



## Conference on ‘Carbohydrates in health: friends or foes’ Plenary Lecture 3: Waterlow lecture

### The rise and fall of gluten!

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Mankind has existed for 2.5 million years but only in the last 10 000 years have we been exposed to wheat. Wheat was first cultivated in the Fertile Crescent (South Western Asia) with a farming expansion that lasted from about 9000BC to 4000BC. Thus it could be considered that wheat (and gluten) is a novel introduction to man’s diet! Prior to 1939 the rationing system had already been devised. This led to an imperative to try to increase agricultural production. Thus it was agreed in 1941 that there was a need to establish a Nutrition Society. The very roots of the society were geared towards necessarily increasing the production of wheat. This goal was achieved and by the end of the 20th century, global wheat output had expanded 5-fold. Perhaps as a result the epidemiology of coeliac disease (CD) or gluten sensitive enteropathy has changed. CD is a state of heightened immunological responsiveness to ingested gluten in genetically susceptible individuals. CD now affects 1 % or more of all adults, for which the treatment is a strict lifelong gluten-free diet. However, there is a growing body of evidence to show that a far greater proportion of individuals without coeliac disease are taking a gluten-free diet of their own volition. This clinical entity has been termed non-coeliac gluten sensitivity (NCGS), although the condition is fraught with complexities due to overlap with other gluten-based constituents that can also trigger similar clinical symptoms. This review will explore the relationship between gluten, the rising prevalence of modern coeliac disease, and the new entity of NCGS along with its associated uncertainties.

#### Gluten: Gluten-related disorders: Coeliac disease: Non-coeliac gluten sensitivity

#### What is gluten?

Gluten is the main storage protein used by some classes of flowering plants to nourish seeds during development and germination<sup>(1)</sup>. It is a high molecular weight protein found in the endosperm of grass-related grains, including wheat, barley and rye. It is the composite of two classes of protein, a glutenin and a prolamin (gliadin in wheat), which can be fractionated to produce  $\alpha$ ,  $\beta$  and  $\gamma$  peptides. As plant seeds are the plant tissue most consumed by men, seed storage proteins have been long studied and characterised. Wheat gluten was first isolated in 1745<sup>(2)</sup> and since then further advances in the knowledge of protein structure have

established that the prolamin components of gluten are responsible for the ability to process wheat to form dough by means of creating a viscoelastic network<sup>(3,4)</sup>.

#### History of gluten and mankind

Mankind has existed for about 2.5 million years but cereal crops were introduced as a component of the human diet about 10 000 years ago during the Neolithic Revolution. This saw a transition from hunting and gathering of food to settled agriculture. The first signs of cultivation have been found in the Fertile Crescent in South

**Abbreviations:** CD, coeliac disease; GFD, gluten-free diet; IBS, irritable bowel type syndrome; NCGS, non-coeliac gluten sensitivity.

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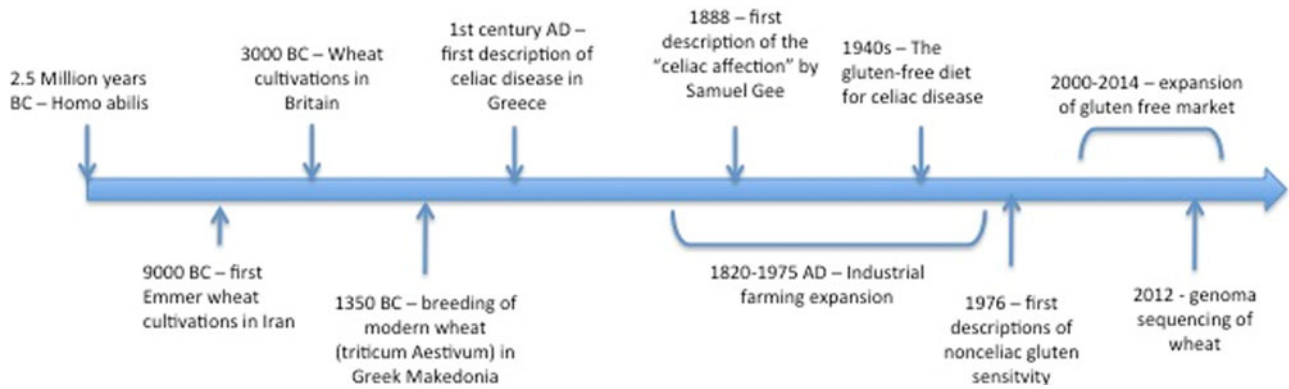


