Effects of a diet based on inulin-rich vegetables on gut health and nutritional behavior in healthy humans

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ABSTRACT

Background: Inulin-type fructans (ITFs) are a type of fermentable dietary fiber that can confer beneficial health effects through changes in the gut microbiota. However, their effect on gut sensitivity and nutritional behavior is a matter of debate.

Objective: We evaluated the impact of consuming ITF-rich vegetables daily on gut microbiota, gastro-intestinal symptoms, and food-related behavior in healthy individuals.

Methods: A single group-design trial was conducted in 26 healthy individuals. During 2 wk, the participants were instructed to adhere to a controlled diet based on ITF-rich vegetables (providing a mean intake of 15 g ITF/d). Three test days were organized: before and after the nutritional intervention and 3 wk after returning to their usual diet. We assessed nutrient intake, food-related behavior, fecal microbiota composition, microbial fermentation, and gastrointestinal symptoms.

Results: The major microbial modifications during the intervention were an increased proportion of the *Bifidobacterium* genus, a decreased level of unclassified Clostridiales, and a tendency to decrease *Oxalobacteraceae.* These changes were reversed 3 wk after the intervention. The volunteers showed greater satiety, a reduced desire to eat sweet, salty, and fatty food, and a trend to increase hedonic attitudes towards some inulin-rich vegetables. Only flatulence episodes were reported during the dietary intervention, whereas intestinal discomfort, inversely associated with *Clostridium* cluster IV and *Ruminococcus callidus*, was improved at the end of the intervention.

Conclusions: A higher consumption of ITF-rich vegetables allows a substantial increase in well-tolerated dietary fiber, which may in turn improve food-related behavior. Moreover, it leads to beneficial modifications of the gut microbiota composition and function. This trial is registered at clinicaltrial.gov as NCT03540550. *Am J Clin Nutr* 2019;109:1683–1695.

Keywords: inulin-rich vegetables, nutrition, gut health, nutritional behavior, healthy humans, gut microbiota, microbial fermentation

Introduction

For several years, the gut microbiota has been pointed out as an attractive ecosystem that plays a key role in host physiology. Alterations of the gut microbiota composition have been associated with a wide variety of conditions such as obesity (1), type 2 diabetes (2), inflammatory bowel disease (3), autism (4), and behavioral disorders (5). In the diet, some nondigestible carbohydrates called prebiotics are fermented by the gut microbiota, thereby conferring potential health benefits (6, 7). Dietary supplementation with purified inulin-type fructans

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Supplemental Figures 1–7, Materials and methods and Supplemental Tables 1–4 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: BCFA, branched-chain fatty acid; BSS, Bristol Stool Scale; FODMAPs, fermentable oligo-, di-, monosaccharides, and polyols; GI, gastrointestinal; ITF, inulin-type fructan; OTU, operational taxonomic unit; PLS-DA, partial least squares discriminant analysis; SCFA, short-chain fatty acid; T0, first test day, taking place before the nutritional intervention; T1, second test day, taking place 14 d after the start of the nutritional intervention; T2, third test day, taking place 3 wk after the return to normal eating habits; VAS, visual analog scale.

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(ITFs) has been shown to exert positive health effects in humans, namely an improvement in intestinal permeability (8), a decrease in fat mass (9), an increase in the production of incretin gut peptides acting on satiety (10), and an improvement in appetite control (11). However, little is known about the effect of ITF on behavior and appetite feelings, when considering consumption of vegetables naturally rich in ITF. Moreover, recent data suggest that fermentation of dietary fiber may lead to gut discomfort, in particular in patients with inflammatory bowel syndrome (12). Surprisingly, only a few studies have attempted to analyze the impact of naturally occurring prebiotics in food products on gut microbiota composition and function, and gastrointestinal tolerance (13, 14). ITFs mostly occur in plant roots, such as Jerusalem artichokes, leeks, or salsify, where they act as a storage and stress-preservative polymer. In this study, we developed a protocol for a daily dietary intervention that lasted 2 wk with ITF-rich vegetables to reach a minimum intake of at least 9 g ITF/d in healthy volunteers. The aim of the study was to evaluate the effect of the nutritional intervention on gut microbiota composition and activity, nutrient intake, food-related behavior, and gastrointestinal symptoms. The composition of the gut microbiota was related to the gastrointestinal symptoms. We also evaluated the persistence of the effects 3 wk after completion of the dietary intervention.

