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# Efficacy of a Gluten-Free Diet in Subjects With Irritable Bowel Syndrome-Diarrhea Unaware of Their HLA-DQ2/8 Genotype

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**BACKGROUND & AIMS:** **A gluten-containing diet alters bowel barrier function in patients with irritable bowel syndrome with diarrhea (IBS-D), particularly those who are positive for HLA allele DQ2/8. We studied the effects of a gluten-free diet (GFD) in patients with IBS-D who have not previously considered the effects of gluten in their diet and were unaware of their HLA-DQ2/8 genotype.**

**METHODS:** **We performed a prospective study of 41 patients with IBS-D (20 HLA-DQ2/8-positive and 21 HLA-DQ2/8-negative) at the Royal Hallamshire Hospital in Sheffield, United Kingdom, from September 2012 through July 2015. All subjects were placed on a 6-week GFD following evaluation by a dietician. Subjects completed validated questionnaires at baseline and Week 6 of the GFD. The primary endpoint was mean change in IBS Symptom Severity Score; a 50-point reduction was considered to indicate a clinical response. Secondary endpoints were changes in hospital anxiety and depression score, fatigue impact score, and Short Form-36 results. Clinical responders who chose to continue a GFD after the study period were evaluated on average 18 months later to assess diet durability, symptom scores, and anthropometric and biochemical status.**

**RESULTS:** **A 6-week GFD reduced IBS Symptom Severity Score by ≥50 points in 29 patients overall (71%). The mean total IBS Symptom Severity Score decreased from 286 before the diet to 131 points after 6 weeks on the diet ($P < .001$); the reduction was similar in each HLA-DQ group. However, HLA-DQ2/8-negative subjects had a greater reduction in abdominal distention ($P = .04$). Both groups had marked mean improvements in hospital anxiety and depression scores, fatigue impact score, and Short Form-36 results, although HLA-DQ2/8-positive subjects had a greater reduction in depression score and increase in vitality score than HLA-DQ2/8-negative subjects ($P = .02$ and $P = .03$, respectively). Twenty-one of the 29 subjects with a clinical response (72%) planned to continue the GFD long term; 18 months after the study they were still on a GFD, with maintained symptom reductions, and demonstrated similar anthropometric and biochemical features compared with baseline.**

**CONCLUSIONS:** **A dietitian-led GFD provided sustained benefit to patients with IBS-D. The symptoms that improved differed in magnitude according to HLA-DQ status. Clinical trials.gov no: NCT02528929.**

*Keywords:* Clinical Trial; Gastrointestinal Symptoms; Food; Carbohydrates.

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder characterized by recurrent abdominal pain, or discomfort, associated with an alteration in bowel habit. Despite being a benign disorder by definition, IBS leads a chronic remitting-relapsing course with associated fatigue, depression, anxiety, and diminished quality of life (QOL).[1]

Prospective cross-sectional studies suggest that IBS with diarrhea (IBS-D) accounts for almost a third of all subjects with IBS and, moreover, is the predominant subtype encountered in clinical practice.[2] Various medications have been proposed to help alleviate the symptoms of IBS-D, although these agents carry potential side effects and may not be the desired option for long-term use.[3] Dietary manipulation has also been

*Abbreviations used in this paper:* CD, celiac disease; FIS, Fatigue Impact Scale; GFD, gluten-free diet; HADS, Hospital Anxiety and Depression Scale; IBS, irritable bowel syndrome; IBS-D, irritable bowel syndrome with diarrhea; IBS-SSS, Irritable Bowel Syndrome–Symptom Severity Score; QOL, quality of life; SF-36, Short Form-36.

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suggested, particularly because up to 84% of patients with IBS believe that food items trigger their gastrointestinal symptoms.[4,5] Of these, gluten-based products are commonly cited as an offending culprit by roughly 1 in 4 patients.[5] Indeed, there is growing evidence to show that individuals are placing themselves on a gluten-free diet (GFD) of their own volition even in the absence of celiac disease (CD).[6] This clinical entity has been termed nonceliac gluten sensitivity following double-blind placebo controlled studies demonstrating gluten to induce symptoms of IBS, fatigue, and depression.[6] However, nonceliac gluten sensitivity is not without its controversies because coexisting nongluten components, such as FODMAPS (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols), can also induce IBS symptoms through mechanisms of gaseous production and osmotic diarrhea.[7,8] Nevertheless, amid this cloud of uncertainty, there remains a paucity of data on whether a GFD can be empirically recommended to patients with IBS-D previously naive to the effects of gluten-based products.

With this in regard, it has recently been demonstrated that patients with IBS-D who are HLA-DQ2/8-positive have accelerated small bowel transit times and altered bowel-barrier function on exposure to a gluten-containing diet compared with those who are HLA-DQ2/8-negative.[9,10] Indeed, previous groups have attempted to evaluate the clinical benefits of a GFD in IBS-D, yielding promising results in the HLA-DQ2/8-positive cohort, but have been limited by patient selection; many of those recruited with IBS-D had potential CD as evidenced by positive celiac-specific antibodies and raised duodenal intraepithelial lymphocytes on histology.[11]

Therefore, to address these uncertainties we aimed to evaluate the clinical response to a GFD in a rigorously defined cohort of patients with IBS-D blinded to their HLA-DQ status. We also assessed whether maintaining a GFD was safe, sustainable, and beneficial.

