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Effect of Gluten Ingestion and FODMAP Restriction on Intestinal Epithelial Integrity in Patients with Irritable Bowel Syndrome and Self-Reported Non-Coeliac Gluten Sensitivity

*Mary Ajamian, Gennaro Rosella, Evan D. Newnham, Jessica R. Biesiekierski, Jane G. Muir, and Peter R. Gibson**

Scope: Since epithelial barrier dysfunction has been associated with gluten and fermentable oligosaccharide, disaccharide, monosaccharide, and polyols (FODMAPs), the effect of alterations in FODMAP a gluten intake on epithelial barrier function in patients with irritable bowel syndrome (IBS) who self-reported gluten sensitivity.

Methods and results: Circulating concentrations of markers of epithelial injury (syndecan-1 and intestinal fatty acid-binding protein) and bacterial translocation (lipopolysaccharide-binding protein and soluble CD14) are measured while consuming habitual gluten-free diet and during blinded challenges with gluten or placebo on a background of low FODMAP intake. In 33 patients, only syndecan-1 concentrations during their habitual diet are elevated (median 43 ng mL^{-1}) compared with 23 ng mL^{-1} in 49 healthy subjects ($p < 0.001$). On a low FODMAP diet, symptoms are reduced and levels of syndecan-1 (but not other markers) fell by a median 3335% ($p < 0.001$) irrespective of whether gluten is present or not.

Conclusion: Gluten ingestion has no specific effect on epithelial integrity or symptoms in this cohort, but reducing FODMAP intake concomitantly reduces symptoms and reverses apparent colonic epithelial injury. These findings highlight the heterogeneity of populations self-reporting gluten sensitivity and implicate FODMAPs in colonic injury in IBS.

1. Introduction

Non-coeliac gluten sensitivity (NCGS), also called non-coeliac wheat sensitivity, is a controversial diagnosis in which individuals lack the genetic, immune, and serological biomarkers of coeliac disease or wheat protein allergy, yet present with similar symptoms that appear to resolve when following a gluten-free diet.[1] In the available literature, NCGS is defined by improvement in gastrointestinal symptoms on a gluten-free diet followed by induction of symptoms by gluten when re-challenged. Unfortunately, the interpretation of such studies has been hampered by nocebo effects, whereby the frequency, and severity of gluten-specific effects in some individuals have been matched by placebo-specific effects in others.[2–6] Whilst there are currently no established biomarkers of disease, some pathophysiological insights have been obtained in recent studies. A study of an Italian cohort of patients who fulfilled criteria for NCGS reported a pattern of elevated markers associated with intestinal barrier dysfunction and microbial translocation that were distinctive from those associated with treated or untreated coeliac disease and healthy controls.[7] Moreover, those with NCGS had a significant decline in levels in these markers in conjunction with symptom improvement when gluten-containing foods were omitted from their diet. The results implicated dysregulation at the intestinal barrier that may provide clues into pathogenic mechanisms underlying NCGS and potential biomarker utility. However, such findings are yet to be reproduced.

In the first randomized controlled re-challenge clinical trial that investigated the effects of carbohydrate-depleted gluten in patients with irritable bowel syndrome (IBS) with self-reported gluten sensitivity, subjects showed no difference in symptom responses to 7-day dietary challenges featuring high gluten (16 g per day), low gluten (2 g per day), or placebo (whey protein isolate).[8] All participants were adherent to a gluten-free diet prior to entering the study, but they were instructed during a run-in period to reduce the intake of fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs). This measure aimed to avoid the confounding effects of altering FODMAP intake during the challenge periods. All patients improved their overall symptom level during the run-in period. As

Dr. M. Ajamian, Dr. G. Rosella, Dr. J. G. Muir, Prof. P. R. Gibson
Department of Gastroenterology
Monash University and Alfred Health
Melbourne, Victoria 3004, Australia
E-mail: peter.gibson@monash.edu

Dr. E. D. Newnham, Dr. J. R. Biesiekierski[+], Dr. J. G. Muir, Prof. P. R. Gibson
Department of Gastroenterology and Hepatology
Eastern Health
Victoria 3128, Australia

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/mnfr.201901275

[+]Current address: Department of Dietetics, Nutrition and Sport, La Trobe University, Bundoora 3086, Australia

