No Effects of Gluten in Patients With Self-Reported Non-Celiac Gluten Sensitivity After Dietary Reduction of Fermentable, Poorly Absorbed, Short-Chain Carbohydrates

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BACKGROUND & AIMS: Patients with non-celiac gluten sensitivity (NCGS) do not have celiac disease but their symptoms improve when they are placed on gluten-free diets. We investigated the specific effects of gluten after dietary reduction of fermentable, poorly absorbed, short-chain carbohydrates (fermentable, oligo-, di-, monosaccharides, and polyols [FODMAPs]) in subjects believed to have NCGS. **METHODS:** We performed a double-blind cross-over trial of 37 subjects (aged 24–61 y, 6 men) with NCGS and irritable bowel syndrome (based on Rome III criteria), but not celiac disease. Participants were randomly assigned to groups given a 2-week diet of reduced FODMAPs, and were then placed on high-gluten (16 g gluten/d), low-gluten (2 g gluten/d and 14 g whey protein/d), or control (16 g whey protein/d) diets for 1 week, followed by a washout period of at least 2 weeks. We assessed serum and fecal markers of intestinal inflammation/injury and immune activation, and indices of fatigue. Twenty-two participants then crossed over to groups given gluten (16 g/d), whey (16 g/d), or control (no additional protein) diets for 3 days. Symptoms were evaluated by visual analogue scales. **RESULTS:** In all participants, gastrointestinal symptoms consistently and significantly improved during reduced FODMAP intake, but significantly worsened to a similar degree when their diets included gluten or whey protein. Gluten-specific effects were observed in only 8% of participants. There were no diet-specific changes in any biomarker. During the 3-day rechallenge, participants' symptoms increased by similar levels among groups. Gluten-specific gastrointestinal effects were not reproduced. An order effect was observed. **CONCLUSIONS: In a placebo-controlled, cross-over rechallenge study, we found no evidence of specific or dose-dependent effects of gluten in patients with NCGS placed diets low in FODMAPs. www.anzctr.org.au. ACTRN12610000524099**

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There is an emerging belief that gluten can mediate the symptoms of at least some patients with irritable bowel syndrome (IBS),[1] and the avoidance of wheat- and gluten-containing products continues to increase worldwide.[2] The clinical entity of non-celiac gluten sensitivity (NCGS) has been defined as those without celiac disease but whose gastrointestinal symptoms improve on a gluten-free diet (GFD).[3,4] Since its original description in 1980,[5] reports of NCGS have not taken into account the presence of other components of wheat, particularly fructans, that might have been pathogenically responsible for the symptoms. The first evidence that gluten might specifically induce symptoms in patients with IBS derived from a randomized, placebo-controlled trial of a single dose of carbohydrate-deplete gluten in 36 patients remaining on their habitual GFD in parallel groups.[6] Although there is some evidence of the effects of gluten in animal models or cancer cell lines,[7–9] little else is known about this entity. For example, mechanisms have not been identified and dose dependence has not been demonstrated.

To further evaluate this concept of NCGS, the current study aimed to examine the hypotheses that, in subjects who report to have NCGS, gluten induces dose-dependent, reproducible gastrointestinal and systemic symptoms. To do this, a randomized, double-blind, cross-over controlled feeding trial of 3 diets differing in gluten content was conducted in patients with IBS fulfilling the definition of NCGS, followed by a rechallenge trial in the same patient cohort. In order to control other potential triggers of gut symptoms, all diets had reduced content of fermentable, poorly absorbed short-chain carbohydrates (ie, fermentable, oligo-, di-, monosaccharides, and polyols [FODMAPs])[10] and, in the second, dairy products and food chemicals were additionally controlled.

Patients and Methods

Patients

Patients were recruited between January 2010 and January 2011 via advertisements in e-newsletters and community newspapers in metropolitan Melbourne, Australia and by referrals from private dietetics practice or gastroenterology clinics. The inclusion

Abbreviations used in this paper: D-FIS, Daily-Fatigue Impact Scale; FODMAP, fermentable, oligo-, di-, monosaccharides, and polyols; GFD, gluten-free diet; IBS, irritable bowel syndrome; NCGS, non-celiac gluten sensitivity; VAS, visual analogue scale.

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criteria were age older than 16 years; symptoms of IBS fulfilling Rome III criteria that self reportedly improved with a GFD; symptoms well controlled on a GFD; and adherence to the GFD for at least 6 weeks immediately before screening as assessed at an interview by a trained nutritionist (JRB). Celiac disease was excluded either by absence of the HLA-DQ2 and HLA-DQ8 haplotype or by a normal duodenal biopsy (Marsh 0) performed at endoscopy while on a gluten-containing diet in individuals expressing the HLA-DQ2 or HLA-DQ8 haplotype. Patients with significant gastrointestinal disease (such as cirrhosis or inflammatory bowel disease), excessive alcohol intake, intake of nonsteroidal anti-inflammatory agents, use of systemic immunosuppressant medication, poorly controlled psychiatric disease, and those unable to give written informed consent were excluded.

