

# Increased fermentable carbohydrate intake alters colonic mucus barrier function through glycation processes and increased mast cell counts

J. Kamphuis, Laurent Lionel Reber, Hélène Eutamène, Vassilia Théodorou

## ▶ To cite this version:

J. Kamphuis, Laurent Lionel Reber, Hélène Eutamène, Vassilia Théodorou. Increased fermentable carbohydrate intake alters colonic mucus barrier function through glycation processes and increased mast cell counts. FASEB Journal, 2022, 36 (5), pp.e22297. 10.1096/fj.202100494RRR . hal-03646954

## HAL Id: hal-03646954 https://hal.inrae.fr/hal-03646954

Submitted on 20 Apr 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



# Increased fermentable carbohydrate intake alters colonic mucus barrier function through glycation processes and increased mast cell counts

## J. B. J. Kamphuis<sup>1,2</sup> | L. L. Reber<sup>2</sup> | H. Eutamène<sup>1</sup> | V. Theodorou<sup>1</sup>

<sup>1</sup>Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE) Toxicologie alimentaire (Toxalim), UMR1331, INRAE/INP/Université de Toulouse III, Toulouse, France

<sup>2</sup>Institut national de la santé et de la recherche médicale (INSERM), Toulouse Institute for Infectious and Inflammatory Diseases (Infinity), INSERM UMR1291 - CNRS UMR5051 - Université de Toulouse III, Toulouse, France

#### Correspondence

J. B. J. Kamphuis, Infinity; INSERM UMR1291 - CNRS UMR5051 -Université de Toulouse III, CHU Purpan - BP 3028, 31024 Toulouse, France.

Email: jasper.kamphuis@inserm.fr

H. Eutamène, Toxalim; UMR 1331 INRAE/INP/Université de Toulouse III, 180 Chemin de Tournefeuille, 31027 Toulouse, France. Email: helene.eutamene@inrae.fr

#### **Funding information**

The research leading to these results has received funding from the People Programme of the EU's 7th Framework Programme under REA grant agreement no. 607652 (ITN NeuroGut). JBJK was supported by fellowships from the Institut National de la Santé et de la Recherche Médicale (INSERM) ATIP-Avenir program, and from the French Medical Research Foundation (FRM) (SPF202005011962). LLR acknowledges support from the INSERM, the French Medical Research Foundation (FRM EQU202103012566), ATIP-Avenir program and the French National Research Agency (ANR18-CE18-0023 and ANR-20-CE15-0026)

#### Abstract

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder for which dietary interventions can be a useful treatment. In recent years, the low-FODMAP approach is gaining traction in this regard. The fermentation of these non-absorbed carbohydrates by the gut microbiota can generate toxic glycating metabolites, such as methylglyoxal. These metabolites can have harmful effects by their role in the generation of advanced glycation end products (AGEs), which activates Receptor for AGEs (AGER). Mast cells can be stimulated by AGEs and play a role in IBS. We have treated mice with lactose or fructo-oligosaccharides (FOS), with or without co-administration of pyridoxamine and investigated the colonic mucus barrier. We have found that an increased intake of lactose and fructo-oligosaccharides induces a dysregulation of the colonic mucus barrier, increasing mucus discharge in empty colon, while increasing variability and decreasing average thickness mucus layer covering the fecal pellet. Changes were correlated with increased mast cell counts, pointing to a role for the crosstalk between these and goblet cells. Additionally, AGE levels in colonic epithelium were increased by treatment with the selected fermentable carbohydrates. Observed effects were prevented by co-treatment with anti-glycation agent pyridoxamine, implicating glycation processes in the negative impact of fermentable carbohydrate ingestion. This study shows that excessive intake of fermentable carbohydrates can cause colonic mucus barrier dysregulation in mice, by a process that involves glycating agents and increased mucosal mast cell counts.

**Abbreviations:** AGE, advanced glycation end products; AGER, advanced glycation end product receptor; CML, Carboxy-methyl lysine; DSCG, disodium cromoglicate; FODMAPs, fermentable oligo-, di-, mono-saccharides and polyols; FOS, Fructo-oligosaccharides; IBS, irritable bowel syndrome; SCFAs, short-chain fatty acids.

H. Eutamène and V. Theodorou equally supervised.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. The FASEB Journal published by Wiley Periodicals LLC on behalf of Federation of American Societies for Experimental Biology.