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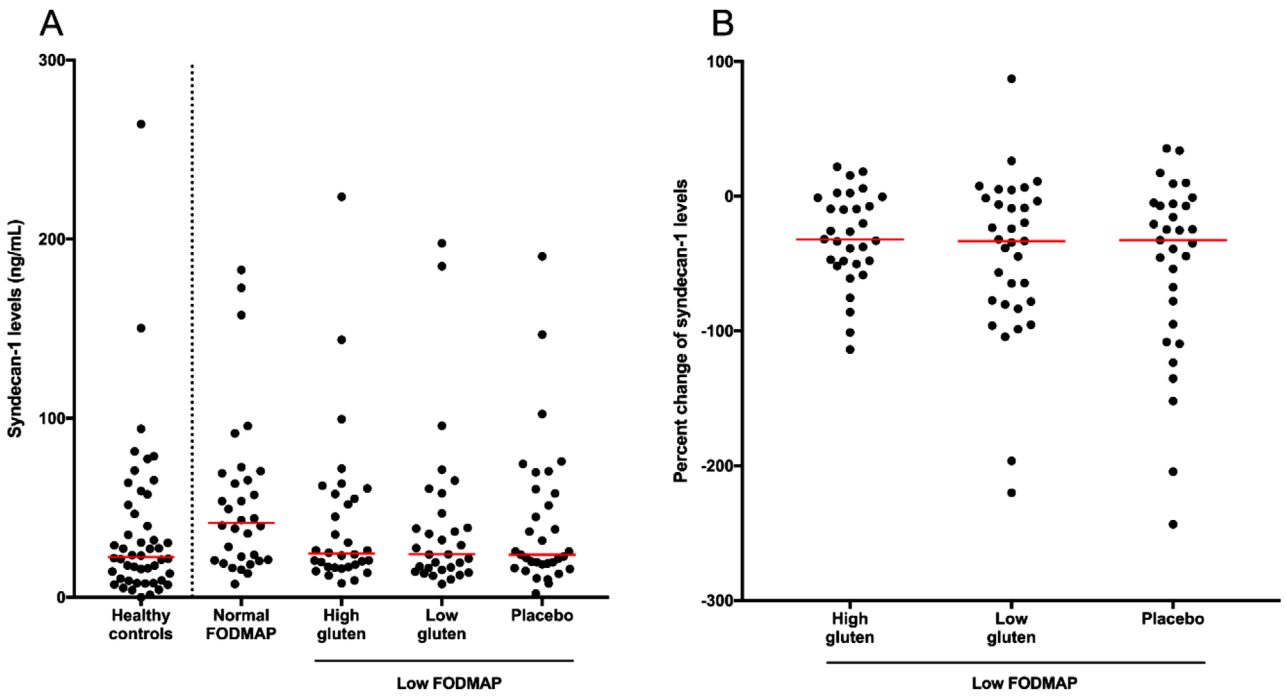

Figure 1. Absolute and percent changes of serum syndecan-1 levels in healthy controls and in individuals with IBS and self-reported gluten sensitivity during the baseline, or normal-FODMAP period, and subsequent high-gluten, low-gluten, and placebo dietary interventions on a background low-FODMAP diet. A) Serum syndecan-1 levels. Outliers comprising ≈400 ng mL^{-1} in one subject during the normal-FODMAP intervention, one during the high-gluten intervention, and two during the low-gluten intervention have been omitted. Significant pairwise comparisons (Wilcoxon test) were found between normal-FODMAP and high-gluten ($p < 0.0001$), normal-FODMAP and low-gluten ($p = 0.0006$), and normal-FODMAP and placebo ($p = 0.0001$) interventions. Levels in healthy controls were different from study participants in the normal-FODMAP dietary intervention ($p = 0.012$; Mann–Whitney test). B) Percent change in syndecan-1 levels relative to normal-FODMAP period after omitting two outliers showing >300% reduction in the placebo group; no significant differences were detected in pairwise comparisons. Red bars represent medians. The vertical broken line separates healthy controls from the experimental cohort.

additional dietary control, patients were provided all their food during the interventional periods. Peripheral blood was collected prior to the run-in period (i.e., after the 1-week observation period on a gluten-free diet) and during each high gluten, low gluten, or placebo intervention. Analysis of this cohort provided an opportunity to assess the biomarkers shown to be abnormal in the other cohort of patients with NCGS and the effects of gluten.

While reduction of dietary intake of FODMAPs is associated with alleviation of gastrointestinal symptoms in patients with IBS and in those with self-reported NCGS,[6] there is a body of data that indicates high intake of non-digestible FODMAPs might have deleterious effects that include impairment of barrier function, epithelial injury and induction of mucosal inflammation, and heightening of visceral sensitivity.[9–15] While much of the information has been generated from the intake of high doses in experimental animals, there are also data implicating similar effects in humans. The gluten re-challenge study outlined above provided the opportunity to determine whether reducing FODMAP intake might influence epithelial integrity and barrier function using circulating markers associated with epithelial injury in general (syndecan-1),[16] small intestinal epithelial injury (intestinal fatty acid-binding protein or I-FABP),[17] and bacterial translocation (human lipopolysaccharide binding protein or LBP, and human soluble CD14 or sCD14).[7,18]

Hence, we aimed to assess the effect of reducing FODMAP intake and reintroducing gluten on markers of intestinal epithelial injury and barrier function in patients with self-reported NCGS who partook in a randomized, double-blinded, placebo-control, dose-ranging gluten re-challenge study in which FODMAP intake was reduced prior to the re-challenges.

2. Results

2.1. Participants

37 subjects completed the interventions. As outlined elsewhere,[8] the mean age was 45 years (range 24–61 years), 31 were female. All fulfilled Rome 3 criteria for IBS. 57% were HLA-DQ2 or DQ8 positive. All were also negative for coeliac serology. A complete set of 4 sera were available for 33 patients and these were evaluated in the present study. 49 healthy controls were recruited. The mean age of this cohort was 39 years (range 22–64 years) and 32 were female.

