Accepted Manuscript

Fructan, Rather Than Gluten, Induces Symptoms in Patients With Self-reported Non-celiac Gluten Sensitivity

Gry I. Skodje, Vikas K. Sarna, Ingunn H. Minelle, Kjersti L. Rolfsen, Jane G. Muir, Peter R. Gibson, Marit B. Veierød, Christine Henriksen, Knut E.A. Lundin

PII: S0016-5085(17)36302-3
DOI: 10.1053/j.gastro.2017.10.040
Reference: YGAST 61516

To appear in: *Gastroenterology*
Accepted Date: 19 October 2017

Please cite this article as: Skodje GI, Sarna VK, Minelle IH, Rolfsen KL, Muir JG, Gibson PR, Veierød MB, Henriksen C, Lundin KEA, Fructan, Rather Than Gluten, Induces Symptoms in Patients With Self-reported Non-celiac Gluten Sensitivity, *Gastroenterology* (2017), doi: 10.1053/j.gastro.2017.10.040.

Manuscript Number: GASTRO 17-00949

Title: Fructan, Rather Than Gluten, Induces Symptoms in Patients With Self-reported Non-celiac Gluten Sensitivity

Short title:

Fructans induce symptoms in NCGS

Gry I. Skodje[1,2,4], Vikas K. Sarna[2,3], Ingunn H. Minelle[4], Kjersti L. Rolfsen[4], Jane G. Muir[5], Peter R. Gibson[5], Marit B. Veierød [4,6], Christine Henriksen[2,4], Knut E.A. Lundin[2,3,8,]

[1]Division of Cancer Medicine, Oslo University Hospital, Rikshospitalet, 0424 Oslo, Norway

[2]K. G. Jebsen Celiac Disease Research Centre, University of Oslo, Norway

[3]Department of Immunology and Transfusion Medicine, Oslo University Hospital, 0424 Oslo, Norway

[4]Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Norway

[5]Department of Gastroenterology, Monash University and Alfred Hospital, Melbourne, Victoria, Australia

[6]Oslo Centre for Biostatistics and Epidemiology, Department of Biostatistics, Institute of Basic Medical Sciences, University of Oslo, 0317 Oslo, Norway

[7]Department of Gastroenterology, Oslo University Hospital Rikshospitalet, 0424 Oslo, Norway

[8]Centre for Immune Regulation, University of Oslo, 0424 Oslo, Norway

Abbreviations:

ATI, amylase trypsin inhibitor

CI, confidence interval

DBPCFC, double blind placebo controlled food challenge

FODMAP, fermentable oligo-, di-, monosaccharides and polyols

GSRS, gastrointestinal symptom rating scale

IBS, irritable bowel syndrome

IEL, intraepithelial lymphocytes

IQR, interquartile range

NCGS, non-celiac gluten sensitivity

SD, standard deviation

Grant support: The study was funded by the Extra Foundation Health and Rehabilitation, the Norwegian Celiac Association, the Throne Holst Foundation for Nutrition Research and the Wedel Jarlsberg Foundation.

Correspondence:

Gry I. Skodje

Department of Clinical Nutrition, Division of Cancer Medicine

Oslo University Hospital, PB 4950 Nydalen, 0424 Oslo, Norway

Phone: +47 23 07 19 15

E-mail: g.i.skodje@medisin.uio.no

Disclosures:

Peter Gibson has published an information/recipe book on the low FODMAP diet, and his University and Department receive royalties from the sale of The Monash University low FODMAP Diet App. The remaining authors have nothing additional to disclose.

Author contributions:

Study concept and design (KEAL, GS, CH, MBV, PRG, VKS), acquisition of data (GS, CH, KLR, IHM, VKS), analysis and interpretation of data (GS, MBV, VKS), drafting of the manuscript (GS, KEAL, CH, MBV, VKS), critical revision of the manuscript for important intellectual content (all co-authors), statistical analysis (GS, MBV), obtained funding (KEAL, GS, MBV), administrative (GS, CH), technical (GS, VKS), material support (JGM, PRG), study supervision (KEAL, CH, MBV).

Acknowledgements:

We thank the Department of Clinical Research Support, the endoscopy and laboratory unit at Department of Gastroenterology, the Proteomics Core Facility and the Nutrition Outpatient Clinic at Department of Clinical Service for their contribution, all located at Oslo University Hospital. We also thank Martha Colban and Anne Beate Hvinden for administrative assistance, and Merete G. Gedde-Dahl, Jorunn Bratlie and Carina Hinrichs for technical assistance. Thanks to all study participants for their effort and contribution.

Abstract:

Background & Aims: Non-celiac gluten sensitivity is characterized by symptom improvement after gluten withdrawal in absence of celiac disease. The mechanisms of non-celiac gluten sensitivity are unclear, and there are no biomarkers for this disorder. Foods with gluten often contain fructans, a type of fermentable oligo-, di-, monosaccharides and polyols. We aimed to investigate the effect of gluten and fructans separately in individuals with self-reported gluten sensitivity.

Methods: We performed a double-blind crossover challenge of 59 individuals on a self-instituted gluten-free diet, for whom celiac disease had been excluded. The study was performed at Oslo University Hospital in Norway from October 2014 through May 2016. Participants were randomly assigned to groups placed on diets containing gluten (5.7 g), fructans (2.1 g), or placebo, concealed in muesli bars, for 7 days. Following a minimum 7-day washout period (until the symptoms induced by the previous challenge were resolved), participants crossed over into a different group, until they completed all 3 challenges (gluten, fructan, and placebo). Symptoms were measured by gastrointestinal symptom rating scale irritable bowel syndrome (GSRS-IBS) version. A linear mixed model for analysis was used.

Results: Overall GSRS-IBS scores differed significantly during gluten, fructan, and placebo challenges; mean values were 33.1±13.3, 38.6±12.3, and 34.3±13.9, respectively (P = .04). Mean scores for GSRS bloating were 9.3±3.5, 11.6±3.5, and 10.1±3.7, respectively, during the gluten, fructan, and placebo challenges (P = .004). The overall GSRS-IBS score for participants consuming fructans was significantly higher than for participants consuming gluten (P = .049), as was the GSRS bloating score (P = .003). Thirteen participants had the highest overall GSRS-IBS score after consuming gluten, 24 had the highest score after consuming fructan, and 22 had the highest score after consuming placebo. There was no difference in GSRS-IBS scores between gluten and placebo groups.

Conclusions: In a randomized, double-blind, placebo-controlled crossover study of individuals with self-reported non-celiac gluten sensitivity, we found fructans to induce

symptoms, measured by the gastrointestinal symptom rating scale irritable bowel syndrome version.Clinicaltrials.gov no: NCT02464150

KEY WORDS: FODMAP, NCGS, wheat, intestine, challenge

Introduction

The interest in gluten-free diet and self-diagnosis of gluten sensitivity has risen worldwide.[1] International consensus statements have defined non-celiac gluten sensitivity (NCGS) as a condition in which ingestion of gluten induces gastrointestinal and extra-intestinal symptoms in absence of celiac disease or wheat allergy.[2, 3]

The condition represents a diagnostic problem as there are no reliable biomarkers and the clinical picture overlaps with irritable bowel syndrome (IBS).[2] A standardized double-blind placebo-controlled food challenge (DBPCFC) has been proposed as a diagnostic tool to confirm the NCGS.[4] However, the clinical value of DBPCFC is questionable.[2, 5, 6]

The pathogenesis of NCGS is incompletely understood. Negative serology for specific antibodies and lack of association with HLA DQ2/DQ8 suggest a limited involvement of adaptive immune mechanisms.[7] A higher expression of toll-like receptors in intestinal mucosa of NCGS patients compared to celiac disease patients, indicate a stronger role of innate immune mechanisms in NCGS.[7] Studies have shown increased intraepithelial lymphocytes (IEL), changes in intestinal permeability and cytokine response after challenge, but all findings have been considered unreliable as diagnostic biomarkers.[7, 8] Thus, the diagnosis is predominantly based on exclusions and self-statements.

Gluten-containing cereals can induce symptoms, but the culprit molecule is unknown. Wheat contains more than one potential symptom inducer such as gluten, fructans (an oligosaccharide of the FODMAPs) and soluble proteins.[8, 9] Gluten has shown to induce symptoms in some studies,[10, 11] but not in placebo-controlled cross-over studies.[12-15] Further, α-amylase trypsin inhibitors (ATI) have been proposed as possible symptom triggers although there are no supporting data in humans.[16] FODMAP restriction in study diets has resulted in symptom reduction,[12, 17] but FODMAPs alone have not been re-introduced in any study of participants with self-reported NCGS. In this randomised double-blind, placebo-controlled, cross-over study we aimed to investigate the effect of gluten and fructan separately on gastrointestinal symptoms in non-celiac individuals with self-reported gluten sensitivity.