## Materials and Methods

### Participants and Setting

This prospective study was carried out at the Royal Hallamshire Hospital, Sheffield, United Kingdom. The hospital provides secondary-care services to a local population of 500,000 people. Following ethical approval the study was conducted from September 2012 through July 2015. All authors had access to the study data and reviewed and approved the final manuscript.

The inclusion criteria were consecutive British adults attending a gastroenterology out-patient clinic department who fulfilled the Rome III criteria for IBS-D. We excluded CD as per negative serum endomysial/tissue transglutaminase antibodies and normal duodenal biopsies. Additional exclusion criteria were as follows: individuals referred with self-reported gluten sensitivity; patients already on a GFD; and individuals with conditions known to mimic IBS-D, such as idiopathic bile acid diarrhea, pancreatic insufficiency, microscopic colitis, and inflammatory bowel disease.[12–15]

### Study Protocol

Eligible patients were given verbal and written information at the time of their first gastrointestinal follow-up clinic consultation. They were told they have a diagnosis of IBS-D (with no evidence of CD) and that the study aimed to evaluate the clinical benefits of a GFD. Willing participants were subsequently referred to 1 of 2 senior dietitians who provided uniform information on how to undertake a GFD.

The subjects were given validated questionnaires to self-complete at Week 0 (the day before commencing a GFD), and then during the GFD period. Information concerning the questionnaires can be found in the Supplementary Materials but they include IBS-Symptom Severity Score (IBS-SSS), Hospital Anxiety and Depression Scale (HADS), Fatigue Impact Scale (FIS), and Short-form 36 (SF-36) QOL. A 6-week GFD duration was chosen because at the time of commencing this study the clinical entity of nonceliac gluten sensitivity was in its infancy and so we used the example of CD to gauge our estimate. It has previously been shown that in CD most patients (77%) resolve their clinical symptoms within a month after commencing a GFD.[16] However, a small percentage linger on for longer and it was believed that 6 weeks was an adequate time to capture most responding patients while maintaining compliance within the study. After 6 weeks all patients were followed up by the dietitians where they returned questionnaires and adherence to the GFD was assessed using a simple, rapid, reliable, and validated tool.[17] The final score of the adherence tool is made up of 5 levels (0–4), which from a clinical perspective can be grouped into 3 levels. Patients scoring 0 or 1 do not follow a strict GFD. Patients scoring 2 follow a GFD but with errors necessitating correction. Finally, patients scoring 3 or 4 follow a strict GFD.[17]

Following these assessments patients in whom IBS symptoms had improved were asked whether they planned to continue with a GFD for the foreseeable future (yes/no answer). In those who answered “yes” a further reconsultation was initiated on average 18 months later to assess sustainability of the diet, symptom scores, and anthropometric/biochemical status.

### Blinding of HLA-DQ2/8 Genotype

The HLA-DQ2/8 typing was performed during the initial investigation period using the polymerase chain reaction and sequence-specific primers. Patients were grouped as HLA-DQ2/8-positive or HLA-DQ2/8-negative. Only the gastroenterologists were aware of

the HLA-DQ2/8 status for the purpose of patient recruitment. Importantly, dietitians and patients were unaware that HLA-DQ2/8 status was being used as the comparative factor.

### *Sample Size Calculation and Statistical Analysis*

The sample size calculation was based on the primary endpoint, which was to detect differences between HLA groups with regard to mean change in IBS-SSS after a 6-week GFD. In a previous study from Sheffield, United Kingdom, the mean IBS-SSS was roughly 270 with a standard deviation of 60.[18] Based on this information a sample size was calculated using computer software PS Power. To detect a clinically relevant change of 50 points on IBS-SSS, with a power of 80% at the 5% level of statistical significance, we estimated 24 participants in each arm. The secondary endpoints were to assess changes in specific IBS symptoms, HADS, FIS, and SF-36 QOL between the HLA-DQ2/8-positive and -negative groups. Other secondary endpoints were whether IBS-SSS clinical responders planned to continue with a GFD and if so whether this was sustainable and its effect on clinical status. Statistical analysis was carried out using SPSS version 21.0 software (SPSS Inc, Chicago, IL), with significance set at a $P$ value of $< .05$. Further statistical details are provided in the Supplementary Materials.

## Results

### *Recruitment*

After approaching 78 subjects with IBS-D (35 HLA-DQ2/8-positive and 43 HLA-DQ2/8-negative) eligible for the study the required sample size of 48 patients (24 HLA-DQ2/8-positive and 24 HLA-DQ2/8-negative) was reached (Figure 1). The main data presented are that of the per-protocol analysis in which 41 patients (20 HLA-DQ2/8-positive and 21 HLA-DQ2/8-negative) completed the 6-week GFD period and showed a mean GFD adherence score of 3.

