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Conflict of interest statement

The authors do not have any conflicts of interest to declare.

Author Contributions

JBJK, FG, LD, MO, HE, and VT designed the experiments; JBJK, BG, ML, IJ, SY, VTo, PR, SC, M-HN-M performed the experiments and analysed data; MO, J-FM analysed data; JBJK, HE, and VT analysed data and wrote the manuscript; HE and VT supervised the project.

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Abstract

Background and Aims: Irritable bowel syndrome (IBS) is characterized by abdominal pain, bloating, and erratic bowel habits. A diet low in fermentable oligo-, di-, mono-saccharides and polyols (FODMAPs) can reduce symptoms of IBS, possibly by reducing microbial fermentation products. We investigated whether ingestion of FODMAPs can induce IBS-like visceral hypersensitivity mediated by fermentation products of intestinal microbes in mice.

Methods: C57Bl/6 mice were gavaged with lactose, with or without the anti-glycation agent pyridoxamine, or saline (controls) daily for 3 weeks. A separate group of mice were fed a diet containing fructo-oligosaccharides, with or without pyridoxamine in drinking water, or a normal chow diet (controls) for 6 weeks. Feces were collected and analyzed by 16S rRNA gene sequencing and bacterial community analyses. Abdominal sensitivity was measured by electromyography and mechanical von Frey filament assays. Colon tissues were collected from some mice and analyzed by histology and immunofluorescence, to quantify mast cells and expression of advanced glycosylation end-product specific receptor (AGER).

Results: Mice gavaged with lactose or fed fructo-oligosaccharides had increased abdominal sensitivity compared with controls, associated with increased numbers of mast cells in colon and expression of the receptor for AGER in proximal colon epithelium. These effects were prevented by administration of pyridoxamine. Lactose and/or pyridoxamine did not induce significant alterations in the composition of the fecal microbiota. Mass spectrometric analysis of carbonyl compounds in fecal samples identified signatures associated with mice given lactose or fructo-oligosaccharides vs controls.

Conclusion: We found that oral administration of lactose or fructo-oligosaccharides to mice increases abdominal sensitivity, associated with increased numbers of mast cells in colon and expression of AGER; these can be prevented with an anti-glycation agent. Lactose and/or pyridoxamine did not produce alterations in fecal microbiota of mice. Our findings indicate that preventing glycation reactions might reduce abdominal pain in patients with IBS with sensitivity to FODMAPs.

KEY WORDS: functional bowel disorder, mouse model, mastocytes, advanced glycation end products

Introduction

Irritable Bowel Syndrome (IBS) is a functional gastrointestinal disorder (FGID) characterized by abdominal pain, bloating, erratic bowel habits, and variable changes in the consistency of stools [1, 2]. It is a heterogeneous disorder, with 4 defined sub-types; Diarrhoea (IBS-D) or Constipation Predominant IBS (IBS-C), Mixed bowel habits (IBS-M), or Unclassified (IBS-U) [3]. Because the underlying causes for IBS are not well understood, it has proven difficult to design evidence-based therapies with a clear mechanism of effect. However, in recent years a low-FODMAP (Fermentable Oligo-, Di-, Mono-saccharides And Polyols [4]) diet has been successfully used to reduce symptoms of IBS [5-8]. Dietary FODMAPs have properties that can lead to distension; they are poorly absorbed in the small intestine, osmotically active, and are fermented by the gut microbiota upon reaching the colon [9]. These dietary components, characterized for IBS patients, can also induce gastrointestinal symptoms in healthy subjects. For example, inulin, a fructo-oligosaccharide, can lead to gastro-intestinal symptoms such as flatulence and gut cramps[10].

At first sight, evasion of dietary FODMAPs helps to prevent problematic gut distension, thus alleviating symptom generation in IBS patients[11], but it is not necessarily expected to ameliorate underlying reasons for the increased sensitivity to distension. Dietary carbohydrate fermentation has been shown to influence seemingly unrelated symptoms as well. A link between perceived food intolerances (mostly to fermentable carbohydrates, or gluten) and IBS symptoms, but also musculoskeletal pain, and fatigue has been described [12]. Lactose and fructose intolerant FGID patients report headaches and tiredness in response to a challenge with these carbohydrates at an even higher rate than the more classical symptoms, such as diarrhoea or gastrointestinal cramps [13]. This indicates that dietary carbohydrate fermentation might promote symptoms through other pathways than intestinal distension alone. According to our knowledge, it has not been shown yet that the efficacy of the low-FODMAP diet is higher in IBS-patients presenting visceral hypersensitivity, which is not omnipresent in IBS patients[14, 15]. Apart from gas production and osmotic distension due to FODMAP ingestion, microbial fermentation products have been raised as factors involved in symptom generation.

The bacterial metabolic toxin hypothesis, proposed by Campbell et al.[16] poses that harmful bacterial fermentation products, particularly those produced in anaerobic fermentation of unabsorbed carbohydrates by the gut microbiota, are responsible for effects observed from food intolerances such as lactose intolerance. Particularly such metabolites as alcohols, ketones, and aldehydes are held responsible. Indeed, methylglyoxal, a highly reactive dicarbonyl compound, increases visceral sensitivity when administered by enema to female Wistar rats, as well as inducing behaviour indicative of headache [17]. The idea that increased methylglyoxal concentrations can lead to

increased sensitivity is supported by the finding that methylglyoxal drives neuropathic pain in diabetic patients, in part through the activation of transient receptor potential ankyrin 1 (TRPA1)[18, 19].

Moreover, reactive carbonyl compounds like methylglyoxal or glyoxal are major precursors to Advanced Glycation End Products (AGEs) [20]. Protein conformation modifications by formation of dicarbonyl adducts could interfere with the function of host proteins, and AGEs are recognized by the innate immune system as damage-associated molecular patterns (DAMPs), which activates a pro-inflammatory signaling pathway [21]. An increase in such glycating agents during microbial processing of FODMAPs in the gut could be expected to enhance the formation of AGEs, and in that way, support a pro-inflammatory state. Interestingly, mast cells can be activated by AGEs through advanced glycosylation end-product specific receptor (AGER) activation[22], or even by aldehydes (acetaldehyde) directly [23]. These processes should not be exclusive to FGID patients, so to explain the specific symptom generation in these patients, we propose that increased susceptibility due to genetic or environmental factors, variations in intestinal permeability and microbiota, and differences in the handling of carbohydrates are likely responsible.

In this study, we have tested the hypothesis that production of carbonyl compounds responsible for increased non-enzymatic glycation reactions produced during fermentation of certain FODMAPs can directly or indirectly induce symptoms of IBS. We used lactose and fructo-oligosaccharides as representatives of FODMAPs in an animal model, to evaluate the effects of chronic increased FODMAP intake on visceral sensitivity and low-grade inflammation through activation of AGER. Finally, we investigated whether mast cells, known to be key players in IBS symptoms and susceptible to AGER activation, were implicated in the effects of this chronic intervention.

Materials and Methods

Animals and sample collection

Lactose experiments: 32 adult male C57Bl/6 mice (Janvier, Le Genest St Isle, France) of 6 weeks old were housed in polypropylene cages in groups of 8 without mixing experimental groups, mice were distributed randomly to groups upon arrival, and offered unlimited access to standard rodent food (Mucedola Global Diet 2018, Harlan, Italy) and water. After a 4 days adjustment period, the lactose-treated group received a daily oral gavage, every morning, of 3mg, 5mg, or 15mg lactose (β-lactose, Sigma Aldrich, France) in 200 µl saline solution, the control group received only saline, for 3 weeks.

Lactose-pyridoxamine experiment: 80 adult male C57Bl/6 mice (Janvier, Le Genest St Isle, France) of 6 weeks old were housed in polypropylene cages in groups of 8 without mixing experimental groups, mice were distributed randomly to groups upon arrival, and offered unlimited access to standard rodent food (Mucedola Global Diet 2018, Harlan, Italy) and water. After a 4 days adjustment period, the lactose-pyridoxamine treated group received a daily oral gavage, every morning, of 5mg lactose (β-lactose, Sigma Aldrich, France) and/or 5mg pyridoxamine (pyridoxamine dihydrochlorate, Sigma Aldrich, France) in 200 µl saline solution, the control group received only saline, for 3 weeks.

Fructo-oligosaccharide experiments: 50 adult male C57Bl/6 mice (Janvier, Le Genest St Isle, France) of 6 weeks old were housed in polypropylene cages in groups of 10 without mixing experimental groups, mice were distributed randomly to groups upon arrival, Animals received a custom modified AIN-93M diet ad libitum, containing 0% or 10% fructo-oligosaccharides (corn-starch partly substituted for fructo-oligosaccharides) (supplementary data table S1), complemented with or without 1mg/mL pyridoxamine in drinking water.

Mice scheduled for immunofluorescence assays 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. Caeca were resected and weighed. Mouse fecal pellets were collected directly from the anus on the last day of the experiment, and collected in 0,5 mL Eppendorf tubes, frozen in liquid nitrogen, and stored at -80°C for downstream analyses.

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).

Abdominal sensitivity

Visceral sensitivity (Electromyography (EMG))

Lactose/ Lactose-pyridoxamine experiments: Under xylazine/ketamine anaesthesia (both 1.2 mg, subcutaneously), two nickel–chrome electrodes were implanted into the abdominal external oblique

muscle and a third into the abdominal skin and exteriorised on the back of the neck. On the fifth to seventh postoperative day, colorectal distensions were used as noxious stimuli to evaluate visceral hyperalgesia by electromyographic (EMG) recording. Polyethylene perfusion and distension catheters (Fogarty catheter for arterial embolectomy, 4F, Edwards Lifesciences, Nijmegen, The Netherlands) were inserted into the colon. The colorectal distension procedure started 60 min after habituation to the tunnel, progressively increasing in 0.02 mL steps, from 0 to 0.08 mL, each step lasting 10 s with 5 min non-distension periods in between. During the distension periods, the striated muscle's EMG activity was recorded and analysed according to Larsson, Arvidsson, Ekman, *et al.* [24]. Basal EMG activity was subtracted from the EMG activity registered during the periods of distension. Method adapted from Gecse, Roka, Ferrier, *et al.*[25]. Statistical analysis: Two-way ANOVA, multiple comparisons between all groups of the same distension, Tukey's correction for multiple comparisons.