Fig. 1. (Colour online) Timeline of the history of gluten and mankind.

West Asia and the subsequent farming expansion lasted until 4000 BC<sup>(5)</sup>.

Cereal harvesting and consumption has gradually increased since then, until its major outbreak in the 20th century. Between the two World Wars, the need to develop a more efficient rationing system and increased agricultural production became evident. The improvement of wheat cultivation became one of the main objectives of the Nutrition Society which was founded in 1941 in Britain to advance the scientific study of nutrition and its application to the maintenance of health<sup>(6)</sup>. This goal was achieved, with modern day global wheat production amounting to over 700 million tonnes per year (<http://faostat.fao.org>).

Moreover, the need to ensure an efficient agricultural production has led to the artificial breeding and selection of wheat variants with better adaption to extreme climate conditions, bread-making qualities and resistance to diseases<sup>(7)</sup>. This has contributed to a dramatic change in the genetic variety and possibly immunogenic qualities of wheat over time<sup>(7)</sup>. Currently, about 95 % of the wheat grown worldwide is bread wheat (*Triticum aestivum*), a hexaploid species which resulted from the spontaneous hybridisations between more ancient tetraploid (Emmer) and diploid species (Wild grass) and was then selected by farmers for its superior qualities and yields, such as higher number and bigger seeds<sup>(8)</sup>. Furthermore, the awareness of the potential role of gluten in processing food has led to the industrial extraction of gluten from plant seeds and its use in the baking industry as an additive with various functions, such as increasing elasticity and stability of food products or as protein supplement to low-protein food<sup>(9)</sup>.

It is therefore believed that the rate of increase in gluten exposure, from the development of wheat cultivation to modern intensive farming, along with its genetic modification, has been too high to give our immune system the time to develop optimal adaptive mechanisms, though this 'evolutionary theory' has yet to be fully clarified<sup>(10)</sup>. Nevertheless, perhaps as a result of all these factors has come the changing epidemiology of coeliac disease (CD) and other gluten-related disorders (Fig. 1).

### Gluten-related disorders: not only coeliac disease

As a counterpart of its nutritional properties and wide availability, the consumption of cereals has been associated with the development of several symptoms and disorders, whose responsibility have been attributed to the gluten component contained in wheat and related grains. A recent consensus has classified gluten-related disorders into three broad categories: allergic reactions, such as wheat allergy; autoimmune, which includes coeliac disease, dermatitis herpetiformis and gluten ataxia; immune-mediated, in the form of non-coeliac gluten sensitivity (NCGS)<sup>(11)</sup>. The purpose of this review is to specifically focus on CD and NCGS.

#### Coeliac disease

CD is a chronic inflammatory enteropathy caused by dietary exposure to gluten<sup>(12)</sup>. Although the manifestations of CD may have been described more than 100 years ago, it is only from the 1940s that the relationship between gluten and CD has been established<sup>(13)</sup>. However, more than 70 years later, the pathogenesis of CD has yet to be fully elucidated, but it is agreed that the ingestion of gluten in genetically predisposed individuals carrying the human leucocyte antigen DQ2 and/or DQ8 alleles can arise in a T-cell mediated immune reaction, leading to small bowel villous atrophy and subsequent clinical manifestations<sup>(14,15)</sup>.

