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Agave Inulin Supplementation Affects the Fecal Microbiota of Healthy Adults Participating in a Randomized, Double-Blind, Placebo-Controlled, Crossover Trial[1–3]

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Abstract

Background: Prebiotics resist digestion, providing fermentable substrates for select gastrointestinal bacteria associated with health and well-being. Agave inulin differs from other inulin type fibers in chemical structure and botanical origin. Preclinical animal research suggests these differences affect bacterial utilization and physiologic outcomes. Thus, research is needed to determine whether these effects translate to healthy adults.

Objective: We evaluated agave inulin utilization by the gastrointestinal microbiota by measuring fecal fermentative end products and bacterial taxa.

Methods: A randomized, double-blind, placebo-controlled, 3-period, crossover trial was undertaken in healthy adults (n = 29). Participants consumed 0, 5.0, or 7.5 g agave inulin/d for 21 d with 7-d washouts between periods. Participants recorded daily dietary intake; fecal samples were collected during days 16–20 of each period and were subjected to fermentative end product analysis and 16S Illumina sequencing.

Results: Fecal Actinobacteria and *Bifidobacterium* were enriched ($P < 0.001$) 3- and 4-fold after 5.0 and 7.5 g agave inulin/d, respectively, compared with control. *Desulfovibrio* were depleted 40% with agave inulin compared with control. Agave inulin tended ($P < 0.07$) to reduce fecal 4-methyphenol and pH. Bivariate correlations revealed a positive association between intakes of agave inulin (g/kcal) and *Bifidobacterium* ($r = 0.41$, $P < 0.001$). Total dietary fiber intake (total fiber plus 0, 5.0, or 7.5 g agave inulin/d) per kilocalorie was positively associated with fecal butyrate ($r = 0.30$, $P = 0.005$), tended to be positively associated with *Bifidobacterium* ($r = 0.19$, $P = 0.08$), and was negatively correlated with *Desulfovibrio* abundance ($r = -0.31$, $P = 0.004$).

Conclusions: Agave inulin supplementation shifted the gastrointestinal microbiota composition and activity in healthy adults. Further investigation is warranted to determine whether the observed changes translate into health benefits in human populations. This trial was registered at clinicaltrials.gov as NCT01925560. *J Nutr* 2015;145:2025–32.

Keywords: prebiotics, agave inulin, fiber, microbiota, bifidobacteria, butyrate

Introduction

The gastrointestinal (GI)[7] microbiota plays a crucial role in human health, affecting metabolism, physiology, and immune function (1–3). Recent advances in sequencing technologies have allowed researchers to gain a better understanding of the thousands of different microbial taxa in the GI tract (4). Increasingly, perturbations in the GI microbiota are being associated with complex diseases, including obesity, diabetes, cardiovascular disease, inflammatory bowel disease, and autism (3, 5–8).

Epidemiologic evidence suggests there are inverse associations between dietary fiber intake and obesity (9), diabetes (10, 11), and coronary heart disease (12–14). Inadequate fiber consumption is a recognized problem in the United States (15), with average intakes barely surpassing 50% of the Adequate Intake recommendation (25–38 g/d) (16). Because inadequate

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[3] Supplemental Tables 1–6 and Supplemental Figures 1–3 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
[7] Abbreviations used: BCFA, branched-chain FA; DP, degree of polymerization; FOS, fructooligosaccharide; GI, gastrointestinal; GOS, galactooligosaccharide; OTU, operational taxonomic unit.
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fiber intake is also associated with increased risk of obesity, diabetes, and cardiovascular disease (9, 17–19), the role of fiber in GI microbial metabolism, function, and disease prevention is of particular interest.

Prebiotics are a promising dietary strategy by which the GI microbiota can be modified for health promotion. Prebiotics are selectively fermented food ingredients that promote specific changes in the composition and/or activity of bacteria already present within the GI tract, thus promoting host health and well-being (20). Bacterial fermentation of prebiotics results in production of SCFAs, lactic acid, gases (hydrogen, methane, and carbon dioxide), and reduced luminal pH (21). SCFAs and particularly butyrate benefit host health by regulating fluid and electrolyte uptake, influencing epithelial cell cytokinetics and barrier function, and exerting anti-inflammatory effects (22–32). Inulin and fructooligosaccharides (FOSs) were shown to promote the growth of bifidobacteria in infants and adults (20, 33, 34). Suggested health benefits of bifidobacteria include production of acetic and lactic acids, synthesizing B vitamins, excreting antimicrobial substances that reduce pathogenic bacteria, and influencing maturation of the immune system (35–40). However, uncertainties in this field of research warrant further study. Until recently, most studies on dietary modulation of the GI microbiota have relied on culture-based methods or molecular methods such as fluorescent in situ hybridization and quantitative real-time PCR, which are restricted to specific bacterial groups. As such, our understanding of how prebiotics affect the entire community structure of the microbiota is relatively unknown.

Agave inulin, which was investigated in the present study, is composed of linear and branched fructose chains, connected with β-2,1 and β-2,6 linkages, and a degree of polymerization (DP) between 25 and 34 (41). In comparison, chicory inulin is linear with β-2,1 linkages and a DP that ranges from 2 to 60 (42). In vitro experimentation has demonstrated that agave inulin is readily fermented by bifidobacteria and lactobacilli (43, 44). In addition, rodent studies have provided evidence that the botanical origin and chemical structure of different inulin-type fibers (e.g., agave inulin and chicory inulin) induce variable effects on body composition, blood cholesterol, and blood glucose concentrations (45–47). The prebiotic effects of agave inulin in healthy adults, however, are currently unknown. Therefore, translational studies to investigate the influence of agave inulin on the human GI microbiota are warranted.

Previously, our laboratory conducted a randomized, double-blind, placebo-controlled, crossover study to assess tolerance and utilization of agave inulin in healthy adults (48). The primary objectives of that study were to determine GI tolerance via subjective daily and weekly questionnaires and fermentation profiles via 8-h breath hydrogen testing after treatment boluses. The study demonstrated that agave inulin was well tolerated up to 7.5 g/d and improved laxation. This report details the secondary objectives of the study to assess *1*) agave inulin utilization by the GI microbiota through measurements of fecal fermentation end products and *2*) amplicon-based bacterial community analysis from the same individuals.