### K E Y W O R D S

advanced glycation end products, fermentable carbohydrates, FODMAPs, goblet cells, intestinal barrier, mast cells, mucosal irritation, mucus barrier dysregulation

### 1 | INTRODUCTION

FASEB IOURNAL

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by abdominal pain, erratic bowel habits, and variable changes in stool consistency.<sup>1</sup> It is a common affliction, with a global prevalence of 11.2% according to a 2012 meta-analysis.<sup>2</sup> Because IBS is a functional disorder, and causes are incompletely understood, treatment has proven difficult. Dietary interventions prove useful, with particularly the low-FODMAP (low in fermentable oligo-, di-, monosaccharides and polyols) approach<sup>3</sup> gaining traction in recent years. Apart from gas production and osmotic distention due to fermentable carbohydrate ingestion, microbial fermentation products have been raised as factors possibly involved in symptom generation. The bacterial metabolic toxin hypothesis proposed by Campbell et al.<sup>4</sup> poses that harmful bacterial fermentation products are responsible for the wide range of systemic effects observed from food intolerances such as lactose intolerance. Anaerobic fermentation of unabsorbed carbohydrates, and the production of metabolites such as 1,2 dicarbonyls, are likely culprits. These reactive dicarbonyls are potent glycation agents which generate advanced glycation end products (AGEs) in reaction with amino acid containing molecules.5,6 Increasing AGE concentrations lead to activation of the Receptor for advanced glycation end products (AGERs), which in turn leads to ROS generation and a pro-inflammatory state through NF-κB pathway signaling.<sup>7</sup> A potential increase in dicarbonyls and other glycating agents during microbial processing of fermentable carbohydrates in the gut can be expected to enhance the formation of AGEs, and in that way, support a pro-inflammatory state. Mast cells too can be activated by AGEs<sup>8</sup> through AGER activation, or by aldehydes (acetaldehyde) directly.9 Our recently published work shows that glycating agents produced in the microbial processing of lactose and fructo-oligosaccharides (FOS) can be responsible for the emergence of abdominal hypersensitivity in mice, by increasing AGER expression and mucosal mast cell counts in the colon.<sup>10</sup> A low-grade inflammation caused by AGE-AGER interaction and generating oxidative stress, supported by mast cell activation, could also have negative effects on the epithelial barrier function. For example, mast cells are responsible for the increased paracellular permeability in the colon of rats subjected to water avoidance stress,<sup>11</sup> and mice subjected to nematode infection.<sup>12</sup> In humans, similarly, it has been shown that psychological stress increases

small intestinal paracellular permeability, which can be prevented by treatment with the mast cell stabilizer disodium cromoglycate (DSCG),<sup>13</sup> implying the involvement of mast cells in the induced epithelial barrier dysfunction. Alternatively, mast cells can impact mucus barrier function, for example by inducing mucus release from goblet cells during immobilization stress in mice.<sup>14</sup> Jalanka-Tuovinen et al.<sup>15</sup> have found that post-infectious-IBS and IBS-D patients report passage of mucus per rectum, and the IBS-type microbiota profile that they describe was positively correlated with both mast cell and goblet cell counts in rectal biopsy samples. It has also been established that diet by itself can modulate intestinal mucus barrier function, in the absence of pathophysiological processes. Male rats on 6% FOS supplemented fiber-free semi-purified diets show heavily impaired cecal epithelial mucus barrier function.<sup>16</sup> On the other hand, dietary fiber deficient diets decrease the colonic mucus barrier,<sup>17,18</sup> an effect which is mediated by the intestinal microbiota.<sup>18</sup> Inulin supplementation given to rats immediately after weaning modifies mucin gene expression, colonic crypt depth and goblet cell numbers.<sup>19</sup>

We used an animal mouse model to investigate whether an increased intake of dietary fermentable carbohydrates is implicated in mucus barrier particularities, which could be involved in further epithelial barrier dysfunction. We sought to characterize the mouse colonic mucus barrier function by considering the influence of the colonic contents on this physiological feature. We have previously described that the mucus layer separating contents from host covers the fecal pellet in mice, instead of being attached to and covering the colonic epithelium,<sup>20</sup> a point of view that was spotlighted in recent work by Bergstrom and colleagues.<sup>21</sup> In a novel approach to reflect this revised understanding, we measured this mucus layer and its variability, as well as the number of discharging goblet cells in empty conditions, when no mucus layer is formed, as an indication of mucosal irritation.

### 2 | MATERIALS AND METHODS

### 2.1 | Animals and sample collection

Adult male C57BL/6 mice (Janvier, Le Genest St Isle, France) were housed in polypropylene cages in groups of eight and offered unlimited access to standard rodent food (Mucedola Global Diet 2018, Harlan, Italy) and water. Lactose-pyridoxamine treated group received a daily oral gavage of 5 mg lactose and/or 5 mg pyridoxamine in 200  $\mu$ l saline solution, the control group received only saline, for 3 weeks. The FOS-pyridoxamine treated group received a custom modified AIN-93 diet, with or without 10% fructooligosaccharides (composition in Table 1), complemented with or without 1 mg/ml pyridoxamine in drinking water. Mice were euthanized by cervical dislocation, after which both 1.5 to 2 cm of distal colon and of proximal colon covering regions with and without contents were removed and stored in Carnoy's fixative overnight. All animal experiments were performed in accordance with EU directive 2010/63/EU and approved by the local Animal Care and Use Committee of Toulouse Midi-Pyrénées (agreement CEEA-86).

