



Applied nutritional investigation

Symptoms of gluten ingestion in patients with non-celiac gluten sensitivity: A randomized clinical trial



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ABSTRACT

Objectives: Non-celiac gluten sensitivity (NCGS) is the presence of symptoms induced by gluten and relieved by a gluten-free diet (GFD) in patients without celiac disease or wheat allergy. Studies are mixed as to whether gluten is the main symptom trigger in patients with NCGS. Gluten immunogenic peptides (GIPs) in stool and urine are novel methods to monitor GFD compliance. Few studies have investigated their use in patients with NCGS. The aim of this study was to assess whether patients with NCGS have increased symptoms with gluten ingestion and to assess compliance with the GFD using stool and urine GIPs.

Method: This was a prospective, randomized, double-blinded crossover trial evaluating symptoms in patients with NCGS. Thirty patients with NCGS and 43 healthy controls were placed on a GFD. Patients received 0.5 or 2 g/d of gluten for 7 d each. The remaining weeks, they received placebo for a total of 4 wk. Symptoms were evaluated weekly using the Celiac Symptom Index (CSI). Urine and stool samples were collected weekly and measured for the detection of GIPs to detect exposure to gluten.

Results: There was no difference in symptom severity within the NCGS group whether receiving placebo or gluten (32.69 versus 31.54, $P = 0.64$). Patients with NCGS had significantly higher CSI scores at baseline than healthy controls. Patients with NCGS were less likely to have stool and urine GIPs than healthy patients.

Conclusion: Patients with NCGS were more adherent to the GFD based on stool and urine GIP results. Patients with NCGS had increased symptom severity at baseline compared with healthy controls. Neither group had significantly increased symptoms after ingestion of gluten.

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Introduction

There has been a recent increase in interest in gluten-related disorders [1]. The spectrum of gluten-related disorders includes celiac disease, wheat allergy, and non-celiac gluten sensitivity (NCGS). NCGS is defined as the presence of intestinal and extraintestinal symptoms induced by the ingestion of gluten-containing food and relieved by a gluten-free diet (GFD) in patients without celiac disease or wheat allergy [1–3]. The wide array of systemic manifestations and the common overlap of confounding diseases like celiac disease and irritable bowel syndrome (IBS) can cloud the diagnostic process [4,5]. Studies have shown that when exposed to gluten, patients with NCGS, may develop an array of intestinal

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symptoms including abdominal pain, bloating, diarrhea, alternating bowel habits, nausea, gastroesophageal reflux disease, or aphthous stomatitis. The most common extraintestinal symptoms include fatigue, weight loss, headache, “foggy mind,” depression, muscle or joint pain, dermatitis, and other skin rashes [4]. These symptoms generally resolve when gluten is excluded from the diet, leading to a diagnosis of NCGS. The mechanism of NCGS also is not well understood. To our knowledge, there currently are no available biomarkers to diagnose NCGS, thus making the diagnosis difficult for clinicians and patients [1].

In this randomized double-blind, crossover placebo-controlled study, we aimed to investigate the effect of gluten on gastrointestinal symptoms in non-celiac individuals with self-reported gluten sensitivity compared with healthy controls. We measured gluten immunogenic peptides (GIPs) in urine and stool, a novel method used to monitor compliance with the GFD [6]. This technique detects gliadin 33-mer equivalent peptide epitopes (33 EPs) in the stool. These peptides show significant resistance to digestion and previous studies have shown they are detected in individuals after normal gluten-containing diet ingestion. Importantly, these peptides usually are not detected in patients on a strict GFD, and there appears to be a correlation between the amount of gluten intake and the levels of these peptides. We used a similar test for GIP detection in urine. Finally, we evaluated how reliable participants were to assess when they had received gluten and their adherence to the GFD.

Study protocol

Eligible participants were 18 to 80 y of age with self-described gluten sensitivity and healthy volunteers in whom gluten sensitivity was excluded. Table 1 presents the demographic characteristics of the participants. Celiac disease was considered adequately excluded if serum antibodies against tissue transglutaminase were negative and duodenal biopsy was normal while on a gluten-containing diet or if the individual was negative for both HLA-DQ2 and HLA-DQ8. Exclusion criteria were pregnancy, use of immunosuppressive agents, type 1 diabetes mellitus, inflammatory bowel disease, or acute gastroenteritis. The study took place at the Jefferson Celiac Center at Thomas Jefferson University Hospital in Philadelphia, Pennsylvania, from October 2014 to November 2018. Participants were recruited through the Jefferson Celiac Center as well as by advertisements featured on the university's web page, radio broadcasts, and in local newspapers.