Methods

Subjects. Healthy adults were recruited for this study via an e-mail list server from the University of Illinois. Participants were screened to ensure general health and to collect demographic information. The inclusion criteria included participants 1) be 20–40 y of age; 2) have BMI (kg/m^2) > 18.5 and < 29.5; 3) be free of metabolic and GI diseases, with no history of such diseases; 4) avoid medications known to affect intestinal function; 5) be free of antibiotic use for at least the past 8 wk; 6) limit alcohol consumption to 2 servings/d (e.g., <28 g ethanol/d); 7) avoid taking prebiotics or probiotics; 8) consume a moderate fiber diet; 9) continue to consume the same dose of vitamin and/or mineral supplements, if applicable; 10) maintain current level of physical activity; 11) agree to keep detailed dietary and stool records; and 12) meet with study personnel weekly. Female participants were excluded if they had menstrual cycles < 27 d or > 29 d in length, were pregnant, or were lactating. Before study initiation, all participants voluntarily signed a written informed consent as approved by the University of Illinois Institutional Review Board. This study was conducted from January 2013 to May 2013 and was registered with clinicaltrials.gov as NCT01925560.

Experimental design and treatments. This study was a randomized, double-blind, placebo-controlled, 3-period, crossover design with 1 7-d baseline period and 3 21-d treatment periods, followed by 1-wk washouts between each period (**Supplemental Figure 1**). This experiment was part of the tolerance study conducted by our laboratory (48). Agave inulin (BIOAGAVE agave inulin fiber; Ingredion Incorporated) and control treatments were provided as chocolate chews (Bruce's Candy Kitchen) in identical wrappers in coded boxes. Chews were formulated to provide 0, 5.0 or 7.5 g fiber in 3 chews. Researchers and participants were blinded to treatment codes. Study participants received instructions on completing a detailed dietary journal from a registered dietitian before study initiation and had weekly 1-on-1 meetings with a study dietitian and/or dietetic interns throughout the trial to ensure record completeness. Dietary intake data were assessed with Nutritionist Pro (Version 5.2, 2012; Axxya Systems). Participants completed daily and weekly GI intolerance questionnaires and stool records throughout the study.

Stool collection and analysis. During days 16–20 of each treatment period, participants brought 3 fresh (within 15 min of defecation) fecal samples to the laboratory by using Commode Specimen Collection Systems (Sage Products) on ice packs within coolers. Samples were homogenized on arrival, a pH measurement was taken (Denver Instrument), and then samples were divided into aliquots for individual experiments. The samples for microbial analysis were flash-frozen in liquid nitrogen and stored at −80°C until analysis. The aliquot for SCFAs (acetate, propionate, butyrate), branched-chain FAs (BCFAs; valerate, isovalerate, isobutyrate), and ammonia was immediately acidified with 2N-HCl (10% wt:vol) and frozen at −20°C until analysis. Phenol and indole aliquots were weighed and then stored at −20°C until analysis.

Fecal dry matter was measured according to the methods of the Association of Official Analytical Chemists (1984) (49). Ammonia concentrations were determined with methods described by Chaney and Marbach (50). Fecal SCFA and BCFA concentrations were analyzed with GC as previously described (51). Phenol and indole concentrations were assessed according to Flickinger et al. (52).

Fecal bacterial DNA was extracted according to the manufacturer's instructions by using the PowerLyzer PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc.) with bead beating for 20 min by using a vortex adaptor. After extraction, a 250-bp region from the V4 region of the 16S rRNA gene was amplified according to Caporaso et al. (53). Sequencing was performed at the WM Keck Center for Biotechnology at the University of Illinois by using an Illumina MiSeq2000 with the use of v3 reagents (Illumina Inc.).

High-quality (quality value > 25) sequence data derived from the sequencing process were analyzed with QIIME 1.8.0 (54). Briefly, sequences were clustered into operational taxonomic units (OTUs) by using closed-reference OTU picking against the Greengenes 13_8 reference OTU database (99% similarity threshold). After quality filtering, weighted and unweighted UniFrac distances were computed at an even sampling depth of 33,388 sequences per sample (55, 56). To create a visual illustration of the responses to agave inulin supplementation, bubble plots that depicted the differences in each study participant's fecal *Bifidobacterium* proportion after 20 d of consumption

of 5.0 and 7.5 g agave inulin/d were created by comparing each treatment dose with the control period (0 g/d).

Statistics. Fecal fermentation end products (SCFAs, BCFAs, phenols, indoles, and ammonia), pH, dry matter, and bacterial sequence percentages were analyzed with the Mixed models procedure of SAS (version 9.3; SAS Institute, Inc.) with treatment as a fixed effect and participant and period as random effects. Post hoc Tukey adjustments were used to control for multiple comparisons. The UNIVARIATE procedure and Shapiro-Wilk statistic were used to test for data normality, and log transformations were used as needed. The Mann-Whitney test was used when normality was not achieved with transformations. Bivariate correlations (Pearson's *r*) between *Bifidobacterium*, *Desulfovibrio*, agave inulin dosage in relation to daily caloric intake, total dietary fiber intake (dietary fiber plus 0, 5.0, or 7.5 g agave inulin/d) in relation to daily caloric intake, BMI, and fecal fermentation end products were assessed. A probability of $P < 0.05$ was accepted as statistically significant, and $P < 0.10$ was considered a trend.

Results

Twenty-nine of the 30 enrolled participants completed the study. One participant was removed from the trial because of consumption of a medication restricted by the study. Participant's baseline characteristics are listed in **Supplemental Table 1**. Agave inulin up to 7.5 g/d was well tolerated with no adverse events (48). Participants' dietary intake and body weights did not differ among treatment periods ($P > 0.05$; **Supplemental Table 2**).

The fecal pH ($P = 0.06$) and 4-methylphenol concentration ($P = 0.07$) tended to be lower after agave inulin supplementation compared with control (**Table 1**).

Illumina MiSeq sequencing of the 87 fecal samples generated >10 million total sequences. Overall, 11 bacterial phyla, 94 families, and 227 genera were identified in the participants (**Supplemental Tables 3** and **4**). Although a number of taxa were identified at each National Center for Biotechnology Information taxonomic hierarchy level, only a few accounted for the majority at each level. Bacteroidetes and Firmicutes represented ~90% of the sequences at the phyla level. Twelve families and 18 genera represented >90% of the sequences. Conversely, 20 phyla, 72 families, and 188 genera made up <1% of total sequences at each respective taxonomic level.

TABLE 1 Fecal fermentation end products of healthy human participants who consumed 0, 5.0, or 7.5 g agave inulin/d in a crossover design[1]

	Treatment with agave inulin				
Item	0 g/d	5.0 g/d	7.5 g/d	SEM	*P*
End product, μmol/g DM feces					
Ammonia	96.2	99.7	99.7	6.78	0.82
4-Methylphenol	1.87	1.83	1.59	0.168	0.07
Indole	0.944	1.01	0.907	0.094	0.17
Isobutyrate	6.49	6.39	6.56	0.342	0.92
Isovalerate	8.25	8.37	8.45	0.437	0.92
Valerate	6.64	7.15	7.52	0.649	0.12
Total BCFAs	21.4	21.9	22.6	1.16	0.59
Acetate	237	254	262	18.7	0.12
Propionate	62.9	68.5	67.3	7.99	0.18
Butyrate	49.9	55.9	55.8	4.98	0.14
Total SCFAs	350	379	385	29.4	0.12
SCFA molar ratio					
Acetate	0.684	0.681	0.686	0.009	0.68
Propionate	0.176	0.174	0.172	0.007	0.63
Butyrate	0.141	0.145	0.143	0.007	0.59
pH	6.88	6.77	6.74	0.078	0.06

[1] Values are least squares means with pooled SEMs, $n = 29$. BCFA, branched-chain FA (isobutyrate + isovalerate + valerate); DM, dry matter.