Diets were prepared and mixed at the UE300 'Unité de Préparation des Aliments Expérimentaux' (UPAE) INRAE Jouy-en-Josas, France.

### 2.2 | Histological sample preparation

Collected tissues were rinsed in 100% ethanol after 1 day in Carnoy's fixative and automatically processed using a Shandon Excelsior ES Tissue Processor by the following program:  $2 \times 60$  min 100% ethanol,  $2 \times 60$  min butanol, 480 min butanol,  $3 \times 80$  min paraffin at 60°C. Tissue samples were included in paraffin blocks using a Thermo Scientific HistoStar Embedding Workstation. 5 µm tissue sections were made using a Microm HM 340 E microtome and attached to Superfrost Plus microscope slides (Thermo Scientific, USA).

TABLE 1 Composition custom AIN93-M +/- FOS diets (g/kg)

AIN-93M	AIN93M-FOS
465.692	365.692
0	100
140	140
155	155
100	100
40	40
50	50
35	35
10	10
1.8	1.8
2.5	2.5
0.008	0.008
	AIN-93M 465.692 0 140 155 100 40 50 35 35 10 1.8 1.8 2.5 0.008

*Note: Reference:* Reeves PG, Nielsen FH, Fahey GC, Jr. *J Nutr.* 1993;123(11):1939–1951.<sup>22</sup>

### 2.3 | Visualization

# 2.3.1 | Mouse mucosal mast cell protease staining

5  $\mu$ m sections were deparaffinated by using 3× 5 min baths of American Mastertech Clearify followed by 3× 5 min 100% ethanol, 3× 5 min 95% ethanol, 2× 5 min 70% ethanol, 5 min demineralized water. Slides were washed 2× in PBS for 5 min, followed by a 2-h blocking step with 10% donkey serum in PBS, and washed 3× 5 min under light agitation in PBS. Slides were incubated overnight at 4°C with primary antibodies (Sheep anti-mMCP1 (MS-RM8 (Moredun Group, UK)) diluted 1:400), followed by  $2 \times 5$  min rinsing steps in PBS. Secondary antibody (Alexa Fluor 594 Donkey-anti-Sheep (A-11016 (Molecular Probes, USA)) diluted 1:400 in 1% donkey serum PBS) incubation was performed for 2 h, followed by  $3 \times 5$  min washing steps in PBS, a quick rinse with tap water, followed by mounting using ProLong Gold® antifade reagent with DAPI (Thermo Fisher Scientific, USA).

### 2.3.2 | Carboxy-methyl lysine staining

5  $\mu$ m tissue sections were deparaffinated by using 3× 5 min baths of xylene followed by  $3 \times 5$  min 100% ethanol, 3× 5 min 95% ethanol, 2× 5 min 70% ethanol, 5 min demineralized water. Slides were washed 2× in PBS for 5 min, followed by a 2-h blocking step with 1% bovine serum albumin (BSA) in PBS, and washed 3× 5 min in PBS. Slides were incubated overnight at 4°C with anti-CML mAb ((clone 6C7) (MABN1837, Millipore, USA) diluted 1:75), followed by  $2 \times 5$  min rinsing steps in PBS. Secondary antibody (Alexa Fluor 647 Goat-anti-Mouse (A32728, Thermo Fisher Scientific, USA)) diluted 1:250 in 1% BSA PBS) incubation was performed for 2 h, followed by 3× 5 min washing steps in PBS, a quick rinse with tap water, followed by mounting using ProLong Gold® antifade reagent with DAPI (Thermo Fisher Scientific, USA).

# 2.3.3 | Alcian blue/hematoxylin/eosin staining

5  $\mu$ m sections were deparaffinated by using 3× 5 min baths of American Mastertech Clearify followed by 3× 5 min 100% ethanol, 3× 5 min 95% ethanol, 2× 5 min 70% ethanol, 5 min demineralized water. Staining was performed by 5 min in hematoxylin, 10 min in running tap water, 30 min in Alcian Blue solution (pH 3.0) followed by 5 min in running water, 3 min in Eosin, 10 min in 95% ethanol, followed by dehydration  $(2 \times 4 \text{ min } 70\% \text{ ethanol}, 2 \times 5 \text{ min } 95\% \text{ ethanol}, 2 \times 5 \text{ min } 100\% \text{ ethanol}, 3 \times 5 \text{ min}$ American Mastertech Clearify), and finally mounted using Diamount mountant.

