

# Intestinal Permeability In Vivo in Patients With Inflammatory Bowel Disease: Comparison of Active Disease and Remission

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**Background and Aims:** Inflammatory bowel disease (IBD) is associated with altered mucus and increased intestinal permeability (IP). Prior reports on permeability in IBD typically used lactulose-to-mannitol ratio (LMR). Food contamination with <sup>13</sup>C-mannitol is a significant potential confounder in IP assessment. We aimed to compare small intestinal (SI) and colonic (COL) permeability in IBD, both active (ACT) and in remission (REM), to normal healthy volunteers (NHV).

**Methods:** Inflammatory bowel disease activity was based on Simple Endoscopic Score for Crohn's Disease (SES-CD) and Mayo endoscopy score for ulcerative colitis (UC). We performed 24-hour IP test using 100 mg <sup>13</sup>C-mannitol and 1000 mg lactulose with urine collected during 0-2, 2-8, and 8-24 hours. The primary endpoint was mg excretion of <sup>13</sup>C-mannitol and lactulose during 2-24 hours reflecting SI and COL permeability.

**Results:** Among 17 CD patients, 7 were ACT (SES-CD >6), and 10 REM (SES-CD 0-2). Among 20 UC patients, 10 had ACT (Mayo score 2-3), and 10 REM (Mayo score 0-1). Urinary excretions over 2-24 hours were higher for IBD than NHV: <sup>13</sup>C-mannitol (13.8 [IQR 8.8, 18.7] NHV; 18.4 [15.6, 29.9] REM; 19.7 [13.8, 23.6] ACT, *P* = .003) and lactulose (1.8 [1.3, 3.1] NHV; 3.6 [2.0, 5.0] REM; 3.5 [2.0, 6.6] ACT, *P* = .006). There was no difference between ACT and REM for any timed urine collection. LMR at 2-24 hours (or 2-8 and 8-24 hours) were not statistically significant between the 3 groups (0.014 [0.010, 0.021] NHV; 0.016 [0.010, 0.023] REM; 0.016 [0.012, 0.038] ACT, *P* = .237).

**Conclusions:** Intestinal permeability is increased in IBD using validated in vivo assay relative to NHV; increased IP in IBD persists during remission.

## Lay Summary

This paper uses validated methods and documents intestinal barrier dysfunction in patients who have Crohn's disease or ulcerative colitis and documents the dysfunction when the disease is active or inactive.

**Key Words:** IBD, barrier, lactulose, mannitol

## Introduction

The pathogenesis of inflammatory bowel disease (IBD) is complex and multifactorial with disturbed interaction between the gut immune mechanisms and intestinal microbiota. The intestinal barrier is composed of several essential elements including luminal enzymes, mucus, unstirred water layer, epithelial layer, and immune mechanisms operating in the lamina propria.<sup>1</sup> The barrier acts as a dynamic interface between the luminal contents including food, commensal and pathogenic bacteria, and the gastrointestinal (GI) tract. The mucosal barrier constitutes the first boundary between intraluminal injurious dietary, metabolic, or infectious factors and the gut mucosa. A defective barrier may result in increased intestinal permeability, which promotes the exposure of the mucosa to luminal content and triggers an immunological response leading to intestinal inflammation. The role of intestinal permeability is of significant interest in the context

of chronic inflammatory GI conditions and the potential for the development of novel approaches to treatment in IBD.<sup>2</sup> Understanding intestinal permeability has relevance in addition to exploring potential treatment targets. Other possible clinical applications would include assessment of disease activity and correlation of permeability to persistent symptoms, whether associated with or without inflammation.

Prior research reviewed elsewhere has shown that patients with IBD display altered composition of mucus and intercellular adhesion molecules that regulate paracellular intestinal permeability.<sup>3</sup> Prior measurements of permeability in IBD in vivo used the ratio of urinary excretion after oral intake of mono- and disaccharides, typically the lactulose-to-mannitol ratio (LMR). For example, studies have documented increased permeability in human and animal models of IBD, in predicting relapse of Crohn's disease (CD), and as a pre-clinical predictor for first-degree relatives of patients with

**Key Messages****What is already known?**

- Urine excretion of orally administered sugar molecules, lactulose, and <sup>13</sup>C-mannitol are used to measure intestinal and colonic permeability. Aspects of intestinal permeability are altered in inflammatory bowel disease with or without inflammation.

**What is new here?**

- In this study of 20 inflammatory bowel disease (IBD) patients in remission and 17 with active disease, IBD is associated with increased intestinal permeability. When assessed, no differences in intestinal permeability were found for disease activity, which was limited by a small sample size. The study was conducted on 37 patients in a single center with limited racial and ethnic diversity, 17 had active IBD reducing the ability to assess correlation between disease activity and intestinal permeability measurements.

**How can this study help patient care?**

- Using the most robust in vivo tests of intestinal permeability available for longitudinal use in large numbers, the study shows the importance of increased permeability both in active IBD and in remission. This points to opportunities to further research the role of barrier dysfunction and develop therapeutic approaches to restore the intestinal barrier.