Subjects and Methods

Subjects

Twenty-six healthy males and females were recruited at the Université catholique de Louvain (Louvain-la-Neuve, Belgium). The inclusion criteria were: men or women, aged 18–85 y, BMI of 20–25, Caucasian, and H_2 producers. The candidates were screened for H_2 production in a breath test 5 h after the ingestion of a high-fiber breakfast (Lactotest 202 monitor, Medical Electronic Construction). The minimum level of expired H_2 required for inclusion in the study was set at 12 ppm (15). Healthy subjects were chosen in this study to investigate the relevance of implementing an ITF-rich diet in the context of a healthy lifestyle. The exclusion criteria were: smoking, use of antibiotics or pro/prebiotics (as a dietary supplement) within 6 wk before starting the study, use of drugs that modify the composition of gut microbiota (antidiabetic drugs, cholesterol-lowering drugs, and proton pump inhibitors), use of laxatives within 4 wk before starting the study, presence of chronic or intestinal disease, pregnancy, presence of psychiatric problems, following a special diet (e.g., vegetarian, high-fiber, or high-protein diets), and excessive alcohol consumption (more than 3 units/d).

The study was approved by the "Comité d'éthique Hospitalo-facultaire de Saint-Luc" (reference B403201627275). Written informed consent was obtained from all participants before inclusion in the study.

Experimental procedure

The study was a single group-design trial and lasted 33 d. Between the first and the fourteenth day, subjects were instructed to consume a hot meal for lunch and a soup for dinner, prepared with ITF-rich vegetables to ensure an uptake of at least 9 g of fructans per day (mean intake of 15 g/d). Recipes were developed and cooked by the university catering services. The compositions of the meals were determined based on the results previously obtained by Kalala et al. (16) and are provided in **Table 1**. The ITF content of the meals was measured with an enzymatic method, AOAC Method 999.03, using the Megazyme fructans HK Assay kit (Megazyme), as previously described by Kalala et al. (16).

To ensure compliance, the hot meals were consumed every day at the university, whereas soup was eaten at home in the evening. Concerning other meals (i.e., breakfast, snacks), subjects were instructed to eat whatever they liked. During weekends, subjects received meal trays to take home. Between days 15 and 33, subjects were asked to return to their usual food habits. Three test days were organized at day 0 (T0), 14 (T1), and 33 (T2). To facilitate the organization of the test days, volunteers were separated into 2 groups, and test days were organized twice in a row. Apart from the first and last days of the dietary intervention, volunteers ate the same recipes each day throughout the intervention. The detailed protocol is schematized in **Supplemental Figure 1**. For test days, subjects were asked to avoid a high-fiber diet the evening before and were asked to fast for a minimum of 10 h. They received a standardized breakfast (water and 2 pancakes) and a standardized lunch. Within 2 d before each test day, all subjects were asked to provide fresh stool samples.

The volunteers were their own control in the design of the study. Indeed, blinding toward a placebo was impossible because the volunteers received ready-made meals to ensure an adequate inulin intake. The vegetables used as a source of inulin in the recipes were presented to the volunteers before the protocol (questions were asked about the previous intake, or acceptability of those vegetables). The objective of the study was not to compare the effect of a placebo diet versus a test diet, but to unravel how behavioral, microbial, and gastrointestinal fermentation evolve with time, taking into account the periods of food changes. The primary outcome was to achieve a significant increase in bifidobacteria after the nutritional intervention. The secondary outcomes were to study the change in microbial activity and composition, evaluate nutrient intake, examine changes in nutritional behavior and gut health, and relate microbiota modifications to gastrointestinal symptoms.

Dietary energy and nutrient intake

Before each test day, the subjects were asked to complete a food diary for 3 d (2 d during the week and 1 d during the weekend) to assess the impact of the nutritional intervention on energy and nutrient intake. The Nubel Pro program and the table of composition from Nubel 2010 were used to assess nutritional and total fiber intake. For some missing data, the online data table Ciqual 2013 was used. Because common tables of composition do not give information about fructans, data collected from the literature were used (17–19), as well as data provided on product labels. When needed, inulin content was measured in foodstuffs as previously described by Kalala et al. (16).

Hydrogen breath test

On test days, following mouth washing, we collected fasting breath samples 2 times in a row to measure baseline expired H_2, following the method prescribed by the Rome Consensus (20).