2.2. Syndecan-1

As shown in **Figure 1**A, healthy controls had a median syndecan-1 level of 23 ng mL^{-1}, which was lower than that of the participants with IBS in the baseline (normal-FODMAP) condition at 43 ng mL^{-1} ($p = 0.018$; Mann–Whitney test), but similar to

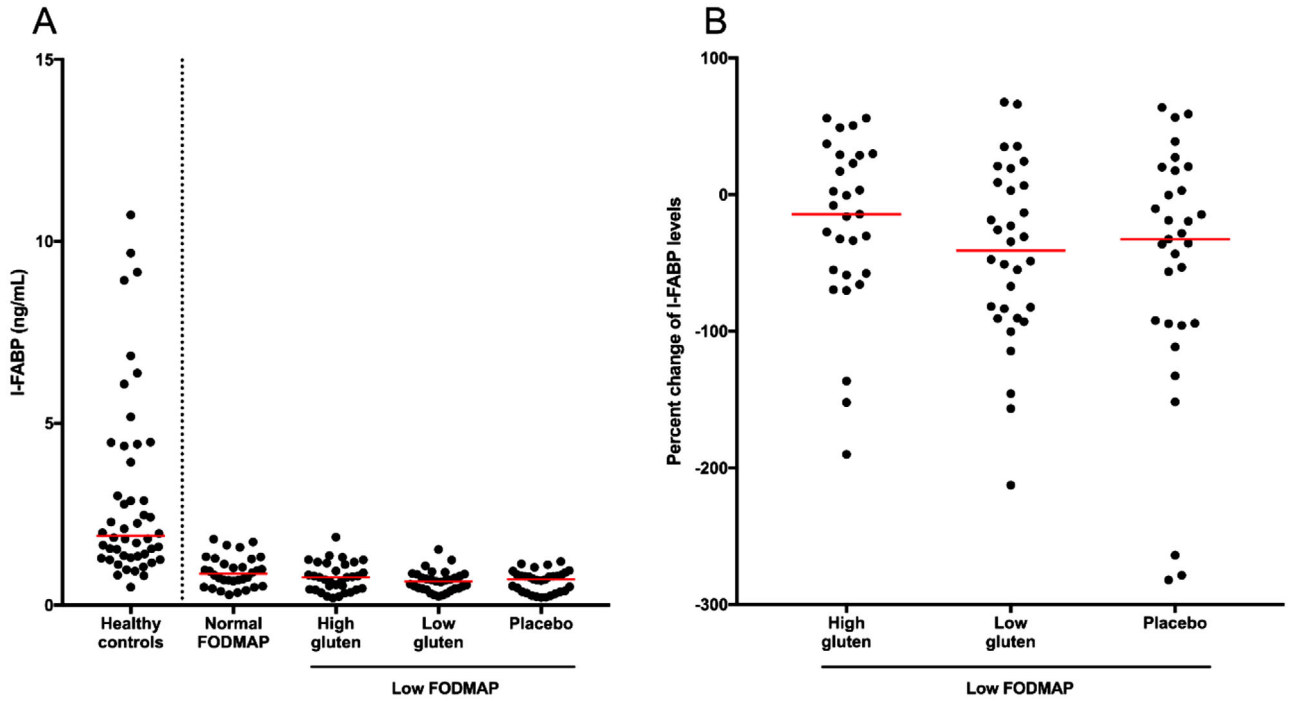

Figure 2. Absolute and percent changes of serum intestinal fatty acid binding protein (I-FABP) levels in healthy controls and in individuals with IBS and self-reported gluten sensitivity during the normal-FODMAP period and subsequent high-gluten, low-gluten and placebo dietary interventions on a background low-FODMAP diet. A) Serum levels of I-FABP. An outlier (>30 ng mL^{-1}) was omitted from the healthy control cohort. There was a significant difference between levels in the normal-FODMAP dietary intervention from the low-gluten ($p = 0.008$) and placebo interventions ($p = 0.015$), as determined by Wilcoxon tests. Levels in healthy controls were different from those in the participants in every dietary intervention arm ($p < 0.0001$; Mann–Whitney tests). B) Percent change in I-FABP levels relative to normal-FODMAP period after omitting outliers showing >300% reduction in the high- and low-gluten groups ($n = 1$ each) and 2 in the placebo group. No significant pairwise comparisons were observed in absolute or percent change levels after correction for multiple comparisons. Red bars represent medians. The vertical broken line separates healthy controls from the experimental cohort.

that during the low-gluten intervention at 28 ng mL^{-1}, the high-gluten intervention at 25 ng mL^{-1}, and the placebo intervention at 24 ng mL^{-1}. There was a significant difference across the paired interventions in the IBS cohort ($\chi^2_F(3) = 24.82$, $p < 0.0001$; Friedman's test). Significant pairwise comparisons, as indicated by Wilcoxon tests, were observed between normal-FODMAP and high-gluten ($p < 0.0001$), normal-FODMAP and low-gluten ($p = 0.0006$), and normal-FODMAP and placebo ($p = 0.0001$) interventions. A significant pairwise comparison was also found by Mann–Whitney test between healthy controls and study participants in the normal-FODMAP dietary intervention ($p = 0.0123$).