Of the 120 participants assessed, 56 were eligible to be placed in the healthy arm and 41 in the NCGS arm. Eleven healthy controls and 11 NCGS participants dropped out of the study because they were not able to adequately comply with the required visits and stool collection. Of the patients with NCGS, 27 had negative HLA-

DQ2 and HLA DQ8. The remaining 3 had positive genetics but negative celiac antibodies to tissue transglutaminase immunoglobulin (Ig)A with a normal IgA level as well as duodenal biopsies that were not consistent with celiac disease while on a gluten-containing diet. Sample size was determined by the number of available patients.

Thirty patients with NCGS and 45 healthy controls were placed on a strict GFD. All participants received education on the GFD from the Jefferson Celiac Center. Participants had a 7-d run-in period with a placebo to establish a symptom baseline before receiving gluten. During the study, the patients with NCGS and the control group received low (0.5 g/d) or high doses (2 g/d) of gluten for 7 d each. Two different doses were used to investigate whether a higher dose was necessary to induce symptoms. For the remaining weeks, participants received placebo for the 4-wk study period. The placebo consisted of corn starch manufactured in an identical capsule to the challenge capsule. Both capsules were gluten free and developed and produced by Thomas Jefferson University.

Stool and urine GIP enzyme-linked immunosorbent assays were collected to detect possible exposure to gluten despite the GFD. This technique has been shown to detect as little as <100 mg gluten daily [6]. The 33-mer peptide used in the study was from Biomedal SL (Sevilla, Spain).

Outcome measures were recorded retrospectively at the end of each week. The primary outcome was symptoms as measured by the Celiac Symptom Index (CSI). The CSI is a validated study to reliably assess relevant symptoms [7]. Table 2 presents the CSI questions answered weekly by each participant. Other secondary outcomes were the Celiac Dietary Adherence Test (CDAT), the Patient Estimation of Gluten Index (PGI), and the GIPs measured in urine and stool samples, which were also collected weekly.

A pharmacist with no clinical involvement in the study prepared the randomization sequence. All participants and study team members were blinded throughout the study. The participants were given the pills (gluten or placebo) at initiation of the study.

Statistical analysis

Ordinal logistic regression was used to model differences in CSI responses by group and exposure to gluten. The independent variables were grouped (healthy control versus NCGS), gluten exposure (yes versus no), and gluten $\times$ group interaction. Generalized estimating equation methods were used to account for correlation among repeated measurements from the same participant. Each question was analyzed separately. The effect of gluten exposure was estimated for each group and differences in groups were estimated for periods with and without gluten exposure. Analysis was performed using SAS PROC GENMOD, SAS/STAT 14.2 (SAS Institute, Cary, NC, USA).

The study was conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from all participants, and the study was approved by the Institutional Review Board at Thomas Jefferson University Hospital. All authors had access to the study.

Results

Primary outcome

Odds ratios (ORs) comparing periods with gluten exposure to those without in each group are shown in Figure 1. ORs >1 mean responses during periods with gluten were higher than those during periods without gluten exposure. There were no significant associations of gluten exposure with CSI question responses. The

Table 1
Baseline characteristics of patients with NCGS and controls

	Patients with NCGS (n = 30)	Controls (n = 45)	P-value
Age, mean (SD) in years	49.17 (16.2)	44.94 (15.5)	0.26*
Sex, n (%)			0.0005 [†]
Male	3 (10)	22 (49)	
Female	27 (90)	23 (51)	
Race, n (%)			0.0001 [‡]
White	27 (90)	22 (49)	
Black	2 (7)	22 (49)	
Asian	1 (3)	1 (2)	