Agave inulin supplementation significantly shifted the relative abundance of fecal Actinobacteria compared with control ($P = 0.002$; 7.5 = 5.0 > 0 g agave inulin/d) (**Table 2**). Compared with control, Actinobacteria were enriched ($P < 0.05$; 7.5 = 5.0 > 0 g agave inulin/d) 3- and 4-fold with 5.0 and 7.5 g agave inulin/d, respectively. These shifts were countered with nonsignificant reductions in Proteobacteria and Bacteroidetes. The relative abundance of the Bifidobacteriaceae family (**Supplemental Table 5**) and *Bifidobacterium* genus (Table 2) were both similarly enriched ($P < 0.001$; 7.5 = 5.0 > 0 g agave inulin/d) by 3- and 4-fold with 5.0 and 7.5 g agave inulin/d, respectively). Four species in the *Bifidobacterium* genera were significantly enriched ($P < 0.005$; 7.5 = 5.0 > 0 g agave inulin/d) after supplementation with 5.0 and 7.5 g agave inulin/d compared with control: *B. adolescentis*, *B. breve*, *B. longum*, and *B. pseudolongum* (**Supplemental Table 6**). Two species, *B. animalis* and *B. bifidum*, were not affected by treatment ($P > 0.05$). The relative abundance of the *Desulfovibrio* genera was reduced ($P < 0.05$; 7.5 = 5.0 < 0 g agave inulin/d) by ~40% with both treatment doses of agave inulin. In addition, the relative abundances of *Lachnobacterium* and *Ruminococcus* were depleted ($P < 0.05$) with 7.5 g agave inulin/d compared with 0 g agave inulin/d; however, 5.0 g agave inulin/d was not different from 0 or 7.5 g agave inulin/d.

Although dietary supplementation of agave inulin resulted in a significant increase in fecal *Bifidobacterium* in the treatment groups, individual responses to the treatments were varied (**Figure 1**). In general, female participants were more responsive to supplementation, demonstrating larger shifts in the abundance of fecal *Bifidobacterium* than for male participants. Two women experienced a 15% increase in fecal *Bifidobacterium* compared with 0 g agave inulin/d, and 3 women demonstrated 5–10% increases in abundance with agave inulin supplementation. Half of the male participants demonstrated increased abundances of ≤5% in fecal *Bifidobacterium* from the 0-g/d treatment period. Alternatively, 5 male participants did not respond to supplementation and were essentially unaffected by agave inulin treatments with 0–1% reductions in fecal *Bifidobacterium* with agave inulin supplementation compared with control.

Bivariate correlations revealed a significant positive correlation between *Bifidobacterium* and grams of agave inulin consumed per kilocalorie ($r = 0.41$, $P < 0.001$; **Figure 2**A). Total dietary fiber intake (total dietary fiber plus 0, 5.0, or 7.5 g agave inulin/d) per kilocalorie, however, only tended to be associated with *Bifidobacterium* abundance ($r = 0.19$, $P = 0.07$; Figure 2B). Total fiber intake was positively associated with fecal butyrate concentration ($r = 0.30$, $P = 0.005$; Figure 2C). Fecal *Faecalibacterium* also was positively associated with butyrate concentrations ($r = 0.29$, $P = 0.007$). Fecal ammonia concentration tended ($r = -0.21$, $P = 0.052$) to negatively correlate with *Bifidobacterium* abundance. No other significant correlations were found between fecal fermentation end products and *Bifidobacterium*. Bivariate correlations revealed several correlations with *Desulfovibrio* abundance, including negative correlations between *Desulfovibrio* abundance and total fiber intake per kilocalorie ($r = -0.31$, $P = 0.003$) and fecal acetate ($r = -0.28$, $P = 0.009$), butyrate ($r = -0.23$, $P = 0.029$), and total SCFA ($r = -0.26$, $P = 0.015$) concentrations. Conversely, positive correlations were found with *Desulfovibrio* abundance

TABLE 2 Predominant fecal bacterial phyla and genera present in healthy human participants who consume 0, 5.0, or 7.5 g agave inulin/d in a crossover design[1]

	Treatment with agave inulin, % of sequences				
Phylum and genus	0 g/d	5.0 g/d	7.5 g/d	Pooled SEM	*P*
Firmicutes	50	51	49	3.5	0.61
Faecalibacterium	12	14	14	1.6	0.40
Eubacterium	0.64	0.84	0.85	0.36	0.28
Clostridium	0.36	0.26	0.27	0.05	0.41
Ruminococcus	5.1[b]	3.1[a,b]	2.3[a]	0.81	<0.01
Roseburia	1.9	1.7	1.6	0.31	0.89
Lachnospira	0.93	0.95	0.87	0.18	0.76
Coprococcus	1.5	1.5	1.5	0.26	0.93
Dialister	0.51	0.76	0.56	0.24	0.29
Dorea	0.43	0.59	0.39	0.14	0.10
Oscillospira	0.34	0.32	0.28	0.04	0.52
Blautia	2.6	2.6	2.3	0.36	0.32
Anaerostipes	0.22	0.16	0.18	0.03	0.42
Lachnobacterium	0.24[b]	0.07[a]	0.08[a,b]	0.06	0.02
Lactobacillus	0.01	0.02	0.05	0.03	0.18
Megamonas	0.02	0.36	0.40	0.24	0.14
Megasphaera	0.14	0.20	0.29	0.15	0.26
Phascolarctobacterium	0.13	0.15	0.14	0.04	0.87
Bacteroidetes	44	42	43	3.5	0.61
Bacteroides	31	32	32	3.5	0.93
Parabacteroides	1.9	1.7	1.4	0.10	0.66
Prevotella	7.7	5.5	6.6	2.7	0.06
Paraprevotella	0.20	0.09	0.19	0.10	0.19
Proteobacteria	3.1	2.8	2.0	0.98	0.61
Sutterella	0.80	1.1	0.92	0.16	0.41
Bilophila	0.21	0.16	0.15	0.03	0.06
Desulfovibrio	0.14[b]	0.08[a]	0.09[a]	0.06	0.01
Succinivibrio	0.75	0.69	0.48	0.65	0.79
Pseudomonas	0.41	0.07	0.01	0.20	0.79
Actinobacteria	1.9[a]	3.6[b]	5.1[b]	0.87	<0.01
Bifidobacterium	1.7[a]	3.2[b]	4.9[b]	0.83	<0.01
Collinsella	0.10	0.22	0.14	0.10	0.17
Verrucomicrobia	0.34	0.50	0.30	0.16	0.23
Akkermansia	0.34	0.50	0.30	0.16	0.23

[1] Values are least squares means with pooled SEMs, $n = 29$. Values in a row without a common letter are significantly different, $P < 0.05$.

and fecal 4-methylphenol ($r = 0.29$, $P = 0.007$) and with fecal pH ($r = 0.24$, $P = 0.02$).