Methods

Participants

Eligible participants were adults aged 18-80 years who self-instituted in gluten-free diet. They were required strict diet adherence for at least six months. They were asked on a re-call basis for relief of gastrointestinal and extra intestinal symptoms. Celiac disease was considered adequately excluded if the duodenal biopsy was normal while on gluten-containing diet or if the individual was negative for both HLA-DQ2 and HLA-DQ8. Wheat allergy was considered excluded if serology showed negative wheat specific IgE levels. Exclusion criteria were pregnancy or lactation, use of immunosuppressive agents, inflammatory bowel disease or other gastrointestinal comorbidity, substantial infection, women of fertile age with inadequate contraceptives, long travel distance or allergy to nuts or sesame seeds.

The study took place at Oslo University Hospital, Rikshospitalet from October 2014 to May 2016. Participants were recruited by advertisements on the web page of the University of Oslo, the Norwegian Celiac Association including their Facebook pages, and by referrals from general practitioners and local hospitals.

Study design and intervention

We recorded the medical background of all participants, including additional diseases, food intolerances and recall of gluten-related symptoms. State of IBS was assessed as defined by the Rome III criteria.[18] Further baseline measurements included gastroscopy with duodenal biopsy, blood tests and a seven-day food record. Nutrient intake was calculated by the nutrition software Diet Planner, Version 1 (Norwegian Food Safety Authority and the Norwegian Directorate of Health, Oslo, Norway). Intakes of total FODMAP and fructans were calculated by the nutrition software Foodworks, Version 7 (Xyris Software Australia Pty Ltd, Highgate Hill, QLD, Australia). FODMAPs were quantified via laboratory analysis using HPLC, UPLC and enzymatic assays as described previously. Gluten-free diet adherence was assessed at baseline by trained dietitians, evaluated by a standardized, locally-developed questionnaire and confirmed by the seven-day food record. Adherence during the study was not re-evaluated, but the participants were asked to keep their diet consistent with the baseline diet throughout the study.

Participants were randomized to one of three seven-day challenges (gluten, fructan or placebo), followed by a minimum of one-week washout period (Supplementary Figure 1). The washout period was extended until the symptoms induced by the previous challenge were resolved before starting the next challenge. This was ensured by a study team member who evaluated the washout symptoms recordings against baseline symptom level and decided prolonging of the washout period when needed.

The challenge vehicle was a 50 g, 220 kcal low-FODMAP gluten-free muesli bar developed and produced by the Monash University, Melbourne (Supplementary Table 1-2), eaten once daily. Fructo-oligosaccharides (Orafti® Oligofructose, Beneo, Tienen, Belgium) 2.1 g was added to the fructan bar, and gluten 5.7 g was added to the gluten bar, both of which mimicked the amount in four slices of sandwich wheat bread. The gluten used was commercially available, carbohydrate-depleted wheat gluten (Vital Wheat Gluten, Manildra Group, Gladesville, New South Wales, Australia). The muesli bars had similar appearance and taste as 12 healthy adults were not able to differentiate their content in a pre-test (data not shown).

To detect and quantify prolamins in the gluten-containing muesli bar, they were analyzed by R5-ELISA Ridascreen® Gliadin (R-Biopharm AG, Darmstadt, Germany) and by mass-spectrometry (nano-LC-MS/MS)(data not shown).[19] Gluten-derived peptides including the 33-mer long peptide described by Shan et al. were confirmed present in the gluten-containing bar, and absent in the fructan-containing or placebo bar.[20] Peptides corresponding to ATIs as described by Junker et al. were not detected.[16] The fructan bar was not analyzed for its fructan content.

Outcomes

Outcome measures were recorded retrospectively at the end of baseline, challenge and washout periods and daily during each period. The primary outcome was gastrointestinal symptoms as measured by the Gastrointestinal Symptom Rating Scale, Irritable Bowel Syndrome-version (GSRS-IBS), recorded retrospectively to reflect the last seven days.[21] GSRS-IBS is a self-administered 13-items questionnaire, with a seven-point Likert scale for each item ranging from 1='no symptoms' to 7='severe symptoms, and with an overall score range of 13-91. There are five GSRS sub-dimensions with their respective score ranges: pain (2-14), bloating (3-21), constipation (2-14), diarrhea (4-28) and satiety (2-14). The secondary outcome was daily gastrointestinal symptoms prospectively measured by a 100 mm visual analogue scale (VAS) for pain, bloating, passage of wind, nausea, stool dissatisfaction and overall gastrointestinal symptoms.

Other secondary outcomes were health-related quality of life measured by Short Form-36 (SF-36) and depression and anxiety symptoms measured by Hospital Anxiety and Depression Scale (HAD).[22, 23] Fatigue was measured by the six complaints within the exhaustion subscale of the Giessen Subjective Complaint List (GBB) and by VAS; weakness, sleepiness, exhaustion, tiredness, dizziness and fatigue.[24]

Sample size

Sample size calculation was based on paired t-test of differences between two challenges within the same subject. The total level of significance was set to *.05* (two-sided), and we used *.02* for the pairwise comparisons (*.05/3*, Bonferroni multiple comparison correction). A previous study found a GSRS-IBS mean difference of 1.5 units and a standard deviation (SD) of 3.2.[25] With 80% power and anticipated drop-out of 30%, 66 participants were required to detect such a difference.

Randomization and blinding

The study statistician with no clinical involvement in the study prepared the randomization sequence for the three challenges to be given in three periods of six sequences (ABC, ACB, BAC, BCA, CAB and CBA) by using a web-based service (http://randomization.com/, second generator, balanced permutations, accessed September 26, 2014). Block size was equal to trial size.[26] All participants and study team members were blinded throughout the study. The allocation concealment was carried out according to a procedure approved by the Department of Clinical Research Support. Seven muesli bars of each type were packed into three separate envelops marked with individual codes 1-66 and week (period) numbers 1-3 according to the randomization sequence. Sealed envelopes were handed out to the participants one week at a time. The participants recorded eaten muesli bars in a diary and returned uneaten bars. Un-blinding was done after the statistical analyses of primary and secondary outcomes.

Statistical methods

Descriptive results are presented as frequency (%), mean (SD) and median (interquartile range, IQR). Differences between the challenge responses were analyzed by linear mixed model. Participants were modelled as random with a random intercept at participant level. Challenge, period and sequence were modelled as fixed effects. Since we found no significant effect of sequence for any of the outcome variables, sequence was removed from the models. Baseline values were included as covariates. Day was included in the analysis of VAS symptom scores. We tested for interaction between challenge and period, and when significant, effect of challenge was analyzed by a linear mixed model within each period. Differences between baseline and washout were analyzed with one way analysis of variance (ANOVA), and differences between participants with and without thyroid disease were analyzed by independent samples t-test. Differences in gluten and fructan response from placebo, were analyzed by paired t-test. Variables with skewed distribution were ln transformed. Multiple pairwise comparisons with Bonferroni correction were performed when appropriate. All analyses were carried out using IBM SPSS, version 24.0 (SPSS Inc, Chicago IL) and a *P-value < .05* was considered statistically significant.

Ethics and approvals

The study was conducted in accordance to the Helsinki Declaration. Written informed consent was obtained from all participants, and the study was approved by the Regional Committee for Medical and Health Research Ethics the 16th September 2014 with the identification 2013/1237 REC South East A. The study is registered in ClinicalTrials.gov (registration number NCT02464150). The manuscript was prepared according to the Consolidated Standards of Reporting Trials (CONSORT) statement (http://www.consort-statement.org). All authors had access to the study data and reviewed and approved the final manuscript.

Results

Recruitment

Of 232 participants assessed, 68 were eligible (Figure 1). Reasons for the 111 participants not meeting the inclusion criteria were: celiac disease not properly excluded (n=61), long travel distance (n=20), not following a gluten-free diet (n=21), symptomatic on gluten-free diet (n=4), celiac disease (2) or already investigated for NCGS (3). Two participants were in excess of the predefined 66 participants needed and excluded from the final analysis to avoid violation of the randomization protocol and the size of the sequences. These two completed the full protocol fully aware from the start that we could not include them in the statistical analysis. Three participants were excluded due to protocol violations. One had a biopsy compatible with active celiac disease at the baseline gastroscopy despite a gluten-free diet and previous negative biopsy on a gluten-containing diet and was later given a celiac disease diagnosis. Two were positive for the celiac disease-associated HLA types (HLA-DQ2 and -DQ8), were on a strict gluten-free diet, but did not have celiac disease ruled out. The remaining 59 participants completed all three challenges and were included in the statistical analysis. Of these, gluten and fructan challenges were prematurely ceased by seven participants each, after 5-6 days. Placebo challenge was prematurely ceased by four participants, after 2-6 days. Cessation was due to omission or unbearable symptoms and did not exclude the participant from analysis. No participants experienced severe adverse effects of the challenges. During the challenges all participants self-reported strict adherence to gluten-free diet, and 98% of the muesli bars were consumed.