NCGS, non-celiac gluten sensitivity

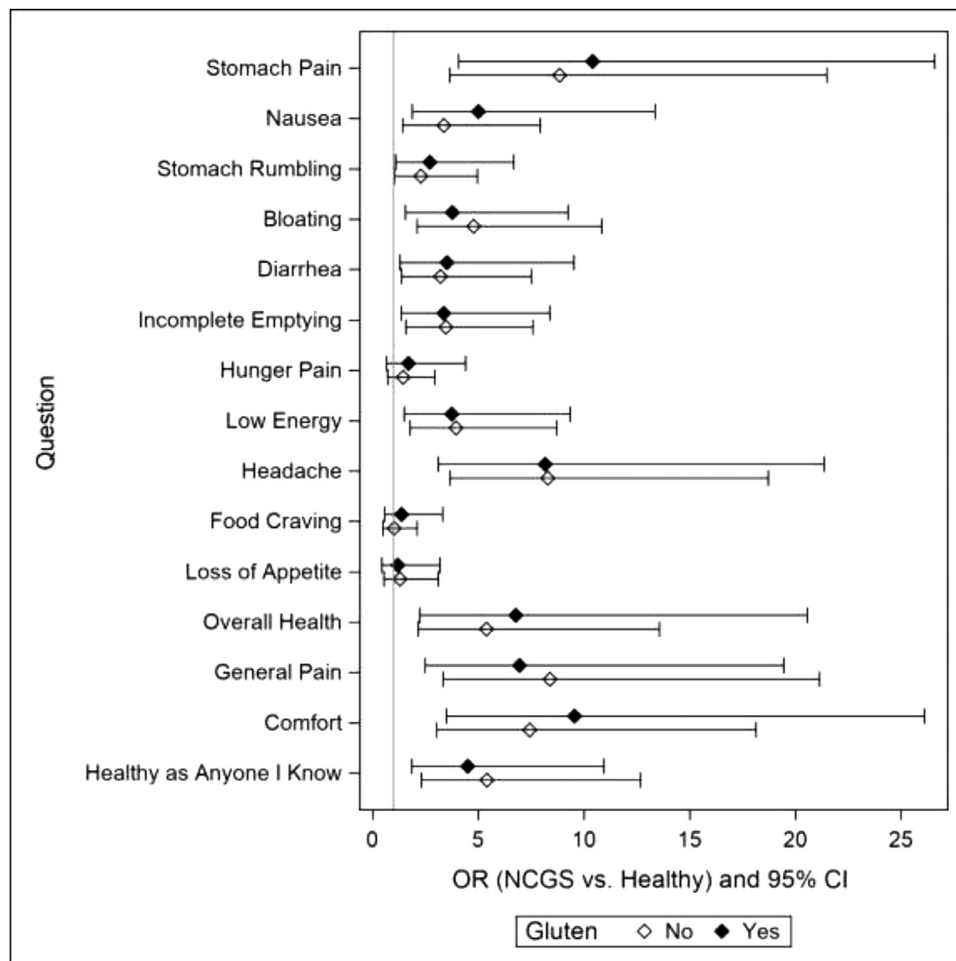
*Two-sample t test.

[†] χ^2 test.

[‡]Fisher exact test.

Table 2
Celiac Symptom Index

Question	1	2	3	4	5
1. Have you been bothered by pain or discomfort in the upper abdomen or in the pit of the stomach during the past 4 wk?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
2. Have you been bothered by nausea during the past 4 wk?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
3. Have you been bothered by rumbling in your stomach during the past 4 wk?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
4. Has your stomach felt bloated during the past 4 wk?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
5. Have you been bothered by diarrhea during the past 4 wk?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
6. When going on the toilet, have you had the sensation of not completely emptying your bowels during the past 4 wk?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
7. Have you been bothered by hunger pains during the last 4 wk?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
8. Have you been bothered by low energy level during the past 4 wk?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
9. Have you been bothered by headaches during the past 4 wk?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
10. Have you had food cravings in the last 4 wk?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
11. Have you had loss of appetite during the past 4 wk?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
12. Related to celiac disease, how is your health?	Excellent	Good	Fair	Poor	Terrible
13. Overall, how is your health?	Excellent	Good	Fair	Poor	Terrible
14. How much physical pain have you had during the past 4 wk?	None	A little	Some	A good deal	Very much
15. I am comfortable	Strongly agree	Somewhat agree	Neither agree nor disagree	Somewhat disagree	Strongly disagree
16. I am as healthy as anybody I know	Strongly agree	Somewhat agree	Neither agree nor disagree	Somewhat disagree	Strongly disagree

**Fig. 1.** Odds ratios comparing groups (NCGS vs healthy controls) during periods with (gluten = 1) or without gluten (gluten = 0). NCGS, non-celiac gluten sensitivity.

only comparisons that came close to being statistically significant were for the headache question in both healthy (OR, 1.45; 95% confidence interval [CI], 0.94–2.23; $P = 0.0934$) and NCGS groups (OR, 1.43; 95% CI, 0.89–2.28; $P = 0.1364$). The sensation of not completely emptying the bowels also had non-significant increased odds of higher response during periods of gluten exposure in both healthy (OR, 1.40; 95% CI, 0.86–2.30; $P = 0.1805$) and NCGS groups (OR, 1.37; 95% CI, 0.90–2.06; $P = 0.1385$).