The changes in syndecan-1 levels from the normal to low FODMAP dietary periods (expressed as percentage change) are shown in Figure 1B. The levels were significantly reduced in all interventions being by a median of 33% for the high-gluten intervention, 33% for the low-gluten intervention, and 35% for the placebo intervention. No difference was observed in the changes across the three interventions.

2.3. I-FABP

As shown in **Figure 2**A, the median I-FABP level in healthy controls was 2.0 ng mL^{-1}, which was higher than 0.9 ng mL^{-1} in the baseline (normal-FODMAP) condition in the participants with IBS, 0.8 ng mL^{-1} for the high-gluten intervention, and 0.7 ng mL^{-1} for the low-gluten and placebo interventions. There was a significant difference across the paired interventions in the IBS cohort ($\chi^2_F(3) = 9.982$, $p = 0.019$). Significant pairwise comparisons, as indicated by Wilcoxon tests, were observed between normal-FODMAP and low-gluten ($p = 0.008$), and normal-FODMAP and placebo interventions ($p = 0.015$). Additional significant pairwise comparisons, determined by Mann–Whitney tests, were observed between healthy controls and study participants in every dietary intervention arm ($p < 0.0001$).

The percentage changes in I-FABP levels from the normal- to low-FODMAP dietary periods are shown in Figure 2B. While I-FABP levels fell across the three dietary interventions, the differences were not statistically significant.

2.4. LBP

As shown in **Figure 3**A, median levels of LBP in healthy controls was 16 µg mL^{-1}, which was similar to 12 µg mL^{-1} in the normal-FODMAP intervention, but greater than 7 µg mL^{-1} that was observed in the high-gluten, low-gluten, and placebo interventions. These differences were significant discoveries when the false discovery rate (FDR) was controlled ($q = 0.0240$ for all p-values). In the participants with IBS, LBP concentrations differed across the

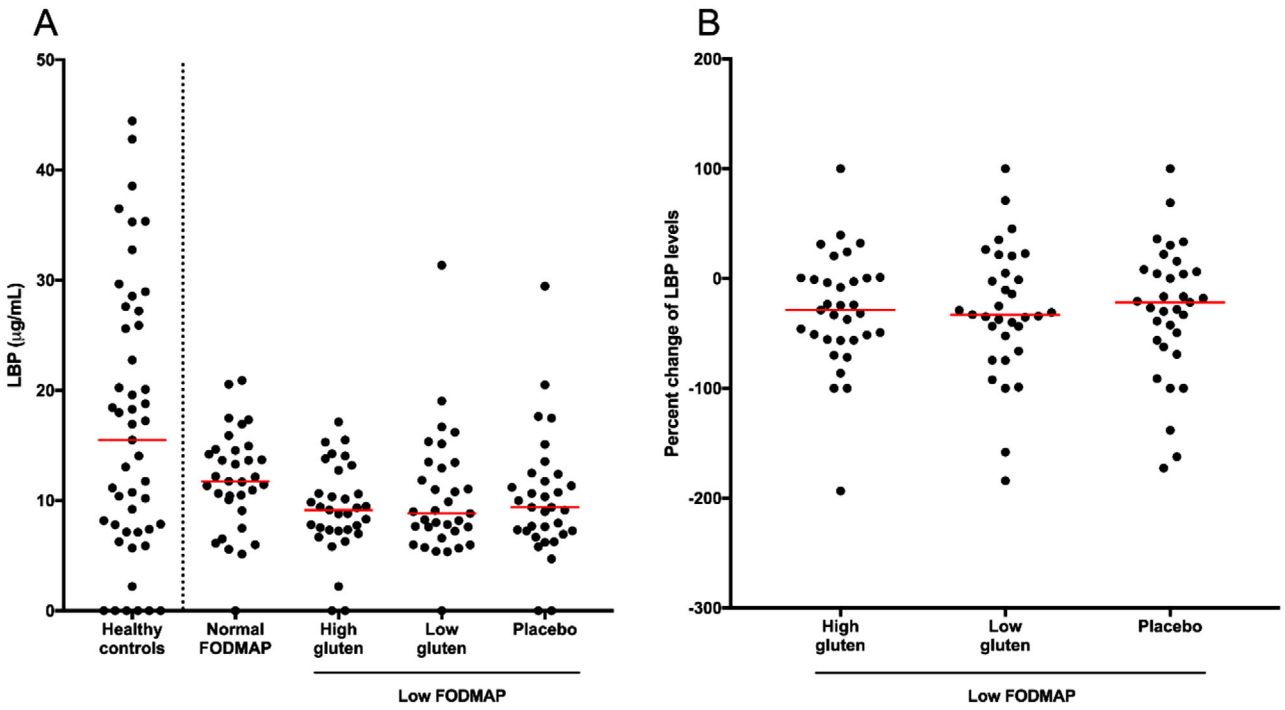

Figure 3. Absolute and percent changes of serum lipopolysaccharide-binding protein (LBP) levels in healthy controls and in individuals with IBS and self-reported gluten sensitivity during the normal-FODMAP period and subsequent high-gluten, low-gluten and placebo dietary interventions on a background low-FODMAP diet. A) Serum LBP levels. Levels during the normal-FODMAP period were different to those in during the high-gluten intervention (p = 0.0017; paired t-test). Levels in healthy controls were different from those in IBS study participants during the high-gluten (p = 0.006), low-gluten (p = 0.017), and placebo (p = 0.018) dietary interventions (Mann–Whitney test). B) Percent change in LBP levels relative to normal-FODMAP period. No significant differences were observed across the dietary intervention groups. Red bars represent medians. The vertical broken line separates healthy controls from the experimental cohort.