Baseline data

Baseline characteristics of the study sample are presented in Table 1. According to recall information the last three months, 18 participants fulfilled the Rome III criteria for IBS, despite reporting symptom relief on gluten-free diet. IBS was not an exclusion criterion. Two participants had IgG-deamidated gliadin peptide above the cut-off (20 U/ml), 22 and 38 U/ml, respectively. They carried the genotype HLA DQ2.5 or DQ8, but had negative duodenal biopsy while on gluten-containing diet. Five participants had changes equivalent to Marsh-Oberhuber type 1 in the baseline duodenal biopsy.[27] Two of these had celiac disease ruled out by negative HLA DQ2/DQ8 and three had previous negative duodenal biopsy.

Self-reported thyroid disease was present in 27 % of the participants, reflected by significantly different thyroid stimulating hormone values in this group compared to the rest (mean (SD) 0.5 (0.8) vs 1.5 (0.9) IU/L, respectively; $P < .001$) but free T_4 levels did not differ significantly (16.7 (4.1) vs 15.0 (2.3) pmol/L, respectively $P = .13$). However, there were no significant differences in gastrointestinal or extra-intestinal baseline symptoms between participants with and without thyroid disease, except that SF-36 general health scale was lower in participants with thyroid disease than in those without thyroid disease, 37 (22) vs 65 (26), respectively ($P = .05$).

Participants adhered strictly to the gluten-free diet at baseline, except one individual who reported one accidental transgression by intake of rye crisp bread and one individual who ate barley porridge on two occasions, both during the seven-day baseline food record. They were otherwise diet-adherent. Based on the seven-day food record the mean (SD) individual fructan intake was 2.5 g (2.1) per day.

Primary outcome

There was a significant difference in mean overall GSRS-IBS across gluten, fructan and placebo challenges, mean (SD) scores were 33.1 (13.3), 38.6 (12.3) and 34.3 (13.9), respectively ($P = .04$, Figure 2). Corrected for multiple comparisons the overall GSRS-IBS was borderline significant for fructan versus gluten ($P < .049$). No significant differences were found for fructan versus placebo ($P = .19$) and gluten versus placebo ($P = .99$).

There was also a significant difference in GSRS-IBS bloating across gluten, fructan and placebo challenge, where mean (SD) scores were 9.3 (3.5), 11.6 (3.5) and 10.1 (3.7), respectively ($P = .004$). Corrected for multiple comparisons the GSRS-IBS bloating was significantly different for fructan versus gluten (*P = .003*), but not for fructan vs placebo (*P = .07*) or for gluten versus placebo (*P = .84*). The fructan challenge induced highest score in the GSRS dimensions pain, diarrhea and satiety, but the differences were not significant (*.07 ≤ P ≤ .15*). No significant difference was found for the dimension of constipation (*P = .93, Figure 2*). There were no significant effects of period *(.23 ≤ P ≤ .81*), and no significant interactions between challenge and period *(.13 ≤ P ≤ .66*). However, when we studied the effect of challenge within each period, mean overall GSRS-IBS was consistently highest after the fructan challenge in all three periods, significantly so in period 2 (*P = .03*, Supplementary Figure 2). In the overall GSRS-IBS in period 2, there was significant difference for fructan versus placebo (*P = .03*), while no significant differences were found for gluten versus fructan (*P = .10*) or gluten versus placebo (*P = .78*).

The difference from placebo was significant for the fructan challenge, but not for the gluten challenge, *P = .04* and *P = .55*, respectively (Figure 3). The difference fructan minus placebo was significantly higher than the difference gluten minus placebo. This difference was found also for the GSRS-IBS dimensions bloating (*P = .002*) and diarrhea (*P = .04,* data not shown).

We did a post-hoc observation of individual courses according to the overall GSRS-IBS stratified by those scoring highest and lowest on gluten, and those who scored highest after fructan and placebo challenge (Figure 4). Thirteen participants scored highest after gluten challenge. Four of these had a difference in score between gluten and placebo above 30%. According to a previously suggested diagnostic tool these four would have been defined as gluten-sensitive.[4] Lowest score after gluten was found in 27 participants. Highest score after fructan and placebo challenge was found in 24 and 22 participants, respectively.

Subject-related factors were added as fixed factors in the linear mixed model, and no effect was found of age, gender, duration of gluten-free diet, BMI, HLA-DQ status, thyroid disease or IBS (*.17 ≤ P ≤ .78*). The mean (SD) duration of the first and second washout periods were 9 (7.2) days and 13 (7.2) days, respectively. There was no significant difference between baseline and washout symptom scores for overall GSRS-IBS ($P = .76$) or GSRS-IBS dimensions ($.38 \leq P \leq .96$).

Secondary outcome

Overall gastrointestinal symptoms scored by VAS were consistently higher after fructan challenge than after gluten and placebo challenge from day one to day seven (Figure 5A). However, there was a significant interaction between challenge, period and day ($P_{\text{interaction}}$ = .01), thus we present results for overall symptoms stratified by period (Figure 5B-D). In period one, there was a significant difference across the gluten, fructan and placebo challenge ($P = .04$), but no pairwise comparisons were significantly different ($.52 \leq P \leq 1.00$). In period two, the fructan scores were highest and placebo scores lowest all days, and, at day 3, 6 and 7, the differences across the three challenges were significant ($P < .008$ for all comparisons). On these days the fructan scores were significantly higher than the placebo scores ($P < .006$). ANOVA also indicated differences between fructan and placebo at day 2 and 4 ($P = .09$ and $P = .07$, respectively). No other comparisons in period two were significantly different ($.07 \leq P \leq .99$). The fructan scores seemed to increase more than gluten and placebo scores from day one to day seven. However, no challenge effect was found by linear mixed model ($P = .48$, Figure 5C). In period three, there was significant interaction between challenge and day illustrated by the crossing lines in Figure 5D ($P_{interaction} = .02$). VAS bloating scores were also consistently higher after fructan challenge than after gluten and placebo challenge from day one to day seven (data not shown). However, there was a significant interaction between challenge, period and day in the VAS measurements of bloating ($P_{interaction} = .02$). There were no interactions for the other VAS measurements ($.06 \leq P_{interacton} \leq .84$). There were no significant challenge by period, challenge by day or period by day interactions for pain, wind and stool dissatisfaction ($.06 \leq P_{interaction} \leq .88$), but for nausea there was a significant challenge by period interaction ($P_{interacton} \leq .02$). There were no significant effects of challenge on abdominal pain, wind and stool dissatisfaction by VAS ($.23 \leq P \leq .88$, data not shown).

In regards to other secondary outcomes, there was a significant difference in SF-36 vitality scale scores across gluten, fructan and placebo challenge, and lowest vitality was found after fructan challenge, mean (SD) 44.3 (25.2), 38.2 (23.4) and 44.4 (24.3), respectively ($P = .04$, Supplementary Table 3). The GBB dimension, weakness, was significantly different across gluten, fructan and placebo challenge, and highest weakness was found after fructan challenge, 32.8 (30.0), 42.5 (26.6) and 33.5 (29.7), respectively ($P = .02$). In the pairwise comparisons, the vitality score was significantly lower and weakness significantly higher after fructan challenge than after gluten challenge ($P = .04$ and $P = .02$, respectively). No significant differences were found for fructan versus placebo or gluten versus placebo for these two variables ($.11 \leq P \leq .99$). No significant differences were found for the other SF-36 scales and measures for fatigue, or for other extra-intestinal symptoms ($.10 \leq P \leq .96$, Supplementary Table 3).

Discussion

This randomized double-blind placebo controlled cross-over study aimed to investigate the effects of gluten (without fructan) and fructan (without gluten) on gastrointestinal symptoms in individuals with self-reported gluten-sensitivity. No significant effect of gluten was found as compared to placebo and fructan. In contrast, a small daily dose of 2.1 g of fructans induced greater symptoms on multiple criteria including the overall GSRS-IBS, after a seven-day challenge. On group level, the difference from placebo was significantly higher after fructan challenge than after gluten challenge. Thirteen participants had their highest symptom score after gluten, while 27 had their lowest score after gluten challenge. Fructan and placebo challenge induced highest score in 24 and 22 participants, respectively.

