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FOOD ALLERGY (D ATKINS, SECTION EDITOR)

Is Gluten a Cause of Gastrointestinal Symptoms in People Without Celiac Disease?

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Abstract The avoidance of wheat- and gluten-containing products is a worldwide phenomenon. While celiac disease is a well-established entity, the evidence base for gluten as a trigger of symptoms in patients without celiac disease (so-called 'non-celiac gluten sensitivity' or NCGS) is limited. The problems lie in the complexity of wheat and the ability of its carbohydrate as well as protein components to trigger gastrointestinal symptoms, the potentially false assumption that response to a gluten-free diet equates to an effect of gluten withdrawal, and diagnostic criteria for coeliac disease. Recent randomized controlled re-challenge trials have suggested that gluten may worsen gastrointestinal symptoms, but failed to confirm patients with self-perceived NCGS have specific gluten sensitivity. Furthermore, mechanisms by which gluten triggers symptoms have yet to be identified. This review discusses the most recent scientific evidence and our current understanding of NCGS.

Keywords Gluten sensitivity · Celiac disease · Irritable bowel syndrome · Wheat · Gastrointestinal symptoms · Fodmaps · Food hypersensitivity

Introduction

Avoidance of wheat-containing products is a worldwide phenomenon. People are avoiding wheat and gluten for putative health benefits. Gluten has been linked to a wide range of conditions including various skin problems [1], fatigue and migraine [2], weight gain [3] and autism [4]. Moreover, wheat and gluten are most often blamed for gastrointestinal symptoms [5]. Unfortunately, this whole area has been complicated by the assumption by many that benefits of a gluten-free or wheat-free diet equate to a problem caused by gluten. The role of dietary components in inducing gastrointestinal symptoms is a complex area. As wheat has multiple constituents, discussion of gluten-mediated problems cannot be divorced from considering the role of other components in wheat. These and other wheat-related conditions will also be considered.

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Components of Wheat

Protein

Gluten is the main storage protein contained within the germ of wheat grains [6]. Gluten is a complex mixture of hundreds of related but distinct proteins, mainly gliadin and glutenin. Similar proteins to the gliadin found in wheat exist as secalin in rye, hordein in barley and avenins in oats, and are collectively referred to as 'gluten'. Derivatives of these grains such as triticale and malt and other ancient wheat varieties such as spelt and kamut also contain gluten. Non-gluten proteins include α-amylase/trypsin inhibitors and have recently been suggested to induce intestinal inflammation [7•].

Carbohydrate

The wheat carbohydrates that have excited most interest with regard to intestinal health are those that are indigestible, because they are a key substrate for bacterial metabolism and growth, and their fermentation releases short-chain fatty acids such as butyrate, which have multiple beneficial effects

on the colonic mucosa. Long-chain carbohydrates, particularly non-starch polysaccharides have important roles in laxation and prevention of colorectal cancer [8•], and wheat bran is often used therapeutically as a rich source of dietary fibre. Wheat is also rich in short-chain carbohydrates. The oligosaccharides, particularly fructans (chains of fructose joined by β(2–1) bonds with a terminal glucose) have emerged as potentially exerting health benefits via a prebiotic effect [9] and as important inducers of functional gastrointestinal symptoms (reviewed in [10]).

The human small intestine does not produce a hydrolase to digest fructans to absorbable single sugars. Ingested fructans, being relatively small molecules, will have an osmotic effect in the small intestine increasing the water content of the lumen [11]. After delivery to the colon, fermentation releases gases, hydrogen, carbon dioxide and methane. These effects result in luminal distension, a major stimulus to the gut's nervous system (see below). Fructans are part of a family of poorly absorbed dietary short-chain carbohydrates (termed fodmaps) [12•]. Fodmaps all behave similarly in the intestine, and their effects on luminal distension and on symptoms are additive. Since fodmaps are found in a wide variety of foods (detailed in Table 1), they are usually consumed in combination. Interestingly, recent grain and cereal composition data has highlighted that wheat- and rye-derived products contain the highest fodmap content, predominantly fructans and galacto-oligosaccharides [14••]. Cereal products with the lowest fodmap contents are mostly gluten-free, based on rice, oat, quinoa and corn ingredients.

Other Components

Little attention has been paid to the lipid component (1–2 %) of wheat, nor to other constituents such as polyphenols contained within whole grains. Wheat also contains lectin activity and wheat germ agglutinin (WGA) is the best studied (reviewed in [15•]). This binds to N-glycolylneuraminc acid found in cell membranes in epithelia of the gut. It can be taken up by the epithelium transcellularly via endocytosis or paracellularly. In studies in vitro, WGA in nanomolar concentrations has several pro-inflammatory effects on a variety of cells in vitro and increases epithelial permeability in cell lines. However, a pathogenic role for WGA in changes in IBS, for example, by changing intestinal permeability or inducing symptoms, is unexplored.

