Nondigestible Fructans Alter Gastrointestinal Barrier Function, Gene Expression, Histomorphology, and the Microbiota Profiles of Diet-Induced Obese C57BL/6J Mice

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Abstract

Background: Obesity is associated with compromised intestinal barrier function and shifts in gastrointestinal microbiota that may contribute to inflammation. Fiber provides benefits, but impacts of fiber type are not understood.

Objective: We aimed to determine the impact of cellulose compared with fructans on the fecal microbiota and gastrointestinal physiology in obese mice.

Methods: Eighteen-wk-old male diet-induced obese C57BL/6J mice (n = 6/group; 40.5 g) were fed high-fat diets (45% kcal fat) containing 5% cellulose (control), 10% cellulose, 10% short-chain fructooligosaccharides (scFOS), or 10% inulin for 4 wk. Cecal and colon tissues were collected to assess barrier function, histomorphology, and gene expression. Fecal DNA extracts were subjected to 16S ribosomal RNA amplicon-based Illumina MiSeq sequencing to assess microbiota.

Results: Body weight gain was greater (P < 0.05) in scFOS-fed than in 10% cellulose-fed mice. Both groups of fructan-fed mice had greater (P < 0.05) cecal crypt depth (scFOS: 141 mm; inulin: 145 mm) than both groups of cellulose-fed mice (5% and 10%: 109 mm). Inulin-fed mice had greater (P < 0.05) cecal transmural resistance (101 U cm⁻³) than 5% cellulose-fed controls (45 U cm⁻³). Inulin-fed mice had lower (P < 0.05) colonic mRNA abundance of Ocln (0.41) and Mct1 (0.35) than those fed 10% cellulose (Ocln: 1.28; Mct1: 0.90). Fructan and cellulose groups had different UniFrac distances of fecal microbiota (P < 0.05) and α diversity, which demonstrated lower (P < 0.01) species richness in fructan-fed mice. Mice fed scFOS had greater (P < 0.05) Actinobacteria (15.9%) and Verrucomicrobia (Akkermansia (17.0%) than 5% controls (Actinobacteria: 0.07%; Akkermansia: 0.08%). Relative abundance of Akkermansia was positively correlated (r = 0.56, P < 0.01) with cecal crypt depth.

Conclusions: Fructans markedly shifted gut microbiota and improved intestinal physiology in obese mice, but the mechanisms by which they affect gut integrity and inflammation in the obese are still unknown.

Introduction

Obesity prevalence remains high in the United States and in the world (1), increasing the risk of other health complications such as insulin resistance, metabolic syndrome, type 2 diabetes, and cardiovascular disease. Obesity has been associated with profound shifts in the gut microbiota communities, including altered Bacteroidetes-to-Firmicutes ratio in leptin-deficient ob/ob mice (2) and in obese human subjects (3). Strikingly, previous studies have successfully induced an obese phenotype by transferring gut microbes from obese mice to lean gnotobiotic mice (2), strengthening the concept that the gut microbiota play an important role in energy homeostasis and metabolic state.

The gastrointestinal tract is an important first line of defense against ingested noxious agents and prevents bacteria and pathogens from entering systemic circulation (4). Intestinal barrier function is known to be compromised with obesity, being most pronounced in obese rodent models (5, 6), whereby elevated plasma concentrations of bacterial LPS promote inflammation mainly by interacting with toll-like receptor 4. In humans, plasma LPS concentrations have been reported to be elevated in obese patients (7), whereas this endotoxin normally remains at low concentration in lean and healthy individuals (8).
Furthermore, reduced expression of tight junction (TJ) membrane proteins, namely occludin (Ocln) and zonula occluden 1 (Zo1), has been reported in ob/ob mice compared with wildtype controls (6). Decreased intestinal barrier function and altered occludin protein distribution has been reported in obesity-prone rats compared with obesity-resistant rats, suggesting that TJ disruption is associated with obesity (9).

Prebiotics, fermentable fibers that selectively stimulate the growth and/or activity of commensal bacteria that exerts benefits to the host, have positive effects on the gastrointestinal tract. A previous study demonstrated that fructan supplementation was able to lower systemic inflammation, enhance intestinal barrier function, and stimulate the growth of beneficial bacteria, including Bifidobacterium spp. and Lactobacillus spp., in ob/ob mice (5). Studies in human subjects also support the impact of fructans on increasing Bifidobacterium; however, research investigating the impact of prebiotic supplementation on the microbiome and intestinal barrier function in preclinical models of obesity are lacking (10–12). Elucidating the potential benefits of prebiotics may result in dietary intervention that enhances gastrointestinal health and ameliorates systemic inflammation in clinically obese patients.