α and β diversity were also assessed and results are included in **Supplemental Figure 2** and **3**, respectively.

Discussion

Prebiotics are selectively fermented ingredients that promote specific changes in composition and/or activity of GI bacteria (20). However, to date, the impact of prebiotics on the microbiota has relied heavily on molecular methods that investigate targeted taxa instead of characterizing the entire community structure. The present study used high-throughput sequencing to characterize the community composition of the fecal microbiota. In addition, we measured fecal fermentation end products, thereby providing both compositional and functional outcomes related to agave inulin fermentation by the GI microbiota. Our data revealed that agave inulin supplementation enriched fecal *Bifidobacterium*. In addition, we found a negative correlation between *Bifidobacterium* and fecal ammonia concentrations. The reduction in fecal pH and phenolic compounds suggests increased saccharolytic fermentation and reduced proteolytic fermentation. Because phenols and ammonia are considered toxic to intestinal epithelial cells, our results indicate a prebiotic effect of agave inulin supplementation.

Because *Bifidobacterium* are not the only bacteria able to use inulin-type fibers and bacterial crossfeeding is particularly important in the complex milieu of the GI tract, an ecologic characterization of the microbiota was necessary. Although *Lactobacillus, Bacteroides*, *Roseburia*, and *Faecalibacterium* have all demonstrated the potential to degrade oligofructose in vitro (57, 58), we found that only *Bifidobacterium* species were selectively enriched in healthy adults who consumed agave inulin. Four species of *Bifidobacterium* were enriched with agave inulin supplementation, *B. adolescentis, B. breve, B. longum,* and *B. pseudolongum,* whereas 2 others were not (e.g., *B. animalis* and *B. bifidum*). In vitro experiments have indicated that *B. adolescentis* is able to grow on FOSs and that its presence contributed to crossfeeding by lactate utilizers, and subsequent butyrate production (35). The presence of various β-fructofuranosidase genes in several strains of these species is supportive of these results (59–62). In addition, in vitro studies demonstrate that *B. bifidum* grows on FOSs but not with inulin (63, 64) and that a commercial probiotic strain of *B. animalis* was also not able to metabolize inulin (59–62). The selective growth inhibition by *B. bifidum* and *B. animalis* may be due to the presence of different β-fructofuranoside genes and also the structural differences between FOSs and long-chain inulin.

The linear relation between agave inulin per kilocalorie and *Bifidobacterium* provides a plausible explanation for the more pronounced effect observed in female as opposed to male participants because agave inulin represented a higher proportion of the dietary intake of women. Dose responses were demonstrated with short-chain FOSs, whereby 2.5 g/d did not increase bifidobacteria counts >0 g/d, but 10 and 20 g/d increased

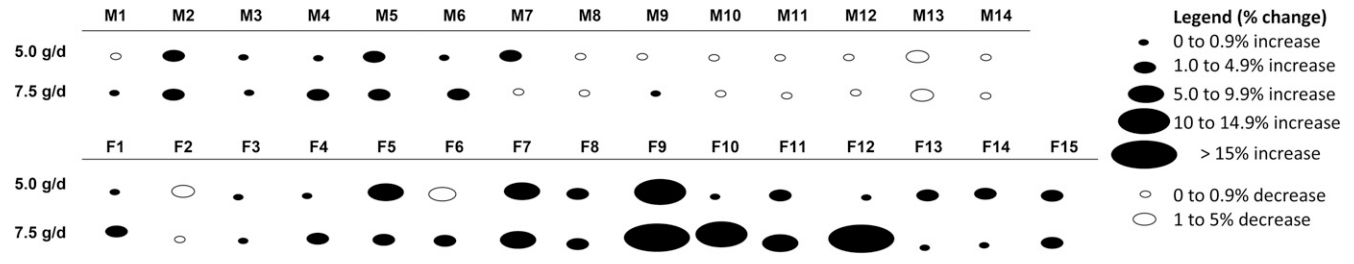

FIGURE 1 Bubble plots show differences in the proportions of fecal *Bifidobacterium* as a percentage in each individual healthy adult male (M) and female (F) participant ($n = 29$) after 20 d of consumption of 5.0 and 7.5 g agave inulin/d compared with the control period (0 g agave inulin/d). The size of the bubbles is representative of the percentage differences. Black bubbles depict increased proportions, and white bubbles depict decreased proportions of fecal *Bifidobacterium* after agave inulin supplementation.

