score and pain.

As shown in **Table 2**, neither treatment group had significant changes from baseline for any of the biomarkers measured. Similarly, no significant differences were observed in the magnitude of changes between the groups, whether assessed using raw data (ANCOVA) or by comparing changes in the indices (t-test). Fecal

Table 1. Patient characteristics according to the dietary treatment group

Patient characteristic	Gluten	Placebo
Number of patients	19	15
Median age (range) in years	40 (29–55)	49 (33–51)
Men	16%	7%
Median body mass index range (range)	23 (18–41)	22 (18–33)
Number with predominant bowel habit		
Constipation in percentage	16	20
Diarrhea in percentage	58	33
Alternating percentage	26	47
HLA type		
DQ2 or DQ8 positive in percentage	53	60
DQ negative in percentage	47	40
Elevated serum celiac antibodies (percentage of patients (mean (s.e.m.) Units/ml))		
Tissue transglutaminase (IgA)	0	0
Tissue transglutaminase (IgG)	0	0
Endomysium (IgA)	0	0
Whole gliadin (IgA)	27 (39 (9))	30 (33 (9))
DQ2/8 positive	5 (33 (0))	7 (24 (0))
Whole gliadin (IgG)	25 (25 (1))	0
DQ2/8 positive	5 (23 (0))	0

HLA, human leukocyte antigen.
There were no significant differences between dietary groups for any index (independent samples t-test and χ^2 test).

lactoferrin was below the detectable level before and after treatment in all but one patient in the placebo arm whose level was 36 mg/ml at both weeks 0 and 6. Removal of this patient's data from the analysis had no effect on the results (data not shown).

Symptomatic responses to gluten did not significantly differ in those expressing HLA-DQ2 and/or HLA-DQ8 ($n = 10$) compared with those who did not ($n = 9$; data not shown). Similarly, no differences in the response of biomarkers to gluten exposure were noted according to HLA-D status (data not shown).

DISCUSSION

Gluten intolerance in individuals without celiac disease is a controversial issue and has recently been described as the "no man's land of gluten sensitivity" (20). The evidence base for such claims is unfortunately very thin with no randomized controlled trials demonstrating that the entity does actually exist. Most published descriptions involve patients with positive serology associated with celiac disease or with intraepithelial lymphocytosis in the duodenum. In other words, evidence of immunological responses seen in celiac disease has been present (21) and this may just represent celiac disease not fulfilling the ESPGHAN (European Society for Paediatric Gastroenterology, Hepatology and Nutrition) criteria for diagnosis. This double-blind, randomized, placebo-controlled rechallenge trial in patients who claim considerable improvement in gut symptoms with the institution of a gluten-free diet does indeed support the existence of non-celiac gluten sensitivity. Gluten specifically induced symptoms including bloating, dissatisfaction with stool consistency, abdominal pain, and tiredness.

Recruitment of patients for this study was not easy mainly because of the failure of most to have celiac disease effectively ruled out. Although the final number ($n = 34$) of participants recruited was less than the *a priori* power calculations suggested and relatively small, our interim power analyses confirmed that the reduced number was adequate to infer a statistically robust result, with unequivocal significant separation of the two groups. All patients developed exacerbation of symptoms in response to gluten and did so within the first week of rechallenge in contrast to the placebo group in which symptom induction occurred more slowly and the level of symptoms reached was less severe. This occurred across the relevant abdominal symptoms of bloating, pain, and satisfaction with stool form, whereas no differences between the treatment groups were shown for the less relevant symptom of nausea. Interestingly, the symptom that quantitatively differentiated the treatment groups to the greatest extent was tiredness, mainly due to placebo having no apparent effect on this end point. Tiredness is a common symptom of IBS (21), and its induction by gluten may provide insights into a mechanism of action.

A key question is by what mechanism symptoms were induced by the ingestion of gluten. It might be anticipated that some patients reporting symptomatic improvement from the gluten-free diet have undiagnosed celiac disease. Celiac disease can be patchy (22) and, although unlikely, it is therefore possible that some patients with undiagnosed celiac disease were included. However, there were no significant changes in celiac antibodies seen in either group. About one-half did not carry *HLA-D* genes believed to be essential for the development of celiac disease. Although the study was not powered to determine differences in responses between genotypes, no clear differences were noted.

