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Systemic symptoms in irritable bowel syndrome: An investigative study on the role of enterocyte disintegrity, endotoxemia and inflammation

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Abstract. Irritable bowel syndrome (IBS) is often accompanied by extra-intestinal symptoms, including fatigue and musculoskeletal pain. The present study aimed to investigate whether these symptoms were associated with markers of enterocyte disintegrity, endotoxemia and inflammation. Patients with IBS were recruited consecutively from our outpatient clinic (n=94) and compared with a group of healthy controls (n=20). Habitual symptoms were assessed using the IBS Severity Scoring System, the Fatigue Impact Scale and Visual Analogue Scales for measuring musculoskeletal pain. A lactulose challenge test was performed to induce post-prandial symptoms, and blood samples were obtained prior to and 90 min following lactulose ingestion to determine levels of intestinal fatty acid binding protein (iFABP), lipopolysaccharide (LPS), the LPS co-receptor soluble cluster of differentiation (sCD) 14, monocyte chemoattractant protein-1 (MCP-1) and calprotectin. Habitual symptom scores were high among the included patients, and lactulose ingestion induced significantly more symptoms in the patient group compared with the healthy control group (P=0.0001). Serum levels of iFABP were reduced in IBS patients compared with healthy controls, prior to and following lactulose ingestion (P=0.0002 and P=0.0001, respectively). Following lactulose ingestion, iFABP levels decreased in IBS patients (P=0.0001) and in healthy controls (P=0.02). Fasting levels of LPS, sCD14, MCP-1 and calprotectin were not significantly different between IBS patients and healthy controls. However, following lactulose ingestion, LPS levels increased in healthy controls (P=0.03), whereas MCP-1 levels decreased in IBS patients (P=0.008). Intestinal and extra-intestinal symptom severities were not correlated with levels of circulating biomarkers. No assessed biomarker in the present study appeared to be associated with symptom development in IBS patients. However, the implications of the low levels of iFABP observed require further investigation.

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Introduction

Patients with irritable bowel syndrome (IBS) often suffer from multiple symptoms in the gastrointestinal tract and in other organ systems (1). A previous study by our laboratory demonstrated high proportions of chronic fatigue and musculoskeletal pain in IBS patients referred to Lovisenberg Diaconal Hospital (Oslo, Norway) for investigation of perceived food hypersensitivity (2). Notably, these extra-intestinal or systemic symptoms may be more debilitating than the intestinal complaints (3). It has been hypothesized that these common comorbid symptoms may share a common pathogenesis (4). However, the cause remains to be elucidated.

A lactulose challenge test to induce symptoms in patients with IBS has been developed by our laboratory (5) and was used as a tool in our previous study (6); other studies have used a modified test (7,8). Ingestion of a small dose of lactulose may elicit abdominal and systemic symptoms, including chills and fatigue. This suggests that the intestines may be the primary site of the ailment, and that blood borne substances may be involved in symptom generation. These putative factors remain unidentified. However, previous studies have indicated a low-grade intestinal inflammation in patients with IBS (9), and consequent increased intestinal permeability (10).

Therefore, the present study aimed to investigate the association between symptoms induced during a lactulose challenge test, and circulating levels of biomarkers indicating enterocyte disintegrity [intestinal fatty acid binding protein (iFABP)], endotoxemia [lipopolysaccharide (LPS) and the LPS co-receptor soluble cluster of differentiation (sCD)14], and inflammation [monocyte chemoattractant protein-1 (MCP-1) and calprotectin]. Alterations in any one of these factors may indicate disruption of the intestinal mucosal barrier and consequently, immune activation, and thus potentially explain systemic symptoms in response to lactulose ingestion.