Fructans are primarily composed of β(2→1) fructosyl-fructose linkages with a terminal glucose unit, which are naturally found in chicory root, artichokes, onions, bananas, garlic, and leeks (13). The nomenclature of fructans is largely based on chain length: inulin has a high degree of polymerization (DP), which is between 10 and 60; oligofructose is a partially hydrolyzed product of long-chain inulin and contains a DP <10; short-chain fructooligosaccharides (scFOS) have a mean DP of 3–6 and are most often synthesized from sucrose (13–15). In vitro fermentation experiments have demonstrated that fructans with DPs <10 exhibit faster fermentation rates than those having a high DP (16, 17).

Herein, inulin and scFOS were investigated to evaluate the effects of fermentable fibers compared with nonfermentable fiber (fructans compared with cellulose) and to determine whether the DP of fructans differentially affected the fecal microbiota and gastrointestinal physiology in obese mice. We hypothesized that fermentable fructan supplementation would lead to greater mRNA expression of TJ proteins and greater cecal crypt depth and improve intestinal barrier function in obese mice, with the effects of scFOS and inulin differing due to their DP. Beneficial shifts in gastrointestinal microbial communities also were expected with fructan supplementation, such as greater relative abundance of Bifidobacterium and Lactobacillus.

### Methods

**Animals and diets.** All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois. Twenty-four 18-wk-old diet-induced obese male C57BL/6 mice (Jackson Laboratory, Bar Harbor, Maine) were used in a completely randomized design. After adjusting to housing (2 wk), mice were fed an AIN-1993 growth (AIN-93G)–based high-fat diet (HFD; 45% kcal from fat) containing 5% cellulose (nonfermentable fiber source) during a 2-wk diet acclimation period. Mice then were randomly assigned to 4 high-fat (45% kcal from fat) AIN-93G–based diets (18) (n = 6/group) containing 1) 5% cellulose, 2) 10% cellulose, 3) 10% scFOS, or 4) 10% inulin for 4 wk. The number of mice used in each group was based on a previously published study by Cani et al. (5). Mice were individually housed in a humidity- and temperature-controlled facility with a 12-h light-dark cycle with ad libitum feeding, free access to water, and weekly body weight measurements. At 26 wk of age, mice were euthanized with CO2 asphyxiation after 6 h of fasting. Cecal and colonic tissues were collected for histomorphology, intestinal permeability measurements, and qRT-PCR analysis. Fecal pellets in the distal colon were collected and stored at −80°C until further analysis.

**Diet analysis.** Ingredient and chemical compositions of the experimental diets are reported in Supplemental Table 1. Diet samples were analyzed for dry matter and organic matter according to AOAC (19). Crude protein was determined using a Leco Nitrogen/Protein Determinator (model FP-2000, Leco Corporation, St. Joseph, Michigan) (19). Fat concentrations were measured by acid hydrolysis (20) followed by ether extraction (21). Gross energy was measured by using a bomb calorimeter (Model 1261, Parr Instruments, Moline, Illinois). Total dietary fiber concentrations were determined using methods described by Prosky et al. (22). Calculated total dietary fiber was based on purities provided by Orafti (Tienen, Belgium) for inulin (Raftiline HP; 95.5%), Research Diets (New Brunswick, New Jersey) for cellulose (Solka-Floc; 99%), and GTC Nutrition (Golden, Colorado) for scFOS (NutraFlora; 95%).

### Table 1

<table>
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<tr>
<th>Characteristic</th>
<th>Cellulose 5%</th>
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<th>scFOS 10%</th>
<th>Inulin 10%</th>
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<sup>1</sup> Values are means with pooled SEs, n = 5–6. Labeled means in a row without a common superscript letter differ, P < 0.05. scFOS, short-chain fructooligosaccharides.

