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Adverse Reactions to Wheat or Wheat Components

Fred Brouns, Gonny van Rooy, Peter Shewry, Sachin Rustgi, and Daisy Jonkers

Abstract: Wheat is an important staple food globally, providing a significant contribution to daily energy, fiber, and micronutrient intake. Observational evidence for health impacts of consuming more whole grains, among which wheat is a major contributor, points to significant risk reduction for diabetes, cardiovascular disease, and colon cancer. However, specific wheat components may also elicit adverse physical reactions in susceptible individuals such as celiac disease (CD) and wheat allergy (WA). Recently, broad coverage in the popular and social media has suggested that wheat consumption leads to a wide range of adverse health effects. This has motivated many consumers to avoid or reduce their consumption of foods that contain wheat/gluten, despite the absence of diagnosed CD or WA, raising questions about underlying mechanisms and possible nocebo effects. However, recent studies did show that some individuals may suffer from adverse reactions in absence of CD and WA. This condition is called non-celiac gluten sensitivity (NCGS) or non-celiac wheat sensitivity (NCWS). In addition to gluten, wheat and derived products contain many other components which may trigger symptoms, including inhibitors of α-amylase and trypsin (ATIs), lectins, and rapidly fermentable carbohydrates (FODMAPs). Furthermore, the way in which foods are being processed, such as the use of yeast or sourdough fermentation, fermentation time and baking conditions, may also affect the presence and bioactivity of these components. The present review systematically describes the characteristics of wheat-related intolerances, including their etiology, prevalence, the components responsible, diagnosis, and strategies to reduce adverse reactions.

Keywords: celiac disease, non-celiac wheat sensitivity, wheat, wheat allergy, wheat intolerances

Introduction

There are no data supporting the suggestion that either wheat-based bread or pasta consumption is directly related to overweight and diabetes, nor that gluten is a cause of addictive overconsumption of food, contributing to overweight (Brouns, van Buul, & Shewry, 2013; Jones, 2012). Nevertheless, it is increasingly suggested that people should avoid eating gluten-containing grains, in particular wheat, because this may cause overweight and a range of related chronic diseases. Much of this information is based on opinions and selective interpretation of limited data available, as presented in social media and some popular books (Davis, 2012).

It is well known, however, that not all individuals are able to tolerate all foods, meaning that they cannot ingest, digest, absorb and/or metabolize some specific foods or food components without adverse physical or mental reactions. Very early observations of wheat gluten intolerance stem from France in 1854 (Peyrat, 1854). Nowadays, celiac disease (CD), in which individuals react adversely to foods containing gluten proteins, is a well-characterized disorder with an auto-immune component. The term gluten intolerance has been used both as a synonym of CD as well as to indicate that a patient experiences a clinical improvement after starting a gluten-free diet (GFD), even when he/she does not have CD. However, according to Ludvigsson et al. (2013), the term gluten intolerance is nonspecific and carries inherent weaknesses and contradictions.

It is often suggested that the prevalence of wheat-related disorders has increased over time, for which various plausible explanations are being given. In addition to CD, some individuals develop allergies to wheat proteins, which differs from CD in the components that cause the reaction and the mechanisms. More recently, a cluster of various intestinal and extra-intestinal symptoms associated with the intake of wheat has been described as Non-Celiac Wheat Sensitivity (NCWS, initially also named as non-celiac gluten sensitivity, NCGS). Perceived adverse reactions to wheat or wheat components can differ significantly with respect to degree of disturbing quality of life, general discomfort, clear diagnostic criteria, and criteria of belief without clear evidence. To give more insight in these matters, the present article discusses aspects of these three types of wheat-related disorders in more

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detail: 1) celiac disease, 2) wheat allergy (WA), and 3) NCWS. For each of these, we will give a brief description followed by discussions of etiology, prevalence, the components responsible, diagnosis, and options for treatment. In an accompanying review (Rustgi, Shewry, Brouns, Deleu, & Delcour, in review), we will address wheat genetics and environmental factors as well as how both impact on compositional differences, which in itself may influence digestion and bioactive responses.

Celiac Disease

CD is defined as a chronic small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals. Due to analytical complexity, the total number of proteins present in the mature grain has never been determined. However, proteomic analysis showed almost 500 proteins present in flour (Dupont, Vensel, Tanaka, Hurkman, & Altenbach, 2011) and over 1,100 present in mature whole grain endosperm (Skylas et al., 2000). Of these proteins, >100 are classified as gluten proteins, which may only differ in composition by a few amino acids. They belong to the prolamins superfamily of plant proteins, which include gliadins and glutenins in wheat, secalin in rye and hordein in barley (Tatham & Shewry, 2008). In the present paper, we focus primarily on wheat. In CD patients, long-term exposure to gluten results in variable degrees of intestinal damage and in most patients, the intestinal mucosa will recover on a GFD. According to the accepted definition, CD is a chronic disease that may present itself in various ways, for which the terms "classical CD," "non-classical CD," "refractory CD," "potential CD," "latent CD," and "transient CD" are being used. For detailed definitions/description of the different terms used, the reader can refer to Ludvigsson et al. (2013). Whether exposure of an individual to gluten results in CD is determined by a combination of a specific genetic predisposition and environmental factors. Approximately 25% to 40% of the population expresses the haplotypes HLA-DQ2 or DQ8, which results in susceptibility to CD. This percentage differs for different regions. It is estimated that only 4% of individuals with the genetic predisposition DQ2 and DQ8 actually develop CD. Seen this relatively small percentage, there must other factors that, when having the genetic predisposition, trigger the initiation of the disease. In this respect, timing of first gluten exposures at a young age (Pinto-Sanchez et al., 2016), the dose of the (initial) gluten exposure (Koning, 2012), disease- or drug/alcohol-related changes in intestinal permeability as well as exposure to antibiotics and viral infections (Lebwohl, Sanders, & Green, 2018) have been proposed. It should however be noted that the exact impact of environmental factors in individual subjects is not yet clear.

CD etiology

The high contents of proline and glutamine in the gluten proteins result in partial resistance to digestion by gastrointestinal peptidases. In case of an altered gut permeability, these undigested gluten peptides can enter the lamina propria of the small intestine via trans-cellular or para-cellular routes, leading to a cascade of reactions causing adaptive immune responses and inflammation as described in detail by (Fasano, 2012; Lebwohl et al., 2018). When undigested gluten peptides enter the lamina propria of the intestinal wall, via transcellular or paracellular routes, an adaptive immune response is initiated after deamidation of the peptides by the enzyme tissue transglutaminase (tTG). This deamidation to glutamate boosts their affinity to the HLA-DQ2/8 receptor. An inflammatory T cell response to HLA-DQ2/8-bound peptides results from the recognition of a specific region (called epitope), which is determined by its amino acid sequence, on the surface of the antigen. The immune and inflammatory responses finally lead to characterized increased numbers of intraepithelial lymphocytes and villous damage (see Figure 1). Many distinct gliadin- and glutenin-derived T-cell epitopes exist, derived from either α-, γ-, and ω-gliadins or from LMW/HMW glutenins. The activation of gluten-reactive intestinal T cells, mediated by recognition of epitopes and enhanced by deamidation by tTG, enhancing receptor affinity, is an decisive step in the development of CD. The characterization of gluten T-cell epitopes, restricted by celiac disease associated HLA-DQ molecules was essential in this respect. It has been shown that not all gliadin epitopes are equally potent in T cell stimulation. Alpha-gliadin (n 57–73), gamma-gliadin (n 139–153), and omega-gliadin (n 102–118) appear to be the most active peptides whereas γ-gliadin shows less activation. In addition, it was shown that individual T cell, immune, and inflammatory cascade responses to different gluten peptides may differ (Camarca et al., 2009; Vader et al., 2003). Inflammatory mediators and substances that can pass through the intestinal wall can cause problems elsewhere in the body such as gluten-induced dermatitis herpetiformis and neurological problems/ataxia (see further below).

The initial development of CD may be related to the food that a child receives early in life. The effects of breastfeeding and the timing of introduction of additional feeds, including bread (that is, time of weaning) on the initiation of CD are not clear (Ludvigsson & Fasano, 2012). It is recommended that the intake of small quantities of gluten (usually bread) should start gradually before the age of 6 months, often simultaneously with breastfeeding (Ivarsson et al., 2013). The reason for this recommendation is that the immune modulatory properties of breastfeeding and the development of the intestinal microbiota would contribute to the prevention of auto-immune diseases (Agostoni et al., 2008). A number of studies have indicated a role of gut microbiota perturbations and related gut-associated immune competence in the etiology of CD (Chander, Yadav, Jain, Bhadada, & Dhawan, 2018; Nadal, Donat, Ribes-Koninckx, Calabuig, & Sanz, 2007; Nistal et al., 2012, 2016; Olivares et al., 2018). Whether this link is truly causal or a consequence of altered dietary patterns in individuals that suffer from CD requires further study.