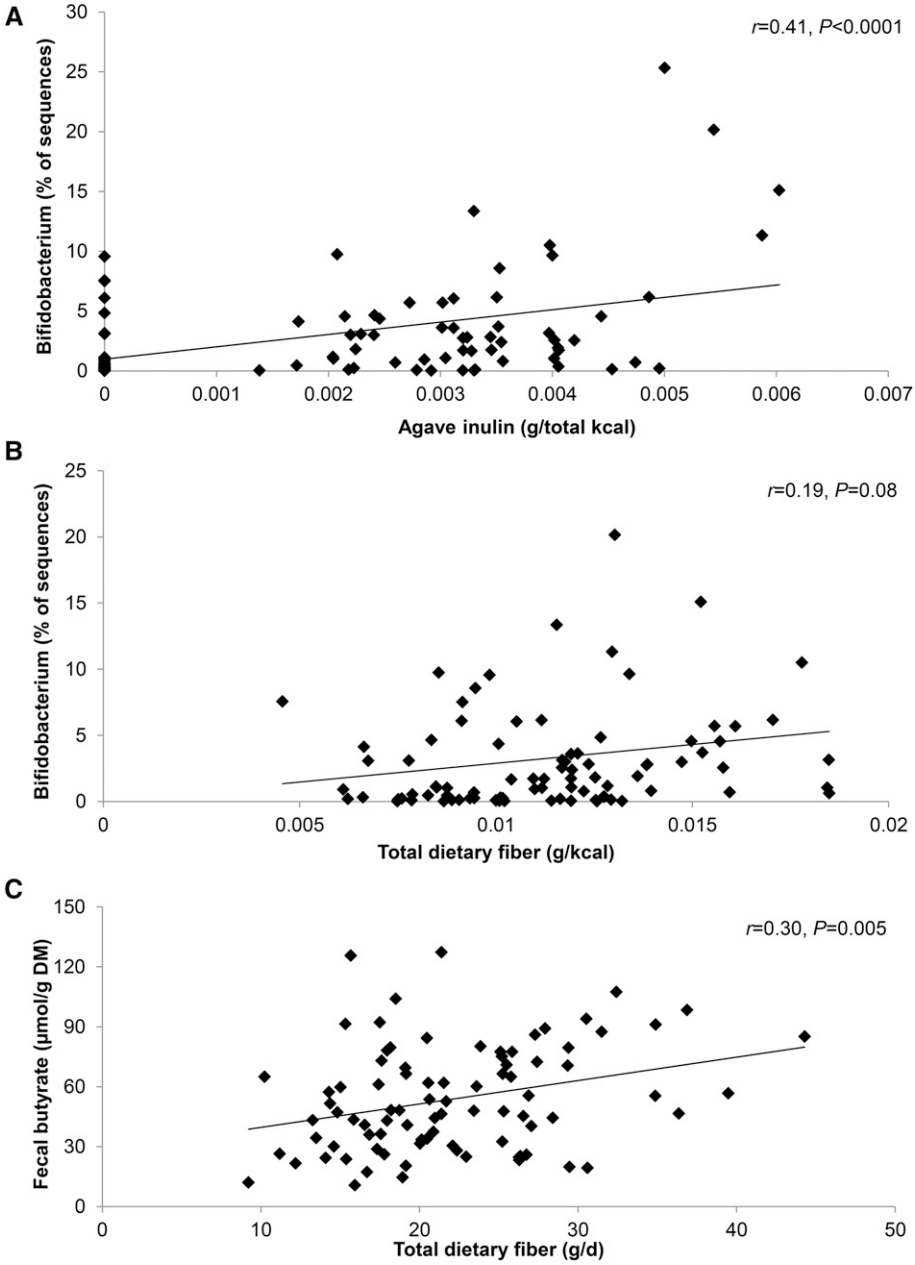

FIGURE 2 Scatterplots depict relations between (A) fecal *Bifidobacterium* and grams of agave inulin consumed per kilocalorie, (B) fecal *Bifidobacterium* and total fiber intake (total dietary fiber plus 0, 5.0, or 7.5 g agave inulin/d agave inulin) per kilocalorie, and (C) total fiber intake (g/d) and fecal butyrate concentrations (μmol/g DM feces) in healthy human participants consuming 0, 5.0, or 7.5 g agave inulin/d in a crossover design. Statistical relations were determined with bivariate correlations (Pearson's *r*), and a probability of $P < 0.05$ was accepted as statistically significant, $n = 29$. DM, dry matter.

fecal bifidobacteria in healthy adults (65). Similarly, galactooligosaccharide (GOS) supplementation followed a dose response curve for enriching bifidobacteria abundance. In that case, supplementation of 2.5 g GOSs/d did not shift fecal microbes in healthy adults compared with control; however, doses of 5.0 and 10 g GOSs/d significantly increased fecal bifidobacteria abundance (66). Host genetics may also contribute to these differential responses (67).

Previously, we reported the breath hydrogen profiles of these same participants after a bolus of 0, 5, or 7.5 g agave inulin/d. The results revealed an early peak (4–6 h) after agave inulin consumption, suggesting fermentation begins more proximally in the GI tract. Breath hydrogen profiles represent 14% of total hydrogen produced in the gut that is subsequently perfused into the lungs (68). By comparison, between 90% and 99% of SCFAs are absorbed by the gut or used by the microbiota (25, 30, 69). As such, fecal SCFAs represent residual fermentation end products, thereby providing a potential explanation for the numeric increase in fecal SCFAs with agave inulin supplementation. Because we previously observed a clear distinction between agave inulin and controls during the 8-h breath hydrogen testing, but only a numeric increase in fecal SCFAs, this suggests that the SCFA measurements were either not sensitive enough to detect the changes in fermentation profiles among treatments or that there was inadequate power.

The fermentation profile in concert with the enrichment of fecal *Bifidobacterium* and depletion of fecal *Desulfovibrio* after agave inulin supplementation is particularly interesting. Proteobacteria, including *Desulfovibrio*, colonize the proximal intestine utilizing mono- and di-saccharides and amino acids as primary energy sources (70). Because saccharolytic fermentation of agave inulin begins 4 h after consumption, this suggests that the impact of supplementation may begin more proximally in the GI tract. Early fermentation could be affecting *Desulfovibrio* by spreading saccharolytic fermentation throughout the GI tract, thereby changing nutrient availability and environmental

conditions along the way. The numeric reduction in the proteolytic fermentation end product, 4-methylphenol, is also supportive of this hypothesis. *Desulfovibrio* is a sulfate-reducing bacteria that uses substrates, including SCFAs and amino acids, to reduce sulfur-containing compounds to hydrogen sulfide, a potential toxin to GI epithelial cells (71, 72). Increased proportions of sulfate-reducing bacteria were noted in individuals with inflammatory bowel disease and autism (7, 8, 73–75). Furthermore, individuals with autism were found to have both increased abundances of *Desulfovibrio* and decreased abundances of *Bifidobacterium* (7, 8, 76). Although the underlying mechanisms of these bacterial shifts in diseased individuals remain unclear, the potential application of agave inulin as a therapeutic agent in individuals with these diseases warrants further investigation.

Our data support the Institute of Medicine's recommendation to consume a high-fiber diet from a variety of sources. Although we did not detect a significant treatment effect of agave inulin supplementation alone, total dietary fiber intake (dietary fiber plus 0, 5.0, and 7.5 g agave inulin/d) was positively correlated with fecal butyrate. The benefits of increased SCFA concentrations and particularly increased butyrate include local and systemic effects. Luminal effects of butyrate include provision of energy for intestinal epithelial cells and effects on enterocyte cell cycle progression, differentiation, and apoptosis via histone deacetylase inhibition; systemically, butyrate was shown to provide immune-modulating functions, influence cholesterol biosynthesis, and improve insulin resistance (23, 24, 26–28, 69, 77, 78).

To our knowledge, this is the first study to use high-throughput sequencing to demonstrate a specific enrichment of fecal *Bifidobacterium* after agave inulin supplementation in healthy adults. The selectivity of other prebiotic fibers was demonstrated in clinical trials by using high-throughput sequencing. Davis et al. (66) reported that 5.0 and 10.0 g GOSs/d specifically enriched fecal *Bifidobacterium*. Resistant starches also were reported to have differential effects on the fecal microbiota. Resistant starch type 4 was previously found to enrich *Bifidobacterium*, whereas resistant starch type 2 selectively enriched *Eubacterium* (79). Other fermentable fibers also have demonstrated more nonspecific shifts, including polydextrose and soluble corn fiber, which were found to enrich several genera in both the Firmicutes and Bacteroides phyla (80, 81).