We deliberately challenged our participants with moderate doses of gluten and low doses of fructans to resemble the clinical situation as closely as possible. The baked muesli bars mimicked gluten-containing food and enabled successful blinding. To date, no studies have used this challenge vehicle. As an evidence of an active immunogenic gluten-component in the musli bars, participants with biopsy-proven celiac disease who were challenged with the gluten bars for 14-days in a related study developed significant increase in IEL-count and significant reduction in villous height to crypt depth ratio in duodenal biopsies by the end of challenge. [28] Further, analysis of the bars confirmed that they specifically contained the food components of interest without other potential culprit food components.

With such confidence in the challenge bars, the lack of gluten-specific responses according to both GSRS-IBS and VAS supports the assumption that gluten plays a less prominent role in symptom generation than initially anticipated.[29] Additional support is that only 13 of 59 participants had their highest symptom score after gluten challenge and 27 had the lowest score after gluten challenge. The moderate dose of 5.7 g gluten is believed to be adequate since previous studies have been able to demonstrate symptom responses on equivalent and lower amounts of gluten.[13, 14] The re-challenge methodology, however, cannot exclude gluten sensitivity in some individuals due to possibly stronger placebo response.

The effect of fructans on overall gastrointestinal symptoms by GSRS-IBS was found both on a group level and in individuals. In the current study, the fructan challenge almost doubled their habitual daily fructan exposure. The effect of FODMAPs on symptoms in patients with IBS is dose-dependent and the doubling of amount received is sufficient to cause symptoms.[30] By comparison, in a recent pilot study, 21 healthy adults did not experience gastrointestinal response to 5 g of fructo-oligosaccharides.[31] Hence, it is likely that the fructan effect in 24 of 59 participants who had their highest symptom score after fructan challenge represents a causal relationship.

However, symptoms may depend on combined exposure to gluten and fructans with synergistic actions. The combination reflects the clinical scenario when patients report symptoms after intake of wheat. This combination has not been studied. It is also possible that fructans present naturally in the food matrix behaves differently to supplements of pure fructo-oligosaccharides added to the diet. Further, the fructo-oligosaccharide added in the muesli bars originated from chicory roots and might have different effect from the fructo-oligosaccharide in wheat. Other components of wheat, such as the ATIs and the lectin, wheat germ agglutinin, were not considered in the current study apart from not being able to detect the ATIs.[16] In vitro studies have found effect on cell activation of these components,[16, 32] but in IBS and NCGS patients the pathogenic role of ATIs and wheat germ agglutinin is unexplored.

Although the differences in the symptoms induced across the challenges were small, the fructan effect was distinct and consistent for many symptoms. Bloating is frequently reported by IBS and NCGS patients and was the only GSRS-IBS sub dimension that showed significant response of the fructan challenge. This result is supported by significant improvement of bloating as a response to low FODMAP diet reduction in IBS patients.[17] Likewise, the present lack of fructan effect on bowel habits supports the lack of effect on appearances and fecal water content in a feeding intervention.[33]

The effect of the fructan challenge was not restricted to abdominal symptoms. The SF-36 vitality scale was significantly lower and VAS weakness significantly increased as response to the fructan challenge compared to gluten and placebo. Improvement in quality of life in IBS patients has been found as an effect of low FODMAP diet.[34] Whether improvement in vitality and weakness are directly related to fructan exposure or secondary to the higher degree of gastrointestinal symptoms cannot be ascertained.

The results of the current study weaken the role of gluten as a symptom inducer in patients with self-reported NCGS, supported the report by Biesiekierski et al. in a blinded re-challenge study where the participants were receiving a low FODMAP diet with tight control of background confounders.[12] In the initial run-in to the blinded re-challenges, Biesiekierski et al. taught the subjects how to minimize FODMAPs in their diets, and this caused a uniform reduction of symptoms. This may have been a placebo effect, but the findings of the present study support that it was a specific effect of the reduction of total FODMAPs. Biesiekierski et al. was not able to find any specific or dose dependent effect of gluten in their randomised double-blind placebo-controlled challenge study.

A possible role of gluten as symptom inducer in participants with self-reported NCGS has been shown in randomised double-blind placebo-controlled challenge studies.[13-15] The authors may conclude justly that some participants are gluten-sensitive, but methodological issues make it difficult to rely on the finding as a correct identification of the gluten sensitive individuals. The current findings contrast these previous studies and weaken the role of gluten as symptom trigger in individuals intolerant of wheat, rye or barley. Rather, the results indicate that fructans are more likely the culprits.[30] The finding raises issue regarding the use of the term “NCGS” and its distinction from IBS. This is consistent with studies that report that some IBS patients do benefit from a gluten-free diet.[11, 35] However, the improvement seen with a gluten-free diet may not be caused by removal of the gluten protein *per se*, but rather the reduction of wheat fructans.

Large placebo response as seen in previous studies demonstrates how difficult it is to correctly identify which patients should be gluten-free.[13-15] Our DBPCFC also resulted in 22 of 59 participants with placebo response. It is therefore appropriate to question whether the DBPCFC in clinical practice is a good tool or even necessary to identify these individuals. Re-challenges of participants with gluten-specific score above a cut-off are usually not done, and not suggested as a diagnostic tool.[4] It was done in the study of Biesiekierski et al., but the gluten specificity was lost.[12]

A common clinical approach when food is suspected to induce symptoms is the elimination of the suspected trigger followed by a clinician-supervised open, systematic re-challenge with symptom monitoring. The method is used for patients on a low FODMAP diet, not for diagnostic purpose, but the approach serves as confirmation of the IBS diagnosis according to the ROME IV criteria. The DBPCFC would not be suitable in a re-challenge of FODMAPs because of the impossibility of blinding. Still, the DBPCFC is currently the preferred method to define food intolerances. It may work for the purpose of proving the existence of a condition, but is less useful as a clinical tool.[5] As long as NCGS is a poorly defined condition with strongly subjective symptoms, standardized open food challenges are meaningful enough for the clinical practice.[5, 36] Followed by long-term monitoring by experienced clinicians, this open-ended perspective could be superior to a conclusive DBPCFC with risk of false negative and false positive results without the possibility to contrast with objective biomarkers.[5]

The general influence of confounding factors in the present study was reduced by using the randomised crossover design.[37] However, the design is complex and demanding. Therefore, dietary and adherence assessment was done before challenge, and not continued through the challenges. Unobserved dietary changes might have occurred during the study. A fructan restriction could have been done in the run-in period to reduce the heterogeneity of the fructan intake before challenge. However, we abstained from manipulating their normal diet to better represent the clinical setting, an approach also used by Laatikainen et al.[38] The heterogeneity of the participants is a common characteristic of the NCGS population,[39] but must also be considered as possible disturbance in interpretation of the results. We deliberately abstained from manipulating the study sample to make the participants present as close to a clinical setting as possible. However, in regards to gender, thyroid disease, IBS and celiac disease in close family our sample was very much alike the samples described in other challenge and cross-sectional studies.[12, 13, 15, 40-42] Further, we did not find any effect of any of these factors on the challenge outcome. Regarding adequate exclusion of celiac disease and celiac disease serology, our sample was more homogenous than in previous challenge studies.[12, 13] Recall bias may occur when recording symptoms seven days retrospectively by GSRS-IBS. However, the method is established as a tool to monitor response during gluten challenge in celiac disease and NCGS patients,[25, 43] Further, the daily scored VAS scales that have been used in similar challenge studies[12, 14] confirmed the main findings of GSRS-IBS in the present study.

The significant interaction effect between challenge, period and day indicated that the effect of challenge differed between periods and days for overall symptoms by VAS. It is not likely that the period effect was caused by a carry-over effect. Washout and baseline symptom scores were similar, indicating that the washout periods were of adequate length. The period effect is a hurdle of the crossover design [12, 13] and might be a cause of participant expectancy commonly observed in participants with a strong preconception of food intolerances.[37] This expectancy is often highest in the first period.[12, 13] Theoretically, repeated placebo-controlled challenges may be an approach to overcome the period effect.

In conclusion, the current randomized, double-blind placebo-controlled crossover challenge in participants with self-reported NCGS found no effect of gluten on group level. The study indicates that fructans are more likely to induce symptoms in those reporting sensitivity to wheat, rye and barley. The finding weakens the use of the term “NCGS” and raises doubts about the need for a gluten-free diet in such patients.