CD prevalence

It is estimated that approximately 1% of the global population suffers from CD. However, a large variation in prevalence among various countries has been reported (Catassi, Gatti, & Lionetti, 2015; Lionetti, Gatti, Pulvirenti, & Catassi, 2015). According to the latest meta-analysis, the global seroprevalence of CD is 1.4% with a range of 1.1% to 1.7%. However, based on analysis of biopsy specimen, it is 0.7% with a range of 0.5% to 0.9% (Singh et al., 2018). CD appears to be more common in women than in men (ratio of 2:1 to 3:1). It is estimated that only one out of eight people with CD has been diagnosed properly following clear symptomatology, which suggests that many individuals remain undiagnosed because of unrecognized (nonspecific) symptoms. This is referred to as latent and silent CD. Rubio-Tapia, Ludvigsson, Brantner, Murray, and Everhart (2012) documented that the prevalence of undiagnosed CD has dramatically increased fourfold in the United States during the past 50 years. The authors comment that the reasons for this increase are unknown and speculate that the most likely explanation may be environmental, such as a change in quantity, quality, or processing of cereals (Rubio-Tapia et al., 2012). Overall 10% to 15% of CD patients

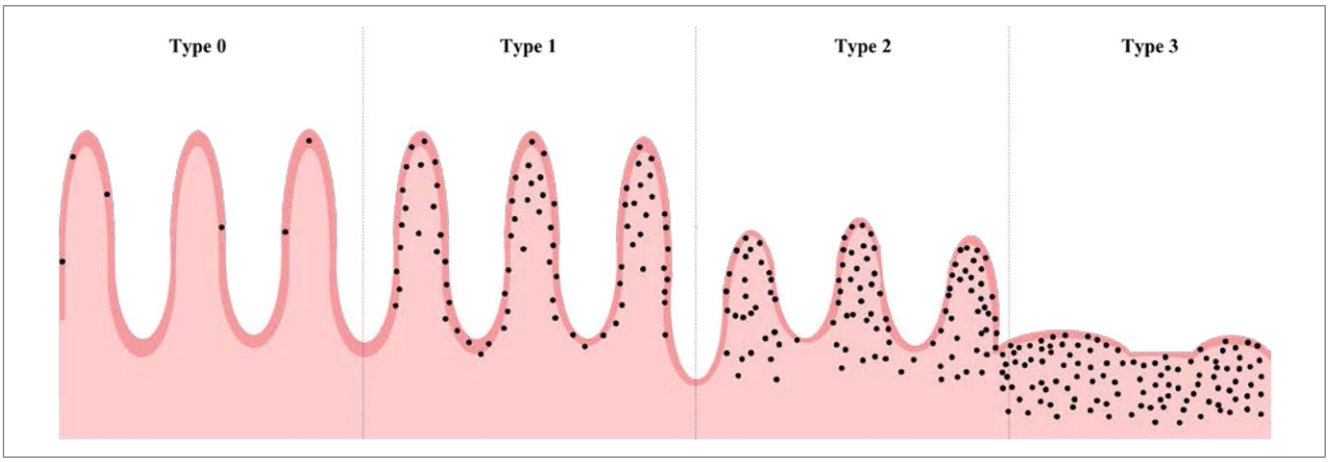

Figure 1—A schematic illustration of progressive tissue changes in the small intestine. Type 0: Normal mucosa with Intraepithelial lymphocyte (IEL) count <25 per 100 enterocytes; Type 1: Normal mucosa with an increased IEL count; Types 2 and 3 show flattening of the villi and increased IEL counts and lymphocytes in the gut mucosa lining. IELs are presented as black dots. (adapted from (Cukrowska et al., 2017). Histological classifications commonly used are Marsh, Marsh–Oberhuber and Corazza. For comparison see Ludvigsson et al. (2013).

appear to suffer from dermatitis herpetiformis, defined as a cutaneous manifestation of small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten. It is characterized by herpetiform clusters of pruritic urticated papules and vesicles on the skin, especially on the elbows, buttocks, and knees as well as IgA deposits in the dermal papillae. Gluten ataxia (GA), defined as "idiopathic sporadic ataxia" is one of a number of neurological manifestations attributed to CD (Ludvigsson et al., 2013). Gluten ataxia has a prevalence of 15% among all ataxias and 40% of all idiopathic sporadic ataxias (Hadjivassiliou, Sanders, & Aeschlimann, 2015). The following factors have also been suggested to play a possible role in CD etiology and modifying our immunological sensitivity to gluten (Olivares et al., 2018): major changes in the overall diet and increased hygiene, changes in our gut microbiota composition and metabolism and related gut-associated immune competence, an overall changed lifestyle (less physical activity, more smoking), and changes in medicine use (notably wider use of antibiotics and vaccines).

CD causing substances

Due to the high contents of proline and glutamine in some parts of the gluten protein sequences, gluten is only partially digestible by the enzymes present in the human intestine. Detailed studies in several countries have led to the identification of the indigested protein fragments that cause intolerance and sensitivity reactions (DiGiacomo, Tennyson, Green, & Demmer, 2013; Ludvigsson et al., 2013; Mamone, Picariello, Addeo, & Ferranti, 2011; Pastorello et al., 2007; Tatham & Shewry, 2008) and allowed the amino acid sequences (epitopes) that stimulate T cells (immunodominance) to be ranked (Anderson, Degano, Godkin, Jewell, & Hill, 2000; Anderson et al., 2005; Shan et al., 2002, 2005; Tye-Din et al., 2010). As a result, a list of internationally accepted gluten fragments, which play a role in CD, was reported (Sollid, Qiao, Anderson, Gianfrani, & Koning, 2012). In addition, there is a collection of >1,000 amino acid sequences of toxic and immunogenic gluten peptides of the FARRP-Program of the University of Nebraska (FARRP 2019). The number, type, and distribution of epitopes, within a wheat type, may play a role in the ability to elicit CD (Shewry & Tatham, 2016). Salentijn and co-workers reported a significantly lower number of indigestible peptides in several tetraploid species (Salentijn et al., 2009, 2013), while a number of other studies have suggested that "modern" hexaploid wheat types may induce more immune and inflammatory reactivity than "ancient" tetraploid and diploid species and hence result in more gastrointestinal problems in wheat-sensitive individuals (Carnevali et al., 2014; Molberg et al., 2005; Pizzuti et al., 2006; Sofi et al., 2010; Sofi et al., 2014; Spaenij-Dekking et al., 2005; Vincentini et al., 2007, 2009). However, other studies have shown that all types of wheat, including "ancient" species and modern cultivars, induce some degree of immune reactivity and thus should be avoided by CD patients (Colomba & Gregorini, 2012; Escarnot et al., 2018; Gregorini, Colomba, Ellis, & Ciclitira, 2009; Suligoj, Gregorini, Colomba, Ellis, & Ciclitira, 2013).

Van den Broeck et al. (2010) used antibodies to the Glia-α9 and Glia-α20 epitopes and immunoblotting to classify modern bread wheat varieties (1986 to 1998) and older types (1863 to 1982) as having low, medium, or high reactivity. They showed that only one of 36 modern wheat varieties had low levels of the 33-mer peptide (containing one of the most harmful CD causing epitopes to which Glia-α9 antibody reacts), compared with 15 of 50 older types and suggested that modern breeding may have contributed to an increased content of highly CD reactive epitopes in modern wheat varieties. However, Ribeiro et al. (2016) reported recently a much more detailed study, comparing bread wheat (*Triticum aestivum* var *aestivum*), spelt (*T. aestivum* var. *spelta*), and durum wheat (*Triticum turgidum* var. *durum*), including modern cultivars and old landraces from different countries and confirmed that there is significant heterogeneity between wheat genotypes in the levels of peptides containing T cell–stimulatory epitopes (Ribeiro et al., 2016). This lead to the conclusion (in line with Spaenij-Dekking et al., 2005) that targeted wheat breeding did not result in increased levels of immunogenic epitopes, thus has not contributed to increases in the incidence of celiac disease.

There is no evidence to support suggestions, particularly in social media, that ancient tetraploid grains and spelt are more tolerable for individuals suffering from CD. (Escarnot et al., 2018; Gregorini et al., 2009; Ribeiro et al., 2016; Suligoj et al., 2013; Vincentini et al., 2007, 2009). The recent comprehensive review of "Peptides from gluten digestion: a comparison between old and modern wheat varieties" by Prandi, Tedeschi, Folloni, Galaverna, and Sforza (2017) concluded that the old varieties may actually contain more immunogenic sequences than modern varieties.

Kasarda (2013) and Shewry, Pellny, and Lovegrove (2016) provide solid evidence that the gluten content in wheat has not increased over time. In fact, total protein content of wheat, measured in whole meal flour (determined as %N × 5.7), slightly decreased, with a parallel increase in starch content and accounts for between 10% and 15% of the dry weight, with gluten proteins accounting for 70% to 75% of the total grain protein (Shewry et al., 2016).