IBD to subsequently develop IBD.<sup>4-10</sup> However, the influence of genetic and environmental factors on intestinal permeability in healthy first-degree relatives of patients with IBD remains unclear, with some studies suggesting a lack of heritability in abnormal permeability.<sup>11-16</sup>

Most observations on intestinal permeability in IBD to date have utilized oral administration of probe molecules that are not metabolized after absorption and are excreted unchanged and can be measured in urine collections.<sup>17,18</sup> However, the methods used for measuring intestinal permeability noninvasively are suspect due to confounding. For example, sucralose may not be the best sugar probe due to small bowel absorption and dietary contamination. Timed collections of diverse studies are variable from 5 to 24 hours. Additionally, lactulose impacts transit at doses greater than 1 g. In previous studies,<sup>17</sup> our group established the use of <sup>13</sup>C-mannitol instead of mannitol, obtained normal values in 60 healthy volunteers aged 18-70 years measured on 3 occasions, and demonstrated no age, sex, or body mass index (BMI) differences. To date, this is the most robust in vivo method to measure intestinal permeability that has been documented with intra- and interindividual coefficient of variation, as well as studied the effect of dietary fiber intake on permeability. Mannitol has a molecular weight of 182 Da and reported or estimated diameters of 6.7-7.2 Å; the corresponding numbers for lactulose are 342 Da, and 9.5-9.7 Å.<sup>19</sup> Our studies also documented the increased permeability in patients with bile acid diarrhea compared to healthy controls and no significant increase in permeability in IBS-D relative to healthy controls.<sup>17,18,20</sup> In another study in patients with IBS-diarrhea, gluten administration increased small intestinal

(SI) permeability compared to a gluten-free diet, especially in patients who were carriers of HLA-DQ2 or 8.<sup>21</sup>

In this study, our first aim was to compare SI and colonic (COL) permeability in patients with IBD to those measurements in normal healthy volunteers (NHV). Additionally, we aimed to compare permeability in patients with active IBD (ACT) and IBD in remission (REM) and each individual group compared to NHV.

**Materials and Methods****Ethical Approval**

The study of IBD patients and NHV included in the pilot assessment of intestinal permeability was approved by the Institutional Review Board (IRB #21-012369). All participants signed written informed consent.

**Participants**

Patients with IBD were identified from a review of medical records (physician assessment, laboratory, radiologic, and endoscopic assessments) from our center's IBD clinic. All IBD patients had undergone colonoscopy within 12 weeks prior to permeability testing. IBD disease activity was based on Simple Endoscopic Score for Crohn's Disease (SES-CD) for CD and Mayo endoscopy score for ulcerative colitis (UC).<sup>22,23</sup> ACT was defined as SES-CD >6 or Mayo endoscopy score 2-3. REM was defined as SES-CD 0-2 or Mayo endoscopy score 0-1. Patient and disease-specific characteristics were collected from the electronic health record. During the period from endoscopy to permeability testing, the IBD participants did not start any new IBD-related treatment including corticosteroids, which were not permitted at least 2 weeks prior to permeability testing.

We excluded patients with prior surgery performed for IBD (ie, total proctocolectomy with ileal pouch-anal anastomosis, ileostomy, colostomy, small bowel or colonic resection, stricturoplasty), daily use of laxatives, non-steroidal anti-inflammatory drugs (NSAIDs), or aspirin. IBD patients and NHV who participated in the study did not have evidence of acute kidney injury or chronic kidney disease. NHV were screened for underlying medical illnesses (diabetes, hypertension, BMI ≥ 30 kg/m<sup>2</sup>, and chronic NSAID use), and screened for GI symptoms with a short-form bowel disease questionnaire (derived from a validated long bowel disease questionnaire) used in prior permeability studies<sup>17,24</sup> to exclude any healthy participants with active GI symptoms. All participants who were unable or unwilling to alter dietary protein or dietary fiber for the permeability testing, pregnant, or planning to become pregnant during the study time were excluded. All participants were required to commit to strict recommendations on dietary and medication exclusions prior to and during the intestinal permeability test.

**IBD Symptoms and Quality of Life**

All IBD patients completed an IBD Smart Form assessing their current symptoms. This patient-reported outcome tool was created for SPARC IBD (Study of a Prospective Adult Research Cohort with IBD) in conjunction with the Crohn's & Colitis Foundation and has previously been validated for quality control across multiple institutions.<sup>25,26</sup> Quality-of-life measures were assessed using the Patient-Reported Outcomes

Measurement Information System Global-10 form and Short Form-12 Health Survey.<sup>27,28</sup>

### In Vivo Measurement of Small Intestinal and Colonic Permeability

The mass (mg) of the oral probes absorbed in the digestive tract and excreted in urine was used to assess the intestinal permeability. This approach is inexpensive and feasible in clinical studies. We performed permeability testing over 24 hours with the established method using 100 mg <sup>13</sup>C-mannitol and 1000 mg lactulose with urine collected in 3 time periods (0-2, 2-8, 8-24 hours). The procedure and technique for permeability testing are detailed in prior publications.<sup>17</sup> In the 2 weeks prior to testing participants were asked to avoid NSAIDs. One week prior to testing, they were asked to avoid strenuous exercise (>5 miles running or equivalent exercise/week). Participants were asked to avoid alcohol, artificial sweeteners, and emulsifiers for 24 hours before and during testing. In prior publications, standardized meals were given avoiding the aforementioned foods, though to help validate this test in a clinical setting, we provided patient education and food menus for a 60- to 70-g fat diet for study participants (Figure S1).

