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REVIEW ARTICLE

Non-coeliac gluten sensitivity

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Abstract

Irritable bowel syndrome-like symptoms in response to wheat ingestion is common and well described, but whether the reaction is due to gluten (i.e., non-coeliac gluten sensitivity), other wheat proteins, or FODMAPs (mostly fructans) alone or in combinations has not been clearly defined. Exclusion of coeliac disease in the presence of negative serology, and normal villous architecture but increased density of intraepithelial lymphocytes on duodenal biopsies, is difficult. Furthermore, the confidence by which a positive diagnosis is made or non-coeliac gluten sensitivity is excluded by blinded placebo-controlled rechallenge with wheat protein is reduced by strong nocebo responses generally found in patients with self-reported non-coeliac gluten sensitivity. The absence of a clear biological mechanism of action and difficulties with the design and interpretation of research studies have plunged this entity into even deeper controversy. In the absence of clarity in its diagnosis, the epidemiology, prognosis, and therapeutic approaches to a patient who may be gluten sensitive remain to be determined. Adequate understanding of the issues surrounding the controversy and further research will slowly unravel the truth behind the problem.

The controversy that is non-coeliac gluten sensitivity

There is no more controversial dietary topic in gastroenterology or in the Western community in general than that of the role of gluten in conditions other than coeliac disease or some wheat allergies. It has received considerable lay press attention and has been the subject of several books, multiple reviews and opinion articles in the medical literature, and now an increasing number of interventional studies addressing the issue. There have been expert consensus meetings that have described and legitimized the condition non-coeliac gluten sensitivity (NCGS) and criteria for its diagnosis reported.[1] Several blinded, crossover studies have claimed in their conclusions that the presence of NCGS is unequivocally present in a proportion of patients believing they are gluten sensitive, and strong recommendations that gluten-free diet (GFD) should be used in patients with irritable bowel syndrome (IBS) have been made on the basis of such data. Yet the topic remains highly controversial. The aims of this review are to define the basis for such controversy and to hopefully assist in evaluating published data.

The root of the controversy

Apart from emotive issues that pervade popular press and the Internet, there are several aspects in the scientific literature regarding the design of studies and interpretation of results that underlie the ongoing controversy.

Attribution of effect. There is little doubt that wheat, rye, and barley can induce symptoms in people without coeliac disease. Population questionnaires have shown that about 10% of non-coeliac Australian adults avoid wheat or are gluten free, mostly because of symptoms they associate with their ingestion (particularly bloating and fatigue).[2] Relatively high proportions of patients with IBS try a gluten-free diet in the UK, with durable benefits in a proportion of them.[3] The major alteration in the gluten-free diet is the exclusion of wheat, rye, and barley. These cereals are composed of several candidate molecules that can potentially be associated with disease or symptom generation. For example, gluten causes coeliac disease, amylase–trypsin inhibitors (ATIs) cause some wheat allergies, and FODMAPs (mostly fructans) can induce symptoms of IBS.[4] These are well-founded causal associations.

When a GFD leads to improvement of symptoms consistent with those described for NCGS and the reintroduction of wheat, rye, or barley leads to the recurrence of the problems, then it is imperative that the causal components of the cereal be identified so that appropriate dietary interventions can be designed as therapy. The general and medical/scientific community has been quick to jump on gluten as the pathogenic molecule before such an association is proven despite the presence of other components with proven or pathogenic capabilities. This has been evident in the scientific literature, both in original studies and propagated in review articles. For example, the effect of gluten-free diet on symptoms and intestinal permeability in a cohort of patients with IBS-D was attributed to removal of gluten specifically,[5] and a recent review of gluten as a dietary trigger in patients with IBS has

attributed effects of gluten-free diets and wheat flour or wheat protein challenges in published interventional and retrospective studies to gluten,[6] despite none of the studies actually demonstrating the specificity to gluten.

Determining gluten-specificity is not easy because it is difficult to purify gluten or gliadin without ongoing contamination with other proteins, particularly ATIs. However, such work is needed to provide the tools to address the issues of mechanisms.