Historically, CD was rare with an incidence of one in 8000 being reported in the 1950s<sup>(16)</sup>. However, contemporary epidemiological studies estimate a worldwide prevalence of approximately one in 100 or 1 %<sup>(17,18)</sup>. Nevertheless, a considerable proportion of patients still remain undiagnosed with estimates that for every patient diagnosed with CD approximately eight cases are yet to be detected. Furthermore, our understanding of the coeliac patient has drastically changed. In fact previously most of the cases that were diagnosed were children, whereas now it has been shown that adult cases (characteristically presenting between the fourth and sixth decades) are more frequently occurring at a ratio of 9:1 compared with the paediatric cohort.



The clinical manifestations of CD are heterogeneous. The classical presentation of malabsorption characterised by chronic diarrhoea, weight loss and failure to thrive is relatively rare. Far more commonly, patients present with non-classical symptoms which include irritable bowel type syndrome (IBS)<sup>(19)</sup>, iron deficiency anaemia<sup>(20)</sup>, osteoporosis<sup>(21)</sup>, ataxia or peripheral neuropathy<sup>(22)</sup>. Indeed, given that IBS is extremely common affecting about 11 % of the population national guidelines now propose that all patients presenting with such symptoms should have CD excluded<sup>(21)</sup>. In fact, a recent meta-analysis has shown that CD accounts for 4 % of those cases presenting with IBS<sup>(23,24)</sup>.

To date, the only therapy for CD is a lifelong gluten-free diet (GFD)<sup>(25)</sup>. Adherence to a restrictive GFD leads to gradual healing of the mucosa of the small bowel and to the resolution of malabsorptive symptoms<sup>(26)</sup>, although there is a consistent proportion of patients who continue to show a low grade of mucosal inflammation even on a GFD<sup>(27)</sup>. The Codex standard (which is used in the UK and Europe), and similarly the Food and Drug Administration in the United States, suggest that foods containing  $\leq 20$  mg/kg or 20 parts per million (ppm) gluten can be labelled as 'gluten-free' and that foods containing between 21 and 100 ppm gluten can be labelled as 'very low gluten'.

#### *Gluten-free diet in the absence of coeliac disease*

Historically, gluten-free products have been of limited availability with knowledge of CD among the general population shown to be lacking<sup>(28)</sup>. This inevitably contributed to the social phobia that individuals with CD experienced when dining out<sup>(29)</sup>. However, over the last decade there has been a paradigm shift with a drastic rise in the availability of gluten-free products paralleled by an increase in awareness among the public<sup>(30)</sup>. Such findings are not only as a consequence of a rise in the incidence and recognition of CD. In fact, surveys conducted among the general population confirm that a greater number of consumers worldwide are following a GFD irrespective of the presence of CD<sup>(31,32)</sup>. Observational studies have reported that up to 13 % of the population may self-report sensitivity to gluten-based products and that up to 5 % of the population may be taking a GFD of their own volition<sup>(31,32)</sup>. In some, the avoidance of gluten-containing food is viewed as a healthier lifestyle change rather than an actual treatment, whereas in others it is a consequence of reporting ill-effects to ingestion of gluten-based products. In fact, the relationship between the ingestion of gluten-containing products and the development of clinical symptoms even in the absence of CD has been described since the late 1970s<sup>(33,34)</sup>. However, it is only in the last few years that the scientific community has focused its attention on this field, prompted by patient demands and by the sudden media pressure regarding the 'gluten-free lifestyle'.

#### *Why is non-coeliac gluten sensitivity different?*

The definition of NCGS encompasses a spectrum of gastrointestinal and extra-intestinal symptoms which are

triggered by the ingestion of gluten-containing food, even in the absence of the serologic and histological hallmarks of CD or wheat allergy<sup>(11,35)</sup>. This terminology was defined following double-blind placebo-controlled studies showing gluten *per se* to induce symptoms in the absence of CD<sup>(36)</sup>. The symptoms reported include abdominal pain, diarrhoea, constipation and bloating, as well as chronic fatigue, behavioural changes, bone or joint pain and muscle cramps<sup>(11,35,36)</sup>. Symptoms typically occur shortly after the ingestion of gluten, resolve on a GFD and relapse after gluten challenge.

