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Research Digest

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Issue 7 ◆ May 2015

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Non-celiac gluten sensitivity: much ado about something?

Small Amounts of Gluten in Subjects with Suspected Nonceliac Gluten Sensitivity: a Randomized, Double-Blind, Placebo-Controlled, Cross-Over Trial

Introduction

Few topics in recent years have polarized the nutrition and health community as much as non-celiac gluten sensitivity (NCGS). Some people believe it is a distinct condition, while others simply do not believe it exists. NCGS has been observed for more than 30 years and refers to a subset of people (often self-diagnosed) who report problems when consuming gluten but don't have any detectable autoimmune or allergic response in their bodies.

NCGS is currently a diagnosis of exclusion. That means it is diagnosed when an individual has had both allergic and autoimmune mechanisms ruled out, and the problems they experienced when eating gluten-containing products go away on a gluten-free diet (GFD). This would ideally in a blinded fashion to avoid any placebo effect of the dietary intervention. One potential classification of the many possible gluten-related disorders and symptomatology is shown in Figure 1.

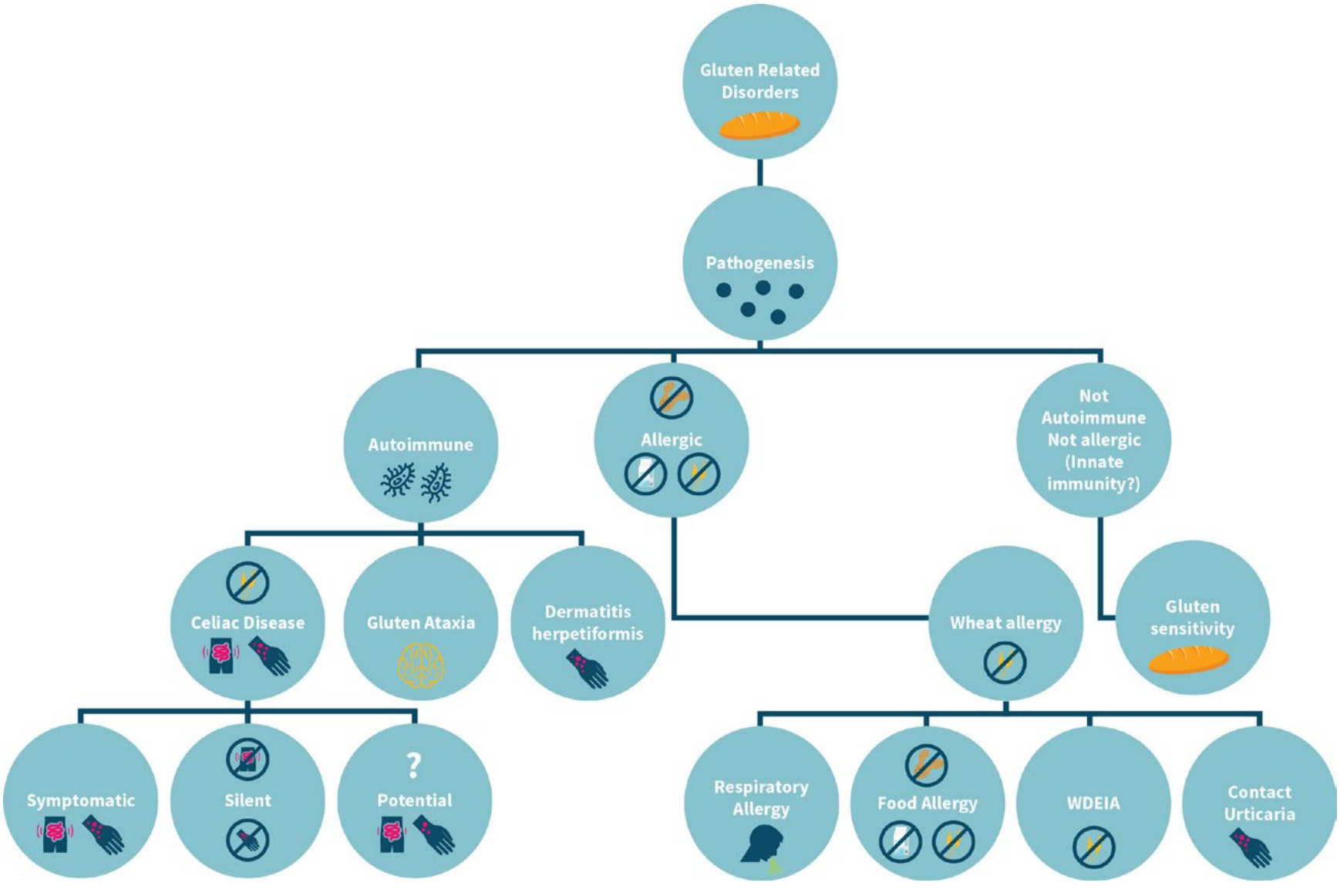
Due to the lack of explicit diagnostic tools, much of the information on NCGS has been gathered from people who have self-reported to be gluten-sensitive. The purpose of this double-blind, placebo-controlled, crossover trial from the University of Pavia in Italy was to study and classify symptoms in people suspected of having NCGS, when given small doses of gluten daily for one week.