It is often suggested that the consumption of vital wheat gluten (a co-product of the wheat starch industry) has increased dramatically because of its incorporation as a functional additive (as a binder and to improve texture) in bread and numerous other food products. However, values for the actual levels of use of vital gluten over the years are difficult to obtain. Based on various assumptions, Kasarda (2013) calculated that the intake of vital gluten has tripled since 1977, from 136 to 408 g per year or 0.37 to 1.12 g per day, per capita of the population. To what extent this relatively small amount of vital wheat gluten is really influential in CD etiology is a matter of debate, given that many times higher intake of gluten consumed in bread (5 to 5.5 kg per year or 13.7 to 15.1 g per day; Kasarda, 2013).

One question that needs to be addressed is the precision of analytical measurements. It should be recognized that the analysis of gluten and gluten epitopes presents challenges due to the difficulty in solubilizing gluten protein/peptides, variation in the compositions of gluten hydrolysates and food matrices, the lack of appropriate reference materials, the specificity of antibodies, and the use of different methods for separation and quantification (Lexhaller, Tompos, & Scherf, 2017; Schalk, Lang, Wieser, Koehler, & Scherf, 2017; Schalk, Lexhaller, Koehler, & Scherf, 2017; Scherf, 2017; Scherf & Poms, 2016; http://www.wgpat.com/aims.html).

Schopf and Scherf (2018) reported that the relative proportions of CD-immunogenic and -toxic peptides in gluten protein fractions vary depending on genetic factors such as species and cultivar in combination with environmental factors such as climate, soil, fertilization, and agricultural practices (Ashraf, 2014; Hajas et al., 2018). Variation in the precise amino acid sequences of gluten proteins, due to substitutions, deletion, and insertions, may affect the number of epitopes that are recognized by the commonly used antibodies. Based on ELISA assays with several antibodies, they concluded that care is required when using ELISA analysis for gluten in foods to estimate their potential activity and clinical relevance in CD (Schopf & Scherf, 2018).

In addition to gluten, it has recently been recognized that non-gluten proteins may also elicit a significant antibody response in patients with CD. Huebener et al. (2015) investigated the level and molecular specificity of the antibody response to non-gluten proteins of wheat in CD. Serum samples from patients and controls were screened for IgG and IgA antibody reactivity to a non-gluten protein extract from the wheat cultivar *Triticum aestivum* -Butte 86. Compared with healthy controls, patients exhibited significantly higher levels of antibody reactivity to non-gluten proteins. The clinical relevance of these observations needs yet to be determined . The main immunoreactive non-gluten antibody target proteins were identified as serpins (a type of serine protease), purinins, globulins, farinins, and α -amylase/protease inhibitors (ATIs). ATIs are a group of enzyme inhibitors present in wheat, in at least 11 different isoforms (Junker et al., 2012). As gluten proteins, ATIs are present primarily in the endosperm. They are also present in isolated vital wheat gluten, which is often added to foods.

The ATI content is affected by the environmental conditions (for example, region, soil, fertilizer, humidity, shade, altitude, and storage conditions). A comparative study of three different durum wheat cultivars grown at three locations in Italy showed that the effect of the growing conditions was greater than the differences between cultivars (Prandi, Faccini, Tedeschi, Galaverna, & Sforza, 2013). Junker et al. (2012) and Zevallos et al. (2017) have built a case for a role of ATIs in inducing inflammation and eliciting an innate immune response, both of which are suggested to potentiate the initiation of CD. These studies were carried out in animal models and *in vitro* assays using isolated protein fractions high in ATIs. These studies gave insights about possible mechanistic effects of isolated ATI fractions on human cell lines and in mice. However, these fractions also contain other unidentified components and have not been exposed to yeast/sourdough fermentation and heat as takes place during food processing. Accordingly, there is a clear need to initiate human trials with oral exposure of 100% ATIs that also have been exposed to food processing. Ultimately the challenge is to isolate ATIs from baked bread. The developments outlined above show that the picture of CD is very complex with many remaining questions.

Zevallos et al. (2017) extracted and characterized ATIs. Their extracts resulted in preparations enriched with >60% bioactive ATIs, in which 10 ATI isoforms were detected (isoforms 0.28, 0.53, 0.19, CM16, CM17, CM1, CM2, CM3, CMX1, and CMX3). The authors studied *in vitro* the biological reponses to exposure of these extracts, determined as release of chemokines and cytokines (IL-8, CCL2/MCP-1, TNF-a, and IL-6) by human THP-1 monocytes/macrophages. It was shown that the bioactivity responses to the most dominantly present isoforms 0.19, 0.28, 0.53, CM2, and CM16, in "ancient wheats" (einkorn and emmer) was significantly lower compared to bread wheat. They also showed in C57BL/6 mice that were previously put on a GFD that ATIs isolated from bread-wheat induced a 30% to 70% higher *in vitro* TLR4 stimulatory bioactivity, as indicated by increased numbers of activated macrophages (F4/80; MHCII) and dendritic cells (CD11c, CD86, CXCR1-CD103). In addition, a significant variation in ATI-related bioactivity was observed among hexaploid wheats purchased from different regions, indicating that genetic, geographic, and environmental growth conditions may play a role. However, this ground breaking work also raised some important questions. For example, the test grains were purchased from food stores or collected at different geographic locations. In the commercial market, grain samples are always mixtures of harvest quantities delivered by different farmers, who may not use the same seed material. To put this more into context, in our current "Well on Wheat?" project, we observed a 28% contamination with other seeds in a 300 kg batch of market purchased spelt wheat (unpublished results). In fact, to be able to conclude about the contents and biological activites of ATIs from any specific type of grain, certified 100% pure seed material should be obtained grown, harvested, and packed under controlled conditions. Another question relates to the purity of the extracted ATI fractions used in the *in vitro* bioactivity essays. According to the authors, there was a significant (approximately 30%) proportion of unknown compounds in the isolated ATI fractions. Accordingly, it is possible that compounds other than ATIs may have played a role in the *in vitro* responses observed. The above-raised concerns point to challenges for sourcing of pure grains and performing careful descriptive analytics as a prerequisite for human intervention trials. More recently, Geisslitz, Ludwig, Scherf, and Koehler (2018) addressed both the issue of environmental effects and of fractionation and quantitative measurement of the predominant ATIs. In their work, the predominant ATIs

0.19 + 0.53, 0.28, CM2, CM3, and CM16 were quantitatively determined in the following wheats: bread-wheat, durum, spelt, emmer, and einkorn. Of each of these wheats, 8 cultivars were grown by the State Plant Breeding Institute, Univ. of Hohenheim (Stuttgart, Germany) under the same environmental conditions with the same agronomic treatments. A targeted LC–MS/MS method using stable isotope labeled peptides as internal standards was used. It was shown that einkorn contained very low to nondetectable amounts of ATIs. However, spelt and emmer had higher ATI contents than bread wheat. In the light of the work of Zevallos et al. 2017, who observed lower bio-reactivity with einkorn and emmer, compared to breadwheat, this raises important questions about the total quantitiy as well the isoform distribution within specific ATI extrtacts that are being used for clinical testing. In this respect, precise analysis and quantification is required before being able to conclude on biological cause-effects of specific grain types.

Lectins are another group of proteins in wheat that have been suggested to be involved in adverse reactions. Based on their cell binding properties, lectins have also been suggested to play a role in CD by causing gut epithelial damage, allowing gluten epitopes to pass the gut barrier (van Buul & Brouns, 2014). Lectins are specific carbohydrate-binding proteins that are present in almost all plants and their seeds, nuts, and fruits. They play a role in important biological processes such as recognition of cells and proteins, and thus protect the plant against external pathogens such as fungi and other organisms. Some cultivated grains and legumes have relatively high concentrations of specific lectins. Lectins are present in the germ of the wheat grain and are called Wheat Germ Agglutinins (WGA). There are no reported comparative studies of the contents of lectins in different wheat varieties, or on possible changes in lectin content or composition since the domestication and intensive breeding of grains. Potential adverse effects of products made of lectin-rich raw materials are based on animal studies and are usually performed with high doses of raw extracted (not heat-exposed) lectins. Because WGA is a heat-labile lectin, it is assumed that it will lose its biological activity as a result of heat exposure, for example, during baking and cooking. Studies using pasta have shown that, although some uncooked whole grain pasta does contain active WGA, cooking the food eliminates all the WGA activity. At present there are no data on the effects of other (heat) treatments such as baking, pasteurization, frying, and extrusion, but it is to be expected that similar effects will be observed as those that occur during the cooking of the pasta. Health effects of wheat lectins were extensively reviewed by van Buul and Brouns (2014).