<sup>3</sup> Abbreviations used: DP, degree of polymerization; HFD, high-fat diet; OTU, operational taxonomic unit; rRNA, ribosomal RNA; scFOS, short-chain fructooligosaccharides; TJ, tight junction.
Intestinal histomorphology and permeability measurements. Cecal and colonic tissue samples were fixed, embedded, and sectioned using a microtome and stained with hematoxylin and eosin at the Veterinary Pathology Laboratory at the University of Illinois at Urbana-Champaign. Cecal and colonic crypt depth were measured using Axiovision AC software and an AxioCam MRc5 (Carl Zeiss, Oberkothen, Germany). Another portion of cecal and colonic tissue samples were resected, cut longitudinally, and mounted into modified Ussing chambers (23) (Physiological Instruments, Inc., San Diego, California) exposing 0.031 cm² of the mucosal and serosal sides to oxygenated modified Krebs buffer solution. After equilibrium was reached, transmural resistance (Ω × cm²) was measured.

RNA extraction and RT-PCR. Total RNA was isolated from cecal and colonic tissue samples using RNeasy Kits (Qiagen, Valencia, California). RNA concentration was determined using a ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, Delaware). RNA integrity was confirmed by gel electrophoresis. TaqMan Gene Expression Assays primer-probe sets (Applied Biosystems, Foster City, California) were used for each gene of interest: monocarboxylic transporter 1 (Mct1), monocarboxylic transporter 4 (Mct4), G protein-coupled receptor 43 (Gpr43), AMP kinase (Ampk), mucin 2 (Muc2), Zo1, and Ocln. Real-time 2-step RT-PCR was performed using the Applied Biosystems 7900HT Real-Time PCR System (Applied Biosystems, Foster City, California). Each gene was tested in triplicate, using eukaryotic 18S ribosomal RNA (rRNA) as a control in

![Image](https://academic.oup.com/jn/article-abstract/146/5/949/4589912)

**FIGURE 1** Colonic gene expression of Zo1 (A), Ocln (B), Mct1 (C), and Ampk (D) of diet-induced obese mice fed a 45% high-fat diet containing 5% cellulose, 10% cellulose, 10% scFOS, or 10% inulin for 4 wk. Data are means ± SEMs, n = 6. Means without a common letter differ, P < 0.05. Ampk, AMP kinase; Mct1, monocarboxylic transporter 1; Ocln, occludin; rRNA, ribosomal RNA; scFOS, short-chain fructooligosaccharides; Zo1, zonula occluden 1.

**FIGURE 2** Species richness and PCoA plots of fecal microbial communities of diet-induced obese mice fed a 45% high-fat diet containing 5% cellulose, 10% cellulose, 10% scFOS, or 10% inulin for 4 wk. (A) α Diversity measures suggested lower (P < 0.01) species richness with fermentable fructan supplementation than with the 2 nonfermentable cellulose groups. PCoA plots of unweighted (B) and weighted (C) UniFrac distances of fecal microbial communities performed on the 97% OTU abundance matrix revealed a distinct separation (P < 0.05) between fructan- and cellulose-fed groups. Each dot represents a sample from each mouse (n = 6/group) fed diets containing 5% cellulose (orange dots), 10% cellulose (green dots), 10% scFOS (blue dots), or 10% inulin (red dots). OTU, operational taxonomic unit; PC, principal coordinate; PCoA, principal coordinates of analysis; scFOS, short-chain fructooligosaccharides.

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parallel with genes of interest. Data were normalized to 18S rRNA and expressed as a ratio to the 18S rRNA signal.

Fecal DNA extraction, amplification, sequencing and bioinformatics. Total DNA from fecal samples was extracted using MO BIO PowerSoil kits (MO BIO Laboratories, Inc., Carlsbad, California) (24). Concentrations of extracted DNA were quantified using a Qubit 2.0 Fluorometer (Life Technologies, Grand Island, New York). 16S rRNA gene amplicons of 250 bp were generated from the V4 region as described by Caporaso et al. (25). Quality of the purified amplicons was assessed using electrophoresis and a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, California). An equimolar amount of the amplicons were sequenced using Illumina sequencing on a MiSeq v2 reagents (Illumina Inc., San Diego, California) at the W. M. Keck Center for Biotechnology at the University of Illinois. QIIME 1.8.0 was used to process the resulting sequence data (26). Briefly, high quality (quality value >25) sequence data derived from the sequencing process were demultiplexed. Sequences were then clustered into operational taxonomic units (OTUs) using closed-reference OTUs picking against the Greengenes 13.8 reference OTU database with a 97% similarity threshold (27). An even sampling depth of 2158 sequences/sample was used for assessing α- and β-diversity measures.