Non-celiac gluten sensitivity (NCGS) is a controversial diagnosis of exclusion. It is only diagnosed when allergic and autoimmune issues are ruled out. The purpose of this study was to describe the symptoms and characteristics of people suspected of having NCGS under blinded and controlled conditions.

Who and what was studied?

The study recruited 61 adults who believed that they experience adverse reactions to gluten. The subjects were screened

Figure 1: Classifying gluten-related disorders



Source: Sapone et al., BMC Med. 2012

Celiac disease vs wheat allergy vs non-celiac gluten sensitivity

	Allergic Reactions	Autoimmune	Immune mediated
Pathogenesis	Wheat allergy	Celiac disease (CD), dermatitis herpetiformis, gluten ataxia	NCGS
Diagnosis	IgE antibodies - Skin prick tests and in vitro IgE assays	Four of the following 5 for CD: 1. Typical symptoms of CD 2. Positivity of serum CD IgA class autoantibodies at high titer 3. HLA-DQ2 and/or HLA-DQ8 genotypes 4. Celiac enteropathy found on small bowel biopsy 5. Response to a gluten-free diet	Excluding allergic and autoimmune reactions, improvement on a gluten-free diet

to be sure they did not have celiac disease or wheat allergy. Potential subjects were also excluded for having lactose intolerance, *H. pylori* infection, and intolerance to various sugars, to prevent possible interference with the study results.

Subjects were given capsules containing either 4.375 grams of gluten a day (roughly the amount in two slices of white bread) or rice starch (placebo) for one week, while otherwise following a GFD. After a one-week washout period, the participants crossed over into the other group, meaning they acted as their own controls, which is effective at eliminating inter-group differences. This study was double-blinded, as neither the researchers or the patients knew who was in each group. By double-blinding the experiment, the scientists ascertained that neither a potential bias on part of the researchers, nor placebo effects on part of the subjects would affect the results.

The intestinal and extra-intestinal symptoms, such as abdominal pain and bloating, brain fog, depression, and mouth sores, were assessed on a subjective basis via daily questionnaires. The change in the weekly overall symptom score (the sum of 15 intestinal and 13 extra-intestinal symptoms) was the main outcome of the study. Secondary outcomes included the change in individual symptom scores that were used to identify subjects with NCGS, as well as serum markers that could predict NCGS (including serum IgG anti-gliadin antibodies, fecal calprotectin, HLA genotyping and intraepithelial lymphocyte density*). Subjects with NCGS were defined as those exhibiting a change in overall score between the placebo and gluten weeks that was greater than two standard deviations away from the mean values.

People suspected of having NCGS were randomized to receive either placebo or a dose of gluten roughly equivalent to two slices of white bread daily for a week. After a week washout period, participants who received placebo were given gluten for an additional week, and vice versa. Subjects filled out questionnaires concerning symptoms to generate an overall symptom score, which was the main outcome measure for this study. Various biochemical markers were also measured to see if any could predict NCGS in patients who reacted strongly to gluten.

What were the findings?

Among the 59 patients that completed the trial, gluten intake was associated with a 30% increase in overall symptoms compared to the placebo. Two participants dropped out of the study due to intolerable abdominal pain during the first week of the study, but only one of them was receiving the gluten while the other was receiving the placebo.

In order to determine who could be labeled NCGS, the researchers analyzed each participant's overall symptom scores (as the change in overall symptoms between the

Lab values that could potentially predict NCGS

- Tissue transglutaminase IgA antibody- "Anti-self" antibody, often abbreviated as "tTG". People with celiac disease make antibodies that attack tissue transglutaminase, an enzyme that repairs damage in the body. The American Gastroenterological Association recommends initial screening for celiac disease with tTG and confirmed by small intestinal biopsy.
- Serum IgG anti-gliadin antibodies (IgA and IgG)- an "anti-gluten antibody," as gliadin is a component of gluten; IgA is made in the small intestine, which can be inflamed by gluten. IgG can also be used in diagnosing autoimmune problems, especially in people who are deficient in IgA (which is associated with celiac disease. Using these along with tTG may be more effective for celiac screening than tTG alone.
- Fecal calprotectin - Stool test which can detect inflammation in the intestines
- HLA genotyping - This test can rule out celiac disease and the genetic susceptibility for it. Approximately 97% of celiac patients have the HLA DQ2 or DQ8 genotype.
- Intraepithelial lymphocyte density - an increase can imply a state of T cell activation, either antigen driven (possibly by gluten).