CD symptoms and diagnosis

The diagnosis of CD is usually based on, blood serology, histological screening of small intestinal tissue obtained by biopsy and eventually also determining the genetic predisposition for CD. As biopsies are rather invasive, especially in children, avoiding endoscopies in both adults and children, when possible, has been proposed (Turner, 2018). In this respect, the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) advises refraining from taking a biopsy in cases where the antibody titers exceed 10 times the normal level (Husby et al., 2012). Below we present some general aspects of CD diagnosis:

- The diagnosis of CD should be considered in patients with:
    1) Symptoms suggestive of CD, such as chronic diarrhea, weight loss, malabsorption, abdominal pain, abdominal distension, and growth retardation in children;
    2) Iron deficiency anemia and/or osteoporosis;
    3) In other diseases, in particular autoimmune diseases associated with celiac disease.
- Diagnosis includes measuring levels of anti-tissue transglutaminase (anti-tTG IgA) and immunoglobulin A (IgA), as well as total IgA and IgA EMA (endomysial antibody).
- In individuals who appear to be IgA-deficient, measuring a combination of IgG-DGP (deamidated gliadin peptide), anti-tTG IgA, and IgA EMA appears to be useful. IgG-DGP is reported to be able to detect some IgA-sufficient patients who are not identified by anti-tTG IgA tests.
- Key diagnostic findings further include the detection of characteristic (albeit not specific) histopathologic changes in intestinal mucosal biopsies. Biopsy analysis should also include intraepithelial lymphocytosis (>25 intraepithelial lymphocytes per 100 enterocytes), crypt hyperplasia, and various grades of villous shortening (see Figure 1). Three histological classifications of CD are being used: Marsh, Marsh–Oberhuber, and Corazza. A direct comparison of these classifications is given by Ludvigsson et al. (2013).
- Finally, conclusive diagnostic evidence that small-intestinal enteropathy is caused by gluten is the histological improvement in response to a GFD.

For a more detailed account of CD diagnosis, see Bai and Ciacci (2017) and Lebwohl et al. (2018).

There are CD associated comorbidities that need to be mentioned. As a consequence of the disappearance of the surface-increasing villi/flattening of the gut mucosa (Figure 1), the intestinal absorptive capacity declines, which will affect the nutritional status. Related symptoms and consequences include (fatty) diarrhea, weight loss, micronutrient deficiencies, and anemia. However, some individuals may not present clear damage to the intestinal mucosa. These people may have one or more general complaints such as chronic fatigue, poor sleep, or headaches. In children, growth retardation, muscle weakness, poor appetite, and a tense abdomen have also frequently been observed. As in adults, children with untreated CD often exhibit mood swings and drowsiness (Lebwohl et al., 2018). The complaints of such people are often not recognized as representing CD and as a result such individuals are not tested or diagnosed, a situation that is called subclinical CD, sometimes also referred to as potential CD.

CD threshold and solutions

The only effective therapy for CD is to go "gluten-free". However, it is not an easy lifestyle to adopt due to the social constraints and ubiquity of gluten in food preparations. A large number of commercial products, especially convenience foods and ready meals, are either prepared using wheat flour or contain one or more of wheat constituents such as wheat proteins, starch, glucose syrup, or maltodextrins, as a filler, processing aid, binding agent, or stabilizing agent. It is important to clarify that "gluten-free" not necessarily means zero gluten, but that the label can be applied to food products for individuals with CD, which carry less than prescribed levels of gluten.

To determine the amount of gluten tolerated by celiac patients in general, a number of gluten challenge studies with a variable number of subjects, gluten doses, and diagnostic methods have been undertaken. For example, working with a single patient (Ciclitira, Evans, Fagg, Lennox, & Dowling, 1984) reached the conclusion that 10 to 100 mg of gliadins induces no or slight changes in the small intestine morphology of celiac patients, whereas higher gliadin doses of 500 mg and 1 g cause moderate to extensive damage. In a later study, Hischenhuber et al. (2006)

reached a similar conclusion that 10 to 100 mg of daily gluten intake is safe for consumption by celiac patients. In separate studies, two Finnish groups also made similar observations and concluded that 20 to 36 mg daily dose of gluten is safe for the celiac patients on GFDs (Kaukinen et al., 1999; Peraaho et al., 2003). On the other hand, Catassi et al. (1993) demonstrated that 100 mg of gliadin intake by celiac patients per day for a month caused deterioration of the small intestinal architecture and a higher dose of 500 mg gliadin per day triggered even more pronounced effects. Later, in a more elaborate double-blind placebo-controlled study performed on 36 celiac patients, the authors concluded that both 10 mg and 50 mg daily gluten doses for 3 months are well tolerated, but that there is a trend for mucosal changes to occur at the 50 mg dose. Reviewing the existing data, Catassi and Fasano (2008) suggested that a conservative threshold for gluten exposure for sensitive individuals would lie between 20 and 100 ppm (=mg/kg).

In a separate study, Collin, Thorell, Kaukinen, and Maki (2004) took a very different approach and analyzed gluten levels in a number of different types of wheat starch ($n = 24$) and naturally gluten-free flours ($n = 59$) consumed by 76 celiac patients who had been on GFDs for 1 to 10 years. The range of gluten found in these products was 0 to 200 ppm and the total daily flour consumption for these individuals was 10 to 300 g. Based on these estimates, they calculated the daily gluten exposure levels and used this data to suggest a threshold of 100 ppm gluten (Collin et al., 2004). However, despite these studies, a consensus on the critical limit or threshold for gluten intake has still not been reached. This is to be attributed to wide variability among celiac patients, as demonstrated by an extensive double-blind placebo-controlled multicenter investigation for the gluten toxicity (10 to 50 mg/day) on 40 celiac patients. In this study, the patients were administered daily a capsule containing 0, 10, or 50 mg of gluten for 90 days and analyzed for clinical, serological, and histological changes in their small intestines. The study reported a high variability among patients in terms of gluten sensitivity. Some patients showed intestinal symptoms after ingesting only 10 mg of gluten a day while other patients showed no histological symptoms even after 3 months daily challenge with 50 mg of gluten (Catassi et al., 2007).

Because of the variability in the results and the wheat consumption levels, different countries allow different gluten levels in the products for consumption by the celiac patients.

In Europe two labeling categories have been defined by the European Food Safety Authority:

1) 'Gluten-free' foodstuffs. These must contain less than 20 mg/kg of gluten in the finished product.This specific labelling applies to all foodstuffs.
2) 'Very low gluten' foodstuffs. These must contain less than 100 mg/kg of gluten in the finished product. This specific labelling applies only to foods for special dietary use.

On the other hand, the Australia and New Zealand Food Agency (ANZFA) recognizes two classes of foods, "gluten-free foods" with no detectable gluten and "low-gluten foods" with no more than 200 ppm gluten. However, the Canadian definition for "gluten-free" is more general, with "gluten-free" meaning a food without wheat, including spelt and Khorasan wheat, or oats, barley, rye, triticale, or any part thereof. On August 5, 2013, the U.S. Food and Drug Administration also set a limit of 20 ppm gluten in allegedly "gluten-free" products.

A decision on the threshold depends not only on the maximum tolerable dose of gluten in food but also on the amount of "gluten-free" product(s) consumed daily, which depends on the eating preferences in a region and per subject. The results of the food challenge study indicated that 200 ppm is not a safe threshold as the gluten intake limit of 50 mg could be reached with the consumption of 250 g of allegedly gluten-free product(s). A 100 ppm limit that allows 100 mg gluten in a kg of food is also impractical, as in European countries consumption of gluten-free products could be as high as 500 g per day (Gibert et al., 2006). However, the threshold of 20 ppm keeps the intake of gluten from 'gluten-free' food well below the 50 mg amount, thus allowing a safety margin for the variable gluten sensitivities and dietary habits of the different patients. This led the Codex Alimentarius Commission (2008) in its current draft proposal to set the following definition for the gluten-free commodities: "a) consisting of or made only from ingredients which do not contain any prolamins from wheat, durum wheat, rye, barley, oats or any *Triticum* species such as spelt (*Triticum spelta* L.,), kamut (*Triticum polonicum* L.) or their crossed varieties with a gluten level not exceeding 20 mg/kg in total based on the foods ready for consumption; or b) consisting of ingredients from wheat, rye, barley, oats or any *Triticum* species such as spelt (*Triticum spelta* L.,), kamut (*Triticum polonicum* L.) or their crossed varieties, which have been rendered "gluten-free", with a gluten level not exceeding 100 mg/kg in total based on the foods ready for consumption; or c) any mixture of the two ingredients as in a) and b) with a gluten level not exceeding (100 mg/kg) in total based on the food ready for consumption."

The immunogenicity of oats is controversial, as it can be tolerated by most but not all individuals with celiac disease, however, to keep the regulations similar throughout the world its use is gluten-free foods for the dietary management of celiac disease is regulated much like wheat by the Codex Alimentarius Commission. It is not clear if the oat tolerance observed in individuals with celiac disease is due to the differences in chemical structure between gliadin and avenin or to the lower avenin content of oat flour in comparison with the high gliadin content of wheat flour. Based on a number of studies, at present, the scientific consensus is 50 g of oats per day are well tolerated by most individuals with celiac disease (Catassi & Fasano, 2008; Gilissen, van der Meer, & Smulders, 2016). In this respect, it should be mentioned that oat is often contaminated with small quantities of either wheat or barley. The contamination may stem from what was still present in the field (due to kernels lost in the past and sprouted and grow again), or during transport and food processing in environments where also wheat and barley was/is present. Such contamination may be sufficient to induce problems in individuals who are sensitive to very small amounts of gluten.