Clinical Problems

There are three clinical conditions in which wheat has a pathogenic role in either the underlying disease or in causing the symptoms.

Irritable Bowel Syndrome

IBS affects approximately 10 % of population across the world [16] and is characterized by abdominal pain, bloating, wind, distension and altered bowel habit but with no abnormal pathology. Although there has been no single biological abnormality identified to explain the recurrence of symptoms in IBS, the physiological bases for the genesis of many functional gut symptoms are likely to be an excessive response to sensory input to the intestine (termed visceral hypersensitivity) and/or abnormal motility responses to such stimulation. Since luminal distension is a major stimulus to the gut via stretch receptors, factors that induce luminal distension, particularly the ingestion of fodmaps, will act as important inducers of symptoms. A diet low in fodmaps provides relief of such functional gut symptoms in three out of four patients with IBS, evidence for efficacy spanning observational, comparative, randomised and blinded placebo-controlled trials (as recently reviewed) [12•]. Indeed, in Australia and other countries, the low fodmap diet is increasingly being accepted as the primary management strategy for IBS, and has been recently adopted by the 2011 Australian National Therapeutic Guidelines [17].

Celiac Disease

Dietary gluten unequivocally causes celiac disease, a common immune-mediated disease that affects 1 % of Western populations and variable proportions across the world [18]. The immune response triggered is specific to toxic peptides within the gliadin fraction of the gluten protein [19] and initiates an immune response causing mucosal inflammation and injury in the small intestine. The damage in the small intestine can cause common functional gastrointestinal symptoms (diarrhea, constipation, excessive wind, bloating) that may or may not relate to malabsorption. Celiac disease patients are exposed to the risk of a wide range of long-term complications from osteoporosis to infertility to lymphoma [20].

As recently reviewed [21•], the pathological process of celiac disease is well understood where, in the small intestine, gliadin escapes degradation and undergoes post-translational modification (deamidation) by tissue transglutaminase (tTG) forming negatively charged amino acids. These bind to the disease-associated human leukocyte antigen (HLA)-DQ2 or -DQ8 receptors on the cell surface of antigen-presenting cells. Once bound, this complex is presented with high affinity to T-cells. The CD4+ T-cell activation leads to the secretion of pro-inflammatory cytokines, inflammation and damaged intestinal villi.

The immunogenicity of α-gliadin is under control of the HLA genes [22], where the genetic susceptibility locus (HLA-DQ2 and/or HLA-DQ8) is expressed in 99.4 % of celiac patients [23, 24]. Given this genetic association, high-risk

Table 1 Dietary FODMAPs and their sources

Food component	Dietary form	Common sources
Fructose	Free monosaccharide constituent (fructose in excess of glucose)	Apple, pear, watermelon, honey, high fructose corn syrup, asparagus, artichoke
Lactose	Free disaccharide	Milk, yogurt, ice cream, soft cheese
Fructans	Fructo-oligosaccharide (FOS) and inulin	Wheat, rye, barley, garlic, leek, onion, asparagus, artichoke, peach, persimmon, watermelon, pistachio, inulin
Polyols	Sorbitol, mannitol, xylitolmaltitol, isomalt	Apple, pear, plum, apricot, nectarine, mushroom, cauliflower, reduced caloric sweetener
Galacto-oligosaccharides (GOS)	Raffinose, stachyose	Legumes, chickpeas, lentils

(*Data from* [13•].)

groups for celiac disease include relatives of celiac patients and those with co-existing autoimmune conditions, including insulin-dependent diabetes mellitus, Down's syndrome, multiple sclerosis, and Williams or Turner syndrome [25].

The prevalence of celiac disease appears to be increasing. The most likely contributors to this are an increase in clinical awareness and an improved definition and improvements in diagnostic tests, although evidence is suggesting there is an unexplained real increase [26]. Despite this, celiac disease remains undiagnosed in the majority of patients, where it is estimated that there are approximately 7–10 undiagnosed cases for each diagnosed celiac patient [27]. As outlined in Table 2, the diagnosis of celiac disease is made using a combination of (1) histological findings from small bowel biopsy (via gastroscopy) including villous atrophy, crypt hyperplasia and intra-epithelial lymphocytosis; (2) raised circulating levels of celiac disease-associated antibodies (serology) such as transglutaminase IgA; (3) histological, serological or clinical improvement after adherence to a gluten-free diet (GFD); and (4) specific allelic variants in the two HLA genes: HLA-DQA and HLA-DQB [28••, 29••, 30–33]. Controversy lies in the 'grey' area where, for example, only duodenal intra-epithelial lymphocytosis is present or serology is suggestive but biopsy is negative.