References

1. Lebwohl B, Leffler DA. Exploring the Strange New World of Non-Celiac Gluten Sensitivity. Clinical Gastroenterology and Hepatology 2015;13:1613-1615.
2. Lundin KE, Alaedini A. Non-celiac gluten sensitivity. Gastrointest Endosc Clin N Am 2012;22:723-34.
3. Ludvigsson JF, Leffler DA, Bai JC, et al. The Oslo definitions for celiac disease and related terms. Gut 2013;62:43-52.
4. Catassi C, Elli L, Bonaz B, et al. Diagnosis of Non-Celiac Gluten Sensitivity (NCGS): The Salerno Experts' Criteria. Nutrients 2015;7:4966-77.
5. Asero R, Fernandez-Rivas M, Knulst AC, et al. Double-blind, placebo-controlled food challenge in adults in everyday clinical practice: a reappraisal of their limitations and real indications. Curr Opin Allergy Clin Immunol 2009;9:379-85.
6. Gibson PR, Skodje GI, Lundin KEA. Non-celiac gluten sensitivity. Journal of Gastroenterology and Hepatology 2017;32:86-89.
7. Sapone A, Lammers KM, Casolaro V, et al. Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: celiac disease and gluten sensitivity. BMC Med 2011;9:23.
8. Brottveit M, Beitnes AC, Tollefsen S, et al. Mucosal cytokine response after short-term gluten challenge in celiac disease and non-celiac gluten sensitivity. Am J Gastroenterol 2013;108:842-50.
9. Carroccio A, Mansueto P, Iacono G, et al. Non-celiac wheat sensitivity diagnosed by double-blind placebo-controlled challenge: exploring a new clinical entity. Am J Gastroenterol 2012;107:1898-906; quiz 1907.
10. **Biesiekierski JR, Newnham ED**, Irving PM, et al. Gluten causes gastrointestinal symptoms in subjects without celiac disease: a double-blind randomized placebo-controlled trial. Am J Gastroenterol 2011;106:508-14; quiz 515.
11. Shahbazkhani B, Sadeghi A, Malekzadeh R, et al. Non-Celiac Gluten Sensitivity Has Narrowed the Spectrum of Irritable Bowel Syndrome: A Double-Blind Randomized Placebo-Controlled Trial. Nutrients 2015;7:4542-54.
12. Biesiekierski JR, Peters SL, Newnham ED, et al. No effects of gluten in patients with self-reported non-celiac gluten sensitivity after dietary reduction of fermentable, poorly absorbed, short-chain carbohydrates. Gastroenterology 2013;145:320-8 e1-3.
13. Di Sabatino A, Volta U, Salvatore C, et al. Small Amounts of Gluten in Subjects With Suspected Nonceliac Gluten Sensitivity: A Randomized, Double-Blind, Placebo-Controlled, Cross-Over Trial. Clin Gastroenterol Hepatol 2015.
14. Elli L, Tomba C, Branchi F, et al. Evidence for the Presence of Non-Celiac Gluten Sensitivity in Patients with Functional Gastrointestinal Symptoms: Results from a Multicenter Randomized Double-Blind Placebo-Controlled Gluten Challenge. Nutrients 2016;8.
15. Zanini B, Baschè R, Ferraresi A, et al. Randomised clinical study: gluten challenge induces symptom recurrence in only a minority of patients who meet clinical criteria for non-celiac gluten sensitivity. Alimentary pharmacology & therapeutics 2015;42:968-76.
16. Junker Y, Zeissig S, Kim SJ, et al. Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. J Exp Med 2012;209:2395-408.
17. Halmos EP, Power VA, Shepherd SJ, et al. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. Gastroenterology 2014;146:67-75 e5.
18. Longstreth GF, Thompson WG, Chey WD, et al. Functional bowel disorders. Gastroenterology 2006;130:1480-91.
19. Cox J, Mann M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. Nat Biotechnol 2008;26:1367-72.
20. Shan L, Molberg O, Parrot I, et al. Structural basis for gluten intolerance in celiac sprue. Science 2002;297:2275-9.

21. Wiklund IK, Fullerton S, Hawkey CJ, et al. An irritable bowel syndrome-specific symptom questionnaire: development and validation. Scand.J.Gastroenterol. 2003;38:947-954.
22. Mykletun A, Stordal E, Dahl AA. Hospital Anxiety and Depression (HAD) scale: factor structure, item analyses and internal consistency in a large population. Br J Psychiatry 2001;179:540-4.
23. Loge JH, Kaasa S. Short form 36 (SF-36) health survey: normative data from the general Norwegian population. Scand J Soc Med 1998;26:250-8.
24. Spangenberg L, Brähler E. Bevölkerungsrepräsentative Neunormierung des Gießen-Tests (14–92 Jahre). Psychotherapie Psychosomatik medizinische Psychologie 2011;61:e15-e18.
25. Brottveit M, Vandvik PO, Wojniusz S, et al. Absence of somatization in non-celiac gluten sensitivity. Scand J Gastroenterol 2012;47:770-7.
26. S. S. Cross-over Trials in Clinical Research. In: Barnett S, S. S, eds. Statistics in Practice. 2 ed. West Sussex, England: John Wiley & Sons Ltd, 2002.
27. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of celiac disease: time for a standardized report scheme for pathologists. Eur J Gastroenterol Hepatol 1999;11:1185-94.
28. Sarna VK, Skodje GI, Reims HM, et al. HLA-DQ:gluten tetramer test in blood gives better detection of celiac patients than biopsy after 14-day gluten challenge. Gut 2017.
29. Molina-Infante J, Carroccio A. Suspected Nonceliac Gluten Sensitivity Confirmed in Few Patients After Gluten Challenge in Double-Blind, Placebo-Controlled Trials. Clin Gastroenterol Hepatol 2017;15:339-348.
30. Shepherd SJ, Parker FC, Muir JG, et al. Dietary triggers of abdominal symptoms in patients with irritable bowel syndrome: randomized placebo-controlled evidence. Clin.Gastroenterol.Hepatol. 2008;6:765-771.
31. Erickson J, Korczak R, Wang Q, et al. Gastrointestinal tolerance of low FODMAP oral nutrition supplements in healthy human subjects: a randomized controlled trial. Nutr J 2017;16:35.
32. de Punder K, Pruimboom L. The dietary intake of wheat and other cereal grains and their role in inflammation. Nutrients 2013;5:771-87.
33. Halmos EP, Christophersen CT, Bird AR, et al. Diets that differ in their FODMAP content alter the colonic luminal microenvironment. Gut 2015;64:93-100.
34. Ostgaard H, Hausken T, Gundersen D, et al. Diet and effects of diet management on quality of life and symptoms in patients with irritable bowel syndrome. Mol Med Rep 2012;5:1382-90.
35. Aziz I, Trott N, Briggs R, et al. Efficacy of a Gluten-Free Diet in Subjects With Irritable Bowel Syndrome-Diarrhea Unaware of Their HLA-DQ2/8 Genotype. Clin Gastroenterol Hepatol 2016;14:696-703.e1.
36. Vazquez-Roque M, Oxentenko AS. Nonceliac Gluten Sensitivity. Mayo Clinic Proceedings 2015;90:1272-1277.
37. Yao CK, Gibson PR, Shepherd SJ. Design of clinical trials evaluating dietary interventions in patients with functional gastrointestinal disorders. Am J Gastroenterol 2013;108:748-58.
38. Laatikainen R, Koskenpato J, Hongisto SM, et al. Randomised clinical trial: low-FODMAP rye bread vs. regular rye bread to relieve the symptoms of irritable bowel syndrome. Alimentary Pharmacology and Therapeutics 2016;44:460-470.
39. Tavakkoli A, Lewis SK, Tennyson CA, et al. Characteristics of patients who avoid wheat and/or gluten in the absence of Celiac disease. Dig Dis Sci 2014;59:1255-61.
40. Volta U, Bardella MT, Calabro A, et al. An Italian prospective multicenter survey on patients suspected of having non-celiac gluten sensitivity. BMC Med 2014;12:85.
41. Aziz I, Lewis NR, Hadjivassiliou M, et al. A UK study assessing the population prevalence of self-reported gluten sensitivity and referral characteristics to secondary care. Eur J Gastroenterol Hepatol 2014;26:33-9.

42. Zingone F, Bartalini C, Siniscalchi M, et al. Alterations in Diets of Patients With Nonceliac Gluten Sensitivity Compared With Healthy Individuals. Clin Gastroenterol Hepatol 2017;15:63-68.e2.
43. Skodje GI, Henriksen C, Salte T, et al. Wheat challenge in self-reported gluten sensitivity: a comparison of scoring methods. Scand J Gastroenterol 2016:1-8.