After an overnight fast, participants ingested 100 mg <sup>13</sup>C-mannitol and 1000 mg lactulose in a 240-mL glass of water; participants fasted for the next 2 hours and received an additional 500 mL of water 30 minutes after the sugars were ingested. Urine samples were collected at 0-2, 2-8, and 8-24 hours. Mass (mg) urinary excretion of the 2 probe molecules, <sup>13</sup>C-mannitol and lactulose, was calculated at 0-2 hours (reflecting upper SI permeability), 2-8 hours (combined SI and COL permeability), and 8-24 hours (reflecting COL permeability) based on previous radiosciintigraphic studies.<sup>29</sup> The ratio of excreted lactulose-to-<sup>13</sup>C-mannitol (LMR) was also calculated for each urine collection. While data in our study are being presented as mass (mg) urinary excretion, simple calculations to convert mass to percentages can be completed by dividing mass (mg) excretion of lactulose by 1000 mg, and mass (mg) excretion of <sup>13</sup>C-mannitol by 100 mg.

For NHV, a measurement of intestinal permeability was used from 59 volunteers evaluated in a previous study, where fiber intake was 16.25 g/day.<sup>17</sup> Fifteen additional NHV were recruited contemporaneously with the IBD patients, so that the total number of NHVs was 74. The urine samples were returned to the lab, volume measured and a 20 mL aliquot was saved and frozen for subsequent batch analysis using liquid chromatography–tandem mass spectrometry (Supplementary Material).<sup>18</sup>

### Endpoints

The primary endpoint was mass (mg) excretion of <sup>13</sup>C-mannitol and lactulose at 2-24 hours reflecting combined SI and COL permeability. This time interval was chosen as 2-24 hours encompasses the most likely anatomical location of the sugar probes in a patient with IBD with current or prior disease activity located in the distal small bowel and/or colon, which would be predicted to be the sites with potentially altered intestinal permeability in patients with IBD. Conversely, the 0-2 hours urine excretion reflects stomach and proximal small bowel permeability.

Secondary endpoints were mass excretion at 0-2 hours (exclusively proximal SI permeability), 2-8 hours (SI and

COL permeability), and 8-24 hours (exclusively COL permeability). LMR (% lactulose/% <sup>13</sup>C-mannitol ratio) was also assessed in the same timed collections, using the equation: lactulose (mg)/1000 mg divided by <sup>13</sup>C-mannitol (mg)/100 mg (where the denominators denote the mass administered orally for each sugar).

### Statistical Analysis

Data from patients with IBD were compared to normal data from NHV. All statistical analyses were conducted using SigmaPlot12. Data shown are median and interquartile range (IQR). The statistical analysis involved 3-group comparisons, using Kruskal–Wallis test (analysis of variance [ANOVA] on ranks) since the majority of data failed the test for normal distribution. If the primary endpoint was statistically significant based on the Kruskal–Wallis test, Dunn's test was performed for 3-group comparisons which were appraised for significance using  $P < .05$  since the program adjusts significance for the multiple comparisons. Values of  $P < .05$  were used for nominal significance for the analyses using the Kruskal–Wallis and Dunn's tests. In addition, since the 3-group comparison may not have been significant and this precluded application of Dunn's test, we also analyzed comparisons between the 3 groups (REM vs NHV; ACT vs NHV; REM vs ACT) using Mann–Whitney Rank Sum test and declared statistical significance for  $P < .017$  (0.05/3) to correct for 3 comparisons. We evaluated the correlation of IBD disease activity with permeability measures using Spearman rank correlation analysis.

### Statistical Power

The statistical power calculation was based on a prediction that the study would include 40 patients with IBD and 60 NHV, using the coefficients of variation previously published in healthy participants using the same method (Table S4).<sup>10</sup>

## Results

### Participant Demographics

Thirty-seven patients with IBD, 17 with CD, and 20 with UC completed the study. Demographics and clinical features of IBD are displayed in Table 1. There were 56.8% female, with a median age of 42 years (IQR 42, 64) and a median BMI of 27.3 kg/m<sup>2</sup> (IQR 22.8, 30.3). At the time of enrollment, median disease duration was 11.0 years (IQR 0.0, 18.5) for CD, and 9.5 years (IQR 3.5, 16.5) for UC. No patients were current smokers at the time of the study. Patient medications at the time of permeability testing are documented in Table S1.

Among the 17 CD patients, 7 (41.2%) were ACT with SES-CD >6, and 10 (58.5%) were REM with SES-CD 0-2. CD disease location was ileal (23.5%), colonic (29.4%), and ileocolonic (47.1%). CD phenotype was inflammatory (76.5%), stricturing (17.6%), and penetrating (5.8%). Among the 20 UC patients, 10 (50.0%) were ACT with a Mayo score of 2-3, and 10 (50.0%) were REM with a Mayo score of 0-1. UC disease location was proctitis (5.0%), left-sided disease (40.0%), and extensive (55.0%).

### Small Intestinal and Colonic Permeability: Primary Endpoint

All measurements are summarized in Table 2. The 2-24 hour urinary mass excretion (prespecified primary endpoint) was significantly higher in IBD for both <sup>13</sup>C-mannitol

**Table 1.** Demographics and clinical features of patients with Crohn's disease and ulcerative colitis undergoing intestinal permeability test.