Study Protocol

The first study was a randomized, placebo-controlled, double-blinded cross-over trial. After an initial 1-week baseline period where the subjects recorded their usual diet and symptoms, participants entered a 2-week run-in period, at the beginning of which all were educated on a diet low in FODMAPs.[10] They were continued on a GFD low in FODMAPs throughout. Patients then received 1 of 3 diet treatments (high-gluten, low-gluten, or placebo) for one week, followed by a washout period of at least 2 weeks and until symptoms induced during the previous dietary challenge resolved, before crossing over to the next diet. Patients were randomized at recruitment according to a computer-generated order, held by an independent observer. Patients unable to continue a treatment due to intolerable symptoms were permitted to cease the study food of that particular arm, but continue to collect data as per day 6 (ie, symptom assessment, physical activity studies, blood and stool samples collected) and collect symptom and food diaries when not on the study diet. Patients then resumed any remaining treatment arms after the allocated washout period.

All participants were invited to return to take part in a rechallenge trial. This was designed and conducted after the initial trial was analyzed. A 3-day challenge period was chosen on the basis of the kinetics of symptom induction in the first trial and a stricter background control of potential triggers of gut symptoms was employed (see Study Food Preparation section). As the time between participation of the 2 trials varied from 8 to 17 months, inclusion/exclusion criteria (as mentioned) were confirmed. Participants were randomly allocated (as for the first study) to receive 1 of the 3 dietary treatments (see Study Food Preparation section) for 3 days, followed by a washout period of minimum 3 days (or until symptoms induced during the previous dietary challenge resolved), before crossing over to the next diet. Patients unable to continue a treatment due to intolerable symptoms were permitted to cease the study food of that particular arm, but continue to collect data as per day 3 (symptom assessment) and go on to resume any remaining treatment arms after the allocated washout period.

Both trials were approved by Eastern Health Research and Ethics Committee and the 7-day protocol also registered with Australia and New Zealand Clinical Trials Register (ANZCTR): ACTRN12610000524099. All authors had access to the study data, and had reviewed and approved the final manuscript.

End Points

The primary end point was the change in overall symptom score measured on a visual analogue scale (VAS) from the run-in period to that at the end of the treatment period. Secondary end points comprised the proportions of participants demonstrating an increase of at least 20 mm on the VAS in overall and individual symptom scores; the change in individual symptom scores compared with run-in; changes in biomarkers and byproducts of protein metabolism; the magnitude of gluten-specific T-cell responses after gluten challenge; change and comparison in scores on fatigue scales and activity levels; and the reproducibility of gastrointestinal symptom scores between the 7-day trial and 3-day rechallenge.

Study Food Preparation

For the initial 7-day trial, the background diet was gluten-free and low in FODMAPs, a major trigger of gut symptoms. During the 3 treatment periods, the background diet had the following incorporated: 16 g/d whole-wheat gluten (high-gluten arm), 2 g/d whole-wheat gluten/d, and 14 g/d whey protein isolate (low-gluten arm) or 16 g/d whey protein isolate (placebo arm).

For the 3-day rechallenge trial, the background diet was gluten-free, and not only reduced in FODMAPs, but also dairy-free and low in naturally occurring and artificially added food chemicals (ie, salicylates, amines, monosodium glutamate, as well as preservatives benzoates, propionate, sulfites, nitrites, sorbic acid, plus added antioxidants and colors), which are all putatively capable of triggering symptoms in some patients.[10,11] During the 3 treatment periods, the study diets had the following incorporated: 16 g/d whole-wheat gluten (gluten arm), 16 g/d whey protein isolate (whey arm), or no additional protein (placebo arm).

All main meals were supplied to the subjects. Detailed food lists of low FODMAP fruit and vegetables were supplied to the participants so they were able to purchase fresh perishable items themselves. The meal plan was adequate in macronutrients, micronutrients, and provided 8 MJ energy daily. Volunteers with smaller energy requirements were given smaller portions, but the same proportion of gluten was added. Volunteers with larger energy requirements were provided with additional low FODMAP, gluten-free meals, and snacks.

Meals in each trial were similar across the 3 diets in texture, taste, and appearance, confirmed with preliminary testing in 5 healthy people where the food containing the gluten could not be differentiated from those that did not. The gluten used was commercially available, carbohydrate-depleted wheat gluten (Vital Wheat Gluten; Penford Australia Ltd, Tamworth, Australia) and contained 75% protein, 1.8% crude fiber, 6.9% lipid, 15.6% starch, and 0.6% ash, as shown on reversed-phase high-performance liquid chromatography. On the basis of size-exclusion high-performance liquid chromatography, the protein content had a distribution of 6.6% non-gluten protein (albumin/globulin), 53.4% glutenin, and 40.0% gliadin. The whey protein isolate (RESOURCE Beneprotein Instant Protein Powder; Nestle Healthcare Nutrition, Inc., Minneapolis, MN) was lactose-free and low FODMAP, as measured following methodologies described previously.[12,13]

The investigator (JRB) and University research chef, assisted by 2 hospitality students, prepared all food in commercial kitchens. Meals were provided as frozen complete meals with instructions to thaw and warm either via microwave or oven. They were free of charge and delivered to participants' homes weekly.

Measurements

Medical history, examination and, if not already done, HLA genotyping were completed at baseline. For the 7-day trial,