The chemical structures of these fibers and the complex GI ecosystem, which provides residence to diverse microbes capable of crossfeeding, should be considered in light of this. Agave inulin is composed of a terminal glucose monosaccharide with linear and branched fructose chains connected with β-2,1 and β-2,6 linkages, and a DP ranging from 25 to 34 (42). GOSs typically contain a terminal glucose with a β-1,4 linkage to galactose polymers linked by β-1,6 covalent bonds; DP generally ranges between 2 and 10 (82). Resistant starch type 2 and type 4 are composed of glucose monomers with α-1,6 glycosidic bonds, with the additional crosslink by phosphorylation of type 4 resistant starches (79). Polydextrose is a highly branched polysaccharide that consists of glucose units linked by α- and β-linked 1,2, 1,3, 1,4, and 1,6 linkages (83). Soluble corn fiber is an oligosaccharide-rich corn starch fraction enriched in α-1,6-glycosidic bonds (84). The distinct molecular structures of these fibers provide a partial explanation for the differences in microbial shifts after supplementation.

Study strengths include the crossover design with washouts, dietary record collection, utilization of state-of-the-art sequencing technology and bioinformatics tools, and assessment of digestive physiologic outcomes. We, however, acknowledge potential limitations, including the lack of biomedical measures such as blood glucose, cholesterol, and TGs. In addition, we aimed to characterize the impact of fiber supplementation on the entire community structure of the fecal microbiota; therefore, a more in-depth examination of the species and strains affected by agave inulin were outside the scope of this research and should be investigated in future studies. Next steps should include assessment of microbial functional capacity and activity through measurement of mRNA or protein expression and further assessment of untargeted bacterial metabolites. Additional characterization of bacterial crossfeeding via in vitro models and computational simulations will also help advance our understanding of the role of diet on the microbiome. Because rodent studies have provided evidence for the benefits of agave inulin supplementation on body composition, blood cholesterol, and blood glucose concentrations (45–47), further investigation is warranted to determine whether these effects translate into health benefits in human populations.

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References

1. Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, Krakoff J. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. Am J Clin Nutr 2011;94:58–65.
2. Goldsmith JR, Sartor RB. The role of diet on intestinal microbiota metabolism: downstream impacts on host immune function and health, and therapeutic implications. J Gastroenterol 2014;49:785–98.
3. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, DuGar B, Feldstein AE, Britt EB, Fu X, Chung Y. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472:57–63.
4. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207–14.
5. Greenblum S, Turnbaugh PJ, Borenstein E. Metagenomic systems biology of the human gut microbiome reveals topological shifts associated with obesity and inflammatory bowel disease. Proc Natl Acad Sci USA 2012;109:594–9.
6. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 2012;490:55–60.
7. De Angelis M, Piccolo M, Vannini L, Siragusa S, De Giacomo A, Serrazzanetti DI, Cristofori F, Guerzoni ME, Gobbetti M, Francavilla R. Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. PLoS One 2013;8: e76993.
8. Finegold SM, Dowd SE, Gontcharova V, Liu C, Henley KE, Wolcott RD, Youn E, Summanen PH, Granpeesheh D, Dixon D. Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe 2010;16:444–53.
9. Liu S, Willett WC, Manson JE, Hu FB, Rosner B, Colditz G. Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. Am J Clin Nutr 2003;78:920–7.