Statistical analyses. All data were analyzed using SAS 9.2 (SAS Institute, Cary, North Carolina) using the Mixed Models procedure. When a main effect was significant, post hoc Tukey test were performed to determine differences among treatment groups. Data normality was checked using the univariate procedure and Shapiro-Wilk statistic, with log transformation being used when normal distributions were lacking. Homogeneity of variance was checked using the general linear model procedure and Levene statistic. Pearson correlations between all OTU in relation to body weight gain, daily food intake, cecal crypt depth, colonic crypt depth, and transmural resistance in cecum and colon were assessed. Data are reported as means ± SEMs with statistical significance set as P < 0.05 and P < 0.10 considered as trends.

Results

Animal characteristics and food intake. Initial (wk 0, before dietary intervention) and final (wk 4) mean body weights of the mice were not different among groups (Table 1). Mean body weight gain, however, was different between groups. Mice fed 10% scFOS gained 1.2-fold more weight than mice fed 10% cellulose (P < 0.05), even though the 10% cellulose-fed mice had higher (P < 0.05) mean daily food intake than the other groups.

Fructans enhance intestinal architecture in obese mice. Cecal crypt depth was greater (P < 0.05) in the mice fed 10% scFOS or 10% inulin than in those fed 5% or 10% cellulose (Table 1). Mice fed 10% inulin had higher (P < 0.05) transmural resistance in the cecum than those fed 5% cellulose, suggesting that this long-chain fructan enhanced cecal integrity. Colonic crypt depth did not differ among dietary groups. However, mice fed 10% scFOS tended to have higher (P = 0.08) transmural resistance in the colon than those fed 5% cellulose, suggesting that this short-chain fructan may benefit the gut integrity of the colon.

Inulin supplementation affects intestinal mRNA expression. mRNA expression of TJ protein ZO1 and Ocln in the colon are shown in Figure 1A, B. ZO1 and Ocln mRNA abundance were not different among dietary interventions in the cecum (data not shown). mRNA abundance of ZO1 was lower (P < 0.05) in the colon of mice fed 10% inulin than in those fed 10% scFOS and tended to be different from the 5% cellulose-fed (P = 0.06) and 10% cellulose-fed (P = 0.05) groups. Similarly, mRNA abundance of Ocln was lower (P < 0.01) in the colon of mice fed 10% inulin than in mice fed 10% cellulose but did not differ from mice fed 5% cellulose or 10% scFOS.

Dietary fiber intervention did not affect mRNA expression of SCFA transporters Mct1 in the cecum (data not shown); however, mice fed 10% inulin had lower (P < 0.05) Mct1 mRNA abundance in the colon than 10% cellulose-fed mice (Figure 1C). Fructan supplementation did not affect SCFA transporter Mct4 and SCFA receptor Grp43 mRNA expression in either the cecum or colon (data not shown). Ampk mRNA abundance was assessed because of growing evidence of its involvement in intestinal barrier function (28). Similar to other genes assessed, dietary intervention did not affect Ampk mRNA expression in the cecum, but mice fed 10% inulin had lower (P < 0.05) Ampk expression in the colon than mice fed all other diets (Figure 1D). The expression of Muc2 was not different in the cecum or colon among dietary treatment groups (data not shown).

Fecal microbiota. A total of 393,167 16S rRNA-based amplicon sequences were obtained, with a mean of 16,381 sequences (range = 2158–35,204)/sample. The data set was rarified to 2158 sequences for analysis of diversity and species richness. α Diversity measures suggested lower (P < 0.01) species richness with fermentable fructan supplementation than with the 2 nonfermentable cellulose groups [observed species at the 97% level (OTUs): 93.0 ± 15.0 compared with 174 ± 20 (fructans compared with cellulose, P < 0.01); Chao 1 index (OTUs): 138 ± 23.0 compared with 233 ± 31.0 (P < 0.01); phylogenetic diversity whole tree matrix (phylogenetic branch distance): 8.40 ± 1.00 compared with 13.6 ± 0.90 (P < 0.01)] (Figure 2A). Species richness did not differ between the 2 cellulose treatment groups (i.e., 5% cellulose compared with 10% cellulose) or between the 2 fructan treatment groups (i.e., 10% scFOS compared with 10% inulin groups).