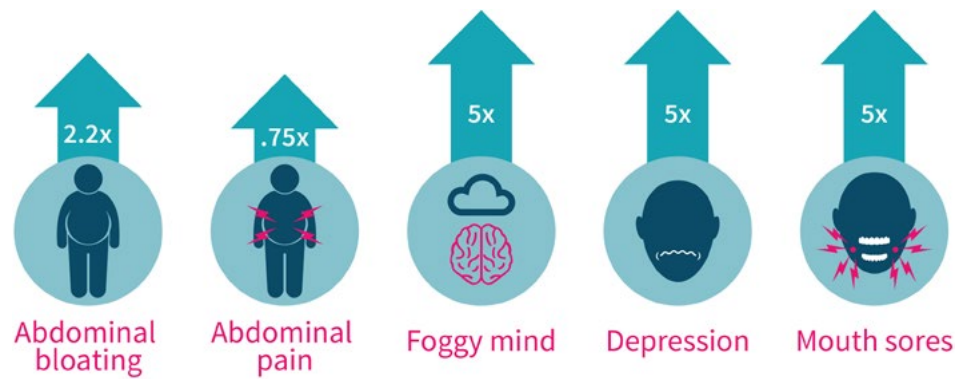
Sensitivity (ability to identify those with the disease) and specificity (ability to identify those without the disease) of the markers is varied... (From Benson et al)

	tTG IgA	AGA IgA	AGA IgG
Reported Sensitivity	90-98%	80-90%	75-85%
Reported Specificity	95-97%	85-95%	75-90%

gluten and placebo ingestion). Interestingly, over half of the subjects (31, or 52%) experienced similar symptoms in the gluten and placebo condition. Only 9 subjects (15%) experienced significantly greater numbers of symptoms in the gluten compared with the placebo condition; and of those, only three subjects fulfilled the previously cited criteria for NCGS.

The researchers also analyzed the intestinal and extra-intestinal scores separately, with the results being comparable to the analysis for overall symptom scores. The main changes are shown in Figure 2. When considering individual symptoms, 15 intestinal symptoms were rated and significant worsening was observed only for abdominal bloating and abdominal pain. Among the 13 extra-intestinal symptoms included on the questionnaire, foggy mind, depression, and aphthous stomatitis (mouth sores) were the only ones that significantly worsened from gluten, compared with placebo. However, a subject-specific analysis was not conducted so it's not clear if the significance was driven exclusively by the low percentage of subjects who actually worsened on the diet.

Figure 2: The main symptoms of non-celiac gluten sensitivity found



Additionally, the researchers attempted to identify lab markers for NCGS, though no significant correlations were found between either IgG anti-gliadin antibodies (IgG AGA), intraepithelial lymphocytes, or HLA-DQ2/DQ8 gene status and the changes in overall symptoms score. However, two of the three participants deemed to have NCGS did have IgG AGA levels above the normal range.

Gluten consumption increased overall symptom scores by 30% compared to placebo, with abdominal bloating and pain, foggy mind, depression, and mouth sores being the worst symptoms. Over half the subjects had similar symptom scores when taking gluten or placebo. Most biomarkers showed no correlation to symptom development, although the IgG AGA antibody was above normal range in two of the three participants determined to have NCGS.

What does the study really tell us?

In a nutshell, the severity of overall symptoms increased with one week of gluten intake compared with one week of placebo. What is more important, however, is that even though the overall mean symptom score was significantly higher for the gluten group in comparison to the placebo group, the result was driven by changes in only a subset of participants. The results suggest that there may have been a nocebo effect in some patients, meaning the mere expectation of potential increases in symptoms triggered a negative effect. Put another way, most of the people who thought they were gluten sensitive were not (or at least it wasn't measurable under these experimental conditions).

With only two out of three identified NCGS patients having IgG levels above the normal range and a lack of significant correlations between NCGS and other potential markers, this study could not find biomarkers that could be predictive of NCGS or shed light on any underlying mechanisms. The authors report that further experiments are currently being undertaken to classify the cytokine response in the intestines of subjects enrolled in the current study, and that early data does not support a role for either the innate or adaptive immune response.

Because a reliable diagnostic tool for NCGS does not currently exist, and the fact that placebo (and nocebo) effects can be very strong, blind challenges are necessary to diagnose this condition. To ensure valid results during this trial, the authors pre-tested the gluten capsules to make sure they had the same taste and appearance as the placebo, and potential participants exhibiting only minor symptoms were excluded. Patient compliance to the supplement regimen and the gluten-free baseline diet was good, and was monitored throughout the trial. The one-week washout period may seem short, but the fact that the scientists were able to detect significant effects suggests that this was a sufficient amount of time.