Figure 1 shows ORs for comparing NCGS to healthy controls during periods with and without gluten. Patients with NCGS had significantly higher scores for most questions with the exception of hunger pain, food craving, and appetite.

Secondary outcomes

Patients with NCGS were less likely to have stool GIP than healthy controls (OR, 0.251; $P < 0.001$), with an increased likelihood in both groups when receiving gluten (OR, 4.815; $P < 0.001$). Patients with NCGS were less likely to have urine GIP than healthy controls (OR, 0.230; $P < 0.001$).

Participants were asked if they had received gluten that week. Their response was then compared with the stool GIP. There was no statistically significant correlation between a positive stool GIP and the Patient Estimation of Gluten Index (PGI).

Discussion

The aim of this randomized double-blind, crossover placebo-controlled study was to investigate the effects of gluten on symptoms in individuals with self-reported gluten sensitivity compared with healthy controls. No significant worsening of symptoms after gluten was found in patients with NCGS compared with healthy controls. Individuals with NCGS did report higher symptoms at baseline, whether or not they were exposed to gluten. Furthermore, participants with NCGS were less likely to have positive stool and urine GIPs indicating that they are more likely to be compliant with the GFD.

Studies looking at gluten as a trigger of symptoms in patients with NCGS date back to as early as 1980, but true interest in the topic increased after Biesiekierski's double-blind, randomized placebo-controlled trial showing that a gluten muffin caused gastrointestinal symptoms in individuals who did not have celiac disease [8,9]. Many subsequent studies supported the idea that gluten can trigger symptoms in patients without celiac disease with as little as <5 g/d of gluten ingested [10–13].

Other studies have questioned the role of gluten as a trigger of patients' symptoms. Biesiekierski et al. reported fewer symptoms with gluten and considered Fermentable Oligo-, Di-, Mono-saccharides And Polyols (FODMAPs) as triggers when she studied patients who were placed on a low FODMAP diet and then given gluten [14]. Similarly, Skopje et al. found that fructan, rather than gluten, induced symptoms in patients with NCGS [15]. Most recently, Caminero described amylase trypsin inhibitors as the etiology of inflammation and intestinal dysfunction in response to gluten in wild-type susceptible mice [16]. Although not identifying a source, the present study supported the findings that gluten may not be the initiating source for symptoms in patients with self-reported NCGS.

The results of the present study weaken the claim that those sensitive to gluten are aware of exposure. Similar results were seen in a double-blind placebo controlled study by Cartee, in which patients with celiac disease were given 6 g of gluten versus placebo. They found no statistical difference in symptoms in the gluten-exposed celiac disease group compared with placebo after 3 d of follow-up. The participants were also asked if they thought they

had received gluten. Only two of the seven patients with celiac disease who received gluten were able to correctly identify the gluten exposure [17].

Limitations of this study included that participants were not given a standardized diet as all participants' food was not provided during the study. They did receive education on the GFD, but they may have had contamination during the study period. Recall bias may have occurred when recording symptoms 7 d retrospectively by CSI. Additionally, individuals had self-reported, not specialist-confirmed NCGS. As a result, the study did not include a pre-enrollment evaluation based on symptomatic response to a GFD and worsening on gluten rechallenge. Without an objective marker for NCGS, participants may have, in fact, had other diagnoses such as IBS or small intestinal bacterial overgrowth. Finally, the only extra-intestinal symptoms monitored were low energy, fatigue, headache, generalized pain, and discomfort. Other extraintestinal symptoms of NCGS such as rash or neurologic symptoms were not included as the validated assessment tool, CSI, was used to monitor symptoms.

Conclusion

The present randomized, double-blind placebo-controlled challenge in participants with self-reported NCGS found no effect of gluten on symptoms. The study indicated that patients with NCGS do have more symptoms at baseline but that gluten may not be a trigger for these symptoms. In the present study, stool GIP appeared to adequately detect exposure to gluten; however, the urine test was found to be less reliable. Finally, patients with NCGS in the present study were not able to adequately determine if they had received gluten. This lack of awareness of gluten ingestion is in keeping with the concept that gluten is not likely the only cause of symptoms in NCGS.

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