The only available treatment for celiac disease is lifelong strict avoidance of all gluten-containing foods. As little as 50 mg (present in 1/80th of a slice of wheat bread) is generally considered to be the minimum quantity of gluten needed to induce damage to the lining of the small intestine [34]. Clinical effectiveness of the GFD as treatment for celiac disease is well documented, including for clinical improvement of symptoms and nutrition, healing of the intestinal lesion in many and prevention of long-term complications [20]. Recent guidelines for patient management with celiac disease have been published by the British Society and the American College of Gastroenterology [30, 32, 33].

Food Hypersensitivity

There is little doubt that wheat can cause food hypersensitivity reactions, but how commonly this occurs in controversial. There are two apparent varieties of wheat hypersensitivity.

- ***'Wheat allergy'***: This has been classified into classical food allergy affecting the skin, gastrointestinal tract or respiratory tract; wheat-dependent, exercise-induced anaphylaxis (WDEIA); occupational asthma (baker's asthma) and rhinitis; and contact urticaria [28••]. There has been little progress in understanding the mechanisms of food allergy, particularly those involved at the molecular level. It is thought that wheat allergy involves cross-linking of immunoglobulin E (IgE) by repeat sequences in gluten peptides, which triggers the release of chemical mediators including histamine from basophils and mast cells [35]. There is no direct evidence to suggest that classical IgE-mediated type 1 allergic reactions to food antigens play a role in the symptoms of IBS [36].
- ***'Wheat sensitivity'***: This has been defined in 30 % of a large cohort of patients with IBS by an Italian group according to exclusion diet, double-blind, placebo-controlled, re-challenge methodology [37••]. Evidence that this is an inflammatory/immune-mediated phenomenon has comprised the association with atopic phenotype, the induction of symptoms with only small amounts of wheat (i.e. could not be fodmaps), the finding of eosinophilic infiltration of the small and large intestinal mucosa and, interestingly, the intraepithelial compartment in about one-third of patients, the presence of anti-gliadin antibodies in about 40 %, and the presence of eosinophilic cationic protein in faeces and a positive basophil activation test in the majority of patients [38, 39, 40•]. The authors identified two varieties—those whose hypersensitivity was specific to wheat and those who had multiple food

Table 2 Features of tests for the diagnosis of celiac disease

Test	Outline	Admissions
HLA typing	Expression of alleles encoding HLA-DQ2 and/or HLA-DQ8 genes	• Expression of the HLA-class II haplotypes, DQ2 and DQ8 is necessary, but not sufficient to develop the disease • Highly sensitive as >99 % celiac patients carry these alleles • Independent of disease activity or diet
Serology	Elevated antibody titres measured at diagnosis and then confirmed with disappearance after treatment (e.g. deamidated gliadin peptides antibodies (IgA and IgG) and anti-tissue transglutaminase)	• Titres can show false negative and false positive results • Although serological tests are predictive, they alone are not sufficient for diagnosis • A single serological test for celiac disease is inadequate to exclude celiac disease for life • Affected by immunosuppressants and additional disorders • Selective IgA deficiency incidence 1:400–500 • Adequate gluten intake required[a]
Intestinal biopsy	Multiple endoscopic biopsies of duodenum to detect partial to total villous atrophy and decreased villous:crypt ratio, elongation of crypts (crypt hyperplasia), and increased intraepithelial lymphocyte (IELs) count	• Villous atrophy can be patchy • 1st part duodenal biopsies may increase yield • Partial villous atrophy can be undetected • Invasive and expensive • Remains gold standard for both celiac disease diagnosis and in determining adequacy of mucosal remission on GFD • Adequate gluten intake required[a]

[a] Adequate dietary gluten intake is required prior to having the gastroscopy and serology. If gluten intake has already been removed or reduced, gluten challenges should be implemented and should comprise a daily intake of at least 10 g of gluten for a minimum 4 weeks. Data from [28••, 29••, 30–33]

hypersensitivities. Reservations about these data include the almost lack of placebo responses in these patients during food challenges (unusual in this patient population), and the lack of information about the effectiveness of wheat-free dietary approaches in the short or long term. The data need confirmation by an independent group.