10. Schulze MB, Liu S, Rimm EB, Manson JE, Willett WC, Hu FB. Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women. Am J Clin Nutr 2004;80:348–56.
11. Montonen J, Knekt P, Jarvinen R, Aromaa A, Reunanen A. Whole-grain and fiber intake and the incidence of type 2 diabetes. Am J Clin Nutr 2003;77:622–9.
12. Wolk A, Manson JE, Stampfer MJ, Colditz GA, Hu FB, Speizer FE, Hennekens CH, Willett WC. Long-term intake of dietary fiber and decreased risk of coronary heart disease among women. JAMA 1999;281:1998–2004.
13. Kromhout D, Bosschieter E, Coulander CDL. Dietary fibre and 10-year mortality from coronary heart disease, cancer, and all causes. The Zutphen Study. Lancet 1982;2:518–22.
14. Streppel MT, Ocke MC, Boshuizen HC, Kok FJ, Kromhout D. Dietary fiber intake in relation to coronary heart disease and all-cause mortality over 40 y: the Zutphen Study. Am J Clin Nutr 2008;88:1119–25.
15. US Department of Agriculture and US Department of Health and Human Services. Dietary guidelines for Americans. Washington (DC): US Government Printing Office; 2010.
16. King DE, Mainous, III AG, Lambourne CA. Trends in dietary fiber intake in the United States, 1999–2008. J Acad Nutr Diet 2012;112:642–8.
17. Cho SS, Qi L, Fahey GC, Klurfeld DM. Consumption of cereal fiber, mixtures of whole grains and bran, and whole grains and risk reduction in type 2 diabetes, obesity, and cardiovascular disease. Am J Clin Nutr 2013;98:594–619.
18. Trock B, Lanza E, Greenwald P. Dietary fiber, vegetables, and colon cancer: critical review and meta-analyses of the epidemiologic evidence. J Natl Cancer Inst 1990;82:650–61.
19. Ludwig DS, Pereira MA, Kroenke CH, Hilner JE, Van Horn L, Slattery ML, Jacobs DR, Jr. Dietary fiber, weight gain, and cardiovascular disease risk factors in young adults. JAMA 1999;282:1539–46.
20. Gibson GR, Scott KP, Rastall RA, Tuohy KM, Hotchkiss A, Dubert-Ferrandon A, Gareau M, Murphy EF, Saulnier D, Loh G. Dietary prebiotics: current status and new definition. Food Sci Technol Bull Funct Foods. 2010;7:1–19.
21. Cummings JH. Short chain fatty acids in the human colon. Gut 1981;22:763–79.
22. Berni Canani R, Di Costanzo M, Leone L. The epigenetic effects of butyrate: potential therapeutic implications for clinical practice. Clin Epigenetics 2012;4:4.
23. Canani RB, Costanzo M, Leone L, Pedata M, Meli R, Calignano A. Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. World J Gastroenterol 2011;17:1519–28.
24. Chen YX, Fang JY, Lu J, Qiu DK. Regulation of histone acetylation on the expression of cell cycle-associated genes in human colon cancer cell lines. Zhonghua Yi Xue Za Zhi 2004;84:312–7.
25. Binder HJ, Mehta P. Short-chain fatty acids stimulate active sodium and chloride absorption in vitro in the rat distal colon. Gastroenterology 1989;96:989–96.
26. Comalada M, Bailon E, de Haro O, Lara-Villoslada F, Xaus J, Zarzuelo A, Galvez J. The effects of short-chain fatty acids on colon epithelial proliferation and survival depend on the cellular phenotype. J Cancer Res Clin Oncol 2006;132:487–97.
27. Davie JR. Inhibition of histone deacetylase activity by butyrate. J Nutr 2003;133:2485S–93S.
28. Lührs H, Gerke T, Müller J, Melcher R, Schauber J, Boxberger F, Scheppach W, Menzel T. Butyrate inhibits NF-κB activation in lamina propria macrophages of patients with ulcerative colitis. Scand J Gastroenterol 2002;37:458–66.
29. Rabbani GH, Albert MJ, Rahman H, Chowdhury AK. Short-chain fatty acids inhibit fluid and electrolyte loss induced by cholera toxin in proximal colon of rabbit in vivo. Dig Dis Sci 1999;44:1547–53.
30. Scheppach W. Effects of short chain fatty acids on gut morphology and function. Gut 1994;35:S35–8.
31. Schwab M, Reynders V, Loitsch S, Steinhilber D, Stein J, Schröder O. Involvement of different nuclear hormone receptors in butyrate-mediated inhibition of inducible NFκB signalling. Mol Immunol 2007;44:3625–32.
32. Schaafsma G, Slavin JL. Significance of inulin fructans in the human diet. Compr Rev Food Sci Food Safety. 2015;14:37–47.
33. Holscher HD, Faust KL, Czerkies LA, Litov R, Ziegler EE, Lessin H, Hatch T, Sun S, Tappenden KA. Effects of prebiotic-containing infant formula on gastrointestinal tolerance and fecal microbiota in a randomized controlled trial. JPEN J Parenter Enteral Nutr 2012;36 1 Suppl:95S–105S.
34. Brownawell AM, Caers W, Gibson GR, Kendall CW, Lewis KD, Ringel Y, Slavin JL. Prebiotics and the health benefits of fiber: current regulatory status, future research, and goals. J Nutr 2012;142:962–74.
35. Belenguer A, Duncan SH, Calder AG, Holtrop G, Louis P, Lobley GE, Flint HJ. Two routes of metabolic cross-feeding between Bifidobacterium adolescentis and butyrate-producing anaerobes from the human gut. Appl Environ Microbiol 2006;72:3593–9.
36. Dong P, Yang Y, Wang W. The role of intestinal bifidobacteria on immune system development in young rats. Early Hum Dev 2010;86:51–8.
37. López P, González-Rodríguez I, Gueimonde M, Margolles A, Suárez A. Immune response to Bifidobacterium bifidum strains support Treg/Th17 plasticity. PLoS One 2011;6:e24776.
38. Menard O, Butel MJ, Gaboriau-Routhiau V, Waligora-Dupriet AJ. Gnotobiotic mouse immune response induced by Bifidobacterium sp. strains isolated from infants. Appl Environ Microbiol 2008;74:660–6.
39. Santacruz A, Collado MC, Garcia-Valdés L, Segura MT, Martin-Lagos JA, Anjos T, Marti-Romero M, Lopez RM, Florido J, Campoy C, Sanz Y. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. Br J Nutr 2010;104:83–92.
40. Ventura M, Turroni F, Bottacini F, Giubellini V, van Sinderen D. Bifidobacterial ecology and comparative genomics: perspectives. Bifidobacteria Genomics Molbi Aspects 2010:31.
41. Lopez MG, Mancilla-Margalli NA, Mendoza-Diaz G. Molecular structures of fructans from Agave tequilana Weber var. azul. J Agric Food Chem 2003;51:7835–40.
42. Roberfroid MB, Van Loo JA, Gibson GR. The bifidogenic nature of chicory inulin and its hydrolysis products. J Nutr 1998;128:11–9.
43. Allsopp P, Possemiers S, Campbell D, Oyarzábal IS, Gill C, Rowland I. An exploratory study into the putative prebiotic activity of fructans isolated from Agave angustifolia and the associated anticancer activity. Anaerobe 2013;22:38–44.
44. Gomez E, Tuohy K, Gibson G, Klinder A, Costabile A. In vitro evaluation of the fermentation properties and potential prebiotic activity of Agave fructans. J Appl Microbiol 2010;108:2114–21.
45. Márquez-Aguirre AL, Camacho-Ruíz RM, Arriaga-Alba M, Padilla-Camberos E, Kirchmayr MR, Blasco JL, González-Ávila M. Effects of Agave tequilana fructans with different degree of polymerization profiles on body weight, blood lipids and fecal Lactobacilli/Bifidobacteria in obese mice. Food Funct 2013;4:1237–44.
46. Rendón-Huerta JA, Juárez-Flores B, Pinos-Rodríguez JM, Aguirre-Rivera JR, Delgado-Portales RE. Effects of different sources of fructans on body weight, blood metabolites and fecal bacteria in normal and obese non-diabetic and diabetic rats. Plant Foods Hum Nutr 2012;67:64–70.
47. Urías-Silvas JE, Cani PD, Delmée E, Neyrinck A, López MG, Delzenne NM. Physiological effects of dietary fructans extracted from Agave tequilana Gto and Dasylirion spp. Br J Nutr 2008;99:254–61.
48. Holscher HD, Doligale JL, Bauer LL, Gourineni V, Pelkman CL, Fahey GC, Swanson KS. Gastrointestinal tolerance and utilization of agave inulin by healthy adults. Food Funct 2014;5:1142–9.
49. Association of Official Analytical Chemists. Official methods of analysis. Washington (DC): AOAC; 1984.
50. Chaney AL, Marbach EP. Modified reagents for determination of urea and ammonia. Clin Chem 1962;8:130–2.
51. Boler BM, Serao MC, Bauer LL, Staeger MA, Boileau TW, Swanson KS, Fahey GC, Jr. Digestive physiological outcomes related to polydextrose and soluble maize fibre consumption by healthy adult men. Br J Nutr 2011;106:1864–71.
52. Flickinger EA, Schreijen EM, Patil AR, Hussein HS, Grieshop CM, Merchen NR, Fahey GC, Jr. Nutrient digestibilities, microbial populations, and protein catabolites as affected by fructan supplementation of dog diets. J Anim Sci 2003;81:2008–18.
53. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 2012;6:1621–4.
54. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 2010;7:335–6.
55. Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, Mills DA, Caporaso JG. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat Methods 2013;10:57–9.