Coeliac contamination of the studied population. For symptomatic patients to be studied, it is important that those with coeliac disease be excluded, because rechallenge with wheat protein induces symptoms in many patients with coeliac disease who are gluten free. There are clinical guidelines for the diagnosis of coeliac disease in clinical practice. These are designed to ensure a very high positive predictive value because the implications of lifelong GFD are major to the patient. These criteria have been applied to research studies, where negative predictive value is far more important. Hence, if the population studied has been "contaminated" with a few patients with coeliac disease, results can be skewed. This was particularly evident in early descriptions of NCGS[7] but is also relevant to more recent studies as recently reviewed.[8] This is of particular relevance where a small proportion of cohorts studied are generally found to have putative NCGS.

In the scientific literature, many "non-coeliac" populations studied have had a significant proportion of patients with duodenal lymphocytosis indicating some type of immune activation occurring in the mucosa.[8] While there are causes for this other than coeliac disease, some do have coeliac disease. Up to 10% of patients with coeliac disease do not have positive coeliac-specific serology. In some study cohorts, coeliac-specific antibodies (to deamidated gliadin) have been positive, but the patients have remained within the study. One review suggested that up to 20% of patients in many cohorts probably had coeliac disease.[8] One solution to this problem may be to restrict the study populations to those with histocompatibility locus antigen (HLA)-DQ genotypes that have an extremely low chance of coeliac disease and/or to insist on absolutely normal duodenal histology in adequate biopsies in those with an at-risk HLA genotype. Where these rules have applied, negative blinded, crossover rechallenge experiments have ensued.[9] If the mechanism underlying NCGS is inflammatory, however, this approach might also eliminate most with the condition.

Interpretative issues in blinded crossover rechallenge studies. The gold standard for determining food intolerance or hypersensitivity in clinical practice is to perform a double-blinded, placebo-controlled rechallenge study after symptoms have abated on an elimination diet. This methodology has been applied in four studies, where the potential for FODMAP-induced symptoms (which is of concern in using wheat flour as challenge agent) has been eliminated by using carbohydrate-depleted wheat protein that was either measured or assumed to be low in FODMAPs.[9–12] This can then be incorporated into food, sprinkled on food, or put in capsules. Placebos can be non-gluten-containing flours (which are invariably very low in FODMAPs). A nocebo response is likely when a patient who believes he or she is gluten sensitive is asked to take something regularly that might contain gluten. This is the opposite of the placebo response when a patient is asked to take a therapy that may well help his or her disease. The problem then arises of how the investigator can pick between a nocebo and specific response with confidence. Statistically, this can be carried out using, for example, a difference between the two challenges (gluten and placebo) that is at least 2 SD of the placebo responses. When this type of analysis is applied, only three of the 61 patients were qualified as having gluten-specific responses in a study where the symptom response to gluten challenge was statistically significantly different from that in response to a blinded placebo in the overall population (per-protocol analysis $n = 59$; $P = 0.034$).[10] Thus, it showed a signal that gluten can be a factor in inducing symptoms, but differentiating which patients should be gluten free (i.e., with NCGS) was very difficult. For this issue to be compounded further, three patients who had specific reactions to gluten (i.e., positive in both high and low gluten arms and minimal response in the placebo arm) in the rechallenge study from Biesiekierski *et al*.[9] were subsequently rechallenged blindly, and the gluten specificity of response was lost. What has been performed in practice is that patients with a greater gluten response have been labelled as having NCGS. For instance, in the study by Zanini *et al*., patients were asked to identify which of the rechallenged substance was gluten, based upon their symptoms.[11] One-third identified gluten and the authors then define that subgroup as having NCGS. However, one-half of the patients identified the placebo, but the authors were reluctant to define those patients as having "non-coeliac placebo sensitivity." The authors believed that they were FODMAP sensitive, but on the basis of the listed ingredients of the placebo, it would have contained minimal FODMAPs.[13] Unfortunately, the same logic was applied by Elli *et al*.,[12] and their conclusions are not, therefore, valid. Until interpretation of such studies is tightened and performed with statistical rigor, confusion will reign.