Mechanical behavioral testing (von Frey)

Fructo-oligosaccharide experiment: Animals were placed upon an elevated mesh floor surrounded by a clear plastic enclosure (10 × 10 × 10 cm). Mechanical sensitivity was assessed using three von Frey filaments with bending force 0.16, 0.6 and 1.4 g (Bioseb Inc., France). In ascending order of force, each filament was applied for a duration of 2 seconds to the mid-plantar area of each hind paw five times, with 3 seconds between each application. Rapid retraction, shaking and/or licking of the hind paw were considered to represent nociceptive specific behaviors and only one of these responses needed to be displayed to be considered as a positive withdrawal response. Applications were applied to both hind paws, counted and then expressed as an overall percentage response. The performing researcher was blind to which experimental groups mice belonged. Statistical analysis: Two-way ANOVA, multiple comparisons between all groups for the same filament, Tukey's correction for multiple comparisons.

Microbiota analysis

Fecal DNA extraction, 16S rRNA gene sequencing, and bacterial community analysis

For materials and methods, please refer to the supplementary data.

Immunofluorescence

Histological sample preparation

Collected tissues from animals that did not undergo the visceral sensitivity protocol 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: 2x 60min 100% ethanol, 2x 60min butanol, 480min butanol, 3x 80min paraffin at 60 °C. Tissue samples were included in paraffin blocks using a Thermo

Scientific HistoStar Embedding Workstation. 5µm tissue sections were made using a Microm HM 340 E microtome and attached to Superfrost Plus microscope slides (Thermo Scientific, USA).

MMCP/AGER: 5µm paraffin embedded sections were deparaffinated by using 3x 5min baths of American Mastertech Clearify followed by 3x 5min 100% ethanol, 3x 5min 95% ethanol, 2x 5min 70% ethanol, 5min demineralised water. Slides were washed 2x in PBS for 5min, followed by a 2-hour blocking step with 10% donkey serum in PBS, and washed 3x 5min 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 or Polyclonal Goat-anti-AGER (ab7764 (Abcam, UK)) diluted 1:400)), followed by 2x 5min rinsing steps in PBS. Incubation with secondary antibody ((Alexa Fluor 594 Donkey-anti-Sheep (A-11016 (Molecular Probes, USA) for MMCP) or (Alexa Fluor 488 Donkey-anti-Goat (A-11055 (Molecular Probes, USA) for AGER)) diluted 1:400 in 1% donkey serum PBS was performed for 2 hours, followed by 3x 5min washing steps in PBS, a quick rinse with tap water, followed by mounting using ProLong Gold® antifade reagent with DAPI (Thermo Fisher Scientific, USA).

Microscopy

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

Immunofluorescence analyses and statistics

Mast cell count analyses: One ratio mastocyte:crypt per mouse based on analysis of 50-250 crypts, dependent on availability of suitable visual material. Image sets were coded, randomised and analysed blindly. Statistical analysis: One-way ANOVA, multiple comparisons with Tukey's correction for multiple comparisons.

AGER expression analyses: Fluorescence intensity of 3 suitable regions of epithelium was analysed per microscope field, 4 microscope fields per mouse were analysed. Only epithelial cells were selected, without goblet cells, because these presented high unspecific staining throughout. Image sets were coded, randomised and analysed blindly. Statistical analysis: One-way ANOVA, multiple comparisons with Tukey's correction for multiple comparisons.

Physiological markers of colonic content transit

Transit time: For this experiment, after 2.5 weeks of intervention, 5 mice per group were temporarily housed in individual cages, 0.15 mL paper-filtered saturated carmine red in physiological salt solution was administered by intragastric gavage, and starting from 2 hours after administration, every 15 minutes or when the researcher noted defecation, the cages were checked for red-

coloured droppings, the time of first appearance of a red dropping was used to determine the transit time. Statistical analysis: One-way ANOVA, multiple comparisons with Tukey's correction for multiple comparisons.

Fecal water content: Droppings were collected in weighted tubes, weighed to determine wet weight, and dried in an oven at 80°C for 48 hours. Tubes were weighed again and used to determine the dry weight. Fecal humidity is the percentage of weight lost between wet and dry weights. Statistical analysis: One-way ANOVA, multiple comparisons with Tukey's correction for multiple comparisons. To compare Ctrl v Fructo-oligosaccharides: Student's t-test.

Verification of intestinal inflammation

Lipocalin-2 ELISA: Fecal supernatants were prepared by grinding 0.2g feces in 1 mL demineralized water with 5 ceramic beads using a Fast-Prep (MP Biomedicals, Illkirch, France) (3x 15sec 6m/s with 1 min breaks on ice) followed by 20min centrifugation at 8000x, supernatants were collected in 1.5 mL Eppendorf tubes and stored at -20°C until use. ELISAs were performed according to instructions provided by the manufacturer (DuoSet ELISA, Mouse Lipocalin-2/NGAL (DY1857)) (R&D Systems, MN, USA). One-way ANOVA, multiple comparisons with Tukey's correction for multiple comparisons.

Microscopic score: For materials and methods, please refer to the supplementary data.

Results

Increase in sensitivity after FODMAP administration

Oral gavage with lactose for 3 weeks increased visceral sensitivity compared to control. These increases were statistically significant for the two highest lactose concentrations for both 0.06 mL and 0.08 mL volume of distension ($P \leq 0.05$) (Supplemental Figure 1). Based on these results and to reflect a modest lactose consumption, subsequent experiments with lactose were performed using 5mg lactose daily. In animals treated with 5mg lactose and/or 5mg pyridoxamine, visceral sensitivity was also increased in the lactose-treated group by ±70 % ($P<0.01$), ±55% ($P<0.0001$) versus control, at a distension volume of 0.06 mL and 0.08 mL respectively (Figure 1). Co-administration with pyridoxamine effectively reversed the effect of lactose to basal visceral sensitivity response, such as observed in the control group (no significant differences between Lact+Pyr, Pyr and control), Lact+Pyr decreased ±38% ($P<0.01$), ±36% ($P<0.0001$) v Lact at a distension volume of 0.06 mL and 0.08 mL respectively, and Pyr was reduced ±30% ($P<0.05$), ±31% ($P<0.0001$) v Lact at distension volumes of 0.06 mL and 0.08 mL respectively.

Fructo-oligosaccharides significantly increased the abdominal sensitivity in response to mechanical stimulation of the hind paw (29% fructo-oligosaccharides v 14% control response rate for 0.6g

filament (P<0.05), and 47% v 30% response rate for 1.4g filament (P<0.05)). These increased abdominal sensitivities were reduced by pyridoxamine (21% fructo-oligosaccharides-pyridoxamine v 29% fructo-oligosaccharides for 0.6g filament, and 39% fructo-oligosaccharides-pyridoxamine v 47% control response rate for 1.4g filament)(Figure 2), but not significantly so.

Mast cell analysis of colonic mucosae

Both lactose and fructo-oligosaccharides significantly (P<0.01 v Ctrl and Lact+Pyr, P<0.001 v Pyr, P<0.0001 v Ctrl and fructo-oligosaccharides+Pyr) increased the number of mucosal mast cells in the proximal colon (Figure 3) versus their respective other groups. Lactose did not significantly increase mast cell counts in the distal colon (Supplemental Figure 4), while the fructo-oligosaccharide diet increased mast cells in both proximal and distal colon (Supplemental Figure 5).

Expression of AGER in epithelial cells of the proximal colon

AGER immunofluorescence intensities in proximal colon epithelial cells were higher for lactose and fructo-oligosaccharides groups (P<0.0001), compared to all other groups (Figure 4). The FOS+pyridoxamine group showed significantly lower AGER expression than the respective control group (P<0.01).

Transit time, fecal water content, and cecal weight

The fructo-oligosaccharide-enriched diet increased the fecal water content and cecal weight and reduced the transit time compared to the control diet. Oral gavage of 5mg/d lactose had no significant impact on these parameters. Addition of pyridoxamine did not impact these characteristics in control or FODMAP-treated animals (Figure 5).

Microbiota

Lactose and/or pyridoxamine did not induce significant alterations in the composition of the fecal microbiota, as demonstrated quantitatively at the OTU level by the prevalence and abundance of the detected OTUs (Figure 6A) and phyla (Figure 6B) respectively. Whichever index of alpha-diversity tested, neither lactose nor pyridoxamine nor a combination of the two significantly altered the number of OTUs, as indicated by the index of richness (chao-1) and evenness (Shannon) (Figure 6C). Multidimensional scaling analysis of unweighted or weighted UniFrac distances revealed no inter-sample difference linked to experimental group (Figure 6D). None of the OTUs agglomerated at the genus rank were significantly affected by FODMAP exposure by using DESeq2.

Intestinal inflammation

Levels of the sensitive inflammation biomarker Lipocalin-2 were at low, non-inflamed, levels in all animals (Figure 7). Similarly, microscopic scoring revealed no inflammation in any of the experimental groups (Supplementary Figures 2 & 3).

Discussion

We have shown that both oral administration of lactose and a high-fructo-oligosaccharides diet can lead to pro-nociceptive effects, as well as an increase in colonic mucosal mast cell counts. Interestingly, these effects were common to the two different FODMAPs at different doses. This indicates that the intake of FODMAPs alone can increase these sensitivities. The dose of lactose (5mg per day) used was chosen to correspond to a modest intake of milk (relative amount of 1 glass of milk for a human of average size), where it has to be noted that mice do not retain lactase activity after weaning and are therefore lactose malabsorbers[26]. For fructo-oligosaccharides, we chose to administer 10% of the total diet based on a previously reported pertinent dose in mice [27]. Another recent study comparing the effects of different fibers on short-chain fatty acids (SCFA), using doses of 10%, showed that fructo-oligosaccharides had different effects on SCFA production than lignin or resistant starch[28]. Because rodent diets naturally contain a great deal more fructans and glycans than a conventional human diet, to be able to see the effects of an oligosaccharide in mice, we chose this higher dose than would be used in humans.

At lower doses of fructo-oligosaccharides (approximately 5% of total dietary intake, half of that used in this work), administered through intragastric gavage, Chen *et al*. observed an increased susceptibility to WAS (water avoidance stress)-induced IBS symptoms, but no difference between fructo-oligosaccharides -treated or control animals in the absence of WAS. This might indicate that WAS, used as a model for IBS, increases the susceptibility to FODMAP induced effects, which could explain why IBS patients but not healthy controls report increased discomfort when eating FODMAPs[29]. Additionally, this indicates that without further challenge mice tolerate this dose of FOS, which would be excessive in humans, without symptom generation.