The amount of gluten used in this study was lower than in some previous trials but certainly a normal physiologic dose (one sandwich per day). It cannot be excluded, though, that higher doses could possibly cause other problems related to fermentation in the gut, even in healthy people. Analyzing

> "The results suggest that there may have been a nocebo effect in some patients, meaning the mere expectation of potential increases in symptoms triggered a negative effect."

overall symptom changes as well as individual symptoms allowed for a robust study on any potential effects of gluten, however the fact that there is no similar analysis of the intra-individual differences for the individual symptoms (there's only group data) is a weakness that can generate the impression that everyone got brain fog, etc.

The fact that, for most subjects, the symptoms worsened to the same extent during the gluten and placebo treatments highlights the importance of nocebo effects in studies on NCGS. Confirmation of NCGS should be interpreted carefully due to the lack of a control group that did not believe gluten was the cause of problems in their bodies. In addition, future studies should consider a potential dose-dependence of the effects. With the equivalent of only one sandwich per day, the gluten load may have been too low to elicit significant differences between the two treatment conditions. It is possible that higher gluten doses could have resulted in a larger group of sensitive patients.

There may be a strong nocebo effect for people claiming to have NCGS, at least at low doses of gluten. This suggests that in real-world practice, blind challenges to gluten would be useful to diagnose NCGS.

"The fact that, for most subjects, the symptoms worsened to the same extent during the gluten and placebo treatments highlights the importance of nocebo effects in studies on NCGS."

The big picture

This study contributes to the growing body of NCGS research. A recent review found prevalence rates between 0.5-13%, with this large discrepancy partly due to studies using a range of populations and partly due to varying inclusion/exclusion criteria. In addition, quantifying the prevalence of NCGS is challenging in the absence of specific biomarkers for diagnosis.

Considering that a family history of celiac disease is prevalent among NCGS patients, it is possible that people classified as NCGS are actually celiac. Additionally, in view of the altered gut permeability in IBS patients after gluten exposures, it is also possible that NCGS could be a subset of IBS. It appears that NCGS does in fact exist, but potentially not in as many people as is sometimes suggested. As is so often the case, more research is needed.

Frequently Asked Questions

I heard NCGS was actually a problem with FODMAP foods?

FODMAPs (Fermentable Oligo-, Di-, Mono-saccharides And Polyols) refer to some types of carbohydrates that are found in foods. These are found in some types of fruits, dairy, legumes, garlic, onion, and wheat. An often cited study from 2013 suggested that a FODMAP intolerance, not gluten itself, was the problem for people. What the media usually failed to report was that the participants in that study all had IBS, a pathology that has been shown to

respond favorably to a low FODMAP diet. Furthermore, the scientists excluded participants if they had intra-epithelial inflammation in the duodenal mucosa (Marsh 1 lesion), which is common in IBS subjects who have responded well to a GFD. Therefore, while FODMAP intolerance may be a cause of symptoms after eating wheat, it doesn't necessarily explain all cases of non-celiac sensitivity to wheat.

Is it possible that something else in wheat, besides gluten, could be the issue?

Non-celiac wheat sensitivity may in fact be a more appropriate label than NCGS, as gluten per se may not be the only problematic component of wheat. A 2013 review titled "Non-celiac gluten sensitivity. Is it the gluten or the grain?" concluded that while there is clearly a connection in some patients between grain ingestion and symptoms in the absence of celiac disease or wheat allergy, whether NCGS is more closely related to the spectrum of celiac disease or inflammatory bowel disorders warrants further study.

Further confounding self-diagnosis is the fact that the usual suspects aren't the only suspects when it comes to causing "gluten" reactions. Figure 3 shows some less obvious sources of gluten as well as a few of the many gluten alternative grains. Some people have fairly simple patterns where the more gluten they eat, the worse they feel. Others may feel somewhat bad after eating bread, but even worse after eating gluten-containing products that may have other components that cause a reaction. Gut disorders (and the huge variety of other health disorders that can appear alongside them) make it difficult to self-diagnose, and thus getting help from a healthcare provider is often essential.

What I should know?

This study adds more weight to the evidence that gluten may cause problems that cannot be ascribed to either an allergic or autoimmune reaction. Furthermore the possible extent of nocebo effects observed in the study at hand suggest that many people who consume a GFD may be doing so unnecessarily. A direct tool for diagnosing NCGS has yet to be determined, and reports of population-wide prevalence remain widely variable. ◆

Are the gluten wars winding down, or just beginning? Discuss the contentious issue of NCGS at our private Facebook forum.

Figure 3: Some common foods that contain gluten