Demographics	Crohn's disease (n = 17)	Ulcerative colitis (n = 20)
Female	8 (47.1%)	13 (65.0%)
Age, years	42.0 (32.5, 65.0)	42.0 (31.0, 64.0)
Body mass index, kg/m <sup>2</sup>	26.6 (22.9, 28.7)	28.2 (22.1, 32.8)
IBD medications		
5-ASA	1 (5.8%)	7 (35.0%)
Azathioprine/6-MP	3 (17.6%)	1 (5.0%)
Budesonide	2 (11.7%)	2 (10.0%)
Anti-TNF	8 (47.0%)	4 (20.0%)
Vedolizumab	2 (11.7%)	2 (10.0%)
Ustekinumab	2 (11.7%)	1 (5.0%)
Upadacitinib	0 (0.0%)	2 (10.0%)
Smoking		
Never	12 (70.6%)	13 (65.0%)
Former	5 (29.4%)	7 (35.0%)
Current	0 (0.0%)	0 (0.0%)
Duration of disease, years	11.0 (0.0, 18.5)	9.5 (3.5, 16.5)
Crohn's disease location		
Ileal (L1)	4 (23.5%)	
Colonic (L2)	5 (29.4%)	
Ileocolonic (L3)	8 (47.1%)	
Isolated upper (L4)	0 (0.0%)	
Crohn's disease phenotype		
Nonstricturing/nonpenetrating (B1)	13 (76.5%)	
Stricturing (B2)	3 (17.6%)	
Penetrating (B3)	1 (5.8%)	
Crohn's disease endoscopy score		
-Remission: SES-CD 0-2	10 (58.8%)	
SES-CD 0	8 (80.0%)	
SES-CD 1	0 (0.0%)	
SES-CD 2	2 (20.0%)	
-Active: SES-CD ≥ 6	7 (41.2%)	
Mild, SES-CD 3-6	2 (28.6%)	
Moderate, SES-CD 7-15	5 (71.4%)	
Severe, SES-CD ≥ 16	0 (0.0%)	
Ulcerative Colitis Location		
-Proctitis (E1)		1 (5.0%)
-Left-sided (E2)		8 (40.0%)
-Extensive (E3)		11 (55.0%)
Ulcerative Colitis Endoscopy Score		
Remission: Mayo 0-1		10 (50.0%)
Quiescent, Mayo 0		7 (70.0%)
Mild, Mayo 1		3 (30.0%)
Active: Mayo 2-3		10 (50.0%)
Moderate, Mayo 2		7 (70.0%)
Severe, Mayo 3		3 (30.0%)

Values are median (interquartile range) or %. Abbreviations: 5-ASA, 5-aminosalicylic; 6-MP, mercaptopurine; IBD, inflammatory bowel disease; anti-TNF, anti-tumor necrosis factor.

(13.8 [8.8, 18.7] NHV; 18.4 [15.6, 29.9] REM; 19.7 [13.8, 23.6] ACT,  $P = .003$ ) and lactulose (1.8 [1.3, 3.1] NHV; 3.6 [2.0, 5.0] REM; 3.5 [2.0, 6.6] ACT,  $P = .006$ ) (Figure 1). Mann-Whitney Rank Sum test ( $P < .017$ ) was significant for <sup>13</sup>C-mannitol 2-24 hours excretion for REM versus NHV ( $P = .003$ ). Lactulose 2-24 hours excretion was significant ( $P = .011$ ) for ACT versus NHV. The 2-24 hour urine excretion in the REM and ACT groups was not statistically different ( $P = .57$  for <sup>13</sup>C-mannitol;  $P = .66$  for lactulose). LMR at 2-24 hours were not statistically significant between the 3 groups (0.014 [0.010, 0.021] NHV; 0.016 [0.010, 0.023] REM; 0.016 [0.012, 0.038] ACT,  $P = .237$ ).

### Small Intestinal and Colonic Permeability: Secondary Endpoints

Secondary endpoints were urinary mass (mg) excretion at 0-2 hours (exclusively upper SI permeability), 2-8 hours (distal SI and COL permeability), and 8-24 hours (exclusively COL permeability).

For the urine excretion during 0-2 hours, there were significantly lower excretions of <sup>13</sup>C-mannitol in the IBD groups relative to controls (14.0 [9.8, 19.4] NHV; 10.1 [6.9, 12.9] REM; 10.8 [7.5, 12.80] ACT;  $P = .003$ ); no such differences were noted for lactulose excretion (1.2 [0.8, 1.6] NHV; 0.8 [0.4, 1.9] REM; 1.0 [0.5, 1.4] ACT;  $P = .247$ ).

The 3-group comparisons showed significantly greater excretion of both sugars in the 2-8 and 8-24 hours collections for ACT and REM compared to NHV (Table 2). However, 0-2, 2-8, and 8-24 hours excretion of both sugars were not significantly different between ACT and REM for any timed collection.

LMR at 0-2, 2-8, and 8-24 hours were not statistically significant between the 3 groups ( $P = .294$ ;  $P = .289$ ;  $P = .571$ , respectively).

### Intestinal Permeability and IBD Disease Activity

For UC patients, we evaluated UC Mayo endoscopy score as a surrogate of disease activity versus urinary mass excretion at 2-24 hours. We did not find a significant relationship for <sup>13</sup>C-mannitol (Rs: -0.315;  $P = .171$ ), lactulose (Rs: -0.078;  $P = .738$ ), or LMR (Rs: -0.039;  $P = .866$ ). When we compared UC patients in REM versus ACT, we found no significant difference for urinary mass excretion at 0-2, 2-8, 8-24, 2-24, or 0-24 hours for <sup>13</sup>C-mannitol, lactulose, or LMR (Table 3).