The observed effects of lactose and the fructo-oligosaccharide diet were prevented by co-administration of with pyridoxamine, a recognized anti-glycation agent [30-35], indicating the involvement of glycation processes in the generation of the effects observed for both FODMAPs. Accordingly, the expression of AGER on colonic epithelial cells increased in response to the FODMAP experiments, and was, likewise, prevented by pyridoxamine co-administration.

The generation of glycating agents in the colonic lumen was evaluated by LC-MS analysis of carbonyl compounds in colonic contents (Supplementary Figure 4). Indeed, a subset of these compounds in colonic contents was significantly increased for FODMAP treated animals versus their respective controls. Taking into account the high chemical reactivity of certain carbonyl compounds with proteins, we can speculate that the amounts of such compounds produced in the colonic lumen of the FODMAP treated animals are sufficient to generate glycation end products, contributing to the

FODMAP-induced effects. Interestingly, despite the prevention of the FODMAP-induced effects by pyridoxamine, no specific differences were detected in the carbonyl compound profiles of the FODMAP versus the FODMAP+pyridoxamine groups, in the colonic content supernatants (Supplemental Figure 3). This can be explained by the strong capacity of the small intestine to absorb pyridoxamine[36], leading to systemic, rather than local protection of the gut tissues against glycating reactions, in a similar way as observed in work studying the prevention of glycation reactions by pyridoxamine in animal models of diabetes[33, 37, 38]. This also implies that the generation of AGEs *in vivo* by interaction with absorbed carbonyl compounds generated during FODMAP fermentation is at the basis of the observed effects, rather than the AGE load of the luminal contents.

We observed an increase in AGER expression in the mucosa of animals treated with lactose or fructo-oligosaccharides, which was, again, reversible by co-administration of pyridoxamine, indicating that glycation processes and activation of advanced glycosylation end-product specific receptor (AGER) are involved in the induction of visceral and abdominal sensitivity in our animal model. *In vitro*, AGEs have been shown to interact directly with mast cells, rapidly triggering mast cell exocytosis dose-dependently [22]. This interaction could be prevented by blocking access to AGER by using an antibody, showing it depends on the interaction between AGEs and AGER [22]. The observed hypermastocytosis in our animals was prevented by co-administration with pyridoxamine too, indicating it is part of the same pathway that is responsible for the increased sensitivity, and indeed an increased mast cell count in tissues is reported as a possible factor in increased visceral sensitivity of IBS patients [39, 40]. In IBS patients on a low FODMAP diet, histamine levels in the urine dropped eight-fold compared to a high-FODMAP intervention[41], supporting the idea that dietary FODMAPs can be responsible for an increase in mastocyte proliferation. Mast cell counts and mast cell mediator production are associated [42], and mast cell mediator release in turn can lead to mast cell hyperplasia [43]. It has been reported that histamine levels are increased in the mucosa of IBS patients [40, 44] and it can be directly involved in the sensitization of TRPV1 by its action on HRH1, which can cause symptom generation in IBS patients [45]. In short, our findings support the idea that anaerobic microbial fermentation of FODMAPs can lead to production of glycating agents, which increase the AGE-load locally in the colon, inducing expansion of the mucosal mastocyte population, and by mastocyte-nerve cell interactions, increasing visceral sensitivity.

Analysis of intestinal microbiota profiles of lactose experiment groups did not uncover significant differences between the profiles of the 4 groups; control, lactose, lactose-pyridoxamine, and pyridoxamine. It is well-known from literature that fructo-oligosaccharides changes the microbiota composition [46, 47], but as indicated by the reversibility of the effects by pyridoxamine in both experimental groups, and the lack of effect of pyridoxamine on microbiota composition, we assess

that the effects of the FODMAPs were mostly due to microbial metabolic changes, rather than a possible dysbiosis. This could alternatively indicate that no significant malabsorption occurs at this modest dose of lactose at 5mg/day, administered by a single gavage, but it's unlikely that we would have observed effects on visceral sensitivity and mast cell numbers if lactose were completely hydrolyzed. Additionally, as mentioned before, we did find differences in carbonyl compound profiles between control and FODMAP-treated animals, which would be surprising if lactose were hydrolyzed and absorbed completely in the small intestine. The generation of dicarbonyl compounds every day immediately after exposition to lactose, while only shortly present due to their reactivity, could lead to cumulative effects over time during the experiment because of generation of longer-lasting AGEs, and an increased RAGE expression, even if lactose at this dose did not lead to alteration in transit markers as could be expected for lactose malabsorption. Conversely, it has recently been reported that a diet high in FODMAPs can induce intestinal inflammation represented by increased mucosal expression of IL-1β, IL-6, IL-17, TNF-α, and IFN-γ, a visceral hypersensitivity, and an increased intestinal permeability in rats, by changing the gut microbiota composition and increasing levels of lipopolysaccharides[48]. We have not observed such changes in the microbiota in our work, nor have we observed intestinal inflammation in either lactose or fructo-oligosaccharides-treated groups, according to fecal lipocalin-2 levels (Figure 7) and general histology (Supplemental Figure 2), and while IBS does not normally present with these kind of inflammatory markers [49], this interesting work of Zhou *et al.* indicates that FODMAPs can induce symptoms in even more ways than previously expected.

We have not observed changes in bowel movement characteristics (fecal water content, output, transit time) in our mice treated with lactose, in contrast to the mice treated with fructo-oligosaccharides, which showed both a higher water content and decreased transit time compared to control, though the basic diet between these 2 groups was not the same, and basal characteristics between the 2 control groups were not identical. In our experiments, the application of the FODMAP representatives is dissimilar between lactose and fructo-oligosaccharides. Lactose was administered once daily, diluted in saline, whereas fructo-oligosaccharides were present in the animal feed, as a percentage of the regular diet. This means that lactose represented chronic acute challenges over 3 weeks, while fructo-oligosaccharides had a permanent and bulkier presence. It is not surprising then, that fructo-oligosaccharides had significantly more effects on transit time and fecal output than lactose. It is also for this reason that we used the alternative analysis of sensitivity in the fructo-oligosaccharide-experiment, as fructo-oligosaccharides lead to increased amounts of intestinal content that prevented emptying of the colorectal cavity even after fasting and habituation periods, impeding a reliable distension procedure. The measure of effects on transit related markers (cecal

weight, fecal water content) in response to exposition to FOS in our experiments indicate that this carbohydrate is not completely fermented and so does not mirror the human situation in a dietary context. However, as mentioned before, administration of FOS at 5% of dietary intake does not induce symptoms in mice without further challenge[29], pointing to the different tolerance to these kind of compounds in a rodent model. In contrast to effects related to aldehyde generation, pyridoxamine did not reverse fecal water content differences between control and fructo-oligosaccharide diets (data not shown), indicating that, like for the lactose-treated groups, differences in transit time were not responsible for the observed symptom generation for this group either, although modification of transit time itself can be seen as a symptom too.

It has been clearly demonstrated that FODMAP ingestion increases water and fermentable material to the proximal colon [50], and can lead to distension through increased chyme volume and production of gas [51]. However, it has been found that increased sensitivity rather than increased distension is the cause for complaints in IBS patients related to FODMAP consumption [11] not taking away the fact that decreasing the FODMAP intake should reduce this distension. The short-term effects observed upon reducing FODMAP intake in patients are probably thanks to a reduction in distension obtained, whereas the effects obtained in our animal model are on a longer time-scale, in which FODMAPs are involved in modulating sensitivity itself. If FODMAP fermentation products increase mast cell numbers, factors that activate mast cell product release such as psychological stress[52, 53] will have a greater effect because there is a larger population to receive these activating signals. It is unlikely that FODMAPs can cause IBS by themselves, but by the effects that we describe, we can conclude that they can cause physiological changes possibly responsible for symptoms of IBS. Recent work by Wilder-Smith *et al.* in a large patient cohort indicates that Central Nervous System (CNS-) as well as gastro-intestinal (GI-)symptoms are increased in the short-term following lactose and fructose ingestion, likely explained by microbial metabolites [54]. This illustrates again that FODMAP intake can be linked to other symptoms of FGIDs besides those induced by increased (osmotic) distension.

Our study shows that the role of FODMAPs in IBS is multifactorial; apart from the previously reported osmolarity and distension related symptom-generation caused by dietary FODMAPs, reactive carbonyl fermentation products of their microbial processing can cause physiological changes in the colon, specifically reminiscent of IBS. These insights may contribute to the development of functional nutritional strategies focused on the prevention of glycation reactions caused by microbial toxic metabolites.

Acknowledgments

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Figure legends

Figure 1: Increase in visceral sensitivity after daily gavage with lactose (Lact) and/or pyridoxamine (Pyr) for 3 weeks, in response to increasing volumes of distension. Sensitivity is expressed as a percentage of the maximum average control response. N=12

Figure 2: Increase in sensitivity to mechanical stimulation of the hind paw, after following a diet high in fructo-oligosaccharides (FOS), with or without pyridoxamine (Pyr) (1mg/mL) in drinking water. N=10

Figure 3: (A) Representative images of mucosal mast cell immunofluorescent staining (mast cells: red; DAPI nuclear counterstain: blue) of proximal colon of animals treated with 5mg lactose and/or pyridoxamine for 3 weeks. (B) Mucosal mast cell (MC) counts of proximal colon in animals treated daily with 5mg lactose (Lact) and/or pyridoxamine (Pyr) for 3 weeks, expressed as MC/crypt. (C) Representative images of mucosal mast cell immunofluorescent staining (mast cells: red; DAPI nuclear counterstain: blue) of proximal colon of animals following a diet high in fructo-oligosaccharides (FOS), with or without pyridoxamine (1mg/mL) in drinking water. (D) Mucosal mast cell (MC) counts of proximal colon of animals following a diet high in fructo-oligosaccharides, with or without pyridoxamine (1mg/mL) in drinking water for 3 weeks, expressed as MC/crypt.