Simple non-invasive studies were performed to look for a signal that inflammation and/or intestinal damage was being induced. This was particularly suspected as evidence of an immune basis for at least a proportion of patients with functional gastrointestinal disorders has already been shown (23,24), and gluten had a prominent effect on tiredness in this population, suggesting a more systemic process. The change in highly sensitive C-reactive protein is considered a marker for systemic circulation of cytokines from a localized site, but no effect on this was observed. Fecal lactoferrin levels increase in the presence of intestinal inflammation due to transepithelial migration of neutrophils to the lumen and the inability of gut bacteria to degrade lactoferrin (25). However, levels were not increased by the interventions. Finally, intestinal permeability, as examined using a dual sugar absorption test, is believed to be a sensitive marker of intestinal injury, but this also did not change overall, and there were no differences between the gluten and placebo groups. Clearly, these markers may not have the required sensitivity to detect subtle inflammation and/or

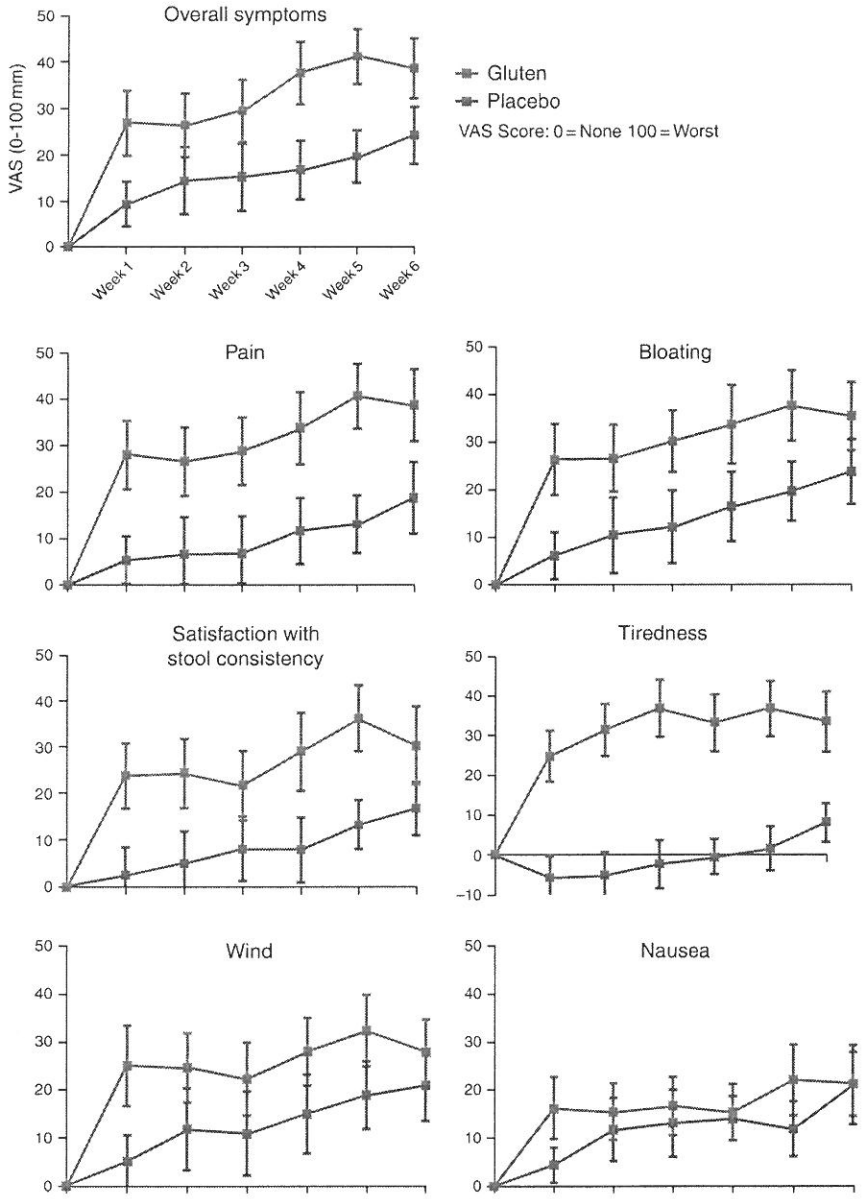

Figure 2. Change in symptom severity from baseline in the gluten and placebo-treated groups over 6 weeks of the study. Data shown represent the mean change for subjects remaining on study therapy at each time point. The differences were compared at week 1 by an independent samples *t*-test, in which overall symptoms ($P=0.047$), abdominal pain ($P=0.016$), bloating ($P=0.031$), satisfaction with stool consistency ($P=0.024$), and tiredness ($P=0.001$) were statistically significant, but wind ($P=0.053$) and nausea ($P=0.120$) were not. The differences were also compared over the entire study period using a linear mixed effects model, in which abdominal pain ($P=0.02$), satisfaction with stool consistency ($P=0.03$), and tiredness ($P=0.001$) were statistically significant, but overall symptoms ($P=0.15$), wind ($P=0.08$), and nausea ($P=0.69$) were not. VAS, visual analog scale.

intestinal damage. Examination at the tissue level is warranted to better address this issue.