NCGS is often self-reported or suspected by the patients themselves and then confirmed by physicians after other forms of gluten-related disorders have been excluded<sup>(35)</sup>. In fact, whilst the diagnosis of CD can be made in most patients on the basis of positive serology (presence of endomysial and/or tissue transglutaminase antibodies) and villous atrophy at duodenal biopsy<sup>(25,37)</sup>; patients with NCGS present with negative serology and absence of villous atrophy<sup>(11)</sup>. However, the presence of antigliadin antibodies has been described in up to 50 % patients with NCGS<sup>(38–40)</sup>, and an increase in duodenal intraepithelial lymphocytes, corresponding to the grade 1 of the Marsh–Oberhuber histologic classification, has been observed in a subset of patients with NCGS in the absence of other criteria for CD<sup>(38)</sup>. Moreover, the prevalence of NCGS seem to be higher in first-degree relatives of subjects with CD, and carriers of human leucocyte antigen DQ2 and/or DQ8 seem to be at greater risk of experiencing symptoms related to NCGS than the general population although these data have not been confirmed in different epidemiological studies<sup>(38,40)</sup>.

The growing interest in this clinical entity has led to the advancing of several hypotheses about NCGS pathogenesis, yet all of them still remain to be fully elucidated. Altered intestinal permeability similar to that involved in the pathogenesis of CD and activation of the innate immune system following gluten exposure have been considered and are under investigation<sup>(11,41–43)</sup>.

#### *How to diagnose non-coeliac gluten sensitivity*

In the absence of clear serologic or histopathologic criteria to orient towards a diagnosis, NCGS has often been perceived as being an IBS-like entity, mainly due to an evident overlap of clinical features between those two syndromes<sup>(44)</sup>. Furthermore, it has also been observed that IBS patients, previously naive to the effects of gluten, may benefit from a GFD<sup>(45)</sup>. To date, the reference standard for the diagnosis of 'true' NCGS is an elimination diet followed by double-blind placebo-controlled gluten challenge, a method which could hardly be introduced into clinical practice<sup>(11)</sup>. Recently, a diagnostic algorithm based on the absence or presence of clinical, serologic and histological criteria has been proposed to diagnose and differentiate NCGS from CD<sup>(46)</sup>. This novel study provides a clinically pragmatic approach as it takes into consideration the difficulties that arise when evaluating patients who present with gluten-based sensitivity and are already taking a GFD, which in cases of CD can lead to negative coeliac

**Table 1.** Main characteristics of the gluten-free diet and the low fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) diet

	Gluten-free diet	Low FODMAP diet
Food component to avoid	Gluten	Fructose Lactose Fructans Galactans Polyols
Food containing the component	Wheat, inclusive varieties (spelt, kamut, farro and durum), barley, rye and triticale. Gluten used as additive in various manufactured food	High fructose: apples, pears, peaches, mango, sugar snap peas, watermelon; honey, sweeteners. High lactose: milk, yoghurt, fresh cheese. High fructans and galactans: artichokes, asparagus, beetroot, brussels sprout, broccoli, cabbage, fennel, garlic, leeks, okra, onions, peas, shallots; cereals (wheat and rye); chickpeas, lentils, red kidney beans, baked beans; watermelon, custard apple, white peaches, rambutan, persimmon. Polyols: apples, apricots, cherries, longan, lychee, nashi pears, nectarine, pears, peaches, plums, prunes, watermelon; avocado, cauliflower, mushrooms, snow peas; sweetener
Minimal threshold of daily intake	≤20 mg/d or 20 ppm; gluten-free 21–100 mg/d or 21–100 ppm; very low gluten	Not clearly defined (wheat and rye: small quantities are allowed)

ppm, parts per million.