Subjects and methods

Study participants. Patients with IBS were recruited consecutively from the outpatient clinic of A.B. at Lovisenberg Diaconal Hospital (Oslo, Norway) between November 2011 and January 2013. The patients were diagnosed with IBS according to the Rome III criteria (11). Patients with organic diseases of the gastrointestinal tract were excluded. Healthy controls were recruited from the staff at Lovisenberg Diaconal Hospital. They were included if they considered themselves to be healthy and were not otherwise medically examined. However, they were excluded if they met the Rome III criteria for IBS, based on questionnaires. Written informed consent was obtained from all participants. The study was performed according to the Declaration of Helsinki and approved by the regional Committee for Medical Research Ethics (REK Sør-Øst; reference number 2011/2474).

Outline of study design. Participants were assessed following an overnight fast. Questionnaires for grading of habitual symptoms were filled in. Subsequently, the subjects underwent a lactulose challenge test, in which a solution of 10 g lactulose dissolved in 200 ml tap water was ingested. The participants filled in questionnaires assessing habitual and lactulose-induced symptoms and were not allowed to eat or drink during the assessment. Blood samples were obtained prior to and 90 min following lactulose ingestion, and were rapidly transferred into a -20˚C freezer for storage until analysis. To minimalize interassay variability, samples from patients and controls were analyzed in the same run.

Assessment of symptoms. Habitual symptoms were assessed in all patients using previously validated questionnaires. IBS severity was measured according to the IBS Severity Scoring System (IBS-SSS) (12). Grading of chronic fatigue was estimated according to the Fatigue Impact Scale (FIS) (13). Severity of musculoskeletal pain was graded using Visual Analogue Scales (VAS) (14). The increase in extra-intestinal symptoms following lactulose ingestion was graded from 0 to 3 for sickness and chills, giving a maximum achievable score of 6 points. Increase in intestinal symptoms following lactulose ingestion was graded from 0 to 3 for borborygmi, bloating and pain, giving a maximum achievable score of 9 points.

Analysis of blood samples. iFABP was analyzed in the serum using a commercial ELISA kit (catalog no. HK 406; Hycult Biotech, Uden, The Netherlands), according to the manufacturer's protocol. LPS was analyzed in plasma using a Limulus Amebocyte Lysate colorimetric assay (Lonza, Walkersville, MD, USA), according to the manufacturer's protocol, with the following modifications: Samples were diluted 10-fold to avoid interference with background color and were preheated to 68˚C for 12 min prior to analysis to dissolve immune complexes, as previously described by Trøseid *et al* (15). sCD14 was analyzed in plasma using a commercial ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA), according to the manufacturer's protocol. MCP-1 was analyzed in serum using a commercial Quantikine® ELISA kit (R&D Systems Europe, Ltd., Abingdon, UK), according to the manufacturer's protocol. Calprotectin was analyzed in serum using a commercial ELISA kit (Bühlmann Laboratories AG, Schönenbuch, Switzerland), according to the manufacturer's protocol.

Statistical analysis. The data were analyzed in GraphPad Prism (version 6; GraphPad Software, Inc., La Jolla, CA, USA). In the text, data are expressed as the mean ± standard deviation. In the figures, data are expressed as mean ± standard error. Paired Student's t-tests were performed to compare means within groups and unpaired Student's t-tests were performed to compare means between groups. Correlations were assessed using Pearson's correlation coefficient. All tests were two-tailed. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Study participants. A total of 94 IBS patients (30 male and 64 female; mean age, 40 years; range, 19-73 years) and 20 healthy controls (8 male and 12 female; mean age, 38 years; range, 25-65 years) were recruited consecutively to participate in the present study. Of the IBS patients, 41 were classified as diarrhea-predominant IBS (IBS-D), 15 as constipation-predominant IBS (IBS-C) and 38 as IBS with mixed stool pattern (IBS-M).

Symptoms. As determined by IBS-SSS, habitual IBS symptoms were classified as mild in none of the patients, moderate in 42 patients and severe in 52 patients. The mean score for musculoskeletal pain according to the VAS was 30.3±25.2 cm, and the mean score for fatigue according to the FIS was 83.4±48.3 points. The mean scores for lactulose-induced symptoms were 4.0±2.4 and 2.0±1.8 points for intestinal and extra-intestinal symptoms in patients. In healthy controls, the scores were 0.4±2.8 and 0±0 points, respectively.