For CD patients, we evaluated the SES-CD score for disease activity versus urinary mass excretion at 2-24 hours. Similarly, we did not find a significant relationship for <sup>13</sup>C-mannitol (Rs: 0.019;  $P = .936$ ), lactulose (Rs: 0.361,  $P = .151$ ), or LMR (Rs: 0.176;  $P = .490$ ). When we compared CD patients in REM versus ACT, the only significant difference was found for lactulose at 8-24 hours (0.5 [0.4, 0.5] REM; 0.9 [0.5, 1.1] ACT,  $P = .013$ ). No additional significant difference was found for urinary mass excretion at the studied endpoints for <sup>13</sup>C-mannitol, lactulose, or LMR (Table 4).

### IBD Symptoms and QOL

Table S2 outlines the differences in self-reported patient responses on an IBD Symptom Survey. Noted differences include increased stool frequency and number of bowel movements per day (0 [0,1] REM; 2.5 [0.5, 3] ACT). Table S3 outlines self-reported quality-of-life measures for IBD patients.

**Table 2.** Urinary excretion of <sup>13</sup>C-mannitol and lactulose for patients with IBD in remission, IBD active, and normal healthy volunteers.

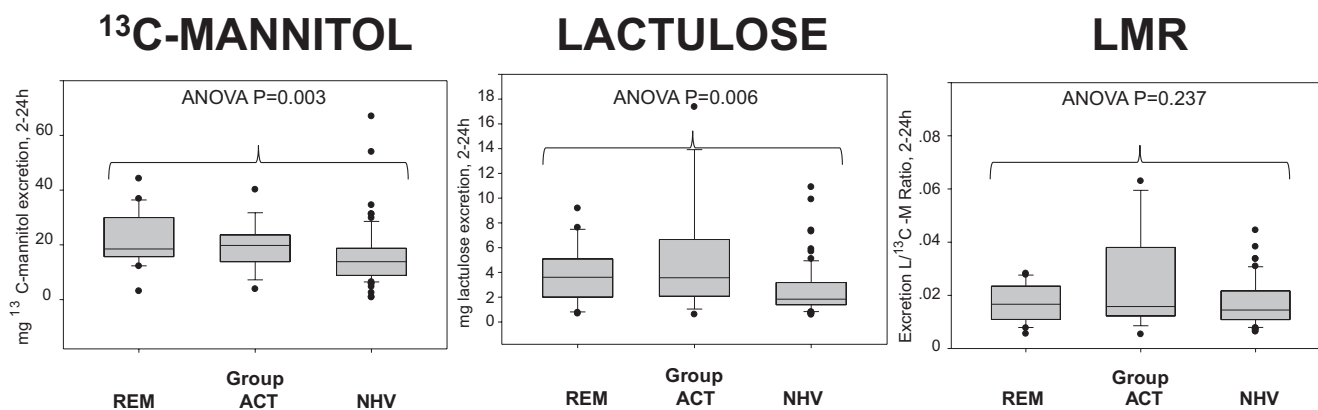
Sugar (dose used)	Timed (h) urine collection	NHV <sup>a</sup> , mg (n = 74)	IBD REM <sup>a</sup> , mg (n = 20)	IBD ACT <sup>a</sup> , mg (n = 17)	P-value K-W	P < .05 by Dunn's multigroup comparison, if significant	P-value M-W RST REM vs. NHV	P-value M-W RST ACT vs. NHV	P-value M-W RST REM vs. ACT
<sup>13</sup> C mannitol 100mg	2-24 h	13.8 (8.8, 18.7)	18.4 (15.6, 29.9)	19.7 (13.8, 23.6)	.003*	REM vs NHV	.003*	.032	.573
	0-2 h	14.0 (9.8, 19.4)	10.1 (6.9, 12.9)	10.8 (7.5, 12.8)	.003*	REM vs NHV	.005*	.017*	.787
	2-8 h	12.0 (7.7, 16.0)	15.8 (13.2, 21.1)	14.9 (11.3, 20.6)	.008*	REM vs NHV	.004*	.102	.337
	8-24 h	1.6 (1.1, 3.1)	3.1 (1.7, 4.7)	3.9 (1.8, 5.2)	.004*	REM vs NHV	.015*	.006*	.772
	0-24 h	29.3 (23.2, 34.8)	30.2 (23.8, 42.7)	29.0 (20.8, 35.2)	.751	NS	.497	.915	.493
Lactulose 1000 mg	2-24 h	1.8 (1.3, 3.1)	3.6 (2.0, 5.0)	3.5 (2.0, 6.6)	.006*	ACT vs NHV	.019	.011*	.659
	0-2 h	1.2 (0.8, 1.6)	0.8 (0.4, 1.9)	1.0 (0.5, 1.4)	.247	NS	.105	.484	.556
	2-8 h	1.5 (1.1, 2.7)	2.5 (1.5, 4.4)	2.6 (1.4, 5.7)	.030*	NS	.047	.035	.796
	8-24 h	0.2 (0.2, 0.3)	0.5 (0.4, 0.7)	0.8 (0.4, 1.3)	.001*	REM vs NHV	.001*	.001*	.279
Lactulose/ <sup>13</sup> C-mannitol ratio	0-24 h	3.3 (2.2, 5.2)	5.1 (2.4, 7.2)	4.5 (2.5, 8.0)	.155	NS	.179	.107	.749
	2-24 h	0.014 (0.010, 0.021)	0.016 (0.010, 0.023)	0.016 (0.012, 0.038)	.237	NS	.602	.104	.241
	0-2 h	0.008 (0.006, 0.012)	0.007 (0.006, 0.013)	0.008 (0.007, 0.019)	.294	NS	.974	.117	.265
	2-8 h	0.014 (0.009, 0.021)	0.016 (0.010, 0.023)	0.017 (0.011, 0.034)	.289	NS	.539	.125	.437
8-24 h	0.018 (0.013, 0.032)	0.016 (0.013, 0.024)	0.030 (0.009, 0.047)	.571	NS	.732	.367	.322	
	0.011 (0.008, 0.016)	0.012 (0.009, 0.018)	0.013 (0.010, 0.033)	.065	NS	.266	.028	.253	