The Not-So-New Clinical Entity of Non-Celiac Gluten Sensitivity

The term 'non-celiac gluten sensitivity' (NCGS), has recently reached consensus agreement in terms of its name and the diagnostic criteria [28••, 29••]. Patients are considered to be NCGS if celiac disease has been excluded and if the gastrointestinal IBS-like symptoms markedly improve on a GFD. Stricter criteria could also be the absence of evidence of allergic or immune mechanisms, or a positive response to blinded, placebo-controlled gluten challenge. Even though the term was only agreed upon recently, the entity has been talked about for decades.

Evaluation of 'exclusion diets' has consistently shown wheat to be one of the most common factors inducing GI symptoms [41]. Dickerson and colleagues first identified that wheat may cause different kinds of chronic ill-health in individuals without celiac disease in 1978 [42]. Ellis and Linaker described normal biopsies and normal lymphocyte counts existing in combination with rapid disappearance of symptoms when their patients withdrew gluten from the diet [43]. Cooper and colleagues also described patients experiencing gluten-sensitive diarrhea, but without celiac disease [44]. These early descriptions did not acknowledge the presence of other components of wheat, particularly fructans, or wheat sensitivity that might be responsible for the symptoms.

The frequency of patients presenting with IBS-type symptoms similar to celiac disease who are reportedly responding well to a GFD, but have no other clinical or diagnostic biomarkers of celiac disease, is increasing and has been suggested to affect up to 15 % of the population [45]. The growing gluten-free market may now be between 15–20 % of the population, those with celiac disease being a minority contributor [46]. Gluten has also been linked to a wide range of conditions other than IBS as above. There is also considerable emotive power behind gluten as a causative agent and now commercial interests add to that pressure. There is an urgent need for quality evidence.

Evidence from In Vitro and Animal Studies

Studies in vitro and animal experiments may provide clues to possible mechanisms that can be applied to subsequent human studies. Gliadin has been shown in vitro to increase epithelial permeability and alter protein expression of components of the tight junctions in Caco-2 cell monolayers, used as a surrogate model for the human gut epithelium [47]. Gliadin also induced apoptosis [48, 49] and increased oxidative stress in that cell line [50]. Studies using animal models of gluten-sensitivity (not celiac disease) have also directly investigated the role of gluten challenge in inducing gut dysfunction [48], and

changes in neuromotor function and microbiota independently of inducing intestinal inflammation or injury were reported [51].

Evidence from Clinical Studies

Studies from several groups have attempted to characterize the phenotypic, genotypic and immune markers of patients with NCGS, where the diagnosis has been based upon a response to GFD, but without evidence of celiac disease. A group from Germany identified a sub-group of patients with diarrhea-predominant IBS who carried the HLA-DQ2 allele, did not have villous atrophy on duodenal biopsy, had varying pathological, immunological (positive IgA anti-gliadin or anti-TTG antibodies and increased density of IELs) and who had symptomatic improvement on a GFD [52, 53]. In two other studies, HLA-DQ2 was not overrepresented in the NCGS cohort studied [54, 55••]. Sapone et al. provided evidence that abnormalities of innate immunity without epithelial barrier dysfunction of the intestinal mucosa were found in NCGS in contrast to patients with celiac disease [56]. A study from Bologna reported 56 % of 78 patients with NCGS had IgG AGA antibodies with mostly negative specific celiac antibodies, although many of the patients had abnormalities on duodenal histopathology [57]. In an older study, immunological markers (including serum IgE, eosinophil counts, histamine release) have appeared normal in NCGS [41]. Finally, a recent comparative study of a cohort of patients with diarrhea-predominant IBS suggested that patients had improved stool frequency on gluten-free, but not gluten-containing, diet and that intestinal permeability was worse on the gluten-containing diet [58•]. This observation was consistent with the effects of gliadin on cell monolayers (see above) but inconsistent with reduced permeability reported in another study where patient selection was quite different [56]. All the studies above have suffered from methodological limitations that include the fact that varying proportions of the patients studied would now be considered to have celiac disease. Furthermore, the specificity of observations to gluten itself (rather than GFD), while implied by the investigational teams, was not demonstrated.