### *Per-Protocol Analysis*

**Baseline Characteristics.** The demographic data for the 41 patients with IBS-D show no differences between the HLA-DQ2/8-positive and -negative groups (Table 1). In terms of baseline symptom questionnaire scores, the mean total IBS-SSS was 286.2, which records as being moderate in severity. This was similar between the HLA-DQ groups. Furthermore, there was no difference in the IBS-SSS subscales other than in pain frequency, which recorded to be higher in the HLA-DQ2/8-positive compared with HLA-DQ2/8-negative group ($P = .04$).

The HADS, FIS, and SF-36 QOL scores were also similar between the HLA-DQ groups. However, HLA-DQ2/8-positive subjects recorded worse physical fatigue (0.02), total fatigue ($P = .05$), and vitality ($P = .05$), with a trend toward worse cognitive fatigue and lower SF-36 mental component score ($P = .06$) (Table 1).

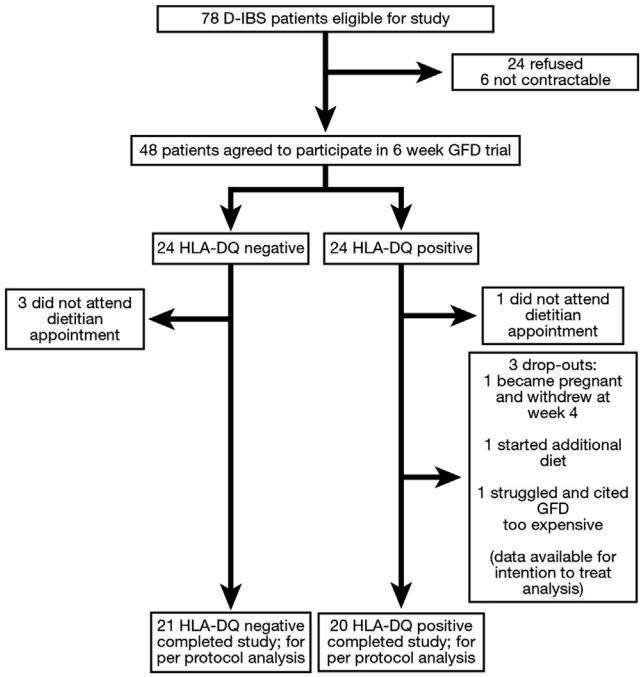


**Figure 1.** Flow chart of subject progression for a 6-week GFD.

**Response to a 6-Week Gluten-Free Diet.** Following a 6-week GFD a reduction of IBS-SSS by ≥50 points, indicating clinical benefit, was seen in 71% (n = 29 of 41) of subjects. This was regardless of mild, moderate, or severe IBS with a favorable response seen in 66.7% (n = 4 of 6), 70.6% (n = 12 of 17), and 72.2% (n = 13 of 18), respectively. Furthermore, there was no difference between HLA-DQ groups, with 70% (n = 14 of 20) of the HLA-DQ2/8-positive and 71.4% (n = 15 of 21) of the HLA-DQ2/8-negative group responding ($P$ = NS).

Figure 2 shows the changes with regard to absolute values. The mean total IBS-SSS dropped from 286.2 at baseline to 131.5 by Week 6 (mean change, -155 points; 95% confidence interval, -213.3 to -96.2 points; $P < .001$). Indeed, a significant symptom reduction was seen as early as Week 2 (mean change, -83 points; $P < .001$) and continued to drop between each interval at Week 4 ($P = .03$) and Week 6 ($P = .03$). The greatest improvements were observed in those who at baseline had severe IBS scores (mean change, -204.5 points), compared with moderate (mean change, -123.8 points) and mild IBS (mean change, -66.3 points) ($P = .04$).

When comparing the response in absolute values according to HLA-DQ subtype, both HLA-DQ2/8-positive and -negative subjects showed a significant reduction in the IBS-SSS ($P < .001$). However, there was no detectable difference between the 2 groups with the mean change in the HLA-DQ2/8-positive group being -153 points (95% confidence interval, -237.6 to -67.7 points) and in the HLA-DQ2/8-negative group being -156.7 points