Recent studies indicate that food processing and events in the gastrointestinal tract may have effects on the gluten proteins. Gianfrani et al. (2015) provided evidence that extensive *in vitro* gastrointestinal hydrolysis drastically reduced the immune stimulatory properties of *Triticum monococcum* gliadin. Mass spectroscopy (MS)-based analysis showed that several *Triticum monococcum* peptides, containing known T-cell epitopes, were degraded during the gastrointestinal treatment, whereas many of *Triticum aestivum* gliadin survived gastrointestinal digestion. More recently, Perez-Gregorio, Dias, Mateus, and de Freitas (2018) monitored the gastrointestinal effects on specific CD epitopes by means of an *in vitro* gastrointestinal digestion model that included incubation with brush-border membrane enzymes. Gluten hydrolysates were characterized by MS and the immunogenic peptides were identified by searching for the main T-cell stimulating epitopes. The immunogenic potential of gluten hydrolysates was further analyzed by enzyme-linked immunosorbent assay (ELISA). The results showed that the composition of the gluten

Table 1–Different features of Celiac Disease (CD), Wheat Allergy (WA), and Non Celiac Wheat Sensitivity (NCWS) (modified from Catassi et al. (2015).

	CD	WA	NCGS
Time interval between gluten exposure and onset of symptoms	Weeks to years	Min to hr	Hr - (days)
Pathogenesis	Autoimmunity (innate and adaptive immunity)	Allergic immune response	Immunity? (innate immunity?)
HLA	HLA-DQ2/8 restricted (~97% positive cases)	Not HLA-DQ2/8 restricted (35-40% positive cases as in the general population)	Not HLA-DQ2/8 restricted (50% DQ2/8-positive cases)
Autoantibodies	Almost always present	Always absent	Always absent
Enteropathy	Almost always present	Always absent (eosinophils in the lamina propria)	Always absent (slight increase in IEL)
Symptoms	Both intestinal and extra-intestinal (not distinguishable between these three gluten-related disorders) Common intestinal symptoms: bloating, abdominal pain, diarrhea, nausea, epigastric pain, alternating bowel habits. Common extra-intestinal symptoms: lack of wellbeing, tiredness, headache, anxiety, foggy mind.		
Complications	Co-morbidities, long-term complications	Absence of co-morbidities, short-term complications (including anaphylaxis)	Absence of co-morbidities and long-term complications (long follow-up studies needed to confirm it)

GI, gastrointestinal; GS, gluten sensitivity; IEL, intraepithelial lymphocytes.

hydrolysates depended on the digestion time and protein structural characteristics. Glutenin oligopeptides were degraded faster whereas oligopeptides from gliadin, mainly from α-gliadin oligopeptides, remained intact for a long time. Peptides containing the α-9 gliadin epitope (PFPQPQLPY) remained intact even after 180 min digestion in intestinal brush-border membrane vesicles. The *in vitro* studies discussed above need to be confirmed *in vivo*. An important conclusion is that not all peptides were degraded, suggesting that they are potentially unsafe for CD patients.

Shewry and Tatham (2016) discussed the possibility of improving wheat by selective breeding and application of modern molecular approaches (transgenesis and gene editing) to remove celiac epitopes but retaining functionality for food processing, however, they concluded that this is a formidable challenge due to the complex multigenic control of the gluten protein fraction. Recently, Sanchez-Leon et al.2018) and Jouanin, Boyd, Visser, and Smulders (2018) have used a CRISPR/Cas9-based approach to induce mutations/small deletions, respectively, in α-gliadin and g-gliadin genes, yielding wheat lines with reduction immunogenicity and acceptable dough quality in laboratory-scale breadmaking trials. However, these genome-edited wheat lines still contain celiac-toxic gluten proteins and therefore cannot be regarded as safe for CD patients. For more detail see the accompanying review by editor should check/include ref details when being published after acceptance in same journal issue Rustgi et al. (in review).

Wheat Allergy

An allergen is defined as a substance that causes an immediate immune reaction upon exposure by ingestion, inhalation or skin contact. Wheat belongs to the eight foods (milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybean) identified by Codex Alimentarius (Codex Alimentarius Commission, 1997 updated 2008) as being responsible for approximately 90% of all food allergies in children (Battais, Richard, Jacquenet, Denery-Papini, & Moneret-Vautrin, 2008). Of these food groups wheat is responsible for causing 11% to 25% of the total food allergy prevalence (Hirschenhuber et al., 2006). Below we will describe briefly the most common forms of wheat related allergies. For an overview of characteristics and symptoms associated with CD, WA, and NCWS, see Table 1.

WA etiology

Allergic reactions to wheat, in contrast to CD, involve IgE (immunoglobulin) antibodies. Depending on the route of allergen exposure and the underlying immunological mechanisms, WA is classified into (1) immediate food allergy; (2) wheat-dependent exercise-induced anaphylaxis (WDEIA), (3) respiratory allergy, and (4) contact urticaria. IgE antibodies play a central role in the pathogenesis of these disorders. In WA, the body reacts to the protein via antigen-presenting cells that activate B-cells to produce allergen-specific immunoglobulin IgE antibodies, which bind to mast cells that are present throughout the body. If two or more IgE antibodies on the surface of a mast cell are linked by an allergen, the mast cell is activated and histamine is secreted. This leads to symptoms such as swollen membranes of the mouth and throat, difficulty in swallowing, shortness of breath, diarrhea, vomiting, abdominal pain, asthmatic reactions, and rashes. In addition, a whole-body reaction leading to a sudden severe drop in blood pressure can lead to anaphylactic shock or even death (Hadjivassiliou et al., 2015). For detailed reviews related to cereal induced allergies see Cianferoni (2016); Gilissen, van der Meer, and Smulders (2014); Inomata (2009); Pasha et al. (2016); Sapone et al. (2012); Scherf (2017); and Tatham and Shewry (2008).

WA prevalence

Food allergy in general occurs in more than 6% of children and in almost 3% of adults. Usually the allergy is to specific proteins in the food consumed. Figures for WA among children vary from <0.1% to 1%, depending on age and country (Hischenhuber et al., 2006; Kotaniemi-Syrjanen et al., 2010; Sapone et al., 2012). However, a large meta-analysis has shown that the general prevalence is at most approximately 0.2% (Zuidmeer et al., 2008).

Generally speaking, about half of children "outgrow a food allergy over time." Depending on the type of allergy, this percentage can be lower (peanut) or higher (milk). With regard to WA, studies show that more than 80% of children have outgrown their WA by their eighth year, and 96% before their 16th year (Kotaniemi-Syrjanen et al., 2010). It explains why the number of adults with WA (0.25%, Zuidmeer 2008) is much lower than the number of people with CD. In adults the prevalence of food allergies appears to be higher in women than in men (Afify & Pali-Scholl, 2017). The prevalence of specific subcategories

of wheat allergy such as WDEIA, bakers' asthma, and contact urticaria is a fraction of the total prevalence of 0.25%, thus, is small. As example, WDEIA was calculated to be 0.017% in Japanese children (Aihara et al., 2001). In adults the prevalence of WDEIA is unknown (Baccioglu, Kalpaklioglu, & Altan, 2017). In recurrent urticaria patients, wheat allergy, diagnosed as wheat-induced urticaria, was observed in 6.8% of this patient group. The prevalence of wheat induced chronic urticaria in adults was estimated at 0.5% to 5% (Bernstein et al., 2014). The incidence of wheat induced asthma is high among bakers with a figure ranging from 1% to 10% and bakers associated rhinitis with an incidence of 18% to 29% (Cianferoni 2016).

WA causing substances

Two proteins types generally recognized to induce allergy in most cases are ATIs and monomeric gliadins (α/β-, γ-, and ω-gliadins) commonly known to be involved in CD etiology but now also recognized for being involved in IgE-modulated allergic reactions (Pastorello et al., 2007; Tatham & Shewry, 2008). To a lesser extent, reactions to LMW-glutenins, lectins (WGA), and possibly also lipid transfer proteins (LTPs) may occur (Baccioglu et al., 2017; Brans, Sauer, Czaja, Pfutzner, & Merk, 2012; Cianferoni, 2016; Gilissen et al., 2014; Mamone et al., 2011; Scherf, Koehler, & Wieser, 2016). Cross-reactions may occur with related proteins present in other grains. For example, Rye γ-70 and γ-35 secalins and barley γ-3 hordein cross-react with wheat ω-5 gliadin (Palosuo, Alenius, Varjonen, Kalkkinen, & Reunala, 2001).