FIGURE 3 Fecal microbial communities at the phyla level of diet-induced obese mice fed a 45% high-fat diet containing 5% cellulose, 10% cellulose, 10% scFOS, or 10% inulin for 4 wk. Data are mean percentages, n = 6. Means ± SEMs of 5% cellulose group are 0.07% ± 3.94% for Actinobacteria; 22.9% ± 5.63% for Bacteroidetes; 76.9% ± 7.80% for Firmicutes; 0.05% ± 0.02% for Tenericutes; 0.08% ± 3.39% for Verrucomicrobia. Means ± SEMs of 10% cellulose group are 0.47% ± 3.94% for Actinobacteria; 26.7% ± 5.63% for Bacteroidetes; 68.8% ± 7.80% for Firmicutes; 0.06% ± 0.02% for Tenericutes; 4.0% ± 3.39% for Verrucomicrobia. Means ± SEMs of 10% scFOS group are 15.9% ± 3.94% for Actinobacteria; 23.9% ± 5.63% for Bacteroidetes; 43.2% ± 7.80% for Firmicutes; 0.00% ± 0.02% for Tenericutes; 17.0% ± 3.39% for Verrucomicrobia. Means ± SEMs of 10% inulin group are 12.1% ± 3.94% for Actinobacteria; 32.1% ± 5.63% for Bacteroidetes; 44.8% ± 7.80% for Firmicutes; 0.00% ± 0.02% for Tenericutes; 11.1% ± 3.39% for Verrucomicrobia. scFOS, short-chain fructooligosaccharides.
TABLE 2  Bacterial phyla, families, and genera of fecal samples of diet-induced obese mice fed a 45% high-fat diet containing 5% cellulose, 10% cellulose, 10% scFOS, or 10% inulin for 4 wk

<table>
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<tr>
<th>Treatment</th>
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<th>10%</th>
<th>10% scFOS</th>
<th>10% Inulin</th>
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<th>P value</th>
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1 Values are means with pooled SEs, n = 6/group. Means in a row without a common superscript letter differ, P < 0.05. scFOS, short-chain fructooligosaccharides. 2 Undefined family within order Clostridiales.

Principal coordinates analysis of weighted and unweighted UniFrac distances performed on the 97% OTU abundance matrix revealed a distinct separation (P < 0.05) on the β diversity of gut microbial communities between fructan- and cellulose-fed groups (Figure 2B, C, respectively). Similar to species richness, β diversity did not differ between the 2 cellulose treatment groups or between the 2 fructans treatment groups. Notably, the first 3 axes of the unweighted and weighted principal coordinates analysis accounted for 57% and 81% of the variation in our study, respectively.

Taxonomic shifts due to prebiotic supplementation. Greengenes classifier assigned usable raw reads to 5 phyla, 39 families, and 66 genera. The most abundant phyla included Firmicutes (58.4% of sequences), Bacteroidetes (26.4% of sequences), Verrucomicrobia (8.0% of sequences), Actinobacteria (7.1% of sequences), and Tenericutes (<0.1% of sequences). Our data demonstrate that fermentable fructans may dramatically impact microbial taxonomy when compared with a nonfermentable fiber source such as cellulose (Figure 3). More specifically, 10% scFOS-fed mice had higher relative abundance of Actinobacteria (P < 0.05) and Verrucomicrobia (P < 0.05) phyla than the 5% cellulose group; 10% inulin-fed mice had a similar numerical elevation in the relative abundance of these 2 phyla; however, the changes were not statistically significant (Table 2). 10% scFOS and inulin supplementation both led to a lower (P < 0.05) relative abundance of Firmicutes than the 5% cellulose group. Relative abundance of Bacteroidetes and Bacteroidetes-to-Firmicutes ratio were not different between groups (data not shown).

Four wk of 10% scFOS supplementation in diet-induced obese mice led to greater relative abundance of Lactobacillus (P < 0.05) than mice fed 10% cellulose. Similarly, inulin supplementation tended to increase the relative abundance of Lactobacillus (P = 0.09) compared with mice fed 10% cellulose, but did not reach statistical significance. 10% scFOS-fed mice had greater relative abundance of Akkermansia (P < 0.05) and an undefined genus within the Coriobacteriaceae family (P < 0.05) than mice fed 5% cellulose (Table 2). Inulin supplementation led to a lower (P < 0.05) relative abundance of Dorea and greater (P = 0.05) relative abundance of an undefined genus within the Erysipelotrichaceae family when compared with 5% cellulose. Both scFOS and inulin supplementation led to a lower relative abundance of Lactococcus (P < 0.01) and an undefined genus within the Peptostreptococcaceae family (P < 0.01) compared with the 10% cellulose group. scFOS- and inulin-fed...
mice had a lower relative abundance of *Oscilllosira (P < 0.01)* and *Ruminococcus (P < 0.01)* than did the 5% cellulose group. Moreover, scFOS and inulin supplementation led to a lower relative abundance of *Turicibacter (P < 0.001)*, SMB53 (*P < 0.05*), and an undefined genus within the *Ruminococcaceae family (P < 0.05)* compared with the 5% and 10% cellulose groups.