**Table 1.** Baseline Characteristics (Mean Scores ± SD)

| | Overall (n = 41) | HLA-DQ2/8 positive (n = 20) | HLA-DQ2/8 negative (n = 21) | *P* value (between HLA-DQ groups) |
|---|---|---|---|---|
| Demographics | | | | |
| Age | 40.4 ± 14.9 | 40.9 ± 16.1 | 40.0 ± 14.1 | NS |
| Females, n (%) | 31 (76) | 15 (75) | 16 (76) | NS |
| White, n (%) | 39 (95) | 20 (100) | 19 (90.5) | NS |
| Employed, n (%) | 30 (73.2) | 13 (65) | 17 (81) | NS |
| IBS-SSS | | | | |
| Abdominal pain | 44 ± 24 | 47.5 ± 20.9 | 40.6 ± 26.3 | NS |
| Pain frequency | 59.3 ± 31.5 | 70 ± 30.6 | 49.0 ± 29.5 | .04 |
| Abdominal distention | 47.9 ± 26.6 | 43.4 ± 27.8 | 52.2 ± 25.3 | NS |
| Stool dissatisfaction | 70.9 ± 23.2 | 71.6 ± 26.2 | 70.3 ± 20.6 | NS |
| Life interference | 60.4 ± 22.5 | 63.7 ± 17.7 | 57.2 ± 26.3 | NS |
| IBS-SSS total | 286.2 ± 97.7 | 299.5 ± 95.6 | 273.6 ± 100.2 | NS |
| Mild IBS, n (%) | 6 (14.6) | 3 (15) | 3 (14.2) | NS |
| Mode rate IBS, n (%) | 17 (41.5) | 8 (40) | 9 (42.9) | NS |
| Severe IBS, n (%) | 18 (43.9) | 9 (45) | 9 (42.9) | NS |
| HADS | | | | |
| Anxiety | 9.9 ± 4.8 | 10.5 ± 4.4 | 9.4 ± 9.1 | NS |
| Depression | 7.0 ± 4.4 | 7.9 ± 3.8 | 6.2 ± 5.0 | NS |
| HADS total | 16.9 ± 8.4 | 18.3 ± 7.2 | 15.6 ± 9.3 | NS |
| FIS | | | | |
| Cognitive | 19.2 ± 13.8 | 22.6 ± 12.5 | 16 ± 14.6 | .06 |
| Social | 15.6 ± 14.9 | 18 ± 12.9 | 13.4 ± 16.6 | NS |
| Physical | 18.5 ± 15.4 | 23.4 ± 14.1 | 13.7 ± 15.5 | .02 |
| FIS total | 53.3 ± 42.1 | 64 ± 40 | 43.1 ± 44.2 | .05 |
| SF-36 QOL | | | | |
| Physical function | 76 ± 25.4 | 71.5 ± 25.5 | 80.5 ± 25.1 | NS |
| Role physical | 50.6 ± 41 | 42.5 ± 39.8 | 58.8 ± 41.6 | NS |
| Bodily pain | 50 ± 25.5 | 50.1 ± 26.4 | 50 ± 25.3 | NS |
| General health | 50.4 ± 17.9 | 46.9 ± 18.2 | 53.9 ± 17.9 | NS |
| Vitality | 39.5 ± 23.4 | 32.3 ± 19.4 | 46.8 ± 25.4 | .05 |
| Social function | 62.1 ± 27.4 | 62.1 ± 22 | 62 ± 32.6 | NS |
| Role emotional | 52.5 ± 42 | 41.7 ± 40.3 | 63.3 ± 41.8 | .09 |
| Mental health | 53.3 ± 20.7 | 50.9 ± 17.9 | 55.6 ± 23.4 | NS |
| SF-36 PCS | 56.4 ± 20.4 | 52.8 ± 20 | 60 ± 20.8 | NS |
| SF-36 MCS | 50.7 ± 23 | 44.7 ± 15.1 | 56.7 ± 27.9 | .06 |

MCS, mental component score; NS, not significant; PCS, physical component score; SD, standard deviation.

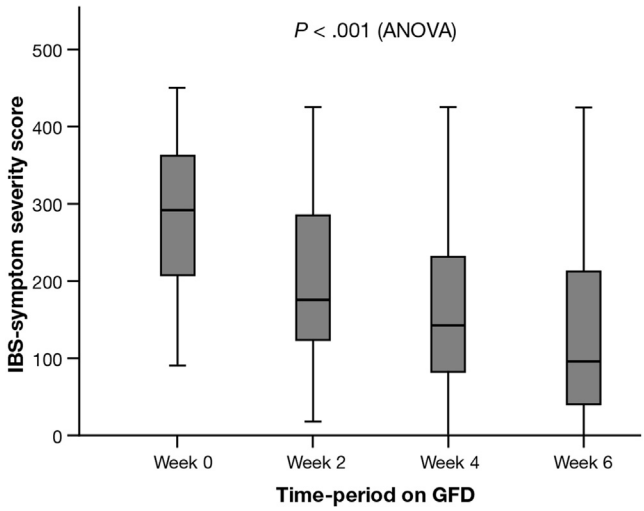


**Figure 2.** Change in IBS-SSS during a 6-week GFD, all subjects. ANOVA, analysis of variance.

(95% confidence interval, -248.6 to -91.2 points) (Figure 3). In terms of the subscales representative of the IBS-SSS there was a significant mean reduction in abdominal pain, pain frequency, stool dissatisfaction, and life interferences in both HLA-DQ groups but no differences between the groups (Figure 3). However, HLA-DQ2/8-negative subjects showed a greater reduction in abdominal distention compared with HLA-DQ2/8-positive subjects ($P = .04$).

There was a significant overall improvement in HADS, FIS, and SF-36 QOL following a GFD, which was seen in both HLA-DQ groups (Table 2). However, the HLA-DQ2/8-positive group experienced a greater improvement in depression ($P = .02$), total HADS ($P = .05$), vitality ($P = .03$), role emotional ($P = .06$), physical fatigue ($P = .07$), and total fatigue ($P = .09$) compared with the HLA-DQ2/8-negative group.