The experience in the trials has implications for clinical practice. The question of whether double-blinded, placebo-controlled challenges are meaningful in an individual must be asked. For an individual undergoing challenge testing, application of rigorous criteria as mentioned earlier would lead to far fewer false positive results, but an increase in false negatives. Repeated testing might improve the accuracy of the results. If responses to the placebo are minimal, then such rechallenge methodology is much more useful in clinical practice. However, in this patient group, minimal responses to placebo have only been reported by one group who uses rechallenge methodology routinely. It enables identification of wheat and other food protein intolerances in a high proportion of patients.[14] In other words, accurate and meaningful use of rechallenge methodology to address food sensitivities in individual patients requires knowledge of the size of the response to the placebo (i.e., nocebo response) in the population being tested.

Lack of biological basis. The biological feasibility of gluten inducing symptoms would be greatly enhanced if mechanisms could be defined. Detailed analysis of published data is beyond the scope of the present review. Induction of inflammatory responses via innate immune activation has been implied by studies *in vitro*, but the data supporting such events have several problems that include reproducibility, difficulties defining patients with NCGS, and lack of healthy controls in many studies (it is quite possible that gliadin induces problems in all). The lack of evidence of

immune activation at the mucosal level in many patients is also an issue not readily consistent with an inflammation-based mechanism. An alternative is the release of exorphins on gluten ingestion. These peptides are well described, and there is some evidence that they can penetrate the mucosa (reviewed elsewhere[4]). However, whether they are responsible for gastrointestinal or extraintestinal manifestations of NCGS awaits further study.

An example of the interpretative problems of published studies was exemplified by a recent publication in which evidence was presented for systemic immune activation and increased intestinal permeability in a population of patients with NCGS.[15] However, the patient populations were poorly defined, and there was no control population with IBS, important because a proportion of patients with IBS have subtle evidence of immune activation. Hence, one must be reserved in reading too much into the findings reported.

Distinction of gluten contributing to symptoms with a condition caused by gluten. One aspect that is more a conceptual issue is that, if gluten is the cause of NCGS, then a GFD should lead to resolution of the symptoms to a level consistent with the healthy population. This has not been the experience when populations with self-reported NCGS have been studied. For instance, two surveys of patients who reported they were gluten sensitive, even those strictly on a GFD, remained moderately symptomatic.[16,17] This was confirmed in the setting of a clinical trial with symptoms assessed by daily entries into a diary using a visual analogue scale.[9] In fact, this group had considerable further improvement of symptoms when taught how to reduce FODMAP intake. The alternative concept is that proteins from wheat, rye, and barley might contribute to symptoms via uncertain mechanisms in patients with IBS but not actually be the principle cause of the IBS, a concept that is similar to the role of FODMAPs in symptom genesis and management.

Current status of non-coeliac gluten sensitivity

There is a general feeling, even amongst the skeptical, that NCGS does exist, but with the difficulty in achieving a diagnosis, its prevalence, natural history, and response to therapy remain speculative only. However, consensus and expert opinion consider that NCGS is not associated with complications that are well described for coeliac disease and that it is unlikely to be associated with malabsorptive problems, given the lack of abnormal small intestinal villi.

There is key issue in patients suspected of having NCGS; there are two major diagnostic interventions to be considered.