Correlations between relative microbial abundance and certain physiological parameters (body weight gain, daily food intake, cecal crypt depth, colonic crypt depth, transmural resistance in cecum, and transmural resistance in the colon) were assessed, and the relative abundance of *Akkermansia* was found to be positively correlated (*r = 0.56, P < 0.01*) with cecal crypt depth (Figure 4).

**Discussion**

Fructans have been shown to accelerate the recovery process, improve intestinal function, and increase colonic SCFA production in piglets with bacterially induced diarrhea (29). Other studies have reported that fructans modulate mucins in the gastrointestinal tract of gnotobiotic rats by increasing beneficial sulfomucins (30, 31). We demonstrated that 4 wk of fructan supplementation affected gastrointestinal health and the fecal microbiota of obese mice, with inulin and scFOS exhibiting differential effects on intestinal transmural resistance and mRNA expression in the cecum and the colon.

Intestinal barrier function was assessed by using an ex vivo Ussing chamber system. Intestinal crypt depth has been shown to be positively correlated to cell proliferation, which ultimately leads to reduced paracellular transport between epithelial cells of the gut (15, 32, 33). In vivo measurements may be useful for assessing impaired intestinal permeability (9, 34), but this approach does not identify the affected segment. Ussing chambers allow for precise assessment and comparison of the physiological intestinal permeability of the each intestinal segment without being influenced by other potential confounding variables (23, 35).

Principal coordinates analysis revealed distinct fecal microbial communities between cellulose- and fructan-supplemented mice. Both fructans led to lower microbial diversity and species richness compared with both cellulose groups but did not differ from one another. Relative abundance of *Akkermansia* was elevated with fructan supplementation and was positively correlated with cecal crypt depth. Overall, our data suggest that fermentable fructans improve gut integrity, and lead to beneficial shifts in the gut microbiota in HFD-fed obese mice, in agreement with previous findings (36).

It has been postulated that prebiotic chain length determines the site of fermentation, such that prebiotics of greater DP reach the distal colon, whereas lower DPs are highly fermented in the proximal colon or terminal small intestine (16, 17). Our crypt depth and transmural resistance data, however, suggest that the greatest benefits of scFOS occurred in the colon, whereas the greatest benefits of inulin occurred in the cecum, which is contradictory to previous findings (37). To better understand the mechanisms by which intestinal resistance was improved after prebiotic supplementation, we examined the mRNA abundance of SCFA transporters and receptors, TJ membrane proteins, *Mic2*, and *Ampk* in the cecum and colon. Our data revealed a lower *Mct1* expression in the colon of inulin-fed mice than 10% cellulose-fed mice, but SCFA transporters and receptors were not affected. SCFA transporters are known to be involved in transporting fermentative intermediates and by-products such as lactate, pyruvate, and ketone bodies (38) and may contribute to these findings.

Lower abundance of TJ membrane proteins *Zo1* and *Ocln* observed in the colon of inulin-fed mice was opposite to our hypothesis and data from previous studies (5). Because *Ocln* acts as a bridge between adjacent epithelial cells, increased expression may strengthen the bond between cells while remaining firmly anchored to *Zo1* proteins (4). *Ampk* appears to be involved in regulating TJ assembly (28), but its colonic expression was lower in inulin-fed mice. Because inulin consistently lowered TJ, SCFA transporter, and *Ampk* gene expression in the colon without deteriorating transmural resistance and intestinal architecture, a more complex mechanism might be involved. Because TJ gene expression and protein localization do not provide information on the passage of small molecules, the transmural resistance data from Ussing chambers are of greater value than gene expression data.