**Aftercare.** At the end of the 6-week GFD period 72% (21 of 29) of IBS-SSS clinical responders planned to

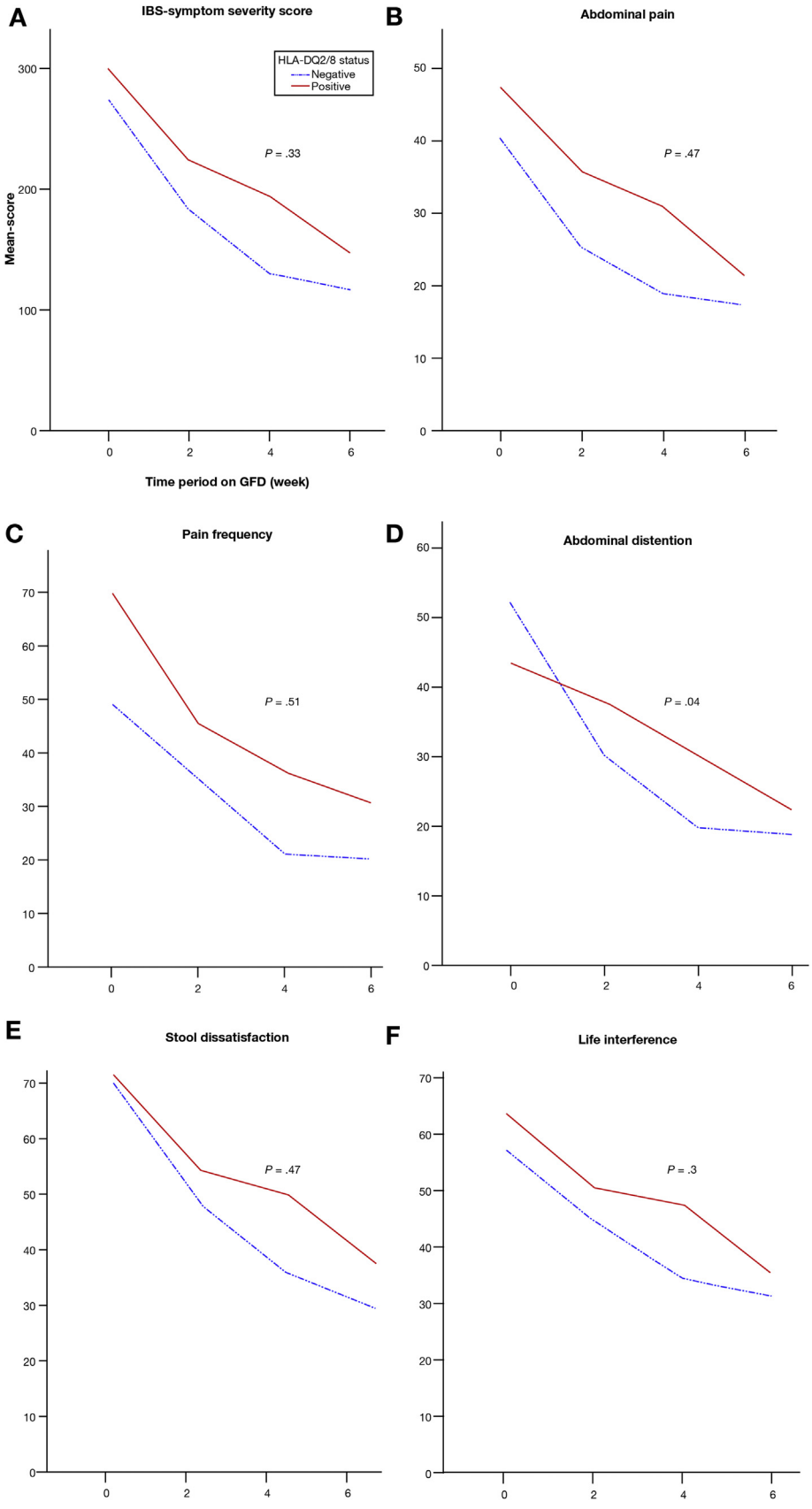


**Figure 3.** (*A–F*) Change in IBS-SSS and subcomponents during a 6-week GFD, according to HLA-DQ status.

continue with a GFD for the foreseeable future; 11 were HLA-DQ2/8-positive and 10 HLA-DQ2/8-negative ($P$ = NS). These 21 patients were reconsulted on average 18 months later (range, 7–26 months) and were still maintaining a GFD with mean adherence score of 3, had ongoing symptom score improvement, and had no