Recently, a series of interventional re-challenge studies from Australia were designed to address the issues of whether gluten can specifically induce symptoms and whether current diagnostic criteria for NCGS are actually identifying patients with NCGS. Two important methodological innovations were included. First, the gluten used was free from contamination by carbohydrates with the potential to induce symptoms (i.e. it was devoid of fodmaps). Secondly, celiac disease had been definitively excluded by accepting only patients with normal duodenal histology and negative serology, or those who were HLA-DQ2/8 negative. Additionally, the second report assessed all patients by utilising an assay that can identify with high sensitivity patients with celiac disease who are currently on a GFD. The first study was a randomised double-blind, placebo-controlled trial of a single dose of gluten (16 g/day) without a controlled dietary background in parallel groups [55••]. Thirty-four patients were randomised to receive two slices of gluten-free bread and one gluten-free muffin, either with or without gluten added, over 6 weeks, whilst continuing their usual GFD. The test foods were indistinguishable and were fodmap-free. The gluten group had greater gastrointestinal symptoms and tiredness induced compared to those induced in the placebo group within the first week. There were no differences for intestinal permeability, faecal lactoferrin and highly sensitive C-reactive protein, and no elevated celiac antibodies. These results were considered the first specific evidence towards the existence of NCGS.

The same research group went onto conduct a follow-up dietary trial in identically selected patients using a crossover design and supplying a controlled diet [59••]. Following a 2-week run-in period on a low fodmap diet, 37 patients with NCGS and IBS who were symptomatically controlled on a GFD underwent a double-blind, placebo-controlled, randomised crossover trial of placebo, low-gluten (2 g/day) or high-gluten (16 g/day) for 1 week, followed by a 2-week washout period, before crossing over to the next diet. All meals and snacks were provided. The food was low in fodmaps and gluten-free, and protein levels were balanced with whey protein. Symptoms consistently and significantly improved on restriction of fodmap intake, but significantly worsened to a similar degree during each dietary treatment period, irrespective of diet. Only six participants (16 % of total cohort) had symptoms significantly induced on the high-gluten arm. A subject-expectancy or order effect was found with the first intervention inducing greater symptomatic changes than the second or third challenges, regardless of what it contained. There were no changes in celiac serology, faecal concentrations of eosinophil cationic protein, calprotectin or human ß-defensin-2, or any gliadin-specific T-cells induced. There were no differences in any end-point in those with and without DQ2/DQ8.

A double-blind, placebo-controlled, randomised cross-over re-challenge was then conducted in 22 of these patients, where they were randomized to receive gluten (16 g/day), whey (16 g/day) or placebo for 3 days each, with a minimum 3-day washout [59••]. All food was provided, but in addition to the food being low in fodmaps and gluten-free, dairy products and food chemicals were also controlled to minimise all potential triggers of gut symptoms. The results showed poor reproducibility of symptom induction to a specific protein. Moreover, only two participants had symptoms

significantly induced on the gluten arm in the 3-day re-challenge, and they were not the same two participants who had a positive response to the gluten (16 g/day) arm in the 7-day trial. A very high nocebo response was found in both trials, regardless of all background dietary triggers being controlled. Either the patients did not have NCGS as self-reported or the trial design precluded its recognition. It may also be possible that gluten may not be a specific trigger of functional gut symptoms once dietary fodmaps are reduced.

Conclusions

Non-celiac gluten sensitivity is an entity awaiting validation, better diagnostic criteria, and, if it does exist, pathogenic mechanisms. Progress will only ensue if the methodologies for the design and execution of studies, and their interpretation markedly improve. The reluctance to acknowledge other components of wheat, such as fructans, non-gluten proteins and WGA, as potential pathogenic factors has often hampered good interpretation of clinical observations. Essential rules for future studies should include the following. First, celiac disease has to be seriously excluded by HLA studies and/or strict histological and immunological criteria. The inclusion of patients with intraepithelial lymphocytosis will always raise the issue of whether they really have celiac disease with a milder intestinal lesion. Secondly, the use of blinded placebo-controlled food re-challenge methodology to prove gluten sensitivity is present is not reliable, especially in patients who believe they have NCGS. Perhaps the selection of patients for study should be those with IBS naïve to a GFD. Thirdly, the trap of assuming that response to a GFD or exacerbation of symptoms due to a gluten-containing diet reflects specific effects of gluten should be outlawed and credence be given to the other wheat-related food constituents that can also cause gastrointestinal symptoms. Perhaps if these rules were followed, we would now be a lot closer to defining mechanisms by which gluten might act, might have developed biomarkers to identify patients who truly do have NCGS and perhaps, most importantly, answered the question of whether NCGS does really exist. On current evidence the existence of the entity of NCGS remains unsubstantiated.

Compliance with Ethics Guidelines

Conflict of Interest Jessica R. Biesiekierski, Jane G. Muir, and Peter R. Gibson declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with animal subjects performed by the authors. With regard to the authors' research cited in this paper, all procedures were followed in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000 and 2008.

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