Author names in bold designate shared co-first authorship

Table 1 Baseline characteristics (n=59)

Female/male, n	53/6
Age (years), mean (SD)	43.7 (12.1)
Body mass index (kg/m^2), mean (SD)	24.4 (4.0)
Duration of gluten-free diet (months), median (IQR)	20.0 (10, 48)
Previous gastroscopy, n (%)	43 (74)
Family member with celiac disease, n (%)	15 (25)
IBS by Rome III, n (%)	18 (31)
Food allergy or intolerance, n (%)	14 (24)
Other food exclusions, n (%)	38 (64)
Additional diseases, n (%)	40 (68)
Thyroid disease, n (%)	16 (27)
Symptoms before gluten-free diet	
Gastrointestinal symptoms n (%)	59 (100)
Extra intestinal symptoms n (%)	45 (76)
Celiac disease characteristics	
HLA DQ2/DQ8 negative, n (%)	25 (42)
Elevated tissue transglutaminase (IgA), n	0
Elevated deamidated gliadin peptide (IgG), n (%)	2 (3)
Study gastroscopy, n (%)	47 (84)
Marsh 0	42 (85)
Marsh 1	5 (11)

NOTE: Marsh 1: >25 IELs/100 EC [29]

SD, standard deviation ; IQR, interquartile range, IBS, irritable bowel syndrome

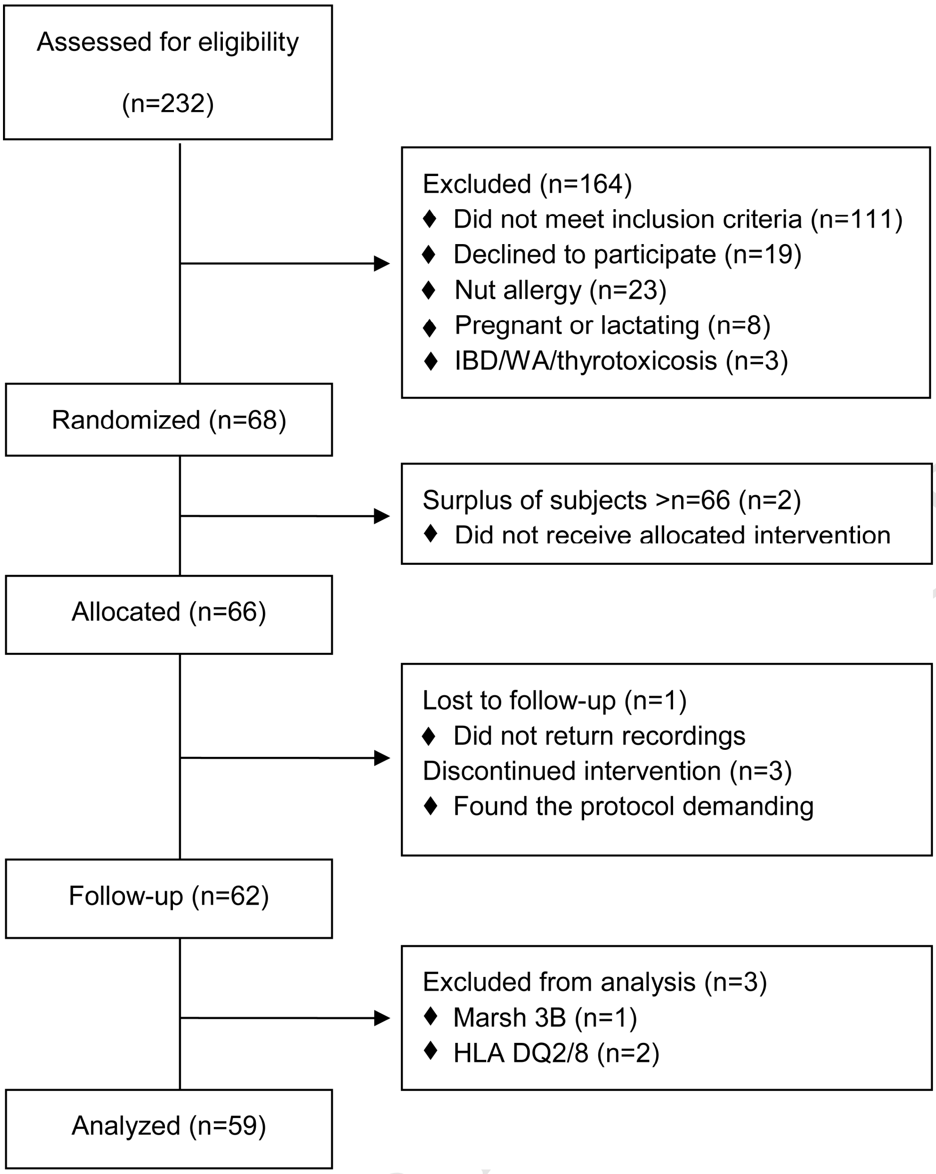
Figure 1 Participant flow. IBD, inflammatory bowel diasease, WA, wheat allergy. HLA, human leukocyte antigen.

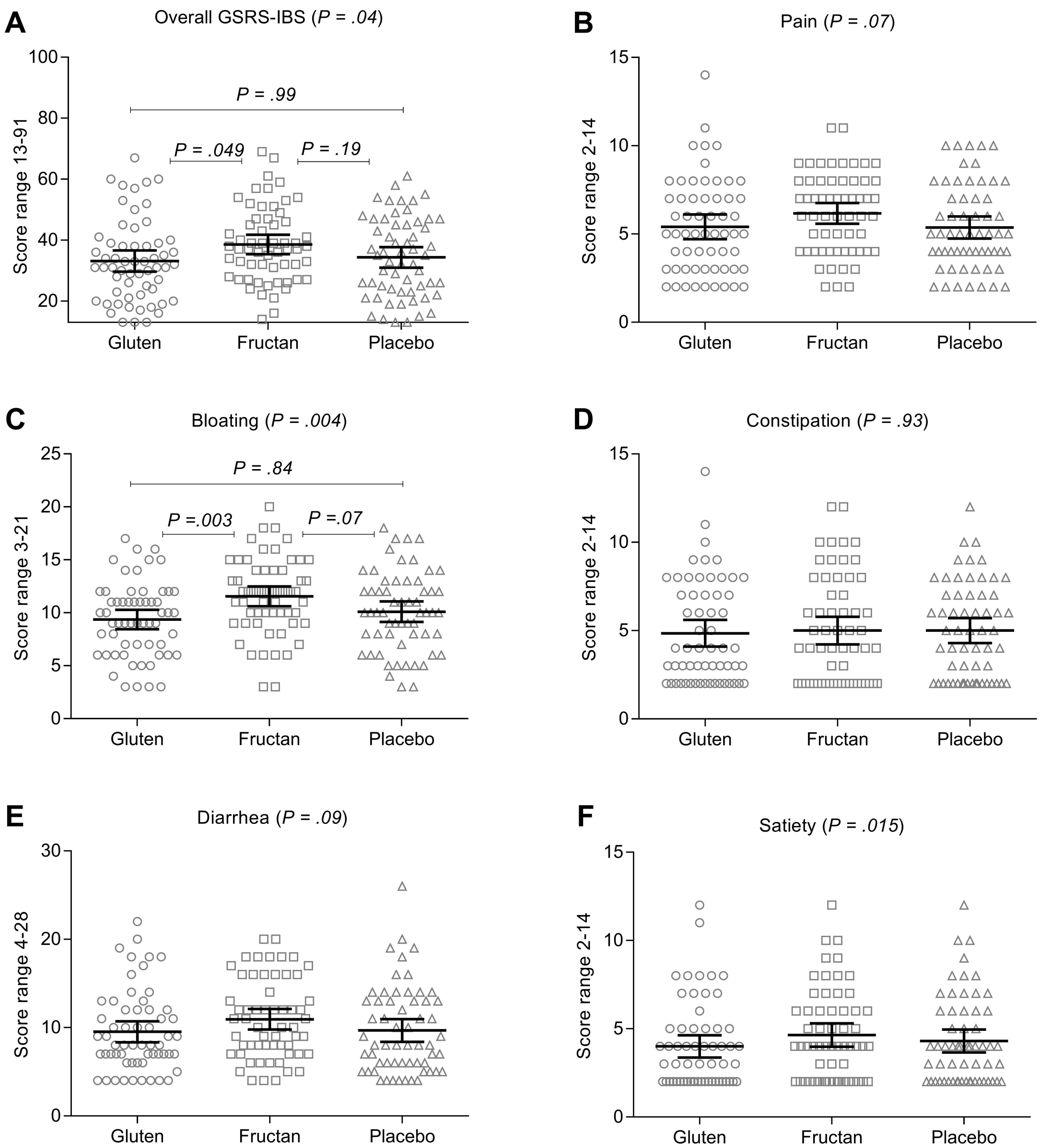
Figure 2 Mean scores (95 % confidence intervals) for overall and sub dimensions of Gastrointestinal Symptom Rating Scale-Irritable Bowel Syndrome version (GSRS-IBS) after gluten, fructan and placebo challenge (n=59). Differences between challenges were analyzed by linear mixed model, and *P*-values are given for the overall test of challenge effect.