### 2.4 | Microscopy and image analysis

# 2.4.1 | Manual ultra-high-resolution composite image overview

EBIOURNAL

Data sets of overlapping microscope views covering entire slides were generated by manual microscope photography (single photo resolution:  $1280 \times 1024$  pixels) and stitched together using Microsoft Image Composite Editor (MICE), as originally described in Kamphuis, Mercier-Bonin, Eutamène, Theodorou (2017).<sup>20</sup> Samples were imaged using a Nikon Eclipse 90i microscope fitted with a DXM 1200 F Digital Camera.

### 2.4.2 | Mucus layer thickness measurements

The fecal mucus layer thickness was measured using ImageJ software; a measurement perpendicular to the surface of the colonic contents was taken every 100 micrometers along the entire imaged surface.

### 2.4.3 | Fluorescence microscopy (MCPT1)

Samples were imaged using a Nikon Eclipse 90i microscope fitted with a DXM 1200 F Digital Camera. Image sets were taken at 200× magnification.

### 2.4.4 | Fluorescence microscopy (CML)

Samples were imaged using a Zeiss Axio-observer microscope fitted with an Axiocam HRm Rev.3. Image sets were taken at 200× magnification.

### 2.4.5 | Active goblet cell count analyses

One ratio discharging goblet cell:crypt per mouse based on analysis of 25–90 crypts in transversal sections of empty colon, depending on availability of suitable visual material was generated. Image sets were coded, randomized, and analyzed blindly. Statistical analysis: One-way ANOVA, multiple comparisons with Tukey's correction for multiple comparisons.

### 2.4.6 | Mast cell count analyses

One ratio mast cell:crypt per mouse based on analysis of 50–250 crypts, dependent on availability of suitable visual material was generated. Image sets were coded, randomized, and analyzed blindly. Statistical analysis: One-way ANOVA, multiple comparisons with Tukey's correction for multiple comparisons.

### 2.4.7 | Quantification of CMLresidue staining

3–4 mean fluorescence intensity values per sample were measured by carefully selecting the epithelial layer and measuring the mean grey value of the proper channel using Fiji (Fiji Is Just ImageJ) software.<sup>23</sup> Image sets were coded, randomized, and analyzed blindly. Statistical analysis: One-way ANOVA, multiple comparisons with Tukey's correction for multiple comparisons.

### 3 | RESULTS

Treatment with 5 mg/day lactose caused a slight reduction of the average fecal mucus layer thickness (-11.9% vs. control, p < .68), but with a markedly altered dispersion of measured values (gray point-cloud, Figure 1A), which shows in the significantly increased coefficient of variation (CoV) for the lactose-treated group (+98% vs. control, p < .01), which was normalized by co-treatment with pyridoxamine (Figure 1A,B). Similarly, treatment with a FOS-enriched diet caused a diminution of the average fecal mucus layer thickness (-33% vs. control, p <.01), with a significantly increased CoV (+26% vs. control, p < .05), which, again, was lowered by co-treatment with pyridoxamine (Figure 1C,D). Measurements were performed using the Manual ultra-high-resolution composite image overview (MUCIO) approach<sup>20</sup> on Alcian blue/hematoxylin/eosin (AB/HE) stained distal colon sections, thus obtaining complete overviews, and numerous measurement values per sample and individual (Figure 1E).

To further investigate the cause of these changes in fecal mucus layer thickness, we next analyzed the activity of goblet cells in empty colon, without the presence of feces, as we previously demonstrated that the colonic mucus layer depends on the presence of luminal contents,<sup>20</sup> and we questioned how the production of mucus in the absence of contents might differ between groups. Elevated intake of lactose or FOS caused an increase in the number of discharging goblet cells in the empty



5 of 11

**FIGURE 1** Fecal mucus layer thickness in distal colon of mice treated with fermentable carbohydrates +/- pyridoxamine. (A) The average colonic fecal mucus layer thickness (black), and the pooled complete dataset that generated these averages (gray) of mice treated daily with 5 mg lactose +/- 5 mg pyridoxamine. (B) The coefficients of variation (CoVs) of the mucus layer thickness averages for mice treated daily with 5 mg lactose +/- 5 mg pyridoxamine. (C) The average colonic fecal mucus layer thickness (black), and the pooled complete dataset that generated these averages (gray) of mice fed with a FOS-augmented diet +/- pyridoxamine-enriched drinking water (1 mg/ml). (D) The CoVs of the mucus layer thickness averages for mice fed with a FOS-augmented diet +/- pyridoxamine-enriched drinking water (1 mg/ml). (E) Representative images showing the fecal mucus layers of all treatment groups