Other potential mechanisms by which a dietary product can induce functional gut symptoms include induction of intestinal distension through the fermentation of poorly absorbed gluten peptides. However, passage of excessive flatus was not a prominent feature (as it is for carbohydrate sources; see the study by Gibson and Shepherd (26) and malodorous flatus might be anticipated because of sulfide production, but was not reported by patients in the study. Indeed, if hydrogen sulfide production was increased, this might potentially alter visceral sensitivity (27). Alternatively, gluten may mediate cholinergic activation as has been shown in murine models of gluten sensitivity (28). This may lead to increased smooth muscle contractility and indirectly have effects on luminal water content. Other functional gut symptoms might also be induced by stimulation of the enteric

Table 2. Celiac serology, intestinal permeability, and C-reactive protein results before and during therapy with gluten or placebo, shown as median (range), and changes in those indices, shown as mean (s.e.m.)

Biomarker	Gluten (n=19)			Placebo (n=15)		
	Baseline	With therapy	Change	Baseline	With therapy	Change
Celiac serology (Units/ml)						
Tissue transglutaminase (IgA)	3.0 (2.0–7.0)	4.5 (2.0–7.0)	0.6 (0.3)	3.0 (2.0–10.0)	3.5 (2.0–10.0)	0.4 (0.5)
Whole gliadin (IgA)	10.8 (3.5–241.5)	4.6 (0.1–51.3)	−29.7 (19.2)	6.6 (0.1–36.6)	5.9 (0.1–36.6)	−4.3 (2.5)
Whole gliadin (IgG)	14.6 (12.1–31.5)	15.5 (11.4–50.6)	2.5 (2.0)	11.9 (10.9–14.6)	11.9 (10.6–15.7)	0.2 (0.3)
Intestinal permeability (L:R ratio)	0.02 (0.01–0.6)	0.01 (0.01–2.4)	0.09 (0.1)	0.04 (0.01–0.15)	0.02 (0.01–0.18)	−0.01 (0.02)
Highly sensitive C-reactive protein (mg/l)	1.4 (0.3–5.3)	0.3 (0.4–19.8)	2.1 (1.4)	1.1 (0.2–8.2)	1.2 (0.3–13.1)	0.5 (0.9)

ANCOVA, analysis of covariance; L:R ratio, lactulose-to-rhamnose ratio

There were no statistically significant differences within each dietary group (paired t-test on log-transformed data) or between treatment groups whether evaluated using baseline and treatment data (ANCOVA) or the changes in indices (independent samples t-test; all $P \geq 0.1$).

nervous system either directly by the supply of neuroactive molecules or by indirect release of neurotransmitters from, e.g., mast cell activation. Neurally active peptides from gluten digestion might potentially gain access to enteric nerve endings, but these are not known to occur and their absorption might seem less likely given normal intestinal permeability. Newer techniques such as examining basophil activation in response to the gluten used might be instructive in this manner (29).