It has long been known that ATIs play a role in bakers' asthma (flour dust allergy) and food allergy to wheat (Pastorello et al., 2007; Tatham & Shewry, 2008). Recently, Zevallos et al. (2018) and Bellinghausen et al. (2018) showed that ATI's exacerbate allergic reactions in mice. ATIs are (cooking, baking) heat resistant and it has been shown that ATIs present in cooked wheat (5 min at 100°C) can still cause an allergic reaction (Pastorello et al., 2007). Interestingly, some people have been reported to show stronger allergic reactions to cooked compared to raw wheat (Tatham & Shewry, 2008). However, Gélinas and Gagnon (2018) provided evidence that ATIs present in thoroughly heated, cereal foods lose their enzymic inhibitory activity. In this respect it should be noted that although inhibitory activity may be lost due to heat denaturation, the intact protein is still present in the food. In other words, a change of α-amylase activity does not necessarily mean that there is also a loss of inflammatory and/or immune reactivity in susceptible individuals. Nonspecific lipid transfer proteins (LTPs) may also play a role in WA, but this is less well known (Gilissen et al., 2014; Pastorello et al., 2007; Tatham & Shewry, 2008). LPTs are low molecular weight proteins present in many plants, including cereal seeds and are well characterized as heat-resistant allergens.

WA symptoms and diagnosis

For the diagnosis of wheat allergy, patients must have reproducible symptoms, which occur quickly (that is, minutes to hours) after wheat exposure. For an overview of characteristics and symptoms associated with CD, WA, and NCWS, see Table 1. Wheat allergy can be diagnosed by a combination of a blood test and a skin test. A blood test will determine whether specific IgE antibodies to allergens (in this case wheat proteins) are present, while a skin test checks for the reaction, after subcutaneous injection, to a very small quantity of wheat proteins.

However, the presence of IgE antibodies against wheat in blood does not always mean that there is an active (food) allergy. Similarly, the skin test does not always result in a conclusive diagnosis (Sapone et al., 2012). Final proof can be obtained by a wheat challenge test, executed in a double blind, placebo-controlled setup.

WA threshold and solutions

People suffering from WA need to completely avoid products containing wheat depending on the severity, and must be aware of possible cross-contamination of foods with traces of wheat. Although rare, in some cases allergic cross-reactivity with other grains (barley, rye), and even with fruits can occur. Children with wheat allergies usually react to smaller amounts of wheat than adults (Gilissen et al., 2014). In the literature, it is reported that 80% of children react to less than 2 g of wheat protein, while for a small subgroup less than 10 mg may even be a problem. On the other hand, the nature of the symptoms in children is often less severe (mainly rashes, respiratory and intestinal cramps) than in adults (more frequent anaphylactic shock, facial edema, and severe intestinal symptoms and esophageal irritation; Hischenhuber et al., 2006). However, it is recommended that self-reported symptoms should be treated with caution. Suspected allergy reactions to plant food should be confirmed with double-blind, placebo-controlled challenge tests (Zuidmeer et al., 2008). Excellent reviews of WA, which cover the background, diagnosis, and management can be found in Battais et al. (2008), Gilissen et al. (2014), and Cianferoni (2016).

Non-Celiac Gluten/Wheat Sensitivity

During recent years a third group of people has been classified who experience symptoms after eating wheat products, but have been diagnosed not to suffer from either WA or CD. Mostly these individuals are self diagnosed wheat intolerant/sensitive. In these individuals, irritable bowel syndrome (IBS)-like gastrointestinal symptoms and extra-intestinal complaints occur, which improve on a gluten-free diet. This group of patients is referred to as "non-celiac gluten sensitivity" (NCGS), or the more recently, "non-celiac wheat sensitivity" (NCWS). Di Sabatino emphasizes that NCWS is not a homogeneous disease syndrome (such as CD and WA), but rather a heterogeneous syndrome (Di Sabatino & Corazza, 2012). It is probable that the underlying causes and mechanisms are not the same for all people with NCWS and that reactions may be caused by different components of wheat or grain (products) and involving different host factors. Ludvigsson et al. (2013) defined NCGS as follows: one or more of a variety of immunological, morphological, or symptomatic manifestations that are precipitated by the ingestion of gluten in individuals in whom CD has been excluded. However, despite the word "gluten" in the currently most cited definition "NCGS," it is far from certain that the gluten is the (main) cause of the symptoms observed. The more recent term "NCWS" was adopted since it was noted that gluten (NCGS) may not be the real cause (Biesiekierski, Peters, et al., 2013; Skodje et al., 2018). For that reason, we will use the term NCWS as most appropriate in the remainder of this article.

NCWS etiology

The etiology of NCWS is not completely clear. Intestinal symptoms of NCWS do overlap with CD, but enteropathy (mucosal damage and flattening the brush border) is absent. Serology is generally negative, though increases in anti-tissue transglutaminase (tTG) antibodies, EMA, and DGP antibodies have been reported (Catassi, Elli, et al., 2015). By contrast to CD, NCWS may show signs of an activated innate immune response, whereas, in line with CD, an increased mucosal permeability, may also occur. Cash et al. (2011) found antibodies that could be related to CD (specifically

against gliadin) in 7.3% of the IBS patients (total: $n = 492$) that they examined. However, in only 0.4% was the diagnosis of CD confirmed. Carroccio et al. (2012) observed in a double-blind study that 276 (30%) from 920 diagnosed IBS patients undergoing an elimination diet and a subsequent double blind placebo controlled challenge of these, were suffering from wheat sensitivity as they became asymptomatic (VAS score <10) on the elimination diet and showed symptoms again after the challenge. Two hundred six of the patients (>22.4%) were diagnosed with multiple food hypersensitivity, as they also reacted to the DBPC challenge with cow's milk protein, egg ($n = 120$), and/or tomato ($n = 112$). Only 70 individuals (7.6%) reacted exclusively to wheat (Carroccio et al., 2012). The group that reacted only to wheat demonstrated more association with CD-related biomarkers. For example, 75% of them had the HLA-DQ2/-DQ8 haplotype and 94% of these were found to have increased numbers of lymphocytes in the duodenum. In addition, in one-third of this group of patients intestinal biopsy was positive for EMA, which may indicate a prestage of CD. An overview of different features of CD, WA, and NCWS is given in Table 1 below. For a detailed overview of characteristics and symptoms associated with CD, WA, and NCWS, see Catassi et al. (2017) and Catassi, Elli, et al. (2015).

NCWS prevalence

An international market research agency reported in 2013 that 35% of adult Americans indicated that they are reducing their gluten intake or following a gluten-free diet because they perceive gluten-free foods to be healthier (Watson, 2013). Such high numbers are, however, in contrast with data from other large population studies and may vary between countries. In the U.S. National Health and Nutrition Examination Survey 2009–2010 ($n = 7,762$, CD patients excluded) only 0.55% answered "yes" to the question "are you on a gluten-free diet?" DiGiacomo et al. (2013) and Rubio-Tapia et al. (2012) reported similar values: according to their research some 0.63% of North Americans follow a gluten-free diet. Between 2004 and 2010, 5,896 patients visited the Centre for CD of the Univ. of Maryland (USA) with CD/WA-like symptoms. Of these patients, 6% met the criteria for NCWS (Sapone et al., 2012). Based on these data, the prevalence in the general population was therefore estimated to be lower than 6%. Two European studies observed a prevalence of respectively 13% self-reported NCWS in a general population cohort of 1,002 men and women and 6.2% self-reported NCWS in a general population cohort of 785 adults (Aziz et al., 2014; van Gils et al., 2016). Seventy-nine percent of these individuals appeared to be females having a significantly increased prevalence of anxiety, depression, chronic fatigue syndrome, and food allergies/intolerances. These observations indicate significant psychiatric comorbidities. In one study, it was noted that hypnotherapy was as effective as a diet low in Fermentable Oligo, Di-, Monosaccharides, and Polyols (FODMAP) in reducing such symptoms (Peters et al., 2016). It should also be noted that all food allergies, including wheat allergy, are more prevalent in women than in men (Afify & Pali-Scholl, 2017). The positive predictive value (PPV) of gluten-related symptoms (defined as the probability that someone with the symptom related to gluten really has NCWS) is very low. For example, Capannolo et al. (2015) studied 392 patients complaining of gluten-related symptoms. It was found that 26 of these (6.63%) were affected by CD, two (0.51%) by WA, and 27 were diagnosed with NCWS (6.88%). The remaining 337 patients (85.96%) did not experience any change of symptoms with a gluten-free diet. The authors conclude that self-perceived gluten-related symptoms are rarely indicative true gluten sensitivity/NCWS. Due to the current broad consensus definition, it is not yet possible to make a reliable estimate of the number of people suffering from NCWS. It is expected that this will be higher than the number of people with CD, but reliable (more or less substantiated) estimates are scarce and range from 0.5% to 10% of the population (Ludvigsson et al., 2013). The discussion above highlights the complexity of wheat-related responses and the need for well-designed studies.