**FIGURE 2** Active goblet cells per crypt in the distal colon of mice treated with fermentable carbohydrates +/- pyridoxamine. (A) Ratio active goblet cells/crypt for distal colon of animals treated with lactose and/or pyridoxamine. In red, the ratio mast cell/crypt is represented. (B) Ratio active goblet cells/crypt for distal colon of animals treated with FOS and/or pyridoxamine. In red, the ratio mast cell/crypt is represented. (C) Representative images showing the increase in active goblet cells in the colon of fermentable carbohydrate treated animals, and its reversal by pyridoxamine. (D) Representative images showing the increased colonic mucosal mast cells in FODMAP-treated animals, and the normalization obtained by pyridoxamine co-treatment

distal colon (lactose: +70% vs. control, p < .0001; FOS: +81% vs. control, p < .0001), and this effect was lost or moderated when cotreated with anti-glycation agent pyridoxamine (lactose-pyridoxamine: -1.7% vs. control, p =.9979; FOS-pyridoxamine: +14.5% vs. control, p = .4622) (Figure 2A–C). At the same time, fermentable carbohydrate treatment caused an increase in colonic mucosal mast cell counts in proximal colon (lactose: +45% vs. control, p < .0001; FOS: +73% vs. control, p < .0001), which was similarly lost through co-treatment with pyridoxamine (lactose-pyridoxamine: -1.2% vs. control, p = .9978; FOS-pyridoxamine: -4.8% vs. control, p =.7663) (Figure 2A,B,D).

The observed effects and their prevention by cotreatment with anti-glycation agent pyridoxamine could be linked to changes in advanced glycation end product (AGE) levels in the intestinal tissue. We stained sections of proximal colon tissue of treated mice for carboxy-methyl lysine (CML), a main AGE residue, by immunofluorescence. Lactose and FOS increased mean fluorescent levels in the epithelial cell layer compared with control (lactose: +21,5% vs. control, p = .0042; FOS: +60% vs. control, p = .0143), which was prevented by co-treatment with pyridoxamine (lactose-pyridoxamine: +0,4% vs. control, p = .9244; FOS-pyridoxamine: +9.2% vs. control, p = .67) (Figure 3A-C) indicating that lactose and FOS treatment have increased CML levels in the colonic epithelium, and pyridoxamine co-treatment was able to prevent this increase.

### 4 | DISCUSSION

We observed an increase in mucus production in both lactose- and FOS-treated animals, as demonstrated by the increased goblet cell discharge in empty distal colon (Figure 2), which has been described before in response to the intake of FOS in rat<sup>24</sup> as well as in man.<sup>25</sup> Ten Bruggencate et al. take this increased mucus production to be an indicator of mucosal irritation in response to an increased luminal concentration of irritants produced by the microbiota,<sup>24,25</sup> a claim that has not been readily accepted by everyone.<sup>26</sup> A mucosal insult by fermentation of fermentable carbohydrates is however supported by our results, and the increased mucus production is somewhat paradoxically accompanied by a reduced fecal mucus barrier formation, and a higher variability in this mucus layer (Figure 1), something more readily understood in the model of colonic mucus organization we previously described,<sup>20</sup> and which has recently been put forth again in an excellent article by Bergstrom et al.<sup>21</sup> In short, the separating mucus barrier is only formed in the presence of colonic contents and covers the fecal pellet within the gut and after defecation. In case

mucus is liberated at an increased rate due to mucosal irritation, even in the absence of feces, there seems to be a hypervariable and insufficient coverage of the feces, and the barrier separating the fecal material from the epithelium of distal colon is compromised. We saw a prevention of these negative effects by co-treatment with pyridoxamine, which is a known anti-glycation agent,<sup>27-30</sup> which points to the involvement of glycation processes in the generation of these effects by the dietary interventions. Recently, elegant work by Kingsley and colleagues show that microbial processing of glucose plays a crucial role in the increased dicarbonyl stress and negative health effects of high sugar intake in a C. elegans model.<sup>31</sup> In our mouse model too, the increase in glycation most likely stems from elevated levels of microbial fermentation products, as proposed in the toxic bacterial metabolite hypothesis by Campbell et al.<sup>4</sup> We have recently published results more broadly in support of this hypothesis; in a mouse model of IBS-symptoms induced by fermentable carbohydrates, the increase in colonic epithelial AGER expression, the increase in mucosal mast cells, as well as the resulting abdominal hypersensitivity induced by elevated intake of the fermentable carbohydrates lactose and FOS could be prevented by anti-glycation agent pyridoxamine.<sup>10</sup> Reactive glycation agents interact with proteins to produce glycated residues, leading to the formation of AGEs,<sup>6</sup> which can directly stimulate mast cells,<sup>8</sup> and mast cells in turn are capable of causing mucus discharge by goblet cells.<sup>14</sup> The aberrant mucus release and passage per rectum of mucus in some IBS patients<sup>15</sup> might be explained in this way too because IBS patients can show increased mast cell numbers.<sup>32-34</sup> Histamine levels are increased in the mucosa of IBS patients, 33,35 while evasion of dietary FODMAPs lowers urinary histamine levels.<sup>36</sup> As shown in our recently published work, lactose and FOS intake can induce an increase in mucosal mast cell numbers in the gut through glycation processes in a healthy mouse model.<sup>10</sup> In this work, we have observed increased levels of CML, an important AGE residue,<sup>37</sup> in lactose and FOS-treated animals, which could be prevented by co-treatment with pyridoxamine (Figure 3). Similarly, we previously reported increased levels of AGER expression in response to these same treatments.<sup>10</sup> Together with the other effects observed in the current work; on goblet cell activity, mast cell numbers, and mucus layer variability, we hypothesize the following cascade: the specific increased carbohydrate fermentation causes production of reactive glycation agents by the intestinal microbiota, which leads to generation of AGEs locally in tissues exposed to these toxic metabolites. This in turn leads to AGER activation and upregulation, activating mast cells and increasing their population locally. At the same time, increased mast cell numbers and mediator production causes goblet cells to discharge more, even in the absence of contents, causing the observed dysregulation (Figure 4).