**Table 2.** Mean Change (95% CI) in Mood, Fatigue, and QOL After a 6-Week GFD Relative to Baseline

| | Overall (n = 41) | HLA-DQ2/8 positive (n = 20) | HLA-DQ2/8 negative (n = 21) | *P* value (between HLA-DQ groups) |
|---|---|---|---|---|
| HADS | | | | |
| Anxiety | -1.85 (-0.8 to -2.9) | -2.5 (-0.6 to -4.4) | -1.2 (-0.2 to -2.3) | NS |
| Depression | -2.1 (-1.0 to -3.2) | -3.4 (-1.6 to -5.2) | -0.86 (+0.4 to -2.1) | .02 |
| Total | -4.0 (-2.1 to -5.8) | -5.9 (-2.6 to -9.2) | -2.1 (-0.3 to -3.9) | .05 |
| FIS | | | | |
| Cognitive | -7.1 (-3.7 to -10.5) | -10.3 (-4.1 to -16.4) | -4.0 (-0.8 to -7.3) | NS |
| Social | -5.95 (-2.6 to -9.3) | -8.1 (-2.3 to -13.9) | -3.9 (-0.2 to -7.7) | NS |
| Physical | -7.1 (-3.1 to -10.6) | -10.9 (-4.3 to -17.4) | -3.5 (-0.8 to -6.2) | .07 |
| Total | -20.1 (-10.3 to -30) | -29.2 (-11 to -47.4) | -11.4 (-3.0 to -19.9) | .09 |
| SF-36 QOL Score | | | | |
| Physical function | +7.5 (+2.1 to +12.9) | +12.0 (+1.5 to +22.5) | +3.0 (+0.1 to +5.9) | NS |
| Role physical | +32.9 (+19.9 to +45.9) | +41.0 (+23 to +59) | +24.7 (+5.0 to +44.5) | NS |
| Bodily pain | +19.1 (+11.3 to +26.9) | +16.9 (+4.8 to +29) | +21.4 (+10.4 to +32.4) | NS |
| General health | +7.7 (+2.1 to +13.2) | +8.35 (-1.5 to +18.2) | +7.0 (+0.95 to +13.0) | NS |
| Vitality | +14.0 (+6.9 to +21.1) | +20.7 (+9.1 to +32.3) | +7.25 (+0.91 to +15.4) | .03 |
| Social function | +13.0 (+3.8 to +22.2) | +14.3 (-1.6 to +30.1) | +11.8 (+0.7 to +22.9) | NS |
| Role emotional | +16.7 (+3.5 to +29.9) | +25.1 (+3.0 to +47.1) | +8.3 (-7.6 to +24.3) | .06 |
| Mental health | +9.95 (+3.4 to +16.5) | +13.0 (+2.3 to +23.7) | +6.9 (-1.4 to +15.2) | NS |
| SF-36 PCS | +14.4 (+7.7 to +21.1) | +14.9 (+4.2 to +25.8) | +14.0 (+4.8 to +23.2) | NS |
| SF-36 MCS | +14.4 (+7.2 to +21.5) | +20.3 (+8.0 to +32.5) | +8.5 (+0.7 to +16.2) | NS |

CI, confidence interval; MCS, mental component score; NS, not significant; PCS, physical component score.

alterations in body mass index or biochemical status relative to baseline (Table 3). All reported that inadvertent gluten exposure triggered IBS symptoms.

### *Intention-to-Treat Analysis*

A total of 44 patients (23 HLA-DQ2/8-positive and 21 HLA-DQ2/8-negative) were available for the intention-to-treat analysis. A GFD led to an overall and within-group improvement in IBS-SSS, FIS, HADS, and SF-36 QOL. However, between-group comparisons revealed that a GFD conferred greater benefit in HLA-DQ2/8-positive subjects with regard to depression ($P = .04$), vitality ($P = .02$), and role emotional ($P = .05$) compared with HLA-DQ2/8-negative subjects. In contrast, HLA-DQ2/8-negative subjects experienced greater resolution in abdominal distention ($P = .03$).

**Table 3.** Comparison of Body Mass Index, Blood Parameters, and Symptom Scores at Mean 18 Months Compared With Baseline in Patients Maintaining a GFD (n = 21)

| | Pre-GFD | On GFD (mean, 18 mo) | *P* value |
|---|---|---|---|
| Mean anthropometric and biochemical status | | | |
| Body mass index ± SD | 27.3 ± 5.4 | 26.7 ± 5.0 | NS |
| Hemoglobin, *g/dL* (% normal) | 14.2 (100) | 13.8 (95) | NS |
| Ferritin, *μg/L* (% normal) | 77.7 (87.5) | 83. 3 (90) | NS |
| Folate, *μ/L* (% normal) | 8.1 (100) | 8.8 (95) | NS |
| Vitamin $B_{12}$, *ng/L* (% normal) | 388.6 (100) | 342.8 (95) | NS |
| Albumin, *g/L* (% normal) | 46.7 (100) | 45.3 (100) | NS |
| Mean symptom scores ± SD | | | |
| IBS-SSS | 313.5 ± 97 | 95.5 ± 75 | <.001 |
| HADS | 17.8 ± 8.6 | 9.35 ± 8 | <.001 |
| FIS | 58.9 ± 45.7 | 20.9 ± 33.5 | <.001 |
| SF-36 PCS | 52.3 ± 19.5 | 79.9 ± 16.9 | <.001 |
| SF-36 MCS | 47.4 ± 23.8 | 76 ± 21.8 | <.001 |

MCS, mental component score; NS, not significant; PCS, physical component score; SD, standard deviation.