NCWS causing substances

Substances that are often suggested to be involved in NCWS are gluten, non-gluten proteins, and FODMAPs. Several intervention studies have been carried out that investigated the reactions of people with (alleged) NCWS when put on a diet with and without gluten and/or wheat, in comparison with a control group. Most of the studies were done in groups of patients with IBS, who reported benefits from avoiding wheat. In the studies that showed significant differences, these mainly involved complaints reported after exposure to wheat/gluten in blind challenges studies (the participant in the test did not know whether wheat/gluten or placebo was present in the food). In most studies, no changes were observed in intestinal permeability or in specific (immunological) biomarkers that could explain a potential underlying cause of NCWS. In a few of the studies, CD could not be ruled out because there was no information about the tissue status of the small intestine (Bucci et al., 2013; Jones, McLaughlan, Shorthouse, Workman, & Hunter, 1982; Sapone et al., 2012) whereas in some other studies, patients showed mild intestinal damage which was consistent with the Marsh 1 stage of CD (Figure 1). These people may therefore have been erroneously labeled as NCWS patients and would probably be diagnosed with CD after further investigation. On the other hand, such slight damage of the intestinal wall and its associated permeability can also occur after extreme physical exercise, drug and/or excessive alcohol use, as well as are reported in people with IBS. According to various studies, there is also a so-called "nocebo" effect (a negative expectation effect). For example, a double-blind placebo-controlled crossover study by Biesiekierski et al. (2013) showed a significant worsening of overall gastrointestinal symptoms irrespective of the intervention (that is, placebo, low-gluten, or high-gluten diet) and interestingly, the symptom scores were highest with the first dietary challenge received. So an order effect was observed, indicating a strong anticipatory symptomatic (that is, nocebo) response, independently of the nature of the dietary challenge (Biesiekierski, Peters, et al., 2013).

It should be noted that a clear relationship of NWCS with gluten alone has not been confirmed by several studies, as they investigated wheat (products) and not gluten per se (Biesiekierski, Muir, & Gibson, 2013; Vazquez-Roque et al., 2013). In these studies, people were also exposed to other components such as LTPs, ATIs, and rapidly fermentable carbohydrates (that is, FODMAPs), normally also present in wheat. To date, there have been no studies carried out in which wheat-sensitive people were tested for their reaction to these individual wheat components or combinations of them. Interestingly, studies using tetraploid durum wheat types of older origin were found to reveal less symptoms in IBS patients when compared to modern wheat (Sofi et al., 2014). It may be that differences in genetics, compared to hexaploid breadwheat, and related differences in the composition of these grains play a rol. (Carnevali et al., 2014; Gélinas & McKinnon, 2016; Sofi et al., 2010, 2014).

FODMAPs are defined as Fermentable Oligo, Di-, Monosaccharides, and Polyols. An increasing number of studies have shown

that a low FODMAP diet is associated with symptom improvement in (subgroups of) patients with IBS (Barrett, 2017; Maagaard et al., 2016; Schumann et al., 2018). A number of studies that looked specifically at the effect of a low FODMAP diet in IBS patients reported significant relief of symptoms, mainly abdominal pain and bloating, likely due to less colonic fermentation and associated gas formation (Barrett & Gibson, 2012; Staudacher, Whelan, Irving, & Lomer, 2011; van der Waaij & Stevens, 2014). Studies on long-term effects are however hardly available. Though, also given the large symptom overlap between IBS and NCWS, wheat-based products are increasingly being listed as foods that contain fermentable carbohydrates (mainly fructose-containing polymers called fructo-oligosaccharides or fructans), being classified as FODMAPs, and may lead to symptoms. However, the quantities of fructans in wheat-based foods are low and far below the levels that may cause abdominal distress in healthy individuals. For example, the total fructans content in two slices of bread amounts to <0.5 g. In a 35 to 50 g cup of breakfast cereals, this is about 1 g and in pasta about 0.5 g per 150 g portion. Furthermore, it should be noted that FODMAPs will be significantly degraded by the yeast and/or active starter culture during dough fermentation which may lead to complete degradation in sourdough systems (Brouns, Delzenne, & Gibson, 2017). It needs to be noted that there is sound evidence that (long-term) avoidance of fermentable dietary fibers can impair favorable gut microbiota composition and metabolism, gut function, and health and that eliminating grains from the diet to avoid FODMAPs also results in the elimination of a wide range of other components that are known to be beneficial (Brouns et al., 2017). In this respect a low FODMAP diet is a clinical diet intended for IBS patients that are carefully guided

NCWS symptoms and diagnosis

Intestinal symptoms commonly reported are as follows: abdominal pain, epi-gastric pain nausea, gastric acid reflux, episodes of constipation, and diarrhea. Most frequent extra-intestinal symptoms are poor sleep, tiredness, lack of well-being, headache, foggy mind, joint/muscle pain, and skin rash/dermatitis. Based on these observations, it is clear that there is significant overlap in symptomatology between NCWS and CD (see also Table 1). At present there is no diagnostic test available for NCWS. Diagnosis is complicated by the fact that people report self-diagnosed symptoms that overlap with CD and WA. Moreover, there is also a significant overlap between symptoms perceived by individuals suffering from IBS. About 70% of IBS patients consider their symptoms to be food related, with wheat ranking in the top five (Rajilic-Stojanovic et al., 2015). In 1982, Jones and colleagues reported that of the 21 IBS patients tested, nine reported symptoms after eating wheat without the presence of CD (according to the then applicable diagnostic criteria; Jones et al., 1982). IBS is known to be associated with psychological factors, altered microbiota composition, motility, and/or visceral perception and high placebo response rates (Borghini, Donato, Alvaro, & Picarelli, 2017; Dolan, Chey, & Eswaran, 2018; Ford & Moayyedi, 2010; see Table 1 and Figure 2). Caio, Volta, Tovoli, and De Giorgio (2014) showed that the 93% of the 44 included subjects that suffered from NCWS test positive for anti-gliadin IgG antibodies and improved significantly following wheat/gluten-free diets. More recently, Uhde et al. (2016) reported comparable results and showed that reported sensitivity to wheat in the absence of CD was associated with significantly increased levels of soluble CD14 and lipopolysaccharide-binding protein, as well as antibody reactivity to microbial antigens, indicating systemic immune activation. It

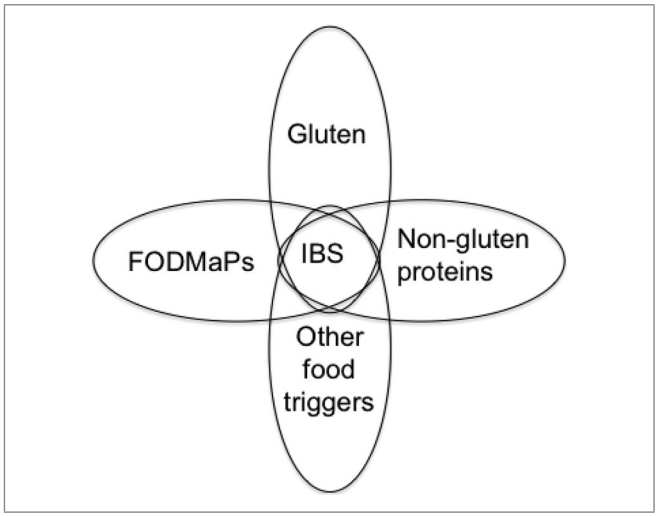

Figure 2–Significant overlap of substances causing symptoms in gluten related disorders and in irritable bowel syndrom (IBS).

should be noted that affected individuals have significantly elevated levels of fatty acid-binding protein 2, a marker of intestinal damage, and correlates with the markers of systemic immune activation. Furthermore, about 50% of NCWS individuals are positive for first-generation anti-gliadin antibodies, mainly IgG, but NCWS is unrelated to the CD genetic markers HLA-DQ2 and -DQ8 (Volta, Caio, Tovoli, & De Giorgio, 2013). The above findings indicate that NCWS is associated with several nonspecific symptoms and factors, complicating an accurate diagnosis (Catassi 2015). At present, suggestions for a possible diagnostic aproach for NCWS have been given by Volta et al. (2013), as follows:

- Gluten ingestion typically elicits the rapid occurrence (in a few hours or days) of intestinal and extra-intestinal symptoms;
- Symptoms disappear quickly (in a few hours or days) after the elimination of gluten from the diet;
- Reintroduction of gluten causes the rapid recurrence of symptoms;
- Celiac disease must be ruled out by means of negative serology (endomysial and tissue transglutaminase IgA antibodies) and a duodenal biopsy on a gluten-containing diet;
- Wheat allergy tests (specific IgE as well as skin prick tests), performed on a gluten-containing diet, must be negative;
- A double-blind, placebo-controlled gluten challenge test is needed in each suspected patient to confirm the diagnosis and to exclude a placebo effect induced by gluten exclusion.