The dose of scFOS and inulin used in current study was comparable to ~40 g fiber/d for women and ~50 g fiber/d for men, which exceeds the adequate intakes of fiber recommended by the Institute of Medicine (39). Both fructans enhanced intestinal architecture, even though greater crypt depth was only observed in the cecum, the segment where the majority of fermentative activity occurs in mice. Previous work has reported that a mixture of oligofructose and long-chain inulin increased distal colon crypt depth in the human flora-associated lean rats (i.e., germ-free rats inoculated with the human microbiota) (30). The different observations might be because of mouse species, the gut microbial communities, or the severity of gut barrier breakdown before prebiotic supplementation.

The gut microbiota has been linked with the development of obesity by altering energy harvest, modulating gut peptides, and regulating lipid metabolism (2, 40, 41). HFD-fed mice have disrupted intestinal barrier function, metabolic endotoxemia, and inflammation (5). Antibiotic use has been shown to lower endotoxemia-induced inflammation and metabolic disorders, suggesting that inflammatory obese phenotypes originate from gut microbiota (42, 43). In the current study, the gut microbial communities of HFD-fed obese mice were dramatically altered with fructans, including greater relative abundances of *Akkermansia, Lactobacillus* and *Coriobacteriaceae*. The relative abundance of *Akkermansia* also was found to be positively associated with cecal crypt depth.
Although we could only identify taxa down to the genus level in the current study, 1 species is commonly detected in the Akkermansia genus, namely Akkermansia muciniphila, a mucin-degrading gram-negative anaerobe (44). Healthy subjects have been reported to have a higher abundance of fecal Akkermansia than obese/diabetic patients or animal models (45–47). A. muciniphila has been positively associated with improvements in lipid metabolism, glucose tolerance, and metabolic disorders (46, 48). Moreover, direct administration of A. muciniphila was able to decrease fat-mass gain, metabolic endotoxemia, adipose tissue inflammation, and insulin resistance in diet-induced obese mice (46). Similarly, administration of Lactobacillus strains has been shown to decrease body weight and fat mass, and improve insulin resistance in obese individuals (49, 50). Beneficial shifts of Akkermansia and Lactobacillus and the enhanced intestinal integrity suggest that fructan supplementation serves as an advantageous strategy to improve gastrointestinal health in the obese population. Because Muc2 mRNA abundance was not different in this study, the greater relative abundance of Akkermansia may not have been in response to elevated host mucin production, which is similar to the response of diet-induced obese mice fed dietary polyphenols (51).

The family Coriobacteriaceae is involved in the conversion of bile acids and steroids and activation of dietary polyphenols and has been strongly associated with greater hepatic triglycerides, glucose, and glycogen concentrations in mice and higher non-HDL plasma cholesterol in hamsters (52, 53). A previous study demonstrated that healthy adult men consuming polydextrose and soluble corn fiber had lower relative abundance of Coriobacteriaceae than the nonsupplemental fiber control group (54). In this study, however, the relative abundance of Coriobacteriaceae was higher in scFOS-fed mice than in those fed 5% cellulose.

Because of the small sample size, we were not able to measure cecal SCFA in the current study. However, fermentable dietary fibers have been shown to increase gastrointestinal fermentation and thus should lead to increased SCFA production. Despite the beneficial shifts of Akkermansia and Lactobacillus observed, the relative abundance of the SCFA-producing family Lachnospiraceae did not differ between treatment groups. Nevertheless, Lachnospiraceae have recently been reported as a contributor of obesity development and metabolic disorder (55). If this finding was true, the unchanged relative abundance of Lachnospiraceae with fructans could be beneficial. On the other hand, the family Ruminococcaceae is also important to dietary fiber degradation and SCFA production. The relative abundance of this family was lower in both fructan-fed groups in the current study, similar to what we found previously in healthy adult men supplemented with polydextrose and soluble corn fiber (54).

The lack of a lean control group and low animal numbers were the main limitations in this study. With limiting tissue and cecal digesta samples available, we also were incapable of measuring TJ and SCFA transporter proteins or SCFA in the gastrointestinal tract. Nevertheless, our ex vivo transmural resistance data did provide physiological assessment of gut barrier function.

In summary, our study demonstrated that fructan supplementation significantly shifted gut microbial communities and improved intestinal physiological outcomes in a diet-induced obese mouse model. Different outcomes obtained from scFOS compared with inulin suggest that fibers and prebiotics that have different DP and/or fermentation rates may impact gut health indexes differently. More research is required to elucidate the mechanisms by which fructans affect gut integrity of obese patients and ameliorate systemic inflammation.

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References