dietary periods ($\chi^2_F(3) = 10.25$, $p = 0.017$). There was a significant difference between the normal-FODMAP arm and high-gluten arms ($p = 0.002$; paired t-test), which was a significant discovery when the FDR was controlled ($q = 0.0102$). Significant pairwise comparisons, as indicated by Wilcoxon tests, were also found between normal-FODMAP intake and low-gluten ($p = 0.02$) and placebo interventions ($p = 0.05$), though these were not significant discoveries when the FDR was controlled ($q = 0.0690$ and $q = 0.0996$, respectively). Significant differences were observed in pairwise comparisons by Mann–Whitney tests between healthy individuals and study participants in the high-gluten ($p = 0.006$), low-gluten ($p = 0.017$), and placebo ($p = 0.018$) intervention arms. These were significant discoveries when the FDR was controlled ($q = 0.0240$).

The change in LBP concentrations from those in the FODMAP arm are shown in Figure 3B. No significant differences were observed in pairwise comparisons.

2.5. sCD14

As shown in **Figure 4**A, sCD14 levels were similar across healthy controls and participants with IBS irrespective of the dietary period when blood was drawn. The percentage change in sCD14 levels from normal-FODMAP arm to the intervention arms are shown in Figure 4B and no significant alterations were observed.

2.6. Correlations between Biomarkers and their Change

A positive, significant correlation was found between LBP and sCD14 levels ($p = 0.020$, $r = 0.403$) in the normal-FODMAP condition. However, no significant correlations were observed between LBP and sCD14 in any low-FODMAP dietary period. There were no significant correlations evident between syndecan-1 and I-FABP levels in any dietary period. There were also no correlations evident between change in syndecan-1 (normal-FODMAP to any of the interventions) with changes in I-FABP and LBP.

3. Discussion

Non-coeliac gluten or wheat sensitivity is controversial since the diagnostic criteria depend largely upon self-reported responses of symptoms to gluten withdrawal and subsequent re-challenge in those where coeliac disease and wheat allergy have been excluded.[1] Several randomized controlled re-challenge studies in adults and children with self-reported NCGS have largely failed to define a sub-group with gluten-specific induction of symptoms.[2–6] The strong nocebo effect in the cohorts in these studies led to similar proportions having marked responses to placebo or to gluten. The findings of a unique combination of biomarkers indicating systemic immune activation and compromised intestinal barrier integrity in an Italian cohort that fulfilled criteria for NCGS raised hope that a pattern of biomarkers