Abbreviations: ACT, active IBD; h, hours; IBD, inflammatory bowel disease; K-W, Kruskal–Wallis test; M-W, Mann–Whitney Rank Sum test; NHV, normal healthy volunteers; NS, not significant; REM, IBD in remission.

The mass of <sup>13</sup>C-mannitol and lactulose ingested with 100 and 1000 mg, respectively. Value of 2-24 data represent sum of mass excreted at 2-8 and 8-24 hours.

<sup>a</sup>Values are median (interquartile range).

\* P < .05 for K-W significance and P < .017 for M-W.



**Figure 1.** Plots depicting Kruskal–Wallis one-way analysis of variance on ranks (ANOVA) comparing urinary mass (mg) excretion of  $^{13}\text{C}$ -mannitol ( $P = .003$ ), lactulose ( $P = .006$ ), and lactulose-to-mannitol ratio ( $P = .237$ ) for urine collected at 2–24 hours in REM and ACT compared to NHV. Mann–Whitney Rank Sum test ( $P < .017$ ) significant for REM versus NHV for  $^{13}\text{C}$ -mannitol ( $P = .003$ ) and ACT versus NHV lactulose ( $P = .011$ ). ACT, active IBD; h, hours; NHV, normal healthy volunteers; REM, IBD in remission.

## Discussion

This study confirms our hypothesis that IBD is associated with increased SI and colonic permeability using an established measurement of SI and COL permeability *in vivo*. The significantly increased SI and COL permeability was found in both active disease and remission for both sugar probes, as measured by the mg excreted for  $^{13}\text{C}$ -mannitol and lactulose, but not for the LMR. This data confirms prior clinical human research and animal models of IBD permeability compared to healthy controls.<sup>30–33</sup> Our results show that  $^{13}\text{C}$ -mannitol has the highest excretion at 0–2 and 2–8 hours; this was expected given the known propensity for bacterial degradation of the sugars that occurs in the colon.<sup>17</sup> The low lactulose absorption (less than 1%) and high colonic bacterial degradation might also account for the low mass excretion and high degree of variability observed in lactulose excretion. Predictably, the low mass excretion also occurs in the presence of active IBD with increased lactulose absorption and excretion, as only 0.7% of the mass administered orally is excreted in the urine. This suggests that LMR may not be as sensitive as the mass excretion of the individual sugars, lactulose, or  $^{13}\text{C}$ -mannitol alone, when evaluating differences in urine excretion as measurements of intestinal or colonic permeability. Recent studies using orally administered fluorophore to quantify permeability in adults with CD characterize the dual sugar absorption test as cumbersome with a long turnaround time, though this has not been our experience in this tertiary care center.<sup>34</sup> Interestingly, that study also found the median permeability to be greater in CD compared to NHV, as we have observed in our current study.<sup>34</sup>

Whereas there were no significant differences between ACT and REM in the current study, this may reflect the relatively small sample size, and further studies may more effectively document significant differences. However, the presence of increased permeability in REM supports the notion that permeability is a mechanistically relevant target for the treatment of IBD.<sup>35</sup> Thus, it is conceivable that increased permeability may play a pivotal role in perpetuating symptoms of disease, and in some circumstances facilitate intraluminal inflammatory triggers. One prior study demonstrated an association in increased intestinal permeability, measured by confocal endomicroscopy, with ongoing bowel symptoms compared

to asymptomatic quiescent IBD and healthy controls (ongoing symptoms 19.0 vs. asymptomatic IBD 7.3 vs. controls 5.9 Confocal Leak Score;  $P = .001$ ).<sup>35</sup> The observation of increased intestinal permeability in remission in IBD also argues for the further testing of the hypothesis that therapeutic strategies to reduce intestinal permeability may have a role in the prevention of disease recurrence or aggravation. The ERIca trial highlighted similar results and found that barrier healing, measured by confocal endomicroscopy, was superior to endoscopic and histologic remission when predicting major adverse outcomes in IBD, including disease flares, IBD-related hospitalization, medication escalation, or steroid initiation.<sup>36</sup> In this context, it has been proposed to use gut permeability as a key target for IBD remission.<sup>36</sup>