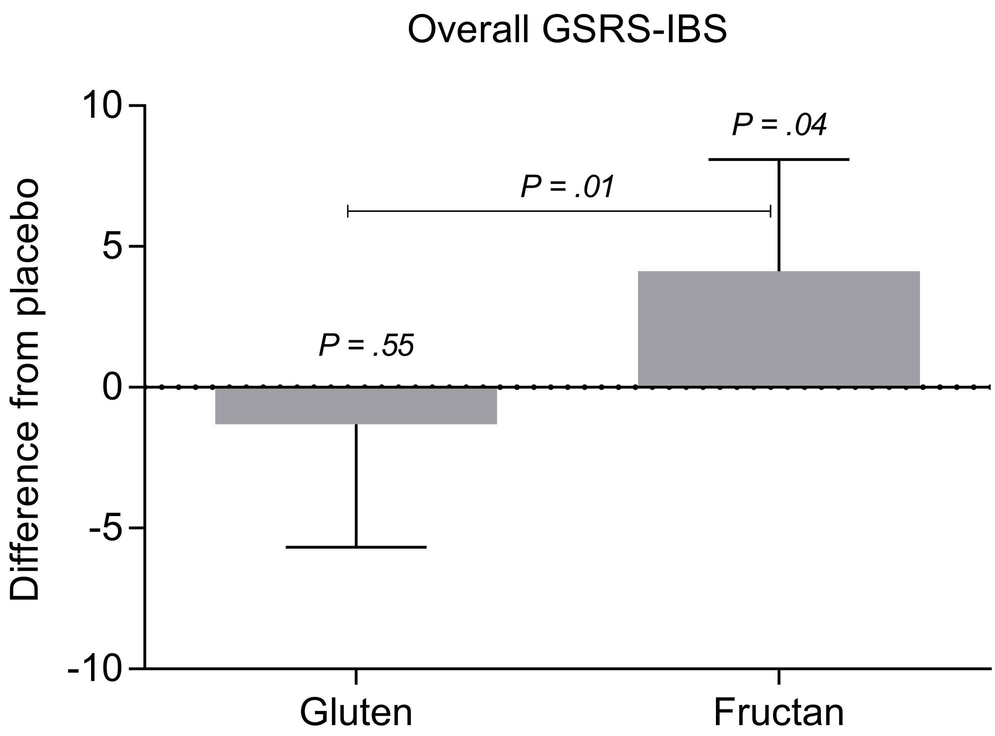
Figure 3 Mean difference in gluten and fructan response from placebo (95 % confidence intervals) for overall Gastrointestinal Symptom Rating Scale-Irritable Bowel Syndrome version (GSRS-IBS) (n=59). Differences were analyzed by paired t-test.

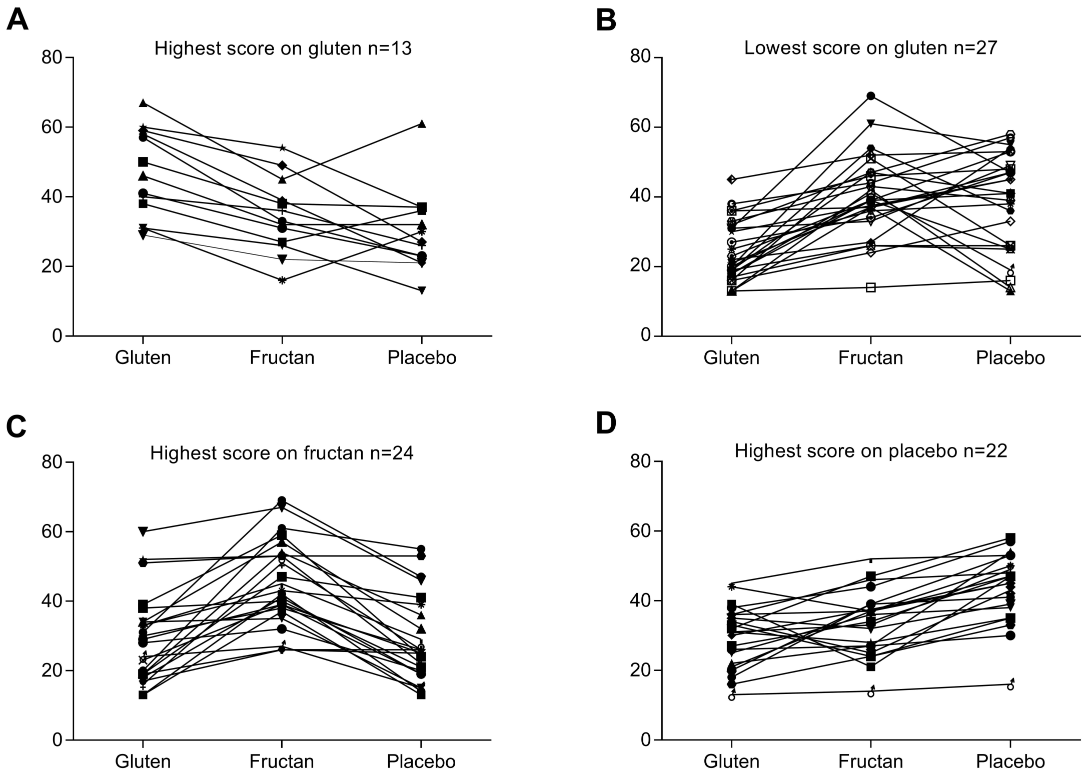
Figure 4 Individual courses according to overall Gastrointestinal Symptom Rating Scale-Irritable Bowel Syndrome version (GSRS-IBS) stratified by those scoring highest and lowest after gluten, and highest after fructan and placebo challenge (n=59).

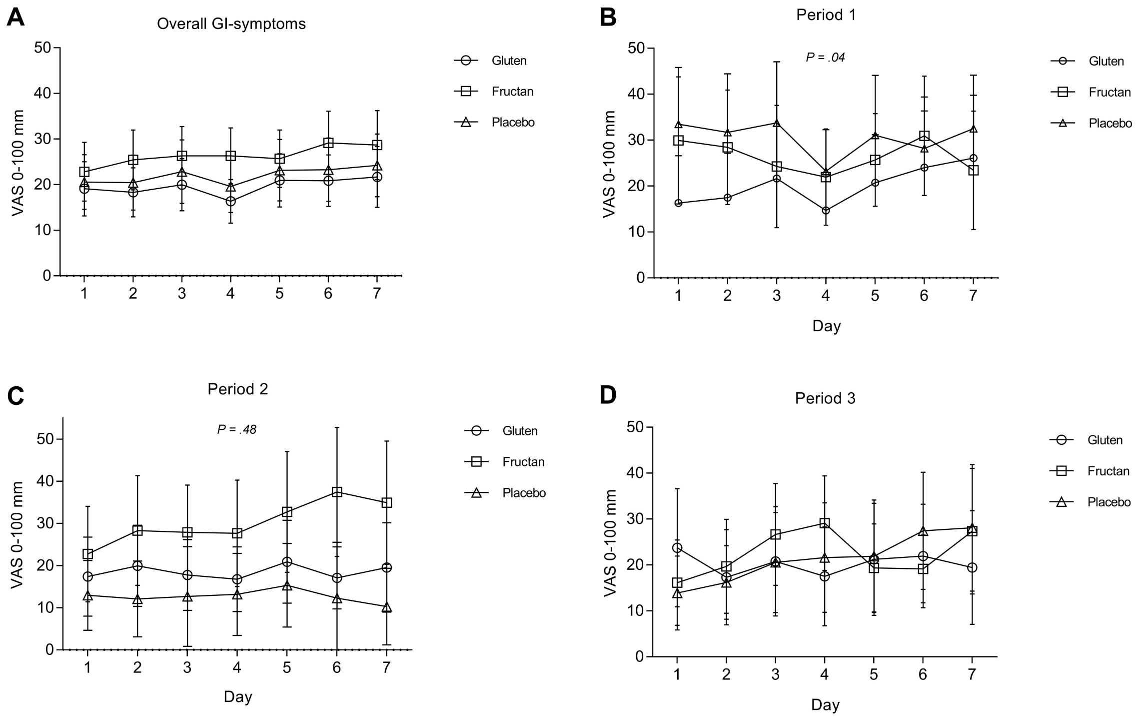
Figure 5 Mean scores (95 % CI) for gastrointestinal symptom measured daily by visual analogue scale (VAS) after gluten, fructan and placebo challenge, shown by the overall result in Figure 5A (n=59) and the result within each period in Figure 5B-D ($18 \leq n \leq 21$). Differences between challenges were analyzed by linear mixed model within each period, and *P*-values are given for the overall test of challenge effect where there was no significant interaction. Day by day differences between the challenges in period 2 were analyzed by independent samples t-test.