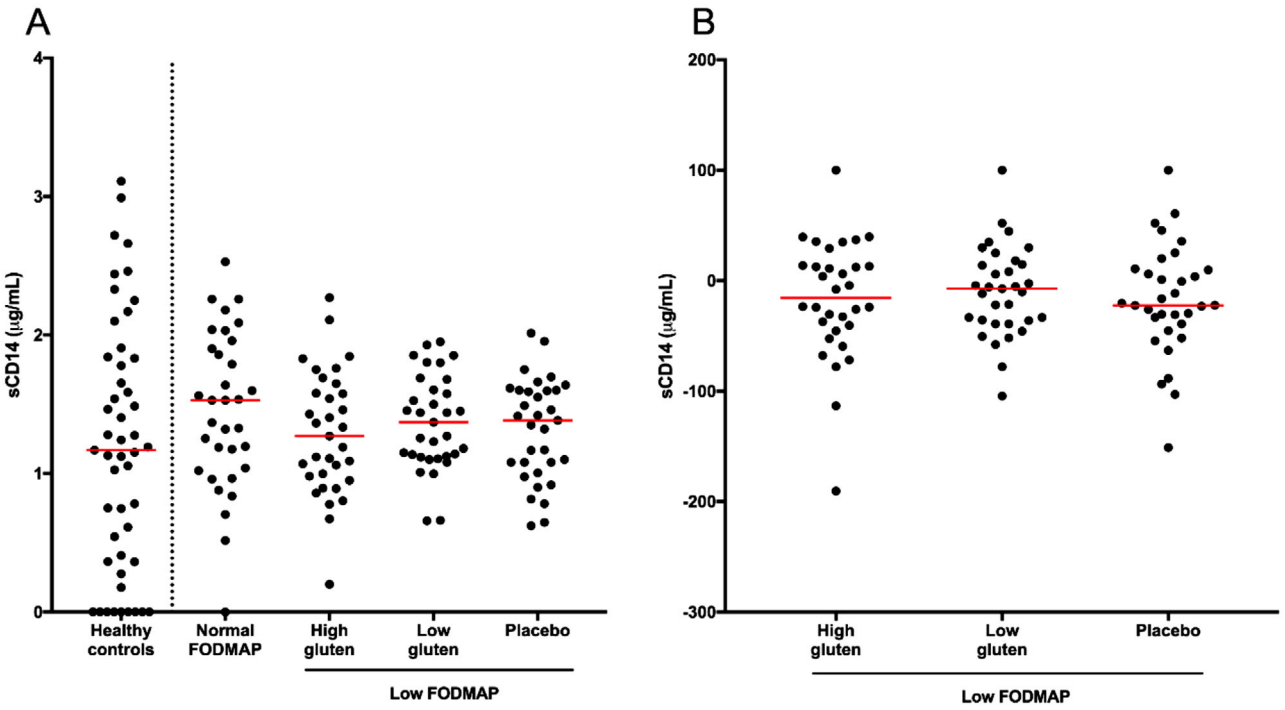

Figure 4. Absolute and percent changes of serum soluble CD14 (sCD14) levels in healthy controls and in individuals with IBS and self-reported gluten sensitivity during the normal-FODMAP period and subsequent high-gluten, low-gluten, and placebo dietary interventions on a background low-FODMAP diet. A) Serum sCD14 levels. B) Percent change in sCD14 levels relative to normal-FODMAP period after omitting one outlier showing >300% reduction in the high-gluten group. No significant pairwise comparisons between means or medians were observed in absolute or percent change levels (analysis performed on complete datasets). Red bars represent medians. The vertical broken line separates healthy controls from the experimental cohort.

that identify the condition pathophysiologically was emerging.[7] However, we were not able to confirm similar abnormalities in these biomarkers nor their induction by the ingestion of gluten. in this cohort of patients with IBS who fulfill the criteria for NCGS. Indeed, the findings have highlighted the potential role of FODMAPs in the pathophysiology of IBS and self-reported gluten sensitivity with the reduction of a marker of intestinal epithelial injury on reducing FODMAP intake in association with improved symptoms. If the change observed is a FODMAP-specific effect, then it supports findings of a blinded triple-arm re-challenge study that implicated fructans, a FODMAP that is generally rich in gluten-containing cereals, as the culprit food component.[6] This was also consistent with improved symptoms in our cohort of patients when these were markedly reduced in their diet as part of the trial, albeit in unblinded observations.

We found that circulating concentrations of the biomarkers of intestinal injury in the subjects with IBS were abnormal when compared with those of a local population of healthy controls. Analysis of blood taken while the subjects were consuming their habitual gluten-free diet revealed concentrations of syndecan-1 to be elevated and I-FABP reduced. In contrast, markers of bacterial translocation were not different. This was in contrast to elevated I-FABP and markers of bacterial translocation observed in the Italian cohort, although syndecan-1 was not measured. This was unlikely to be due to technical issues as the same assays were applied and the same scientist (M.A.) performed most of them. These observations indicate that the Australian and Italian cohorts have very different pathophysiology despite fulfilling similar clinical criteria. This idea has been previously raised.[19] Italian cohorts in whom intestinal histopathology has been reported seem to have a high prevalence of intraepithelial lymphocytosis and eosinophilic infiltration,[20] but whether they are representative of the patients utilized in the biomarker study is not known. In our cohort, about one half had duodenal histology performed (those who were HLA-DQ2 or DQ8 positive) and none had such abnormalities.

Both I-FABP and syndecan-1 are markers of intestinal epithelial injury. I-FABP (also known as fatty acid-binding protein 2) is expressed throughout the small and large intestines,[21,22] but tissue concentrations are more than tenfold greater in the proximal small intestine compared with those in the colon.[23] Upon damage of the enterocyte, I-FABP is released into the circulation. Several studies have observed increased circulating I-FABP levels in patients with coeliac disease compared with those in normal healthy controls [7,24–29] and has been used as a marker of responsiveness to gluten in a study involving adults with coeliac disease undergoing a gluten challenge.[30] Levels of I-FABP may be increased in Crohn's disease, but not in active ulcerative colitis, consistent with its being a marker of small rather than large intestinal epithelial injury.[31,32] Syndecan-1 or CD138, is a transmembrane proteoglycan of epithelial cells involved in adhesion between cells and extracellular matrices and has been implicated to play a key role in maintenance of the intestinal epithelial barrier.[33–35] For example, the loss of syndecan-1 core protein and ectodomain components in an animal study has been shown to increase protein efflux into the gut

lumen,[35] and its overexpression in cell culture prevented bacterial translocation and promoted maintenance of the intestinal barrier.[33] Soluble ectodomains of syndecan-1 are constituently shed and able to migrate into the circulation. The local release of inflammatory cytokines, including tumor necrosis factor alpha and interleukin-1β, enhances such shedding.[36–38] Consistent with such concepts, circulating syndecan-1 levels increase in patients with active Crohn's disease and ulcerative colitis[38,39] and fall in response to anti-tumor necrosis factor therapy.[40,41] Heightened levels of syndecan-1 correlated to mucosal damage in pediatric patients with coeliac disease and not in in children with non-specific abdominal pain.[38]