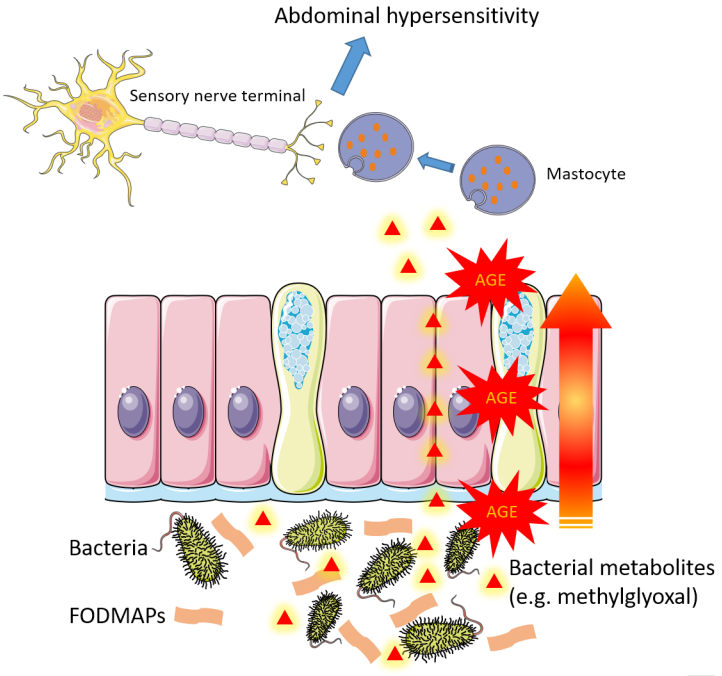
Figure 4: (A) Representative images of epithelial AGER immunofluorescent staining (AGER: green; DAPI nuclear counterstain: blue) of proximal colon of animals treated with 5mg lactose (Lact) and/or pyridoxamine (Pyr) for 3 weeks. (B) Intensity of AGER staining in mucosal epithelial cells of proximal colon of lactose experiment. (C) Representative images of epithelial AGER immunofluorescent staining (AGER: green; DAPI nuclear counterstain: blue) of proximal colon of animals following a diet high in fructo-oligosaccharides (FOS), with or without pyridoxamine (1mg/mL) in drinking water. (D) Intensity of AGER staining in mucosal epithelial cells of proximal colon of fructo-oligosaccharide experiment.

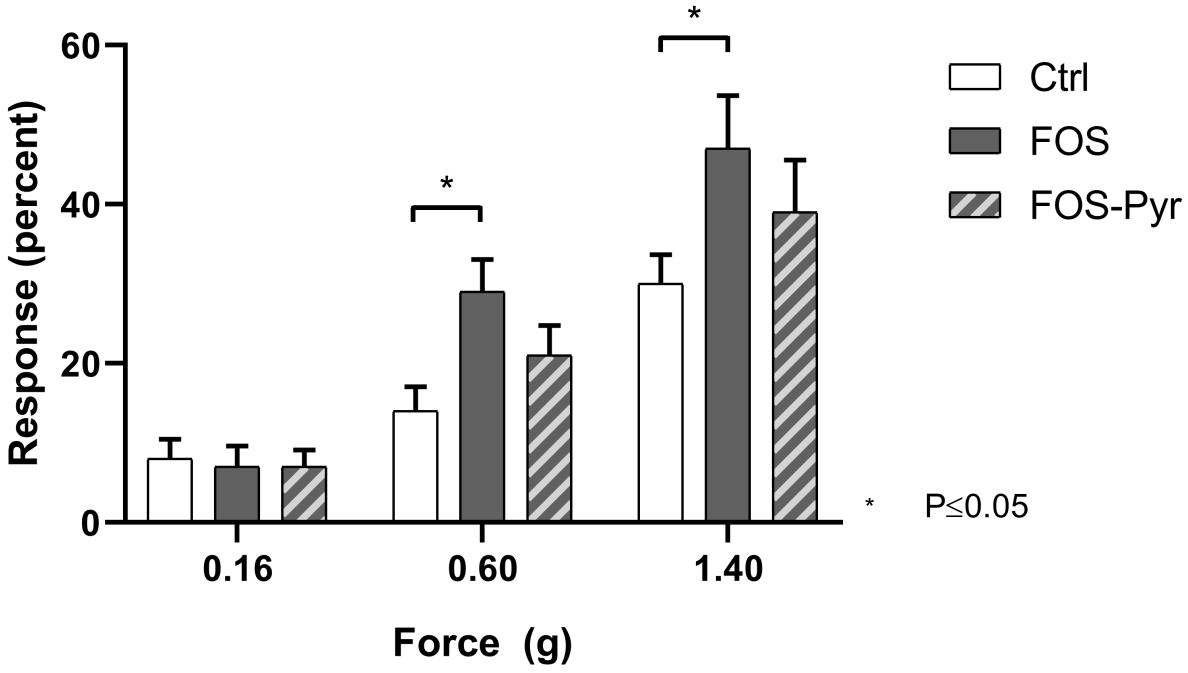
Figure 5: Fecal water contents of animals treated with FODMAPs; lactose (lact), fructo-oligosaccharides (FOS) (A) Fecal water content (B) cecal weight and (C) transit time of lactose groups. (D) Fecal water content (E) cecal weight and (F) transit time of animals following a control, or a fructo-oligosaccharide-enriched diet.

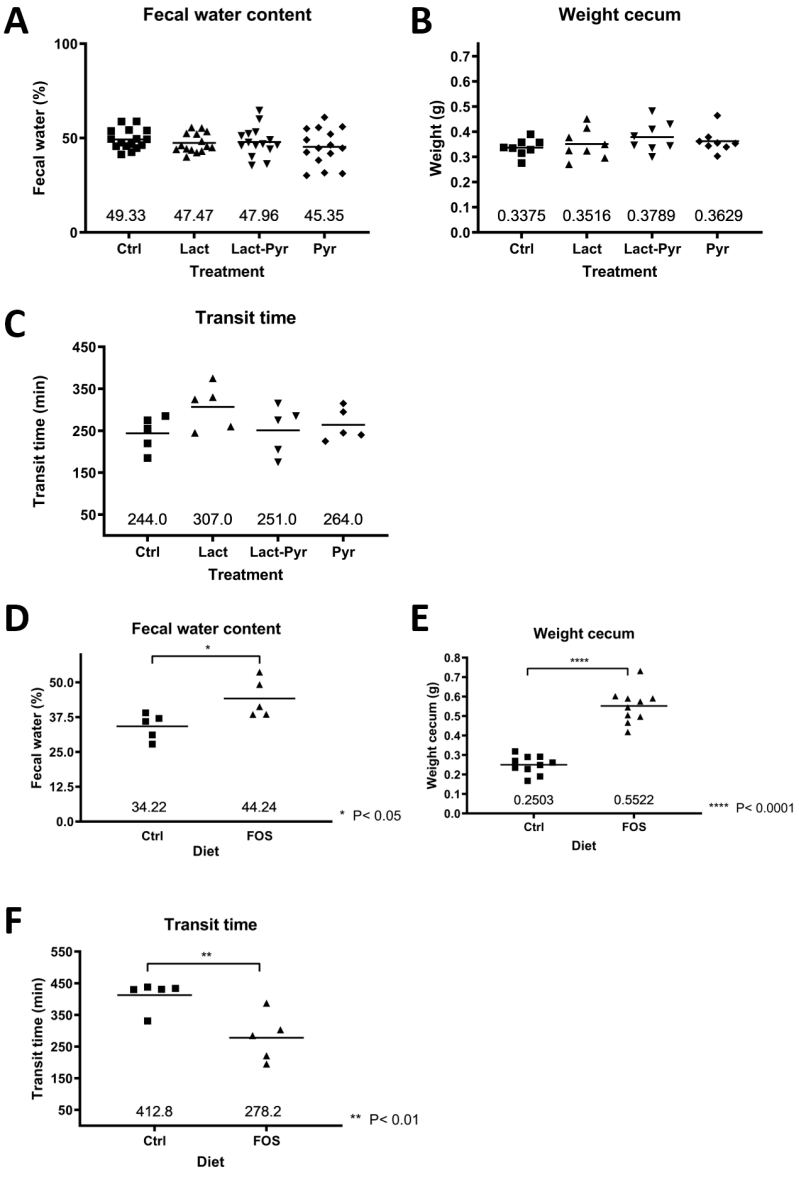
Figure 6: Effect of lactose (Lact) and/or pyridoxamine (Pyr) on the community distribution and diversity of the fecal microbiota as determined by 16S rRNA gene Illumina Miseq sequencing of animals treated with 5mg lactose and/or pyridoxamine daily. (A) Prevalence per OTU in samples according to groups (B) Relative abundance (%) per phylum according to groups (C) Richness (α-

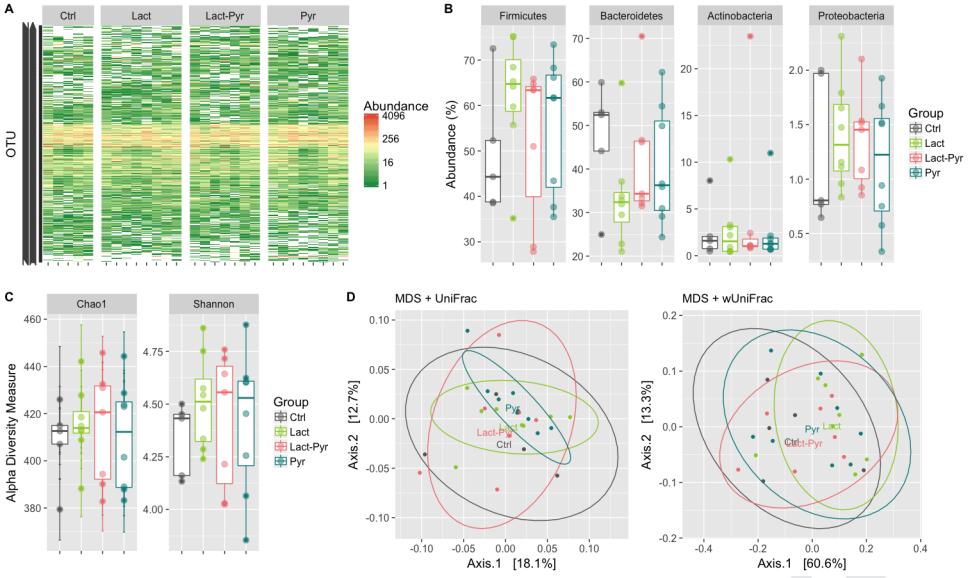
diversity) measured by Chao1 and Simpson Indexes (D) Unweighted (left) and Weighted (right) Unifrac Multidimensional Scaling (MDS) plots representing structural changes between groups (β-diversity). The fraction of diversity captured by the coordinate is given as percentage.