Biomarkers. The numbers of blood samples from analyzed consecutive individuals (patients/healthy controls) were as follows: iFABP (48/19), LPS (26/12), sCD14 (11/11), MCP-1 (10/10), and calprotectin (10/10). Serum levels of iFABP were reduced in IBS patients compared with healthy controls, prior to (702±442 vs. 2,110±2,429 pg/ml; P=0.0002) and 90 min following lactulose ingestion (402±344 vs. 1,820±2,022 pg/ml; P=0.0001). Following lactulose ingestion, iFABP levels decreased in healthy controls (P=0.02) and in IBS patients (P=0.0001; Fig. 1). Fasting levels of LPS were not significantly different in IBS patients (42±15 pg/ml) compared with healthy controls (42±15 pg/ml); however, following lactulose ingestion, LPS levels increased in healthy controls (P=0.03), whereas the levels did not increase significantly in IBS patients (Fig. 2). Fasting levels of sCD14 were not significantly different in IBS patients (2,030±560 ng/ml) compared with healthy controls (2,204±544 ng/ml), and levels were not altered by lactulose ingestion, in IBS patients or in healthy controls (Fig. 3). Fasting levels of MCP-1 were not significantly different in IBS patients (303±98 pg/ml) compared with healthy controls (294±90 pg/ml). Following lactulose ingestion, MCP-1 levels decreased in IBS patients (P=0.008), whereas the levels did not alter significantly in the healthy controls (Fig. 4). No significant differences were observed in the serum levels of

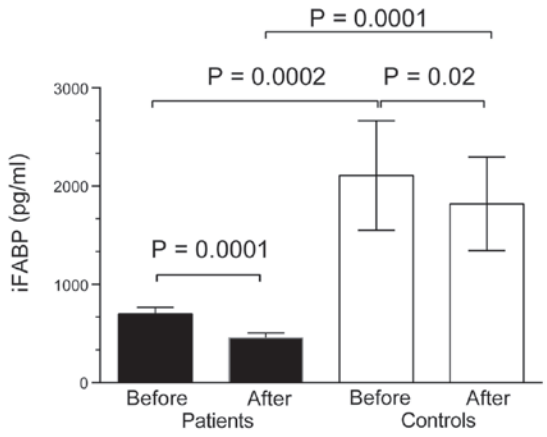

Figure 1. Serum levels of iFABP in IBS patients (n=48) and healthy controls (n=19) prior to and 90 min following ingestion of 10 g lactulose. iFABP levels were reduced in patients compared with healthy controls, and in patients and healthy controls decreased following lactulose administration. The data are expressed as the mean ± standard error. iFABP, intestinal fatty acid binding protein; IBS, irritable bowel syndrome.

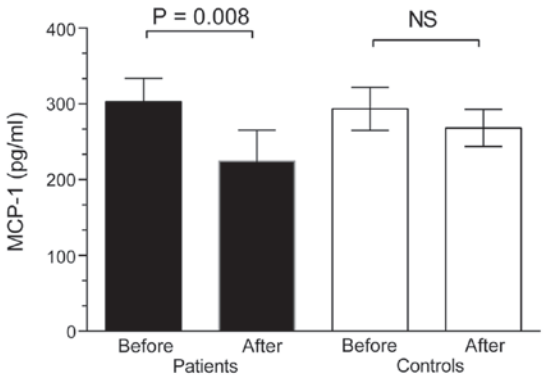

Figure 4. Serum levels of MCP-1 in IBS patients (n=10) and healthy volunteers (n=10) prior to and 90 min following ingestion of 10 g lactulose. Fasting levels of MCP-1 were not significantly different in IBS patients compared with healthy controls. Following lactulose ingestion, MCP-1 levels decreased in IBS patients, whereas levels did not alter significantly in healthy controls. The data are expressed as the mean ± standard error. MCP-1, monocyte chemoattractant protein-1; IBS, irritable bowel syndrome.