Assessed for eligibility
(n=232)
Excluded (n=164)
Did not meet inclusion criteria (n=111)
Declined to participate (n=19)
Nut allergy (n=23)
Pregnant or lactating (n=8)
IBD/WA/thyrotoxicosis (n=3)
Randomized (n=68)
Surplus of subjects >n=66 (n=2)
Did not receive allocated intervention
Allocated (n=66)
Lost to follow-up (n=1)
Did not return recordings
Discontinued intervention (n=3)
Found the protocol demanding
Follow-up (n=62)
Excluded from analysis (n=3)
Marsh 3B (n=1)
HLA DQ2/8 (n=2)
Analyzed (n=59)


A
Overall GSRS-IBS (P = .04)
Score range 13-91
P = .99
P = .049
P = .19
Gluten
Fructan
Placebo
B
Pain (P = .07)
Score range 2-14
C
Bloating (P = .004)
Score range 3-21
P = .84
P =.003
P =.07
D
Constipation (P = .93)
Score range 2-14
E
Diarrhea (P = .09)
Score range 4-28
F
Satiety (P = .015)
Score range 2-14


Overall GSRS-IBS
Difference from placebo
10
5
0
-5
-10
P = .04
P = .01
P = .55
Gluten
Fructan


A
Highest score on gluten n=13
B
Lowest score on gluten n=27
C
Highest score on fructan n=24
D
Highest score on placebo n=22
80
60
40
20
0
Gluten
Fructan
Placebo


A
Overall GI-symptoms
VAS 0-100 mm
Gluten
Fructan
Placebo
B
Period 1
P = .04
Day
C
Period 2
P = .48
D
Period 3

Supplementary Table 1 Muesli bar formulations g per 100 g

Ingredient	Placebo	Gluten	Fructan
Maple syrup	16.8	15.4	12.8
Rice malt	16.4	14.9	16.4
Quinoa flour	15.6	0	15.5
Soft brown sugar	15.4	14.1	15.4
Sesame seeds	7.7	6.8	7.7
Pecans	7.3	6.4	7.3
Quinoa flakes	5.4	4.6	5.4
Pepitas	5.4	4.6	5.4
Puffed quinoa	3.9	3.3	3.9
Macadamia oil	3.1	2.7	3.1
Rice puffs	3.0	2.5	3.0
Gluten flour	0	15.2	0
White chia seeds	0	9.6	0
Fructose	0	0	0.2
Galactooligosaccharides	0	0	0.1
Fructoologisaccharides	0	0	3.8

Supplementary Table 2 Nutritional content of Vital gluten[a] and the muesli bars[b] per 100 g.

Nutrient	Vital gluten	Placebo	Gluten	Fructan
Energy (kcal)		402.3	438.1	393.7
Protein (g)	75	7.3	18.1	7.3
Fat (g)	6	15.8	18.1	15.8
Carbohydrate (g)	9	58.2	49.3	55.9
Sugars (g)	5	33.9	27.9	31.7
Fibre (g)		3	2.5	3.2
Water (g)	9	8.1	10.8	6.8
Ash (g)	1	n/a	1.2	n/a

[a]Analyzed by Dairy Technical Services Ltd Food, Laboratories, Flemington, Australia.

[b]Foodworks, Version 7 (Xyris Software Australia Pty Ltd, Highgate Hill, QLD, Australia

Supplementary Table 3 Mean (SD) scores for Short Form-36 (SF-36) scales, Beck Depression Inventory version two (BDI-II), Hospital, Anxiety and Depression Scale (HAD), Giessen Subjective Complaint List (GBB) and selected extra-intestinal symptoms by 100 mm visual analogue scale (VAS) at baseline and after gluten, fructan and placebo challenge (n=59).

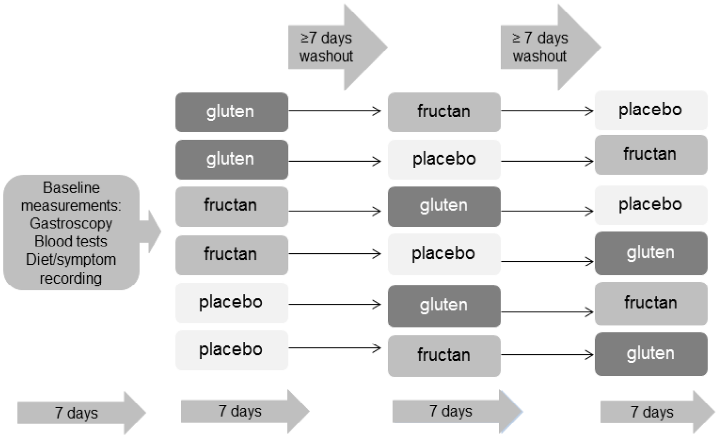
Symptoms	Baseline Mean (SD)	Gluten Mean (SD)	Fructan Mean (SD)	Placebo Mean (SD)	*P*-value
SF-36:					
Mental health	76.2 (15.0)	76.7 (17.4)	74.6 (15.6)	73.5 (17.8)	.36
Vitality	49.4 (25.5)	44.7 (25.3)	38.6 (23.5)	44.0 (24.4)	.04
Bodily pain	62.4 (21.1)	59.5 (22.5)	59.0 (21.1)	56.7 (23.9)	.73
General health	60.8 (26.2)	66.8 (23.6)	65.6 (23.5)	65.2 (24.5)	.62
Social functioning	78.2 (26.9)	78.0 (25.6)	78.2 (23.0)	78.6 (24.2)	.96
Physical functioning	88.2 (15.8)	86.0 (19.5)	86.0 (17.0)	86.6 (16.8)	.94
Role physical	61.6 (41.8)	58.0 (38.2)	59.1 (43.7)	64.7 (39.8)	.63
Role emotional	74.7 (37.1)	82.4 (31.8)	73.9 (38.3)	76.8 (36.5)	.23
BDI-II	9.3 (8.1)	7.5 (8.2)	8.5 (7.7)	9.4 (8.9)	.27
HAD overall	9.1 (6.5)	7.8 (6.4)	9.1 (6.6)	8.9 (7.2)	.39
HAD anxiety	5.5 (3.7)	4.3 (3.6)	5.1 (3.8)	5.3 (4.7)	.40
HAD depression	3.8 (3.6)	3.4 (3.5)	3.8 (3.3)	3.8 (3.7)	.60
GBB	8.0 (6.3)	9.2 (6.4)	9.6 (6.6)	9.4 (6.7)	.71
EI symptoms by 100 mm VAS:					
Weakness	34.1 (29.1)	32.4 (30.0)	41.7 (27.1)	33.4 (29.7)	.02
Sleepiness	30.7 (28.9)	31.5 (28.8)	36.1 (27.3)	30.7 (27.5)	.18
Fatigue	37.0 (30.3)	34.9 (29.7)	39.8 (27.6)	36.9 (29.6)	.28
Tiredness	40.0 (30.5)	39.3 (29.5)	46.4 (29.4)	39.3 (27.7)	.10
Dizziness	27.0 (25.6)	27.7 (28.6)	28.4 (23.5)	27.0 (29.3)	.91

Exhaustion	33.7 (30.0)	34.9 (30.7)	36.6 (27.6)	31.9 (29.9)	.45

NOTE. Higher scores in SF-36 indicate better health. Differences between gluten, fructan and placebo were analysed by linear mixed model and *P*-values are given for the main effect of challenge. SD, standard deviation, EI, extra-intestinal.

Supplementary Figure 1 Study design and time line.

Supplementary Figure 2 Mean scores (95 % confidence intervals) for overall Gastrointestinal Symptom Rating Scale-Irritable Bowel Syndrome version (GSRS-IBS) after gluten, fructan and placebo challenge within each period ($18 \leq n \leq 21$). Differences between challenges were analyzed by one way analysis of variance, and *P*-values are given for the overall test of challenge effect.


≥7 days
washout
≥ 7 days
washout
Baseline
measurements:
Gastroscopy
Blood tests
Diet/symptom
recording
gluten
fructan
placebo
gluten
placebo
fructan
fructan
gluten
placebo
fructan
placebo
gluten
placebo
gluten
fructan
placebo
fructan
gluten
7 days
7 days
7 days
7 days