The other key issue is whether symptoms are being induced by peptide(s) derived from gliadin proteins or non-gliadin parts of gluten, or by a contaminant of the gluten. There is ample evidence *in vitro* that suggest that gluten can induce injury and changes in epithelial cells by non-DQ2-restricted mechanisms. For example, gliadin is able to increase epithelial permeability and alter protein expression of components of the tight junction (16), induce apoptosis (14,30), and increase oxidative stress (15) in Caco-2 (human colon adenocarcinoma) monolayers, a surrogate model for the human gut epithelium. In addition, gliadin may inhibit RNA and DNA synthesis (17). Of non-gliadin components, carbohydrates would be considered a likely candidate, especially as fructans are present in wheat, are poorly absorbed in the small intestine, and do induce functional gut symptoms (6). However, the gluten used was devoid of FODMAPs. Wheat proteins are commonly implicated in food hypersensitivity and it must be considered that the induction of symptoms by gluten in this study might be a wheat-specific phenomenon, and not gluten specific. Such a finding would have implications for the dietary restriction that would be necessary in such patients to attain good symptomatic control.

The prevalence of non-celiac gluten intolerance among patients with functional gut disorders is unknown. Patients in this study were highly selected because of the frequent failure of investigative work-up by health professionals for celiac disease or from self-administered therapy without any investigations at all. Methods to identify these patients are required. At present, they are restricted to ruling out celiac disease, followed by trial of a gluten-free diet, followed by rechallenge. Better diagnostics will only derive from understanding the mechanism of action and what component of gluten is actually inducing the symptoms.

In conclusion, this double-blind, randomized, placebo-controlled rechallenge study of patients with IBS without celiac disease who have reached satisfactory levels of symptom control with a gluten-free diet shows that gluten is indeed a trigger of gut symptoms and tiredness. No evidence for intestinal inflammation or damage, or for latent celiac disease was found to offer a mechanistic explanation for symptom deterioration caused by gluten. How common non-celiac gluten intolerance is, how it can be reliably identified, and what its underlying mechanisms are, warrant further evaluation.

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CONFLICT OF INTEREST

Guarantor of the article: Peter R. Gibson, MD, FRACP.

Specific author contributions: Enrolment of patients and assessment of recruited patients: Jessica R. Biesiekierski, Evan D. Newnham, Peter M. Irving, Jacqueline S. Barrett, and Melissa Haines; analysis of data: Jessica R. Biesiekierski, Evan D. Newnham, and Peter M. Irving; writing of paper: Jessica R. Biesiekierski, Evan D. Newnham, Peter M. Irving, Jacqueline S. Barrett, Melissa Haines, James D. Doecke, Susan J. Shepherd, and Jane G. Muir; approval of the final draft: all authors; design of study: Peter M. Irving, Susan J. Shepherd, and Peter R. Gibson; statistical analysis: James D. Doecke; recruitment of patients: Susan J. Shepherd; supervision of J.R.B.: Jane G. Muir; oversight of study: Jane G. Muir and Peter R. Gibson; mentoring and writing of study: Peter R. Gibson.

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Potential competing interests: Susan J. Shepherd has published cookbooks directed toward issues of dietary fructan restrictions, fructose malabsorption, and celiac disease. She has also published shopping guides for low FODMAPs and low fructose and fructan foods.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ The existence or non-existence of non-celiac gluten intolerance is contentious.
- ✓ Although wheat is one of the most common factors inducing gut symptoms and an existing public perception that gluten intolerance in individuals without celiac disease is common, the published scientific literature is devoid of well-designed studies that directly investigate this area.

WHAT IS NEW HERE

- ✓ This study is significant as it provides for the first time, high-quality evidence that gluten itself may trigger gut symptoms and fatigue in individuals who do not have celiac disease.
- ✓ The findings will stimulate a large body of subsequent work to, e.g., confirm the effect, determine mechanisms, and to define the prevalence of this condition in the community.
- ✓ The impact on clinical practice will be in the use of dietary gluten restriction in the management of patients with functional gut symptoms.

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