The finding of increased intestinal permeability may also be relevant, as a marker of predisposition to develop IBD. Thus, some data demonstrates that increased permeability with abnormal LMR (hazard ratio [HR] 3.03; 95% confidence interval [CI] 1.64–5.63;  $P = 3.97 \times 10^{-4}$ ) may lead to the development of CD in asymptomatic first-degree relatives, and surprisingly these results showed alteration in permeability more than 3 years before diagnosis of CD (HR 1.62; 95% CI 1.051–2.50;  $P = .029$ ).<sup>8</sup> Interestingly, the role of intestinal permeability in a cross-sectional study of first-degree relatives of IBD patients remains unclear, as no association was found between abnormal intestinal permeability and small bowel ulceration in these individuals.<sup>37</sup> Recent data using serum proteomic markers, namely MMP-12 and CXCL9, in conjunction with LMR as a novel biomarker for CD can detect evidence of preclinical CD providing further information on the pathogenesis of CD.<sup>2</sup> Studies have investigated the heritability of abnormal permeability in IBD relatives finding associations with CARD15 genetic mutation.<sup>15</sup> Other studies have shown contrary data indicating environmental factors may play a stronger role than heritability in abnormal intestinal permeability in first-degree relatives of patients with CD.<sup>11–13</sup> Further consensus on standardized measures is needed as these studies used diverse techniques in assessing intestinal permeability. Ultimately, only longitudinal studies with thorough follow-up will provide clarity on whether increased intestinal permeability in relatives of CD patients reflects an early pre-inflammatory change or serves as a marker of subclinical inflammation that compromises the barrier's integrity.

**Table 3.** Urinary excretion of <sup>13</sup>C-mannitol and lactulose for patients with ulcerative colitis in remission and ulcerative colitis with active disease.

Sugar (dose used)	Timed (h) urine collection	UC REM <sup>a</sup> , mg (n = 10)	UC ACT <sup>a</sup> , mg (n = 10)	P-value K-W
<sup>13</sup> C mannitol, 100 mg	2-24 h	25.4 (3.1, 44.2)	19.6 (3.8, 29.6)	.179
	0-2 h	10.8 (5.7, 19.0)	11.1 (1.6, 22.2)	.918
	2-8 h	19.5 (2.4, 34.3)	14.0 (2.9, 20.7)	.162
	8-24 h	3.2 (2.2, 8.5)	3.4 (1.8, 5.7)	.623
	0-24 h	34.3 (11.4, 56.4)	28.0 (14.9, 51.8)	.280
Lactulose, 1000 mg	2-24 h	4.6 (1.2, 6.4)	2.4 (1.9, 5.8)	.970
	0-2 h	1.0 (0.5, 1.6)	1.1 (0.6, 1.6)	.791
	2-8 h	3.1 (0.8, 5.0)	2.0 (1.2, 4.0)	1.000
	8-24 h	0.5 (0.3, 1.4)	0.6 (0.2, 1.6)	.791
	0-24 h	5.4 (1.9, 7.8)	3.7 (2.2, 7.3)	1.000
Lactulose/ <sup>13</sup> C-mannitol ratio	2-24 h	0.017 (0.009, 0.024)	0.014 (0.011, 0.031)	.521
	0-2 h	0.010 (0.005, 0.014)	0.010 (0.007, 0.016)	.571
	2-8 h	0.017 (0.007, 0.025)	0.016 (0.010, 0.029)	.521
	8-24 h	0.017 (0.014, 0.023)	0.024 (0.006, 0.0242)	.677
	0-24 h	0.049 (0.027, 0.057)	0.043 (0.031, 0.088)	.734

Abbreviations: ACT, active IBD; h, hours; IBD, inflammatory bowel disease; K-W, Kruskal–Wallis test; M-W, Mann–Whitney Rank Sum test; NHV, normal healthy volunteers; NS, not significant; REM, IBD in remission.

The mass of <sup>13</sup>C-mannitol and lactulose ingested with 100 and 1000 mg, respectively. Value of 2-24 data represent sum of mass excreted at 2-8 and 8-24 hours.

<sup>a</sup>Values are median (interquartile range).

\*P < .05 for K-W significance.

**Table 4.** Urinary excretion of <sup>13</sup>C-mannitol and lactulose for patients with Crohn's disease in remission and Crohn's disease with active disease.

Sugar (dose used)	Timed (h) urine collection	CD REM <sup>a</sup> , mg (n = 10)	CD ACT <sup>a</sup> , mg (n = 7)	P-value K-W
<sup>13</sup> C mannitol, 100 mg	2-24 h	17.1 (15.3, 32.4)	21.6 (8.0, 40.1)	.714
	0-2 h	8.3 (5.3, 13.2)	9.4 (5.4, 14.0)	.957
	2-8 h	14.3 (12.9, 31.0)	16.1 (6.6, 32.9)	.849
	8-24 h	2.4 (1.4, 3.8)	4.2 (1.7, 5.5)	.262
	0-24 h	26.8 (23.6, 36.9)	30.1 (20.2, 40.1)	.961
Lactulose, 1000 mg	2-24 h	3.2 (2.3, 4.3)	5.0 (2.5, 6.7)	.157
	0-2 h	0.7 (0.3, 2.2)	1.0 (0.4, 1.5)	.704
	2-8 h	2.5 (1.7, 3.8)	3.9 (1.5, 5.8)	.464
	8-24 h	0.5 (0.4, 0.5)	0.9 (0.5, 1.1)	.013*
	0-24 h	5.0 (1.4, 11.7)	4.3 (2.5, 7.4)	.214
Lactulose/ <sup>13</sup> C-mannitol ratio	2-24 h	0.016 (0.012, 0.022)	0.026 (0.015, 0.049)	.188
	0-2 h	0.007 (0.006, 0.012)	0.008 (0.007, 0.025)	.551
	2-8 h	0.016 (0.012, 0.020)	0.017 (0.013, 0.052)	.526
	8-24 h	0.015 (0.013, 0.033)	0.030 (0.020, 0.077)	.260
	0-24 h	0.040 (0.032, 0.073)	0.083 (0.043, 0.118)	.188