One important remark needs to be made here. When stating that symptoms disappear quickly (in a few hours or days) after the elimination of gluten from the diet, one should be aware that gluten avoidance includes avoidance of all other components of wheat (including non-gluten proteins and FODMAPs).

NCWS threshold and solutions

For many cases of "NCWS" in IBS patients, a GFD and/or wheat-free diet is advised (Nijeboer, Mulder, & Bouma, 2013). However, there is no reliable information on the threshold of wheat consumption at which people develop complaints. Furthermore, recent studies suggested that FODMAPs and/or probably ATIs, rather than gluten, may be the cause of intestinal distress. The threshold value will depend strongly on the causative

component, and the sensitivity of the patient to that component. With regard to dietary fiber, which includes some FODMAPs such as fructans, it is essential to understand that for a healthy intestinal microbiome and intestinal function, the consumption of fermentable carbohydrates is generally recommended. The production of short chain fatty acids and gas during the fermentation of indigestible carbohydrates is not an aspect of disease but merely a normal physiological process in the large intestine, with a range of beneficial effects. Whether the consumption of just 0.5 g to a little more than 1 g of FODMAPs, as present in most breads, can indeed lead to disruptive gas formation is still unclear. As mentioned above, spelt wheat has been reported to have a somewhat lower FODMAP content (1.257 ± 0.213ab g/100 g DM) compared to bread-wheat (mean value 1.568 ± 0.204 g/110 g DM) (Ziegler & Steiner, 2016). However, it is doubtful that the small differences in the FODMAP contents of different types or cultivars of wheat have any clinical relevance. To what extent this may play a role for some of people with IBS who say they benefit from eating spelt products instead of modern bread wheat products deserves further investigation. Ziegler and Steiner (2016) commented that for wheat bakery products suitable for consumption by IBS patients, the applied long-lasting dough fermentation method, leading to a potential degradation of 90% of the fructans and raffinose (a trisaccharide that is the second most abundant FODMAP in wheat) in modern and ancient wheat species appears to be substantially more important than the selection of a specific wheat type.

Wheat and Mental-Psychological Disorders

Apart from the disorders mentioned above, effects on gut–brain axis mediated mental–psychological disorders and well-being have also been proposed (Hadjivassiliou et al., 2010). Although proposed adverse effects of wheat or gluten on the brain and its functions are beyond the scope of this review on intestinal related adverse reactions and disorders, it should be emphasized that intestinal events can induce effects on the central nervous system by means of the gut–brain axis (Oriach et al., 2016; Vitetta, Vitetta, & Hall, 2018). For this reason, we present some backgrounds here while referring to selected important papers. The term "follow your gut feeling," when making decisions, appears to have a physiological basis by means of bidirectional communication pathways between gut and brain, in which gut microbiota, the hypothalamic–pituitary–adrenal (HPA) axis, and serotonin metabolism play an important role. There are various lines of growing evidence that the composition of gut microbiota and related metabolism have impacts on brain metabolism and function, including cognition, behavior, mood, and mental disorders (Allen, Dinan, Clarke, & Cryan, 2017; Kennedy, Cryan, Dinan, & Clarke, 2017). Recently, Mu, Yang, and Zhu (2016) describes gut microbiota as a 'peace keeper' in brain health. There are some common neurotransmitter and metabolic pathways that play a role in these disorders (Kennedy et al., 2017), which also can be influenced by diet (Julio-Pieper, Bravo, Aliaga, & Gotteland, 2014). A possible role of gluten and/or wheat has been proposed, based on several single case observations that alleviation of mental disorders can take place after moving to a gluten-free diet (Lionetti, Leonardi, et al., 2015). It has also been reported that consuming wheat and/or gluten is associated with a variety of psychological symptoms in children, such as depression and attentional problems and that mothers who are unaware of their child having CD more often report depression and anxiety, aggression, and sleep problems (Smith et al., 2017). An important question in this respect is whether gluten, in addition to triggering CD, also the cause of these problems or whether these are simply a comorbidity of CD. Along similar lines, changes in intestinal microbiota composition and activity have been associated with CD, pointing to a possible role of microbiota in its etiology. However, there are at present neither studies demonstrating causality (Galipeau & Verdu, 2014), nor address cause or consequence. For example, autism spectrum disorder is known be associated with a high prevalence of both eating problems and gastrointestinal disorders. In children, eating/feeding problems are complex and multifactorial (Chaidez et al., 2014) and in many cases, it can be expected that the related eating disorders themselves impact on nutrient supply and the gut microbiome, rather than these being the cause of the disorders. Thus, many suggestions that gluten or wheat is a direct cause of mental disorders lack a sound evidence base with respect to components, mechanisms, and cause—effect interactions. In addition, many of the behavioral studies in this field are poorly controlled, have small participant numbers and made use of non-validated questionnaires (Holingue, Newill, Lee, Pasricha, & Daniele Fallin, 2018; van De Sande, van Buul, & Brouns, 2014). Nevertheless, studies using microbe-free animal models, using fecal transplantation and/or studies in which antibiotics-induced microbiota depletion has been carried out, show metabolic and related behavioral changes (Johnson & Foster, 2018). It is thought that such changes are modulated by microbiota-associated metabolites, which in turn strongly depend on the available substrates that enter the distal part of the small intestine and subsequently the colon, where most microbial metabolism takes place. According to these observations, the modulation of the microbiota by means of ingestion of pro- and prebiotics that induce favorable effects on mental functions (psychobiotics) has recently been proposed (Sarkar et al., 2016). Recent work in pigs using distal-ileal-antibiotics infusion showed altered neurotransmitter expression occurring simultaneously with changes in both the large intestinal microbiota and the concentration of aromatic amino acids in the colon, blood, and hypothalamus (Gao et al., 2018). According to the researchers, these findings indirectly indicate that intestinal microbiota can affect hypothalamic neurotransmitter expression, which in turn may impact on brain function. These findings point to possible mechanisms and interactions that remain to be shown to play a role in humans. In fact, in humans, so many factors are involved in the day-to-day changes in gut microbiota, their metabolism, and gut barrier function, including alcohol, infection, antibiotics, painkillers, stress, and many dietary components (Rybnikova, 2018; Sandhu et al., 2017), that conclusions about gluten or bread as a causal factor for brain disorders cannot be drawn (Kelly, Clarke, Cryan, & Dinan, 2016). Clearly there is a need for well-controlled studies in this respect (Fasano, 2017; Li & Zhou, 2016).

Conclusions

The prevalence of diagnosed celiac disease and wheat allergy as well as self-perceived sensitivity to wheat and/or gluten appears to be increasing over time. The term non-celiac gluten sensitivity implies that, in the absence of celiac disease, gluten is the cause of the reported symptoms. However, in addition to gluten, other components present in wheat can potentially contribute to the observed symptoms in these individuals. In this respect, there has been a strong focus on rapidly fermentable carbohydrates (FODMAP), leading some groups to conclude that not gluten but FODMAP cause the sensitivity symptoms. In turn, this has lead to changing the name from NCGS to non-celiac wheat sensitivity.

There is a significant overlap between the symptomatology of individuals suffering from NCWS, CD, and IBS. In addition, IBS

and NCWS are both asssociated with high level of psychiatric comorbidities. Many individuals suffering from self-perceived NCWS claim that they benefit from eating ancient tetraploid grains or spelt-wheat products instead of bread-wheat products. However, these are closely related forms of the same hexaploid species (*Triticum aestivum*) and contain gluten as well as ATIs and FODMAPs. Thus, at present, the widely promoted suggestion that ancient grains are preferable for gluten sensitive individuals, compared to modern hexaploid bread wheat, remains unproven. It may be that social media news and related nocebo effects may play a strong role in these perceptions. At present, systematical unraveling of the possible pathophysiology caused by a variety of components present in a variety of wheat types may provide further insights into the mechanisms of wheat-related disorders especially in NCWS. In addition, detailed characterization and quantitative analysis of grain components,based on the availability high quality standards materials, is required to underpin the elimination of undesired components, either by food processing or by targeted breeding including gene editing.

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Author Contributions

Fred Brouns wrote the base manuscript and obtained critical input and complementary paragraphs by Gonny van Rooy and Sachin Rustgi. Peter Shewry and Daisy Jonkers were involved in final review and editing. Fred Brouns did the final editing. All authors contributed to locating and to interpreting the literature sources.

Conflicts of Interest

The authors declare no conflict of interest.

Abbreviations

ATI	amylase trypsin inhibitor
CD	celiac disease
DGP	deamidated gliadin peptide
EMA	endomysial antibodies
FODMAP	fermentable oligosaccharides, disaccharides, monosaccharides and
GFD	gluten-free diet
IBS	irritable bowel syndrome
LTP	lipid transfer proteins
NCGS	non-celiac gluten sensitivity
NCWS	non-celiac wheat sensitivity
tTG	tissue transglutaminase
WA	wheat allergy
WDEIA	wheat-dependent exercise-induced anaphylaxis
WGA	wheat germ agglutin

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