serology and normal duodenal biopsies<sup>(46)</sup>. It has been suggested that where available negative human leucocyte antigen DQ2 and DQ8 genotype is useful, in that it can exclude CD with certainty given its 100 % negative predictive value; this will account for almost half of presenting cases<sup>(31)</sup>. However, if human leucocyte antigen-DQ typing is not readily available, or is positive, then a gluten challenge followed by coeliac investigations is required<sup>(46)</sup>. Traditionally, a gluten challenge has been suggested to be ≥10 g gluten (equivalent to about 4 slices of bread) daily for 6 weeks, prior to formalised testing. More recently this could be as little as ≥3 g gluten (equivalent to 1.5 slices of bread) daily for 2 weeks<sup>(47)</sup>, which may be more suited to patients specifically presenting with gluten sensitivity. By adopting this approach in secondary-care gastrointestinal practice only a minority of adult patients will have a diagnosis of CD (7 %), with the remaining 93 % subsequently diagnosed as NCGS<sup>(31)</sup>. Furthermore, individuals with NCGS do not appear to suffer the nutritional deficiencies (anaemia and haematinic deficiencies) and low mean BMI commonly associated with CD, which is a reflection of the state of normal villi as seen in NCGS as opposed to the villous atrophy in CD<sup>(31)</sup>.

#### *The controversies surrounding non-coeliac gluten sensitivity*

Given the scattered data regarding the pathogenesis of NCGS, it is unclear when faced with a patient diagnosed with NCGS whether gluten is genuinely responsible for their symptom complaint. Firstly, there are no diagnostic biomarkers for NCGS and as previously mentioned carrying out double-blind placebo-controlled gluten challenges is impractical and cumbersome in routine clinical practice. Secondly, evidence concerning the efficacy of a diet low in fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) in controlling IBS symptoms has disclosed the concept that other components of wheat may be responsible for triggering

symptoms instead (Table 1)<sup>(48,49)</sup>. In fact, FODMAP such as fructans, largely contained in wheat and related grains, can trigger gastrointestinal symptoms by means of a combination of osmotic effect and increased gas production from bacterial fermentation<sup>(50,51)</sup>. It has recently been demonstrated that individuals with NCGS on a self-imposed GFD show further improvement when placed on a low FODMAP diet and that subsequent blinded gluten re-introduction shows no specific or dose-dependent effect<sup>(52)</sup>. This has led to the concept of NCGS being challenged although this study actually demonstrated a placebo response to low-dose gluten, high-dose gluten, or whey protein which may be as a consequence of an anticipatory effect in view of the crossover design of the study rather than dismiss the effects of gluten<sup>(52)</sup>. Moreover, it has also been suggested that other proteins contained in wheat, such as lectins, agglutinins and amylase-trypsin inhibitors can trigger the innate immune response and therefore lead to the development of symptoms after ingestion of wheat<sup>(53–55)</sup>. For these reasons, it has been advised that in clinical practice it may be more appropriate to describe patients with NCGS using the preface ‘self-reported’, or alternatively use the term non-coeliac wheat sensitivity<sup>(56,57)</sup>. Nevertheless, this complex field opens a gateway for future studies to identify means of delineating which gluten-based component is responsible for triggering any particular individual’s symptoms.

#### **Conclusion**

The rise in gluten production and consumption has led to the recognition of gluten-related disorders. CD affects 1 % of the population. However, there is a growing body of evidence to show that individuals without CD are taking a GFD of their own volition. This clinical entity is defined as NCGS, although it is not without its controversy and uncertainty given the lack of diagnostic biomarkers and associated conflicting substrates which can provoke similar symptoms.

**Conflict of interest**

None.

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I. A. and F. B. are joint first authors. D. S. S. is senior author.

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