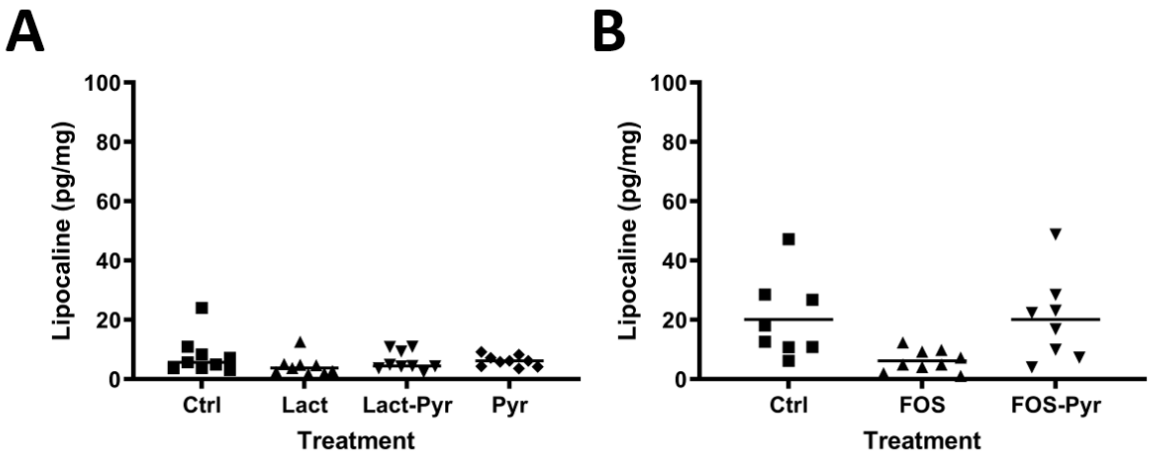
Figure 7: Lipocalin-2 concentrations of feces, expressed as pg/mg feces, of animals treated with lactose (A), or fructo-oligosaccharides (B) and/or pyridoxamine. None of the animals showed increased lipocalin levels indicative of inflammation.