**FIGURE 3** Mean fluorescence level of carboxy-methyl lysine (CML) immunostained colonic epithelium in mice treated with fermentable carbohydrates +/- pyridoxamine. Pictures show CML residues (orange) and cell nuclei (blue). (A) Mean fluorescence level of CML staining of colonic epithelium of animals treated with lactose +/- pyridoxamine. (B) Mean fluorescence level of CML staining of colonic epithelium of animals treated with FOS +/- pyridoxamine. Dotted "Control" line indicates mean fluorescence level of the negative control: stained with only the secondary antibody. (C) Representative images showing the increase in mean fluorescence intensity of colonic epithelial cells of animals treated with fermentable carbohydrates, and the normalization obtained by pyridoxamine co-treatment. White dash-dotted line indicates typical selection of epithelial cells used for mean fluorescence intensity measurements

Previously, it has been demonstrated that a 10% FOS diet decreases the cecal mucus barrier in rats<sup>38</sup> and similarly, a 6% FOS-diet induces an increased cecal permeability in rats on an otherwise fiber-free diet, probably due to a disturbed mucus layer, which caused a transient inflammatory state, which abated in a timely fashion, and was interpreted by the authors as an adaptative response to develop the immune system.<sup>16</sup> We observed no overt inflammation in our mice after 3 weeks of treatment with either 5 mg daily of lactose or diet containing 10% FOS, by fecal lipocalin-2 quantification, macroscopic or microscopic observation.<sup>10</sup> It is likely that an increase in short-chain fatty acids (SCFAs) caused

by our interventions has a significant effect on mucus barrier function as well, as previously reported<sup>39,40</sup> for SCFAs. However, in contrast to our study, these effects are generally described to be positive for mucus function, and if similar processes were responsible in our experiments, the observed reversibility by anti-glycation agent pyridoxamine is not easily explained. The SCFAs acetate, butyrate, and propionate can also directly stimulate mucus release<sup>41</sup>; the periodic arrival of the colonic contents in the distal colon would bring increased levels of SCFAs with it, stimulating release of mucus in the presence of feces. In contrast, we have observed an increased discharge in the absence of



**FIGURE 4** Hypothesized cascade by which FODMAP ingestion could dysregulate the colonic mucus barrier. Toxic bacterial metabolites produced during FODMAP fermentation lead to AGE formation, AGER activation and upregulation, which cause mast cell number increases and activation, interaction between mast cells and goblet cells lead to mucus discharge dysregulation

contents, thus during moments where the SCFAs concentrations were not particularly increased.

The specific predisposition of the immune system to react or to tolerate gut microbes becomes more important in case of a dysfunctional mucus barrier and increased intestinal permeability, and it is indeed known that serum cytokine profiles in a subset of IBS patients have a tendency to show more IL-6, IL-8, and less IFN- $\gamma$ , while mucosal mRNA expression of IL-10 and FOXP3 tend to be decreased.<sup>42</sup> These tendencies could indicate the relatively higher predisposition of the IBS patient intestine to retain low-grade inflammation, in particular in the context of a dysfunctional mucus barrier, which we showed here, in mice, can be caused by a badly tolerated fermentable carbohydrate intake. In our view, the dysregulation of the intestinal mucus barrier observed herein is unlikely to cause systemic or even local symptoms unless other factors are present that exacerbate or exploit this dysregulation, which might prove a fruitful topic for further research. Characterizing the mucus barrier by measuring the fecal mucus barrier in filled, and the discharging goblet cells in empty conditions, gives an indication of the state of the murine colonic mucus barrier using the renascent appreciation of the impact of the colonic content and its microbiota on the colonic mucus barrier and its organization.<sup>20,21</sup> We have shown that FODMAP ingestion can cause mucosal irritation, and dysregulation of the intestinal mucus barrier in mice, by a process that involves glycating agents, increased AGE levels in the colonic epithelium, and a subsequent increase in mast cell counts.