In light of what is known about these two markers, the low to normal levels of I-FABP in association with the habitual diet of the patients with IBS and self-reported gluten sensitivity in our cohort indicate a low likelihood that small intestinal injury is occurring, but the elevated levels of syndecan-1 suggest injury to large intestinal epithelium. Furthermore, the normalization of the syndecan-1 (reducing by about 30%), and lack of significant change in I-FABP during the gluten and placebo challenge periods indicated that gluten was not injurious and that something had changed between habitual and challenge dietary periods. The main difference in food composition between the habitual and challenge periods was the reduction of FODMAP intake, suggesting that the FODMAPs might potentially have been the injurious factors. Such a notion is not new. Ingestion of high doses of fructo-oligosaccharide or lactulose in murine studies is associated with increased epithelial permeability, increased susceptibility to and severity of Salmonella infection, and increased mucus production (assumed a response to epithelial injury).[10–13] In rats, ingestion of a high FODMAP diet induced increased colonic epithelial permeability and mucosal inflammation with heightened visceral sensitivity.[14] When fecal water from patients with IBS on their habitual diet was placed into the colonic lumen in that rat model, similar effects were observed. Moreover, those effects were abolished when the patients were consuming a diet low in FODMAPs. Mechanistically, these deleterious effects could be blocked by the use of antibodies that bind lipopolysaccharide (LPS), giving rise to the concept that the reduced absolute abundance of bacteria (specifically those bearing LPS) that has been consistently documented in patients consuming a low FODMAP diet may be causally related to reduced visceral sensitivity and improved longer term symptom severity. Hence, our findings, in which likely large intestine-specific epithelial injury present in the patients with IBS while consuming their habitual diet reversed when FODMAP intake was reduced, suggest a causal relationship. Only further studies could define whether this association is indeed causal.

No abnormality in the degree of bacterial translocation could be detected under any of the dietary conditions in our study, in contrast to that observed in the Italian cohort. sCD14 and LBP are two endogenous proteins commonly studied together to assess levels of circulating bacterial products, namely LPS (i.e., endotoxin) from gram-negative bacteria and, in this way, act as surrogate markers to identify bacterial translocation. Primarily produced in hepatocytes, LBP has a high affinity for circulating LPS, and is typically upregulated in response to increased concentrations.[42,43] The presence of LBP is necessary to facilitate the binding of LPS to CD14, a glycoprotein that exists as a membrane-associated form (mCD14) primarily on monocytes/macrophages (considered CD14-positive cells) and as a soluble, extracellular form (sCD14) released from the membrane.[44,45] Both forms of CD14 are upregulated by the presence of LPS and other bacterial wall components.[46] Usage of these markers to indicate microbial translocation across the gut barrier have described in diverse settings such as intestinal disease activity in patients with human immunodeficiency virus infection, active inflammatory bowel disease,[47] and following the ingestion of apparently pro-inflammatory diets in healthy subjects.[7,48] Both LBP and sCD14 had significant, positive correlations with I-FABP in the Italian cohort with NCGS,[7] and heightened levels of sCD14 along with I-FABP have been described in both treated and newly diagnosed, untreated patients with coeliac disease compared with those in healthy individuals.[25] Thus, our negative findings indicate that excessive systemic exposure to LPS was unlikely to be occurring in the patients with IBS.

In conclusion, using biomarkers of intestinal epithelial injury and microbial translocation, we have clearly demonstrated the heterogeneity of populations self-reporting gluten sensitivity when defined by symptomatic criteria. Gluten is unlikely to be inducing intestinal injury or inflammation in patients who believe they are gluten sensitive when their small intestinal histology is normal or when they are HLA DQ2 or DQ8 negative. These objective findings support the lack of symptomatic responses specifically to blinded gluten challenge in this cohort with normal duodenal histology and/or not carrying HLA-D haplotypes associated with coeliac disease. The correction of objective of evidence of epithelial injury as shown by normalization of circulating syndecan-1 concentrations with reduction of FODMAP intake supports the clinical observations that FODMAPs play a key role in symptom generation in this cohort. The findings are attributed speculatively to a protective effect on the colonic epithelium of reducing dietary FODMAP intake supporting other data for the concept that symptomatic improvement on a diet low in FODMAPs may be attributed to more than just the reduced stimulation of mechanoreceptors via osmotic and fermentative distension of the intestinal lumen.

4. Experimental Section

Study Participants: Study participants were fully described in the published report of the interventional study.[8] Briefly, subjects, recruited by advertising, had chronic abdominal symptoms that fitted the Rome 3 criteria for IBS, believed themselves to be sensitive to gluten and were adherent to a gluten-free diet as assessed by a nutritionist at the time of recruitment. They were above 16 years of age, none had antibodies to wheat antigens, and coeliac disease had been excluded by the absence of HLA-DQ2 or 8 or by normal duodenal histology when on a gluten-containing diet. They were not taking any drugs or had other illness that was known to compromise intestinal mucosal integrity. Subjects 16–70 years old who believed themselves to be healthy with no known gastrointestinal illness were also recruited by advertising and word-of-mouth. Subjects were selected on the basis of exclusion criteria; they were excluded if consuming a restrictive diet (e.g., gluten free) or if taking any medication or complementary medicine that might potentially affect intestinal barrier function. They were also excluded if they had pre-existing liver disease, diabetes, and autoimmune disorders or evidence of ongoing, active infection.