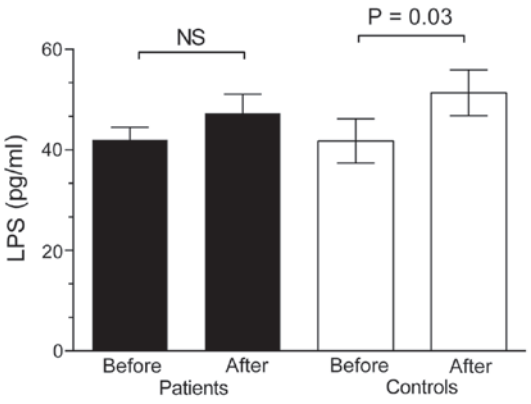

Figure 2. Plasma levels of LPS in IBS patients (n=26) and healthy controls (n=12) prior to and 90 min following ingestion of 10 g lactulose. LPS levels were similar in patients and healthy controls prior to lactulose administration; however, levels increased significantly in healthy controls but not in patients following ingestion. The data are expressed as the mean ± standard error. LPS, lipopolysaccharide; IBS, irritable bowel syndrome.

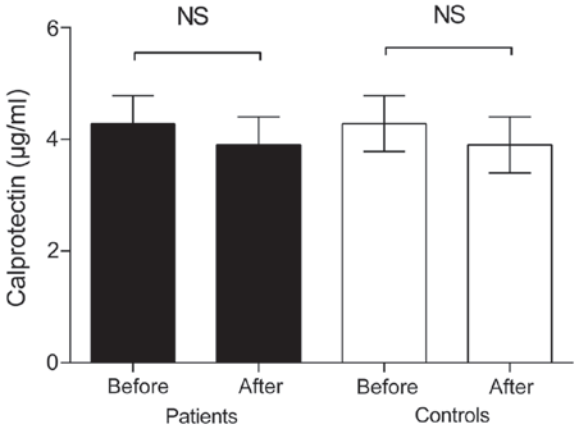

Figure 5. Serum levels of calprotectin in IBS patients (n=10) and healthy controls (n=10) prior to and 90 min following ingestion of 10 g lactulose. No differences were observed in the levels of calprotectin in patients compared with healthy controls, and levels were not significantly altered by lactulose ingestion. The data are expressed as the mean ± standard error. IBS, irritable bowel syndrome.

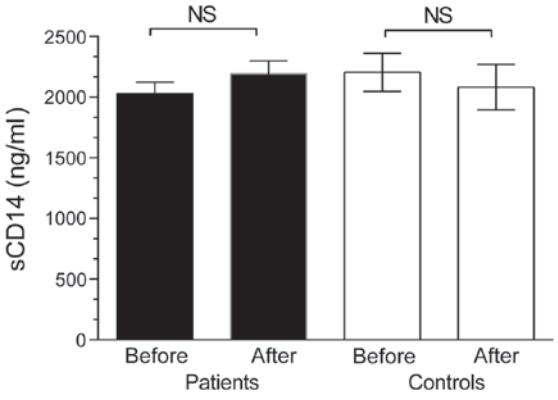

Figure 3. Plasma levels of the lipopolysaccharide co-receptor sCD14 in IBS patients (n=11) and healthy controls (n=12) prior to and 90 min following ingestion of 10 g lactulose. Fasting levels of sCD14 were not significantly different in IBS patients compared with healthy controls, and levels were not altered by lactulose ingestion, in IBS patients or in healthy controls. The data are expressed as the mean ± standard error. sCD14, soluble cluster of differentiation 14; IBS, irritable bowel syndrome.

calprotectin in patients compared with healthy controls prior to (4.2±1.2 vs. 4.2±1.22 μg/ml; P=0.99) or following (P=0.37) lactulose administration (Fig. 5). The levels of biomarkers were similar in patients classified as IBS-D, IBS-C and IBS-M. No correlations were observed between any of the biomarkers and habitual or lactulose-induced symptoms.