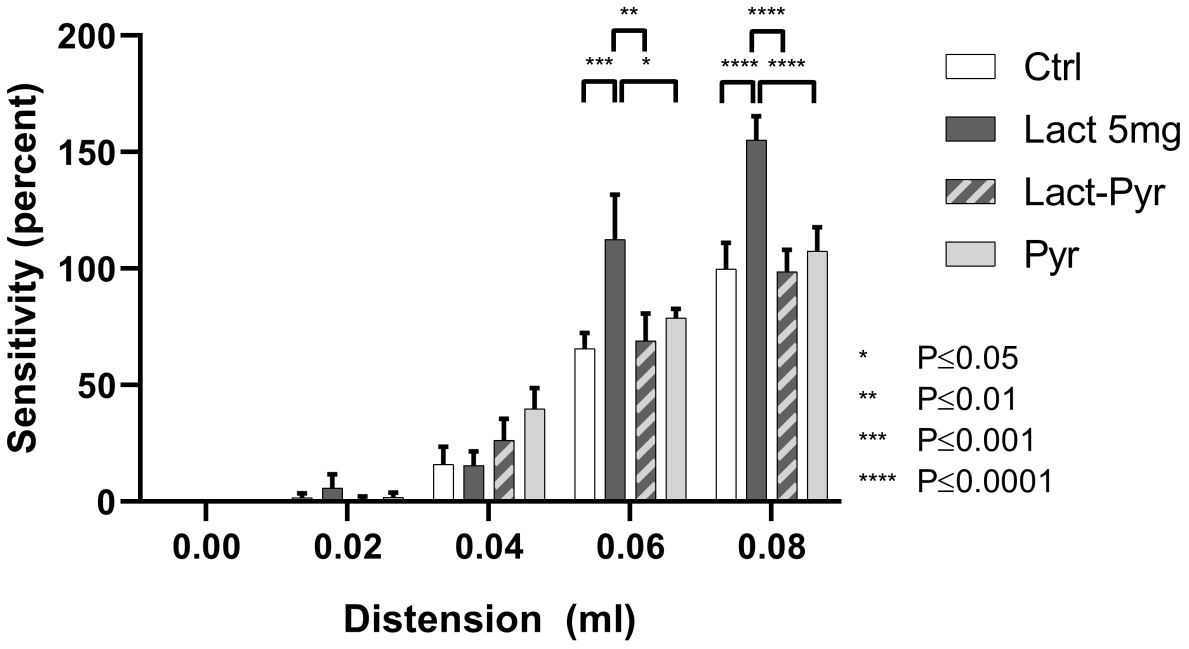


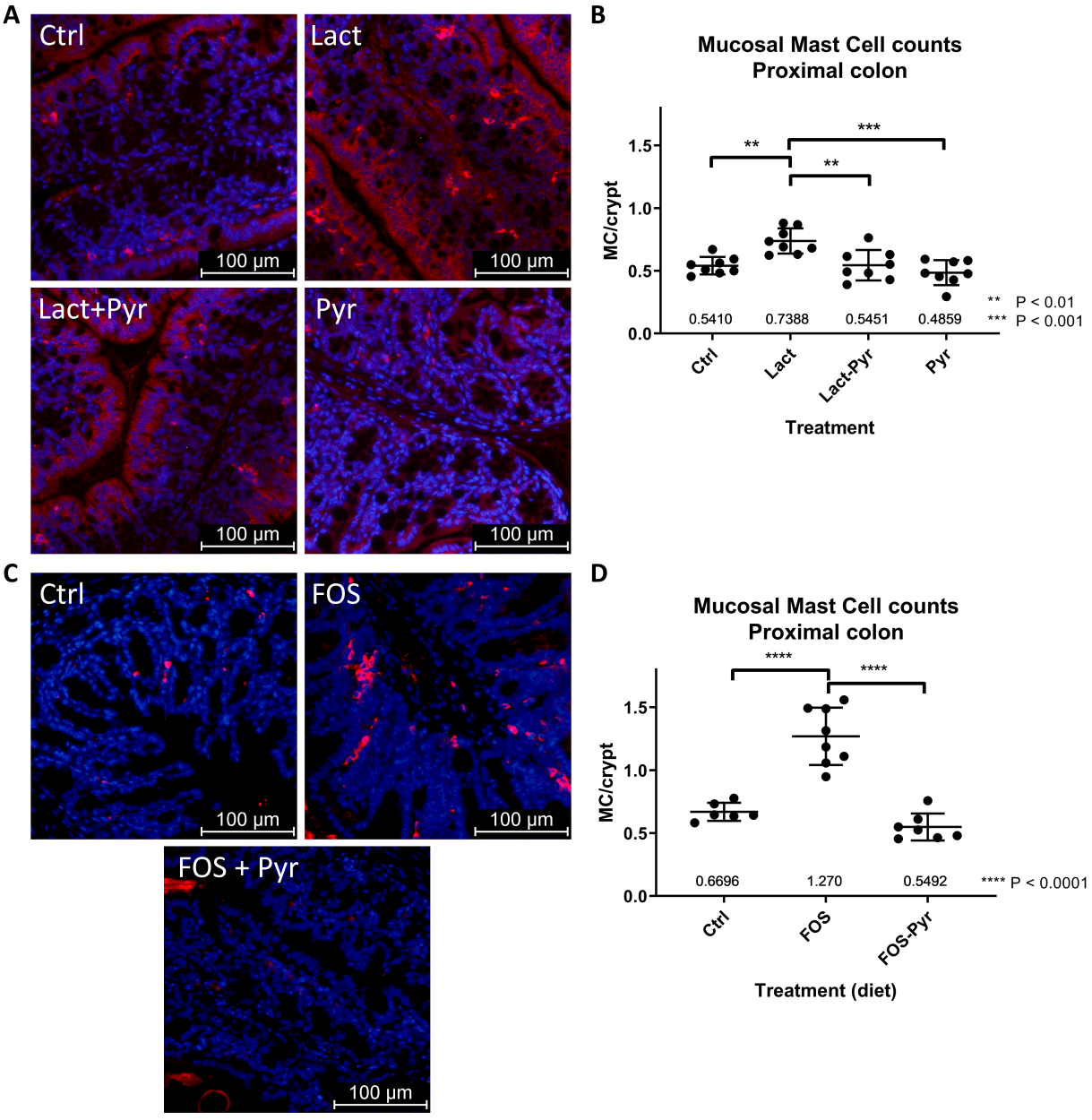


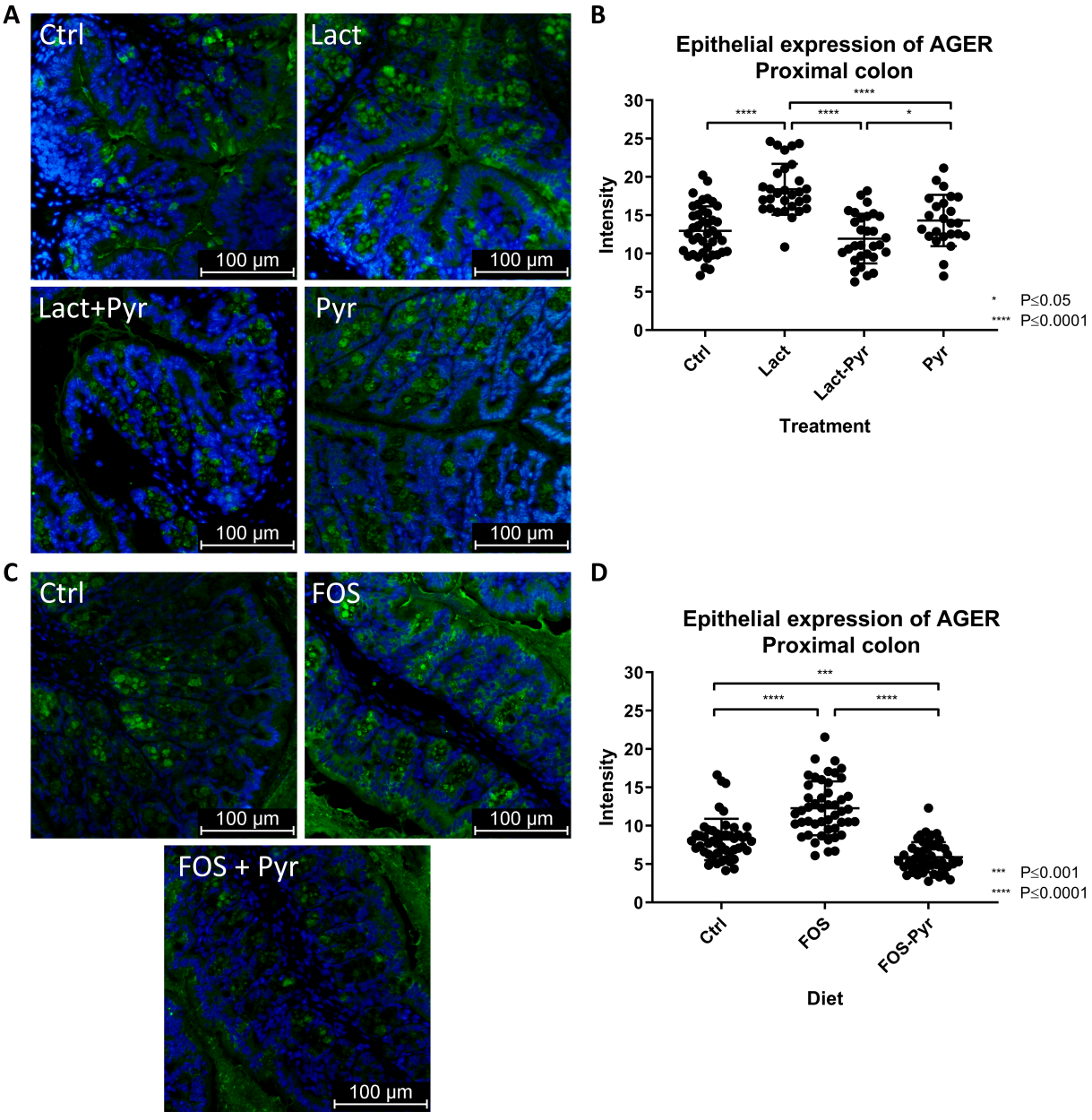

A
Fecal water content
Fecal water (%)
100
50
0
49.33
47.47
47.96
45.35
Ctrl
Lact
Lact-Pyr
Pyr
Treatment
B
Weight cecum
Weight (g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.3375
0.3516
0.3789
0.3629
Ctrl
Lact
Lact-Pyr
Pyr
Treatment
C
Transit time
Transit time (min)
450
350
250
150
50
244.0
307.0
251.0
264.0
Ctrl
Lact
Lact-Pyr
Pyr
Treatment
D
Fecal water content
*
Fecal water (%)
50.0
37.5
25.0
12.5
0.0
34.22
44.24
* P< 0.05
Ctrl
FOS
Diet
E
Weight cecum
****
Weight cecum (g)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.2503
0.5522
**** P< 0.0001
Ctrl
FOS
Diet
F
Transit time
**
Transit time (min)
550
450
350
250
150
50
412.8
278.2
** P< 0.01
Ctrl
FOS
Diet






Sensitivity (percent)
200
150
100
50
0
0.00
0.02
0.04
0.06
0.08
Distension (ml)
**
****
***
*
****
****
Ctrl
Lact 5mg
Lact-Pyr
Pyr
* P≤0.05
** P≤0.01
*** P≤0.001
**** P≤0.0001


A
Ctrl
Lact
Lact+Pyr
Pyr
100 µm
B
Mucosal Mast Cell counts
Proximal colon
MC/crypt
1.5
1.0
0.5
0.0
**
**
***
0.5410
0.7388
0.5451
0.4859
** P < 0.01
*** P < 0.001
Ctrl
Lact
Lact-Pyr
Pyr
Treatment
C
Ctrl
FOS
FOS + Pyr
100 µm
D
Mucosal Mast Cell counts
Proximal colon
MC/crypt
****
****
1.5
1.0
0.5
0.0
0.6696
1.270
0.5492
**** P < 0.0001
Ctrl
FOS
FOS-Pyr
Treatment (diet)


A
Ctrl
Lact
Lact+Pyr
Pyr
100 µm
B
Epithelial expression of AGER
Proximal colon
Intensity
Ctrl
Lact
Lact-Pyr
Pyr
Treatment
* P≤0.05
**** P≤0.0001
C
Ctrl
FOS
FOS + Pyr
100 µm
D
Epithelial expression of AGER
Proximal colon
Intensity
Ctrl
FOS
FOS-Pyr
Diet
*** P≤0.001
**** P≤0.0001

Supplementary data

Materials and Methods - Microbiota analysis

Faecal DNA extraction, 16S rRNA gene sequencing, and bacterial community analysis; Genomic DNA was obtained from frozen faeces using the ZR Faecal DNA Miniprep™ kit (Zymo Research). The microbial 16S rRNA gene was amplified during the first PCR step with adapter fusion primers targeting the V3 to V4 regions (corresponding to a 460-bp region of *Escherichia coli* 16S rRNA gene, GenBank number J01695 with bacterial forward 343F (TACGGRAGGCAGCAG[1]) and reverse 784R (TACCAGGGTATCTAATCCT[2]) primers. Pooled amplicon libraries were sequenced employing an Illumina MiSeq (2 x 250 bp) at the GeT-PlaGe platform in Toulouse (France).

Sequence reads were quality controlled and high quality filtered reads were further processed using FROGS pipeline (Find Rapidly OUT with Galaxy Solution) to obtain OTUs and their respective taxonomic assignment thanks to Galaxy instance (http://sigenae-worbench.toulouse.inra.fr)[3]: an initial FROGS pre-processing step which allows to select overlapping reads with expected length without N. Swarm clustering method was applied by using a first run for denoising with a distance of 1 and then a second run for clustering with a maximal aggregation distance of 3 on the seeds of the first Swarm. Putative chimerae were removed using Vsearch combined with cross-validation (GitHub repository. DOI:10.5281/zenedo.15524). Cluster abundances were filtered at 0,005%[4] and/or had to be present at least in 3 samples. 100% of clusters were affiliated to OTU by using the silva132 16S reference database and a taxonomic multi-affiliation procedure (Blast+ with equal multi-hits[5]). Taxonomic assignment at the lowest phylogenetic level and prevalence-based filtering step allowed to obtain of 468 OTUs (after correcting multi-affiliations and some misleading affiliations). Between 15 000 and 22 518 valid sequences per sample were counted.

Richness and diversity indexes of bacterial community, as well as clustering and ordinations, were computed using the Phyloseq package (v 1.19.1) in RStudio software[6, 7]. Within sample community alpha diversity was assessed using Chao-1 and Shannon indexes. Divergence in community composition between samples was quantitatively assessed by calculating both weighted and unweighted UniFrac distance matrices. Unconstrained ordination was visualized using multidimensional scaling (MDS) and compared using Adonis test (9999 permutations).

In order to evaluate differential abundance in response to experimental treatment and identify important taxa modulated by lactose and prevented by pyridoxamine, OTUs were agglomerated at the genus rank. Univariate differential abundance of taxa was tested using a negative binomial noise model for overdispersion as implemented in the R package DESeq2 (v1.14.1[8, 9]). A 2x2 factor design combined with a Wald test was applied. Taxa were considered significantly differentially abundant

between groups if their adjusted P-value was below 0.05 and if estimated change was log2FC>|1.5|. Tests were corrected for multiple inferences using the Benjamini-Hochberg method to control the false discovery rate.

Sequences are available on MG-RAST[10], Project name JK_Lactose, temporary project ID: mgp 90034.

Dietary information

Composition custom AIN93-M +/- Fructo-oligosaccharide (FOS) diets

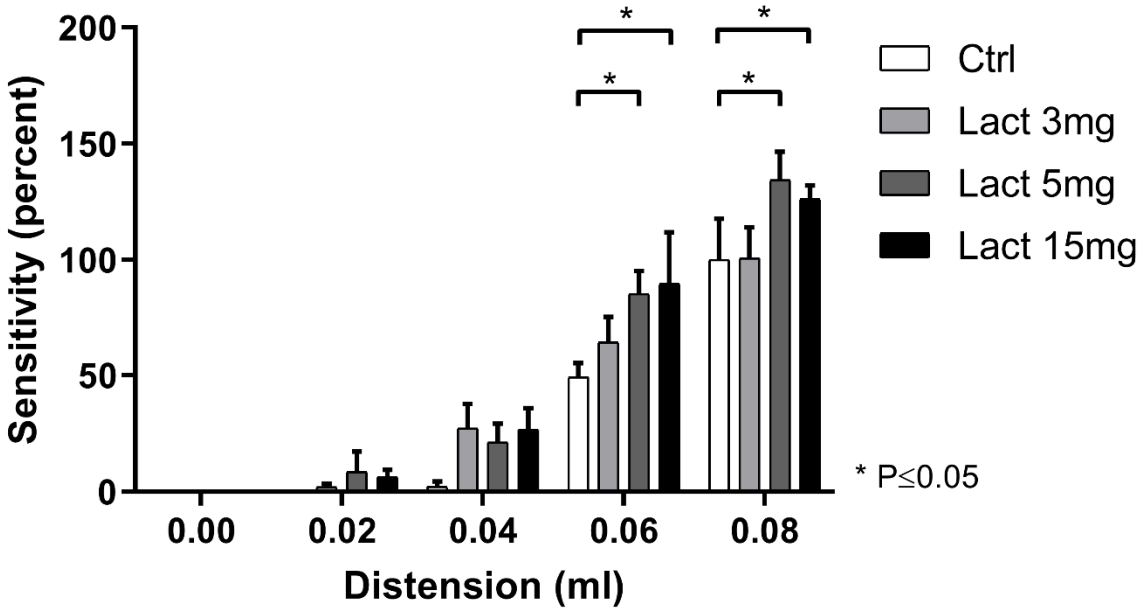
Table S1 - Diet composition AIN93M +/- FOS (g/kg)

	AIN-93M (Energy Density: 3.6 kcal/g)	**AIN93-M-FOS** (Energy Density: 3.4 kcal/g)
Corn-starch	465.692	365.692
Fructo-oligosaccharides	0	100
Casein	140	140
Dextrinized corn-starch	155	155
Sucrose	100	100
Soybean oil	40	40
Powdered cellulose	50	50
Mineral mix (AIN-93M-MX)	35	35
Vitamin mix (AIN-93-VX)	10	10
l-Cystein	1.8	1.8
Choline bitartrate	2.5	2.5
Tert-butylhydroquinone	0.008	0.008

Reference: Reeves, P. G., F. H. Nielsen and G. C. Fahey, Jr. (1993). "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 **123**(11): 1939-1951.[11]

Energy densities based on Reeves *et al.*[11] for AIN-93M, and calculated for AIN93M-FOS using energy densities of 3.7kcal/g for cornstarch, and 1.75kcal/g for FOS[12].