9 of 11

### ACKNOWLEDGMENTS

We would like to thank Mathilde Levèque, Muriel Mercier-Bonin, and Hervé Robert for discussions proven helpful in developing the methods of analysis of mucus layer thickness and variability. We are grateful to all members of the animal facility staff (EZOP, INRAE Toxalim) for housing and animal care, and access to facilities. We are grateful to the staff at the Cell Imaging Facility of Infinity (Inserm UMR1291, Toulouse, France) for technical assistance.

### DISCLOSURES

All authors declare that there are no conflicts of interests related to this work.

### AUTHOR CONTRIBUTIONS

J. B. J. Kamphuis, L. L. Reber, H. Eutamène, and V. Theodorou designed the experiments; J. B. J. Kamphuis performed the experiments, analyzed data, and wrote the manuscript; J. B. J. Kamphuis, L. L. Reber, H. Eutamène, and V. Theodorou corrected and approved the final manuscript; and L. L. Reber, H. Eutamène, and V. Theodorou supervised the project.

### DATA AVAILABILITY STATEMENT

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

### ORCID

J. B. J. Kamphuis https://orcid. org/0000-0002-8494-613X L. L. Reber https://orcid.org/0000-0003-3384-6769

### REFERENCES

- 1. Enck P, Aziz Q, Barbara G, et al. Irritable bowel syndrome. *Nat Rev Dis Primers*. 2016;2:16014.
- 2. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. *Clin Gastroenterol Hepatol*. 2012;10:712-721.e714.
- 3. Gibson PR, Shepherd SJ. Evidence-based dietary management of functional gastrointestinal symptoms: the FODMAP approach. *J Gastroenterol Hepatol*. 2010;25:252-258.
- 4. Campbell AK, Matthews SB, Vassel N, et al. Bacterial metabolic 'toxins': a new mechanism for lactose and food intolerance, and irritable bowel syndrome. *Toxicology*. 2010;278:268-276.
- Rabbani N, Xue M, Thornalley PJ. Dicarbonyls and glyoxalase in disease mechanisms and clinical therapeutics. *Glycoconj J*. 2016;33:513-525.
- Thornalley PJ, Langborg A, Minhas HS. Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. *Biochem J*. 1999;344(Pt 1):109-116.
- Wautier M-P, Guillausseau P-J, Wautier J-L. Activation of the receptor for advanced glycation end products and consequences on health, *Diabetes Metab Syndr: Clin Res Rev.* 2017;11(4):305-309.
- 8. Sick E, Brehin S, Andre P, et al. Advanced glycation end products (AGEs) activate mast cells. *Br J Pharmacol*. 2010;161:442-455.
- 9. Ferrier L, Berard F, Debrauwer L, et al. Impairment of the intestinal barrier by ethanol involves enteric microflora and mast cell activation in rodents. *Am J Pathol.* 2006;168:1148-1154.
- 10. Kamphuis JBJ, Guiard B, Leveque M, et al. Lactose and Fructooligosaccharides increase visceral sensitivity in mice via glycation processes, increasing mast cell density in colonic mucosa. *Gastroenterology*. 2020;158:652-663.e656.
- 11. Santos J, Yang P, Söderholm JD, Benjamin M, Perdue M. Role of mast cells in chronic stress induced colonic epithelial barrier dysfunction in the rat. *Gut.* 2001;48:630-636.
- McDermott JR, Bartram RE, Knight PA, Miller HR, Garrod DR, Grencis RK. Mast cells disrupt epithelial barrier function during enteric nematode infection. *Proc Natl Acad Sci.* 2003;100:7761-7766.