Discussion

The present study revealed no evidence of enterocyte disintegrity, endotoxemia or inflammation in patients with IBS, prior to or following lactulose provocation. Therefore, the results do not support the hypothesis that IBS-associated systemic symptoms are due to mucosal damage, as measured by iFABP, leakage of microbial endotoxin, as measured by LPS/sCD14 or inflammation, as measured by MCP-1 or calprotectin in blood.

The FABP family is comprised of 14-15 kDa intracellular proteins expressed in intestinal epithelial cells (16). iFABP is

present throughout the intestine, with the greatest expression in the jejunum (17). Following intestinal mucosal damage, iFABP is released into the circulation and its plasma concentration increases, for example in patients with untreated celiac disease (18), following surgical trauma, in severe sepsis or in intestinal malignant disease (19). Although various previous studies refer to low-grade inflammation and impaired intestinal permeability in IBS patients (20-22), the present study observed low levels of serum iFABP in patients, and a further decrease following lactulose administration. Therefore, no indication of mucosal damage was determined in patients with IBS, prior to or following lactulose challenge. In accordance with these results, a recent study reported that serum iFABP is not a useful biomarker of intestinal barrier dysfunction driven by gut microbiota changes in obesity (23). Therefore, marginally increased intestinal permeability, as reported in IBS and obesity (21,22), may not be a consequence of mucosal damage measurable by iFABP.

Passage of small molecules (<600 Da) through tight junctions of the intestinal mucosa is regulated by cell-to-cell adhesion proteins (20). The iFABP molecule is too large to cross the mucosal barrier through these intercellular spaces. A molecule that may pass through paracellular spaces without overt epithelial damage, although which still requires impaired barrier function, is LPS from Gram-negative bacteria. Following intestinal-to-blood leakage, LPS activates immune cells via its co-receptor CD14, a surface antigen expressed on monocytes and macrophages. In patients with overt mucosal damage, as in inflammatory bowel disease (IBD), plasma levels of LPS correlate with disease severity (18). In diseases with subtle mucosal damage, associated with insulin resistance and low-grade intestinal inflammation, conditions often referred to as 'metabolic endotoxemia', LPS may be a causal or complicating factor (24). Whether lactulose challenge may impair mucosal barrier function at a tight junction level, without causing 'mucosal damage' as measured by iFABP, remains unclear. Intravenous infusion of LPS in healthy volunteers triggers visceral hypersensitivity with characteristics similar to those observed in IBS patients (25), and elevated plasma levels of LPS were associated with IBS (IBS-D) in a recent study (26). A separate study demonstrated elevated serum immunoglobulin A against LPS in patients with fatigue (27), a condition often linked to IBS (28). In the present study, similar blood levels of LPS in IBS patients and healthy controls were observed, with a slight increase in the mean LPS levels in the two groups following lactulose administration (statistically significant only in the healthy controls). These results suggested that lactulose ingestion may increase intestinal leakage of LPS; however, the lactulose-induced leakage of LPS was not specific for IBS patients, and thus may not explain their systemic symptoms. It is possible that the increase in LPS is associated with the decrease in iFABP. LPS contains fatty acids and low levels of iFABP may be a consequence, or an indication, of a defence reaction to subtle LPS stimulation. Obesity is characterized by subtle leakage of LPS into the circulation (24), and low levels of iFABP have been reported in obese patients (23).

The proinflammatory cytokine, MCP-1, is critical for monocyte recruitment in inflammatory conditions. In the present study, similar levels of MCP-1 in patients and healthy controls were observed, and these levels decreased significantly in patients following lactulose challenge. In addition, calprotectin levels were similar in patients and healthy controls and tended to decrease (non-significantly) following lactulose challenge. A previous study revealed elevated MCP-1 levels in IBS patients (29). The reason for this contradictory data remains to be elucidated.

In conclusion, the results of the present study revealed no indication of mucosal damage, endotoxemia or inflammation in the IBS patients assessed. Notably, patients had abnormally low values of iFABP, a finding that requires further investigation.

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