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



Supplemental figure 1 - Increase in visceral sensitivity after daily gavage with different concentrations of lactose for 3 weeks, in response to increasing volumes of distension. Sensitivity is expressed as a percentage of the maximum average control response. N=8

Histological scoring

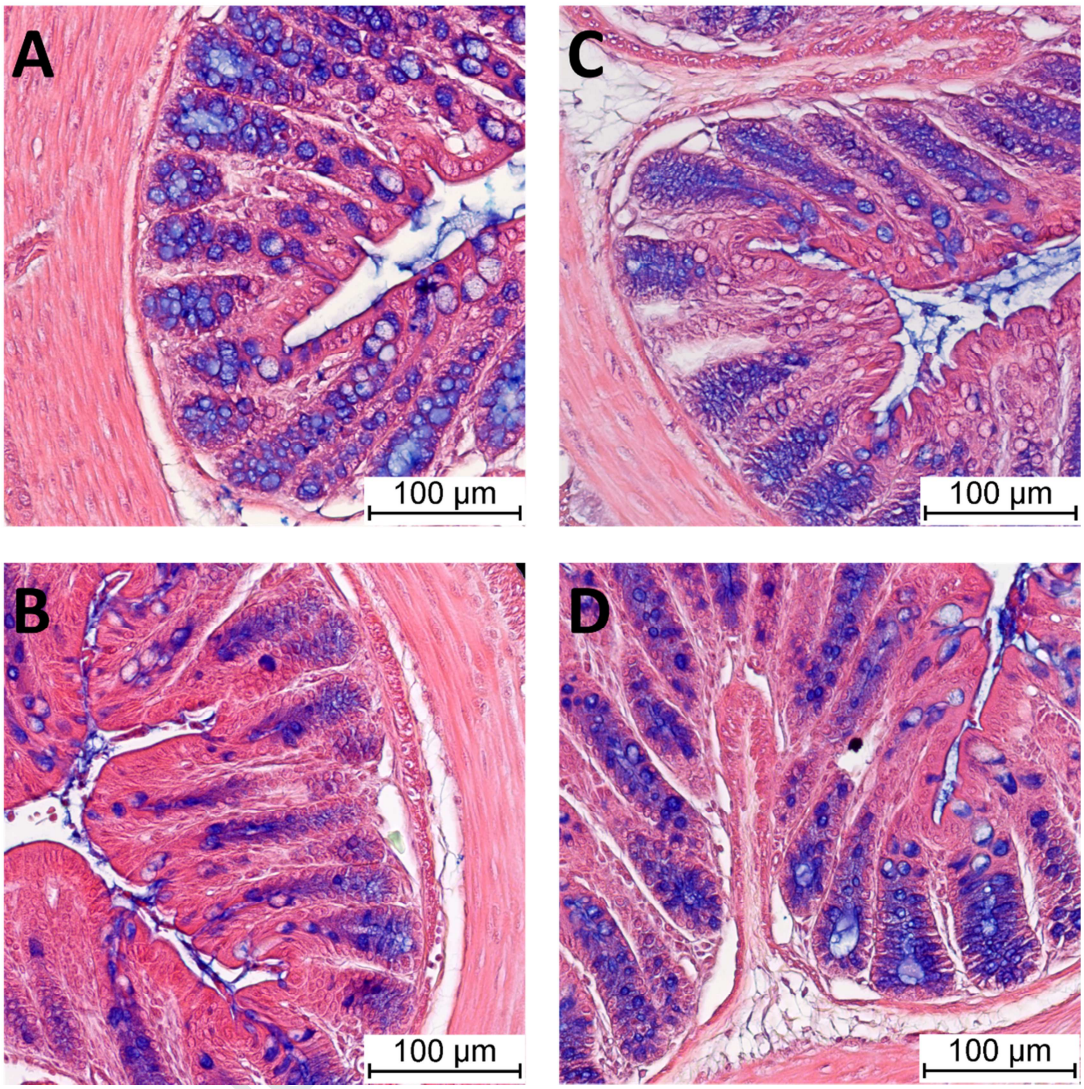
M&M

Histological processing, ABHE staining; 5µm paraffin embedded sections were deparaffinated by using 3x 5min baths of American Mastertech Clearify followed by 3x 5min 100% ethanol, 3x 5min 95% ethanol, 2x 5min 70% ethanol, 5min demineralised 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 (2x 4min 70%ethanol, 2x 5min 95% ethanol, 2x 5min 100% ethanol, 3x 5min American Mastertech Clearify), and finally mounted using Diamount mountant.

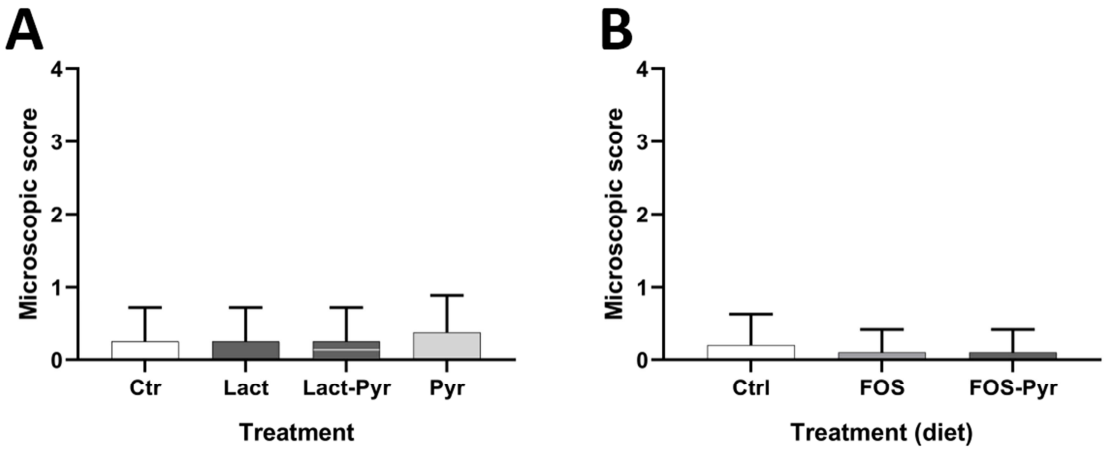
Manual Ultra-high resolution Composite Image Overview (MUCIO); datasets of overlapping microscope views covering entire slides were generated by manual microscope photography (single photo resolution: 1280 × 1024pixels) and stitched together using Microsoft Image Composite Editor (MICE), as originally described in Kamphuis, Mercier-Bonin, Eutamène, Theodorou (2017)[13]. Samples were imaged using a Nikon Eclipse 90i microscope fitted with a DXM 1200 F Digital Camera.

Microscopic scoring; composite micrographs were scored according to protocol[14] on a scale from 0-4; 0: no signs of inflammation, 1: very low level of leukocytic infiltration, 2: low level of leukocytic infiltration, 3: high level of leukocytic infiltration, high vascular density, thickening of the colon wall, 4: transmural infiltrations, loss of goblet cells, high vascular density, thickening of the colon wall. Statistical analysis: Scores were averaged per experimental group; One-way ANOVA, multiple comparisons with Tukey's correction for multiple comparisons.

Results



Supplemental figure 2 - Representative images of sections of colon showing no signs of inflammation. (A,B): Controls of lactose, fructo-oligosaccharides (FOS) respectively. (C,D) Lactose-, and FOS-treated, respectively.



Supplemental figure 3 – Microscopic scores of Lactose-pyridoxamine experiments (A) (N=8), and Fructo-oligosaccharides (FOS)-pyridoxamine experiments (B) (N=10). Apart from a very mild presence of leukocytes in 1 or 2 individuals in each group, no signs of inflammation were observed in any group.

No macroscopic (at moment of tissue collection) or microscopic signs of inflammation have been observed in response to FODMAP administration, further excluding the presence of overt active inflammation in these experiments.

LC/ MS analysis of aldehydes

M&M

Chemicals

Methanol (HPLC grade) and acetonitrile (Optima LC/MS grade) were purchased from Fisher (Illkirch, France), formic acid from Sigma Aldrich (St Quentin Fallavier, France). Ultra-pure water was obtained using a Milli-Q system (Millipore, St Quentin en Yvelines, France).

1-((ammoniooxy)methyl)-2-bromobenzene chloride (BBHA) was purchased from Interchim (Montluçon, France). Piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) and trifluoroacetic acid (TFA) were obtained from Acros organics (Geel, Belgium). Glyoxal, Methyglyoxal and 3-Deoxyglucosone were purchased from Sigma Aldrich (St Quentin Fallavier, France) and benzaldehyde-d5 (Internal standard) from CDN isotopes (Quebec, Canada). BBHA derivatives were synthesized in house according to previously published methods[15]. Briefly, BBHA (50-100 µmol, 1-2 eq) was added to standard solutions of aldehydes (50 µmol) in PIPES buffer (0.1 M, pH 6.5, 1 mL), and the mixture was stirred at 6-8°C for one hour. Each BBHA derivative was then purified by SPE.

Sample treatment

Intestinal content samples were prepared by homogenising 0.2g intestinal content in 1 mL demineralised water with 5 ceramic beads using a Fast-Prep (MP Biomedicals, Illkirch, France) (3x 15sec 6m/s with 1 min breaks) followed by 20min centrifugation at 8000x, supernatants were collected in 1.5 mL Eppendorf tubes and stored at -20°C until use.