## Discussion

This prospective study demonstrates that 71% of subjects with IBS-D show clinical response to a 6-week GFD, with additional improvements in mood, fatigue, and QOL. These findings occurred irrespective of HLA-DQ status, although HLA-DQ2/8-positive subjects experienced greater improvement in depression and vitality compared with their HLA-DQ2/8-negative counterparts. In contrast, HLA-DQ2/8-negative subjects showed a greater reduction in abdominal distention. Finally, 72% of patients planned to continue with a GFD long-term, and on review mean 18 months later were still maintaining the diet, reported ongoing symptom remission, and did not show any detrimental effects toward body mass index or hematinic status.

The strengths of this study include the methodology used. We recruited a rigorously defined cohort of subjects with IBS-D who had no evidence of organic pathologies. In particular, we ensured exclusion of CD to prevent any ambiguity when interpreting the findings. This contrasts to previous studies that on attempting to evaluate the clinical effects of a GFD in IBS-D have been

limited by including those with potential CD, as evidenced by the presence of celiac-related antibodies and/or raised duodenal intraepithelial lymphocytes.[11] We also ensured that patients and dietitians were blinded to the fact that HLA-DQ2/8 status was being used as the comparative factor. Finally, this was a real-life pragmatic study where the onus was left on the patients to take a GFD following a single dietetic clinic appointment, as opposed to being in a heavily controlled research environment where all meals are provided. Furthermore, we were able to demonstrate safety and durability of the diet with ongoing symptom remission. Hence, we believe that our findings can be generalized to patients with IBS-D in clinical practice.

The limitations of this study include the placebo-effect of undertaking a dietary trial. However, systematic meta-analysis of randomized controlled trials in IBS have demonstrated a pooled placebo response rate of 37.5%, with lower responses seen in those who fulfil the Rome criteria on study entry and that used 8 weeks or more of therapy.[19] Indeed, a placebo effect of 33% was found in a recent trial performed on subjects with IBS within Sheffield.[18] This suggests that in our study the 71% response rate to a GFD at 6 weeks is unlikely placebo particularly because well-being was maintained at mean 18 months despite having had no interim office visits. Moreover, other investigators have recently shown a similar response rate with 80 of 102 patients with IBS improving following a 6-week GFD, with subsequent double-blinded exposure to gluten-containing powder leading to significant symptom deterioration in 74.3% compared with 16.2% of those receiving gluten-free powder.[20]

These findings therefore support the use of a GFD in IBS-D. However, we do not know how a GFD directly fares in comparison with other dietary therapies proposed to alleviate the symptoms of IBS-D. For example, the low-FODMAP diet has been shown to effectively reduce gastrointestinal symptoms in 50%–70% of patients with IBS,[7] although most recent data from a 4-week multicenter study suggest that simple dietary interventions (eg, regular meal patterns; avoidance of large meals; and reduced intake of fat, insoluble fibers, caffeine, and gas-producing oligosaccharide-containing foods, such as beans, cabbage, and onions) are equally as effective as a low-FODMAP diet.[21] Further randomized comparative dietary trials are needed to address not only the impact of diet on IBS symptoms but also to assess extraintestinal symptoms, social QOL, day-to-day practicality, sustainability, and safety. Indeed, restriction of oligosaccharide (fructan) content has been shown to alter the composition of beneficial colonic microbiota.[22–25] With regard to the low-FODMAP diet reductions in the proportions of *Bifidobacteria*, butyrate-producing clostidrial groups and mucus-associated bacterium *Akkermansia muciniphila* have been noted in patients with IBS and healthy control subjects.[22,23] A GFD has been shown to reduce *Bifidobacteria* and *Lactobacilli* in healthy subjects.[24,25] The implications of altered microbiome on long-term colonic health are unknown and require elucidation.

Nevertheless, dietary therapies are now on the menu for treating IBS-D,[4] and we speculate that the pathophysiological mechanism by which a GFD improves symptoms may differ according to HLA-DQ status. It has previously been shown that subjects with IBS-D have increased small bowel intestinal permeability compared with control subjects,[26] and that duodenal instillation of dietary food antigens (commonly to wheat) leads to an immediate and transient increase in duodenal intraepithelial lymphocyte density, formation of epithelial leaks/gaps, and widening of intervillous spaces as seen by confocal laser endomicroscopy.[27] Elsewhere, it has been shown that HLA-DQ2/8-positive subjects with IBS-D have faster small bowel transit, with exposure to a gluten-containing diet reducing tight-junction proteins and increasing intestinal permeability, compared with HLA-DQ2/8-negative subjects.[9,10] Furthermore, experimental models in HLA-DQ8 gluten-sensitized mice have provided a mechanistic explanation for symptom induction by demonstrating gliadin to induce immune activation in the absence of intestinal atrophy, paralleled with increased acetylcholine release from the myenteric plexus resulting in enhanced muscle contractility and epithelial hypersecretion, with the abnormalities reversed following gluten withdrawal.[28]