56. Lozupone C, Lladser ME, Knights D, Stombaugh J, Knight R. UniFrac: an effective distance metric for microbial community comparison. ISME J 2011;5:169–72.
57. Ramirez-Farias C, Slezak K, Fuller Z, Duncan A, Holtrop G, Louis P. Effect of inulin on the human gut microbiota: stimulation of Bifidobacterium adolescentis and Faecalibacterium prausnitzii. Br J Nutr 2009;101:541–50.
58. De Vuyst L, Leroy F. Cross-feeding between bifidobacteria and butyrate-producing colon bacteria explains bifdobacterial competitiveness, butyrate production, and gas production. Int J Food Microbiol 2011;149:73–80.
59. Muramatsu K, Onodera S, Kikuchi M, Shiomi N. Purification and some properties of β-fructofuranosidase from Bifidobacterium adolescentis G1. Biosci Biotechnol Biochem 1993;57:1681–5.
60. Falony G, Verschaeren A, De Bruycker F, De Preter V, Verbeke K, Leroy F, De Vuyst L. In vitro kinetics of prebiotic inulin-type fructan fermentation by butyrate-producing colon bacteria: implementation of online gas chromatography for quantitative analysis of carbon dioxide and hydrogen gas production. Appl Environ Microbiol 2009;75:5884–92.
61. Ryan SM, Fitzgerald GF, van Sinderen D. Transcriptional regulation and characterization of a novel beta-fructofuranosidase-encoding gene from Bifidobacterium breve UCC2003. Appl Environ Microbiol 2005;71:3475–82.
62. Van der Meulen R, Avonts L, De Vuyst L. Short fractions of oligofructose are preferentially metabolized by Bifidobacterium animalis DN-173 010. Appl Environ Microbiol 2004;70:1923–30.
63. Falony G, Calmeyn T, Leroy F, De Vuyst L. Coculture fermentations of Bifidobacterium species and Bacteroides thetaiotaomicron reveal a mechanistic insight into the prebiotic effect of inulin-type fructans. Appl Environ Microbiol 2009;75:2312–9.
64. Rossi M, Corradini C, Amaretti A, Nicolini M, Pompei A, Zanoni S, Matteuzzi D. Fermentation of fructooligosaccharides and inulin by bifidobacteria: a comparative study of pure and fecal cultures. Appl Environ Microbiol 2005;71:6150–8.
65. Bouhnik Y, Vahedi K, Achour L, Attar A, Salfati J, Pochart P, Marteau P, Flourie B, Bornet F, Rambaud JC. Short-chain fructo-oligosaccharide administration dose-dependently increases fecal bifidobacteria in healthy humans. J Nutr 1999;129:113–6.
66. Davis LM, Martínez I, Walter J, Goin C, Hutkins RW. Barcoded pyrosequencing reveals that consumption of galactooligosaccharides results in a highly specific bifidogenic response in humans. PLoS One 2011;6:e25200.
67. Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, Beaumont M, Van Treuren W, Knight R, Bell JT. Human genetics shape the gut microbiome. Cell 2014;159:789–99.
68. Levitt MD. Production and excretion of hydrogen gas in man. N Engl J Med 1969;281:122–7.
69. Ruppin H, Bar-Meir S, Soergel KH, Wood CM, Schmitt MG, Jr. Absorption of short-chain fatty acids by the colon. Gastroenterology 1980;78:1500–7.
70. Kamada N, Chen GY, Inohara N, Núñez G. Control of pathogens and pathobionts by the gut microbiota. Nat Immunol 2013;14:685–90.
71. Scanlan PD, Shanahan F, Marchesi JR. Culture-independent analysis of desulfovibrios in the human distal colon of healthy, colorectal cancer and polypectomized individuals. FEMS Microbiol Ecol 2009;69:213–21.
72. Huycke MM, Gaskins HR. Commensal bacteria, redox stress, and colorectal cancer: mechanisms and models. Exp Biol Med (Maywood) 2004;229:586–97.
73. Frank DN, Robertson CE, Hamm CM, Kpadeh Z, Zhang T, Chen H, Zhu W, Sartor RB, Boedeker EC, Harpaz N. Disease phenotype and genotype are associated with shifts in intestinal-associated microbiota in inflammatory bowel diseases. Inflamm Bowel Dis 2011;17:179–84.
74. Gibson G, Cummings J, Macfarlane G. Growth and activities of sulphate-reducing bacteria in gut contents of healthy subjects and patients with ulcerative colitis. FEMS Microbiol Lett 1991;86:103–11.
75. Christl SU, Scheppach W, Kasper H. Hydrogen metabolism in the large intestine–physiology and clinical implications. Z Gastroenterol 1995;33:408–13.
76. Heberling CA, Dhurjati PS, Sasser M. Hypothesis for a systems connectivity model of autism spectrum disorder pathogenesis: Links to gut bacteria, oxidative stress, and intestinal permeability. Med Hypotheses 2013;80:264–70.
77. Alvaro A, Sola R, Rosales R, Ribalta J, Anguera A, Masana L, Vallvé JC. Gene expression analysis of a human enterocyte cell line reveals downregulation of cholesterol biosynthesis in response to short-chain fatty acids. IUBMB Life 2008;60:757–64.
78. Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, Cefalu WT, Ye J. Butyrate improves insulin sensitivity and increases energy expenditure in mice. Diabetes 2009;58:1509–17.
79. Martínez I, Kim J, Duffy PR, Schlegel VL, Walter J. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. PLoS One 2010;5:e15046.
80. Hooda S, Boler BMV, Serao MCR, Brulc JM, Staeger MA, Boileau TW, Dowd SE, Fahey GC, Swanson KS. 454 pyrosequencing reveals a shift in fecal microbiota of healthy adult men consuming polydextrose or soluble corn fiber. J Nutr 2012;142:1259–65.
81. Holscher HD, Caporaso JG, Hooda S, Brulc JM, Fahey GC, Jr., Swanson KS. Fiber supplementation influences phylogenetic structure and functional capacity of the human intestinal microbiome: follow-up of a randomized controlled trial. Am J Clin Nutr 2015;101:55–64.
82. Angus F, Smart S, Shortt C. Prebiotic ingredients with emphasis on galactooligosaccharides and fructo-oligosaccharides. In: Tamime AY, editor. Probiotic dairy products. New York: Blackwell Publishing; 2005. P.120–37.
83. Lahtinen SJ, Knoblock K, Drakoularakou A, Jacob M, Stowell J, Gibson GR, Ouwehand AC. Effect of molecule branching and glycosidic linkage on the degradation of polydextrose by gut microbiota. Biosci Biotechnol Biochem 2010;74:2016–21.
84. Knapp BK, Bauer LL, Swanson KS, Tappenden KA, Fahey GC, De Godoy MR. Soluble fiber dextrin and soluble corn fiber supplementation modify indices of health in cecum and colon of Sprague-Dawley rats. Nutrients 2013;5:396–410.