Protocol: The study protocol of the placebo-controlled, randomized, double-blind crossover intervention was previously described in the

published report.[8] Briefly, participants remained on a gluten-free, normal FODMAP diet, and their intake (via a food diary) and symptoms were documented for 1 week. They were then instructed on reducing FODMAPs in addition to remaining gluten-free for a 2-week run-in period. Patients then received one of the three diets (i.e., low gluten, high gluten, and placebo) on a background low FODMAP diet for 1 week followed by a minimum 2-week washout before the second diet was commenced for 1 week. The same process was followed for the third diet. All food was provided during the interventions and neither the assessing investigator nor the subjects knew the nature of the interventional diets, which differed only in the protein composition—16 g/d gluten/d versus 2 g/d gluten plus 14 g/d whey protein versus 16 g/d whey protein (placebo). Severity of gastrointestinal symptoms were assessed using daily visual analogue scales. Peripheral blood was taken at the end of the 1-week observation period on a gluten-free diet without restriction of FODMAPs after enrolment in the study as well as on day 6 of each dietary intervention. Blood was also collected when a particular dietary intervention was discontinued due to intolerable symptoms if these occurred later than day 6. A single sample of 40 mL of peripheral blood was also taken from the healthy controls. Serum was extracted, placed in aliquots, and stored at −80 °C until used for assays. Thus, serum was available for four dietary regimens: gluten-free, normal-FODMAP; gluten-free low-FODMAP (placebo); low-gluten, low-FODMAP; and high-gluten, low-FODMAP, in addition to the healthy controls who provided normal ranges.

The nutritional composition was evaluated by food diary input and analysis using FoodWorks (Xyris Software, Australia) and, for the provided diets, food content, and laboratory food analysis for FODMAPs as previously detailed.[8] This and food diaries enabled adherence to the diets to be evaluated and has been previously reported. No dietary intake was assessed in the healthy controls.

The modification of the protocol to measure the biomarkers in this study and the taking of blood from healthy controls were approved by the Monash University Human Research Ethics Committee (Approval number 7102). Patients or the public were not involved in the design, or conduct, or reporting, or dissemination of this research

Biomarker Assay Methodology: Concentrations of the biomarkers (below) were measured by commercially available ELISAs, which were all performed according to manufacturers' protocols. The assay kits were for human I-FABP (also known as fatty acid-binding protein 2) (R&D Systems, USA), LBP (Hycult Biotech, The Netherlands), human sCD14 (R&D Systems, USA), and human syndecan-1 (CD138) (Diaclone, France). The average coefficient of variation between duplicates was below 10%. Averages of duplicates were determined and absolute values are expressed in the following units: ng mL^{-1} for I-FABP and syndecan-1, and μg mL^{-1} for LBP and sCD14.

Statistical Analyses: Statistical analyses were performed on complete datasets by IBM SPSS Statistics Version 24 (IBM Corp., USA) and GraphPad Prism 6 (GraphPad Software, USA). Figures were generated with GraphPad Prism 6, outliers being omitted from some graphs for illustrative purposes. Normality of distribution with regards to marker levels and clinical indicators in study cohorts were determined by Shapiro–Wilk tests. For repeated-measures pairwise comparisons, repeated-measures *t*-tests were used for normally distributed marker levels or Wilcoxon tests for nonparametric marker distributions. Friedman's tests were used for repeated-measures comparisons between marker levels with at least one or more nonparametric distributions. All multiple pairwise comparisons, including those featured in figures, met criteria for statistical significance after controlling the FDR at 5% unless otherwise stated.[49] *p*-values of false discoveries are not included in figures. Pearson's *r* correlations between marker data were performed when both variables had normal distributions, whereas Spearman's *r* correlations were performed when either or both variables had nonparametric distributions. All *p*-values were two sided and determined to be statistically significant at $p \leq 0.05$.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

M.A. devised the study, performed assays, interpreted the data, wrote the manuscript, and edited the manuscript. G.R. devised the study, interpreted the data, and edited the manuscript. E.D.N. and J.R.B. devised and performed the original study, and edited the manuscript. J.G.M. devised and performed the original study, interpreted the data, and edited the manuscript. P.R.G. devised the original study and current analysis, interpreted the data, wrote the manuscript, and edited the manuscript

Conflict of Interest

M.A., G.R., E.D.N., and J.R.B. declare no conflict of interest. J.G.M. and P.R.G. declare that Monash University financially benefits from the sales of a digital application, booklets, and an online course regarding the FODMAP diet. P.R.G. has published a recipe and an educational book on the FODMAP diet.

Keywords

bacterial translocation, functional bowel disorders, gluten-free diet, intestinal epithelium

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