It therefore remains to be explored whether in our study the effect of a GFD seen in HLA-DQ2/8-positive subjects can be attributed to the specific removal of gluten protein per se, thereby "switching-off" an HLA-DQ2/8-driven immune-mediated process and restoring gut health. This could account for the improvement in IBS-D symptoms but also explain the marked benefit experienced from a generalized and mental well-being perspective in the HLA-DQ2/8-positive group; in that, gluten-related or equivalent protein-peptides (exorphins) are no longer present or able to cross the intestinal epithelium into the systemic circulation and central nervous system where they may cause symptoms, such as depression.[29] In contrast, for the HLA-DQ2/8-negative IBS-D group the improvement seen with a GFD may not be caused by removal of the gluten protein per se but rather eliminating the oligosaccharide (fructan) component found in wheat, given that there was a rapid resolution of intestinal distention.[8] Future studies should ascertain the effects of specifically isolated gluten-based constituents in subjects with IBS-D according to the presence or absence of HLA-DQ2/8 genotype. Attention should also be paid to differences between specific haplotypes, including the influences of heterozygosity and homozygosity.

In conclusion, a dietitian-led GFD should be considered as a therapeutic option for the management of patients with IBS-D who are previously naive to the effects of gluten. The type of symptom improvement may differ in magnitude according to HLA-DQ status and warrants mechanistic exploration.

## Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at http://dx.doi.org/10.1016/j.cgh.2015.12.031.

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**Conflicts of interest**
These authors disclose the following: David S. Sanders has received an educational grant from Dr Schär (a gluten-free food manufacturer) to undertake investigator-conceived and -led research studies on gluten sensitivity. Dr Schär has no access to study data. The remaining authors disclose no conflicts.

## Supplementary Materials

### *Self-completed questionnaires*

The IBS-SSS[1] is a frequently used assessment in clinical studies where responders rate, over the preceding 10 days, abdominal pain severity, pain frequency, bloating, bowel habit dissatisfaction, and life interferences related to bowel symptoms. The maximum cumulative score available is 500, and subjects can be classified as having no symptoms (<75), to mild (75–175), moderate (175–300), and severe IBS (>300). A reduction of 50 points is considered to confer a clinical improvement. This questionnaire was completed by all patients at Weeks 0, 2, 4, and 6. In those who opted to continue with a GFD it was completed again at 18-month mean follow-up.

The HADS[2] is a psychological screening tool to which there are in total 14 items, 7 each for depression and anxiety. Each item is rated from 0 (not present) to 3 (maximum), giving a cumulative score for each subscale to range from 0 to 21.[2] A subscale score of ≥11 is used to indicate a clinically significant level of anxiety or depression. This questionnaire was completed by all patients at Weeks 0 and 6, and at 18-month mean follow-up in those maintaining GFD.

The FIS[3] consists of a total of 40 questions enquiring for the impact of fatigue over the preceding month. The questions can be subdivided into 3 sections: (1) physical functioning (10 items), (2) cognitive functioning (10 items), and (3) psychosocial functioning (20 items). Each item consists of a statement, being rated by the subjects as 0 (no problem) to 4 (extreme problem). A higher score represents greater fatigue severity. This questionnaire was completed by all patients at Weeks 0 and 6, and at 18-month mean follow-up in those maintaining GFD.

The SF-36 questionnaire[4] measures general health-related QOL over the last month. There are 36 items divided into 8 subsections that include physical functioning, physical role, body pain, general health perceptions, vitality, social functioning, emotional role, and mental health. These can then be further aggregated to form a physical component summary and a mental component summary. For each subscale, the raw scores are transformed into a scale of 0–100, with 100 representing the best possible health-related QOL. This questionnaire was completed by all at Weeks 0 and 6, and at 18-month mean follow-up in those maintaining GFD.

### *Statistical analysis*

Statistical analysis was carried out using SPSS version 21.0 software (SPSS Inc, Chicago, IL), with significance set at a $P$ value of $< .05$. Categorical variables at baseline (Week 0) were summarized by descriptive statistics, including total numbers and percentages, with between-HLA-group comparisons performed using the chi-square test. Continuous variables at baseline were summarized by mean values, standard deviation, with between-HLA-group comparisons made using the unpaired Student $t$ test for parametric data and Mann-Whitney $U$ test for nonparametric data.

Following a 6-week GFD, overall and within-HLA-group changes for IBS-SSS and its associated subscales were analyzed using a repeated-measures 1-way analysis of variance test with post hoc Bonferroni analysis. A mixed-design 1-way analysis of variance was used to compare between-HLA-group changes for IBS-SSS/subscales over the 6-week GFD period.

With regard to overall and within-HLA-group changes in FIS, SF-36 QOL, and HADS, these were analyzed using a paired Student $t$ test for parametric data and Wilcoxon signed rank test for nonparametric data; data presented using 95% confidence intervals. In contrast, between-HLA-group comparisons for these indices were made after computing for differences at Week 6 relative to baseline followed by applying the unpaired Student $t$ test or Mann-Whitney $U$ test as appropriate.

Finally, in those patients opting to continue with a GFD comparisons of their body mass index, hematinics, and symptom scores at 18-month follow-up relative to baseline were made using chi-square, paired Student $t$ test, or Wilcoxon signed rank test as appropriate.

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