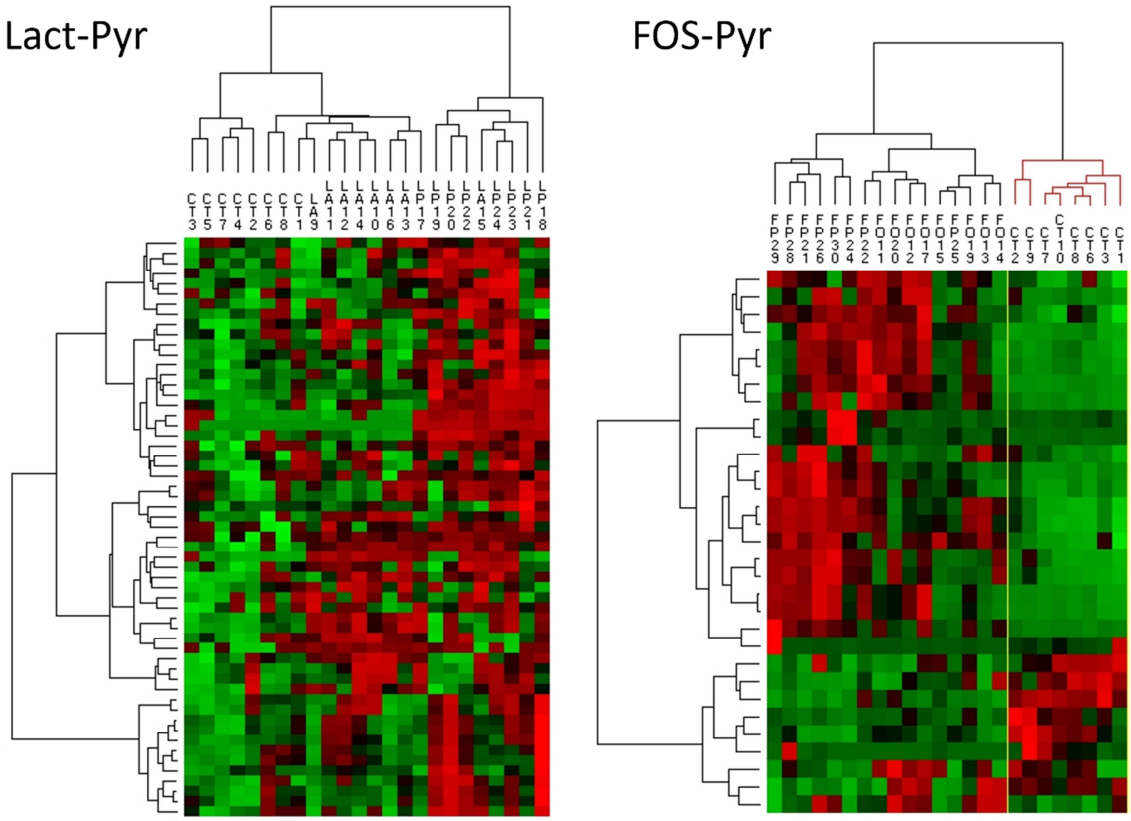
Intestinal content samples (100 µl) were added to 60 µL of PIPES buffer (0.1 M, pH 6.5) and derivatised with 120 µL BBHA (50 nmol / µL) in presence of 20 µL of internal standard (Benzaldehyde-d5, 1 ng/µL). The samples were stirred at 6-8°C for one hour. SPE was conducted on a Visiprep SPE Vacuum manifold (Supelco, St Quentin Fallavier, France), using Agilent C18 Bond Elut (100mg, 1 mL) cartridges. The sorbent was conditioned with 1 mL of CH_3OH, then 1 mL water. The derivatized samples were vortexed and then deposited on the SPE cartridge. Washing was performed with first 1 mL PIPES (rinse the container and deposit on cartridge) and then 0.05% TFA/CH_3OH (3/2). The cartridge was then dried under vacuum (1min), eluted with 400 µL CH_3OH and collected in glass tubes. Finally, the volume was adjusted to exactly 400 µL with CH_3OH, and the extracts were stored at -20°C until analysis.

Liquid chromatography – mass spectrometry

Sample extracts were analyzed by high-performance liquid chromatography coupled to high-resolution mass spectrometry (HPLC-HRMS). The HPLC system consisted in an Ultimate 3000 RS pump fitted with the Ultimate 3000 autosampler (Dionex-Thermo Scientific, Les Ulis, France). The

flow rate was 0.2 mL/min with the following elution gradient program: 0min 0% of B, from 3min to 15min 100% of B, and from 15 to 25min 100% of B. Mobile phases were composed of (A) $H_2O/CH_3CN/HCOOH$ 95/5/0.1 (v:v:v) and (B) $CH_3CN/H_2O/HCOOH$ 95/5/0.1 (v:v:v). 5µL of sample were injected on a Kinetex Core-Shell C18 (150 x 2.1mm, 2.6µm) column (Phenomenex, Le Pecq, France) maintained at 40°C. Detection was achieved on an LTQ-Orbitrap XL hybrid mass spectrometer (Thermo Scientific) equipped with an electrospray ionization source used in the positive mode. Ionization parameters were set at +4.5kV for the spray voltage, 35 arbitrary units (au) for the sheath gas flow rate (N_2), 5 au for the auxiliary gas flow rate (N_2) and 300°C for the capillary temperature. High-resolution mass spectra were acquired at a resolution power of 30,000 from m/z 80 to 800 in centroid mode. Identifications were performed by tandem mass spectrometry experiments (MS^n) using the ion trap mass analyzer of the LTQ-Orbitrap mass spectrometer. Solutions of synthesized standard glyoxal-BBHA, methyglyoxal-BBHA and 3-deoxyglucosone-BBHA at different concentration levels were used to characterize the method in terms of linearity of response, repeatability and sensitivity. Statistical analysis: From the lactose-pyridoxamine experiment raw data files, ions were extracted using xcms software[16]. Signals corresponding to brominated compounds were filtered based on the HRMS signal of the exact mass of each $[M+H]^+$ ion, according to a mass measurement error of ± 5 ppm, and to the occurrence of two signals of equivalent intensities with ΔM = 1.998 corresponding to the mass difference between the two bromine isotopes. Then isotopic ratio between isotopes was checked. For the fructo-oligosaccharides-pyridoxamine experiment, the same extraction process was carried out. Supervised multivariate partial least squared discriminant analysis with orthogonal signal filtration (OSC-PLS-DA) and univariate non-parametric Kruskal Wallis tests were carried out on these ions. Discriminant models were validated if PLS Q2 criterion was greater than 0.4 and a permutation test was validated. Significant potential aldehyde ions were selected if PLS variables importance on projection (VIP) was greater than 1 and univariate Kruskal Wallis p-values with false discovery rate correction was lower than 0.05. Heatmap with hierarchical clustering analysis (HCA) using Euclidian distance and Ward method as aggregation criterion was used to present the results of both experiments.

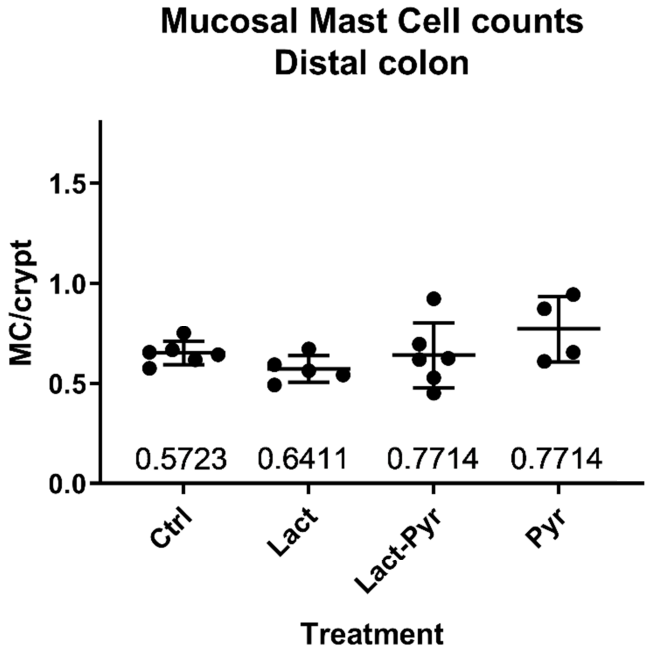
Results



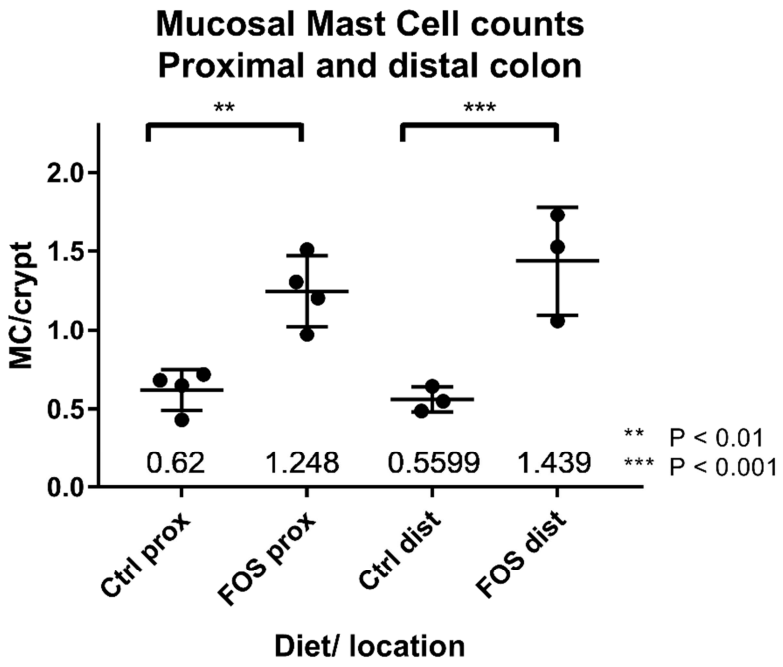
Supplemental figure 3 - Derivatised ions (carbonyl compounds) for which the control group had lower values than the FODMAP treated groups

HPLC/MS analysis of aldehydes in colonic contents

In lactose- and fructo-oligosaccharide-treated animals, a metabolomic analysis by HPLC-MS of colonic contents after BBHA derivatization identified a distinct clustering of global reactive carbonyl compound profiles versus control (Figure S3). Additive administration of pyridoxamine of lactose- and fructo-oligosaccharide -treated animals did not modify internal clustering between these groups.



Supplemental figure 4 - Mucosal mast cell (MC) counts of distal colon in animals treated daily with 5mg lactose (Lact) and/or pyridoxamine (Pyr) for 3 weeks, expressed as MC/crypt.



Supplemental figure 5 - Mucosal mast cell (MC) counts of distal versus proximal colon in animals after following a diet high in fructo-oligosaccharides (FOS), for 3 weeks, expressed as MC/crypt.

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What you need to know

Background and context

A diet low in fermentable oligo-, di-, mono-saccharides and polyols (FODMAPs) can reduce symptoms of irritable bowel syndrome (IBS), possibly by reducing microbial fermentation products. We investigated whether ingestion of FODMAPs can induce IBS-like visceral hypersensitivity mediated by fermentation products of intestinal microbes in mice

New findings

We found that oral administration of lactose or fructo-oligosaccharides to mice increases abdominal sensitivity, which can be prevented with an anti-glycation agent. The lactose or fructo-oligosaccharides did not produce alterations in fecal microbiota composition of mice.

Limitations

This study was performed in mice.

Impact

Agents that prevent glycation reactions might reduce abdominal pain in patients with IBS with sensitivity to FODMAPs.

Lay Summary

Feeding mice lactose or fed fructo-oligosaccharides, which can cause symptoms in patients with irritable bowel syndrome, resulted in an increased abdominal sensitivity in mice. We identified agents that reduced the abdominal pain and changes in the colon that might cause symptoms—these might be developed as treatments for patients.