- *Exclusion of coeliac disease*: Important factors in effectively excluding coeliac disease include ensuring adequate gluten intake (at least 3 g/day for at least 4 weeks[18]) prior to serological testing and duodenal biopsy, the taking of adequate biopsies of both the first and second parts of the duodenum, and HLA-DQ genotyping where the absence of DQ2 and DQ8 will effectively exclude coeliac disease. The presence of an increased density of intraepithelial lymphocytes on duodenal biopsy with normal villous length (Marsh 1 lesion) creates a problem as to whether this is a manifestation of coeliac disease, NCGS, or another condition. Marsh 1 lesion in a seropositive person is generally accepted as representing coeliac disease. However, if seronegative, which is not unusual even in subsequent proven coeliac disease, coeliac disease cannot be effectively excluded. Some authors have referred to this as "coeliac lite" provided that an at-risk HLA-DQ genotype is present.[8] In this situation, gluten loading (e.g., 3 g of gluten per day for 6 weeks) prior to repeated biopsy before a GFD is instituted is a good approach, but one that is often not popular with the patients. Interpretation of repeat duodenal biopsy after 6–12 months of GFD is not easy, unless the lesion has progressed to show villous change.
- *Blinded placebo-controlled food or gluten challenge*: This is the traditional way of determining a specific food intolerance or hypersensitivity but carries issues of interpretation because of nocebo effects as demonstrated in all of the rechallenge randomized controlled clinical trials.[9–12] The use of FODMAP-deplete wheat protein is essential, but the rules by which a positive study can be stated in an individual will depend upon the nocebo effect in the population being tested; for example, if the nocebo effect is weak, there can be greater certainty that positive effects to the wheat protein are meaningful. Another way might be to use repeated placebo and repeated active challenges in a given order with consistent responses/lack of response to both being the readout. However, there are no data to guide clinical practice.

The therapy is, by definition, a GFD, but whether this needs to be strict exclusion of gluten or mild reduction is not known. Dietary analysis has indicated, however, that persons on GFD without coeliac disease are as adherent as coeliac patients.[19] Because it is not believed that the condition is associated with complications if left untreated, there is no reason at present to insist on a GFD apart from achieving symptomatic relief. If the GFD achieves symptomatic relief via reducing FODMAP intake, then it would seem more appropriate to institute a low FODMAP diet initially. Certainly, the study by Biesiekierski *et al*. found in a cohort of patients with self-reported NCGS much improved symptom control with an additional low FODMAP approach than with GFD alone.[9] The need for comparative studies of GFD and low FODMAP diet in this subgroup of patients is evident to guide the therapeutic approach.

In clinical practice, however, many patients who believe that a GFD has considerably helped them with their symptoms continue the diet. There are three key considerations that should guide clinicians towards intervening or supporting continuation of the diet. Firstly, the question of whether the patients are compromising their nutrition. Secondly, the effect that the diet is having on their psychosocial health should be addressed because the use of restrictive diets can lead to or be a manifestation of disordered eating behaviors and the social burden of restrictive diets can be considerable. Thirdly, the actual success of the diet in improving their symptoms should be assessed, as, in one survey, many believed gluten was the cause of their problems, but they remained highly symptomatic despite being gluten free.[20]

The evidence that eliminating gluten/wheat protein might have some therapeutic benefit has been derived from two sources. The first is the results of blinded rechallenge studies where gluten seemed to induce greater symptoms than the placebo in some crossover[10,12] and parallel group studies.[20,21] This is a different issue to defining those who have NCGS, as discussed earlier. The second piece of

evidence comes from a double-blinded 3-day rechallenge of patients with self-reported NCGS where greater current feelings of depression were associated specifically with gluten ingestion and not with placebo, even though abdominal symptoms were not different.[22] The notion here is that the patients feel better in themselves off gluten even though gut symptom levels were similar. This observation needs careful re-examination with longer exposure to gluten.

Definitions

The final aspect is that of what this condition NCGS should be called.[23] There have been many suggestions, and it seems that NCGS is less in favor as uncertainty grows regarding the true role of gluten as opposed, for example, to other cereal proteins (like ATIs). Non-coeliac wheat sensitivity has received some traction, but this forgets the fact that rye and barley appear to have similar actions and that fructan-induced symptoms must be included. Non-coeliac wheat protein sensitivity is more instructive to the clinician but still excluded rye and barley. Self-reported NCGS has also been referred to as "patients who avoid wheat and/or gluten," but this has had less traction.[24] A more correct definition for the time being would be "people who avoid the cereals wheat, rye and barley." The right name will not appear before the condition is clearly defined.

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