- 13. Vanuytsel T, van Wanrooy S, Vanheel H, et al. Psychological stress and corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism. *Gut.* 2014;63:1293-1299.
- 14. Castagliuolo I, Lamont JT, Qiu B, et al. Acute stress causes mucin release from rat colon: role of corticotropin releasing factor and mast cells. *Am J Physiol*. 1996;271:G884-G892.
- Jalanka-Tuovinen J, Salojärvi J, Salonen A, et al. Faecal microbiota composition and host-microbe cross-talk following gastroenteritis and in postinfectious irritable bowel syndrome. *Gut.* 2013;63(11):1737-1745.
- Genda T, Sasaki Y, Kondo T, et al. Fructo-oligosaccharide-Induced transient increases in Cecal immunoglobulin A concentrations in rats are associated with mucosal inflammation in response to increased gut permeability. *J Nutr.* 2017;147:1900-1908.
- 17. Brownlee IA, Havler ME, Dettmar PW, Allen A, Pearson JP. Colonic mucus: secretion and turnover in relation to dietary fibre intake. *Proc Nutr Soc.* 2003;62:245-249.
- Desai MS, Seekatz AM, Koropatkin NM, et al. A dietary fiberdeprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell.* 2016;167:1339-1353.e1321.
- Paturi G, Butts CA, Stoklosinski H, Ansell J. Effects of early dietary intervention with a fermentable fibre on colonic microbiota activity and mucin gene expression in newly weaned rats. *J Funct Foods*. 2012;4:520-530.
- Kamphuis JBJ, Mercier-Bonin M, Eutamene H, Theodorou V. Mucus organisation is shaped by colonic content; a new view. *Sci Rep.* 2017;7:8527.
- 21. Bergstrom K, Shan X, Casero D, et al. Proximal colon-derived O-glycosylated mucus encapsulates and modulates the microbiota. *Science*. 2020;370:467-472.
- 22. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr*. 1993;123:1939-1951.
- Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an opensource platform for biological-image analysis. *Nat Methods*. 2012;9:676-682.
- 24. Ten Bruggencate SJ, Bovee-Oudenhoven IM, Lettink-Wissink ML, Van der Meer R. Dietary fructooligosaccharides increase intestinal permeability in rats. *J Nutr.* 2005;135:837-842.
- Ten Bruggencate SJ, Bovee-Oudenhoven IM, Lettink-Wissink ML, Katan MB, van der Meer R. Dietary fructooligosaccharides affect intestinal barrier function in healthy men. *J Nutr.* 2006;136:70-74.
- 26. Guarner F. Prebiotics and mucosal barrier function. *J Nutr.* 2006;136:2269.
- 27. Cardoso S, Carvalho C, Marinho R, et al. Effects of methylglyoxal and pyridoxamine in rat brain mitochondria bioenergetics and oxidative status. *J Bioenerg Biomembr*. 2014;46:347-355.
- de Arriba SG, Stuchbury G, Yarin J, Burnell J, Loske C, Munch G. Methylglyoxal impairs glucose metabolism and leads to energy depletion in neuronal cells–protection by carbonyl scavengers. *Neurobiol Aging*. 2007;28:1044-1050.
- 29. Illien-Junger S, Grosjean F, Laudier DM, Vlassara H, Striker GE, Iatridis JC. Combined anti-inflammatory and anti-AGE drug treatments have a protective effect on intervertebral discs in mice with diabetes. *PLoS One.* 2013;8:e64302.

- 31. Kingsley SF, Seo Y, Allen C, Ghanta KS, Finkel S, Tissenbaum HA. Bacterial processing of glucose modulates *C. elegans* lifespan and healthspan. *Sci Rep.* 2021;11:1-12.
- 32. Wouters MM, Vicario M, Santos J. The role of mast cells in functional GI disorders. *Gut.* 2016;65:155-168.
- Barbara G, Stanghellini V, De Giorgio R, et al. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology*. 2004;126:693-702.
- 34. Bashashati M, Moossavi S, Cremon C, et al. Colonic immune cells in irritable bowel syndrome: a systematic review and meta-analysis, *Neurogastroenterol Motil.* 2018;30(1):e13192.
- Barbara G, Cremon C, Carini G, et al. The immune system in irritable bowel syndrome. *J Neurogastroenterol Motil.* 2011; 17:349-359.
- 36. McIntosh K, Reed DE, Schneider T, et al. FODMAPs alter symptoms and the metabolome of patients with IBS: a randomised controlled trial. *Gut.* 2017;66:1241-1251.
- Ikeda K, Higashi T, Sano H, et al. N (epsilon)-(carboxymethyl) lysine protein adduct is a major immunological epitope in proteins modified with advanced glycation end products of the Maillard reaction. *Biochemistry*. 1996;35:8075-8083.
- Tsukahara T, Iwasaki Y, Nakayama K, Ushida K. An improved technique for the histological evaluation of the mucus-secreting status in rat cecum. J Nutr Sci Vitaminol. 2002;48:311-314.

- Willemsen LEM, Koetsier MA, Van Deventer SJH, Van Tol EAF. Short chain fatty acids stimulate epithelial mucin 2 expression through differential effects on prostaglandin E1 and E2 production by intestinal myofibroblasts. *Gut.* 2003;52:1442-1447.
- 40. Wrzosek L, Miquel S, Noordine ML, et al. Bacteroides thetaiotaomicron and Faecalibacterium prausnitzii influence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent. *BMC Biol.* 2013;11:61.
- 41. Shimotoyodome A, Meguro S, Hase T, Tokimitsu I, Sakata T. Short chain fatty acids but not lactate or succinate stimulate mucus release in the rat colon. *Comp Biochem Physiol A: Mol Integr Physiol.* 2000;125:525-531.
- Bennet SM, Polster A, Tornblom H, et al. Global cytokine profiles and association with clinical characteristics in patients with irritable bowel syndrome. *Am J Gastroenterol*. 2016;111:1165-1176.

**How to cite this article:** Kamphuis JBJ, Reber LL, Eutamène H, Theodorou V. Increased fermentable carbohydrate intake alters colonic mucus barrier function through glycation processes and increased mast cell counts. *FASEB J.* 2022;36:e22297. doi:10.1096/fj.202100494RRR