Abbreviations: ACT, active IBD; h, hours; IBD, inflammatory bowel disease; K-W, Kruskal–Wallis test; M-W, Mann–Whitney Rank Sum test; NHV, normal healthy volunteers; NS, not significant; REM, IBD in remission.

The mass of <sup>13</sup>C-mannitol and lactulose ingested with 100 and 1000 mg, respectively. Value of 2-24 data represent sum of mass excreted at 2-8 and 8-24 hours.

<sup>a</sup>Values are median (interquartile range).

\*P < .05 for K-W significance.

Additional potential applications include the evaluation of permeability in patients with quiescent IBD who experience persistent GI symptoms including increased stool frequency, looser stool consistency, urgency, and abdominal pain.<sup>3,35</sup> Although other etiologies must be prudently assessed, it is logical to consider subclinical barrier dysfunction as a potential link to persistent symptoms.<sup>38–40</sup> These symptoms are analogous to irritable bowel syndrome, which is associated

with increased permeability especially when associated with increased fecal bile acid excretion.<sup>17,18</sup> Our study did not observe significant correlations, but the analysis was clearly compromised by the small sample sizes, and the categorical nature of the disease activity in contrast to the continuous variables in the measurements of permeability.

Our study has several strengths, including reproducibility of urine excretion of sugar probe molecules making it possible to

accurately measure SI and COL permeability in IBD and NHV. Some limitations include the total number of participants with IBD was 37 and NHV was 74. For the primary endpoint of this study, we included both CD and UC together as a cohort based on disease activity, this may have led to heterogeneity. There was also a lack of ethnic or racial diversity, and difficulty recruiting active IBD patients prior to steroid or biologic initiation. Future studies will be required to establish normal values across diverse cohorts of individuals. Additionally, this small study could not reliably assess the impact of disease location and severity on intestinal permeability or GI transit, the latter being a possible explanation for some of the differences observed in the 0-2 hours sugar probe excretion. Due to sample size, mild, moderate, and severe disease activity could not be adequately examined for UC and CD. Additionally, though previously validated in NHV and other cohorts with diarrhea (IBS and bile acid diarrhea), the impact of interindividual variation in GI transit time is unclear. Therefore, disease location in addition to disease inflammatory activity and transit requires further research to understand these key clinical characteristics and their impact on permeability measurement.

While acknowledging the need for further research as outlined above, the simple, relatively inexpensive, and non-invasive nature of this evaluation of intestinal and colonic permeability provides the basis for future mechanistic studies with longitudinal measurements to better understand the role of permeability in the maintenance of IBD in remission or exacerbations of disease given the expanded armamentarium of targeted IBD therapies. Further studies may also explore the test's potential as a biomarker in identifying the predictors of preclinical IBD.<sup>39,41</sup> Currently, there are no standardized therapies to efficaciously target intestinal permeability, but several pharmacological (*divertin*, inhibition of phosphorylation of myosin light chain kinase) and dietary (eg, glutamine, zinc carnosine, probiotics) interventions exist and may prove beneficial in the clinical setting.<sup>3,9</sup> This research also continues to encourage multidisciplinary collaboration to assess the combined roles in the pathobiology of IBD as well as therapeutic approaches directed at intestinal permeability healing, improved gut microbiota composition, resolved inflammation, and improved gut-brain interaction that may lead to long-term improvement for patients with IBD.<sup>38</sup>

## Conclusions

In conclusion, our study suggests that intestinal permeability is significantly higher in patients with IBD ACT or REM compared to healthy controls. This is consistent with previous observations, which suggest that low-grade inflammation or remodeling of the GI tract leading to severe inflammation can influence long-term permeability despite stable medical management. No serious adverse effects were observed in association with the test itself, and this test was proven to be feasible, inexpensive, and efficacious. These results pave the way for further research into intestinal permeability as a potential biomarker in the development, aggravation, and potentially novel approaches to the treatment of IBD.

## Supplementary data

Supplementary data is available at *Inflammatory Bowel Diseases* online.

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## Author Contributions

K.D.: protocol development, recruitment, conduct of study, grant application, coauthor. C.R., E.O.: study coordination. I.B., D.E., M.R.: lab manager, study administration, coauthor. V.C.: coauthor. L.R.: protocol development, principal investigator, author. M.C.: conceptualization, protocol development, grant application, principal investigator, author

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## Conflicts of Interest

K.D., C.D., I.B., M.R., E.O., V.C., and M.C. have no conflict of interests. L.R. has served as a consultant for Bristol Meyers Squibb Company, Fresenius Kabi USA, Geneoscopy, Janssen, and Roivant Sciences.

## Data Availability

Data will be made available to investigators by direct application to the PI and provision of the summary of the proposed use of the data and specific aims of the study.

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