TABLE 1 Nutritional composition of food provided daily during the intervention[1]

Day	Composition of the meals	Energy, kcal	Energy, kJ	Proteins, g	Lipids, g	Carbohydrates, g	Sugars, g	Starch, g	Water, g	Alcohol, g	Total fiber, g	Fructans, g
Day 1	Turkey, mashed Jerusalem artichoke*, spinach, pumpkin cream	515	1829	40.3	28.6	22.4	4.3	3.4	440	0	15.1	13.8
	Tomato and basil soup	126	527	4.7	5	15.5	8	7.5	437	0	3.6	0.3
	Total	641	2356	45	33.6	37.9	12.3	10.9	877	0	18.7	14.1
Day 2	Epigram of lamb, potatoes with garlic*, salsify* with cream	665	2778	26.6	46.2	35.6	12.9	22.5	372	0	23.5	17.4
	Carrot soup with cumin	67	281	1.6	2.2	10.4	9.7	0.7	444	0	5.8	0.5
	Total	732	3059	28.2	48.4	46	22.6	23.2	816	0	29.3	17.9
Day 3	Sea bass, cooked wheat (Ebly), stuffed artichoke bottoms*, tomato coulis	578	2414	42.1	15	68.3	9.2	59.1	702	0	7.8	12.2
	Garlic* soup	189	788	6.5	9.2	19.9	9.7	9.2	306	0	4.7	2.4
	Total	767	3202	48.6	24.2	88.2	18.9	68.3	1008	0	12.5	14.6
Day 4	Blue cheese and artichoke* quiche	751	3137	37.3	51.6	35	17.2	17.8	277	0	2	7.6
	Onion* soup	111	464	3	8.1	6.5	2.6	3.9	367	0	0.3	2.2
	Total	862	3601	40.3	59.7	41.5	19.8	21.7	644	0	2.3	9.8
Day 5	Chicken breast, gratin dauphinois, green beans, shallot* sauce	684	2856	41	39.8	40.4	15	24.5	483	0	9.8	3.1
	Jerusalem artichoke* soup	221	640	6.2	11	22.3	6.1	3.3	332	0	12.8	10.5
	Total	905	3496	47.2	50.8	62.7	21.1	27.8	815	0	22.5	13.6
Day 6	Grilled burger, French fries, artichoke* salad	545	2280	30.2	31.2	40.1	11	28	260	0	3.8	7.8
	Leek* and chive soup	98	408	2.9	0.2	21.1	12.7	8.4	0	0	6.6	1.7
	Total	643	2688	33.1	31.4	61.2	23.7	36.4	260	0	10.4	9.5
Day 7	Calf crepinette, potatoes with thyme, grilled scorzonera*	553	2311	34.3	28.8	39.3	13	26.1	424	0	30.1	26.8
	Fish soup	192	801	17.4	8.8	10.1	5.6	4.2	489	0	3.6	0.2
	Total	745	3112	51.7	37.6	49.4	18.6	30.3	913	0	33.7	27
Day 8	Rump steak, jacket potatoes, salsify* provencal	366	1529	40	5.8	38.4	13.1	25.4	453	0	26.2	17.8
	Vichyssoise soup	169	706	4.7	7.3	21	13.4	6.6	400	0	5.2	1.2
	Total	535	2235	44.7	13.1	59.4	26.5	32	853	0	31.4	19

(*Continued*)

TABLE 1 (*Continued*)

Day	Composition of the meals	Energy, kcal	Energy, kJ	Proteins, g	Lipids, g	Carbohydrates, g	Sugars, g	Starch, g	Water, g	Alcohol, g	Total fiber, g	Fructans, g
Day 9	Rabbit leg, Jerusalem artichoke gratin*, carrot with thyme	664	2456	53.1	28.8	43	21.1	8	726	2.2	19.9	12
	Cauliflower soup	139	580	5.4	8.4	10.4	6.7	3.8	366	0	4.5	0.6
	Total	803	3036	58.5	37.2	53.4	27.8	11.8	1092	2.2	24.4	12.6
Day 10	Tuna, rye bread, Italian style artichoke*	581	2428	52.9	12	61.1	16.6	43.7	459	1	8.3	8.8
	Onion* soup	111	464	3	8.1	6.5	2.6	3.9	367	0	0.3	2.2
	Total	692	2892	55.9	20.1	67.6	19.2	47.6	826	1	8.6	11
Day 11	Irish steak, mashed Jerusalem artichoke*, ratatouille	612	2236	26.5	44.8	24.2	9.5	0.9	449	0	17.4	13.7
	Squash soup with harissa	59	248	3.1	0.4	10.9	6.5	4.4	422	0	3.4	0.4
	Total	671	2484	29.6	45.2	35.1	16	5.3	871	0	20.9	14.1
Day 12	Turkey breast, gratin dauphinois, salsify* with cream	461	1923	37.5	20.1	32.4	11.4	20.3	382	0	23.1	16.9
	Celery root* soup	81	337	4.8	1.4	12.2	3.1	9.1	471	0	8.5	1.8
	Total	542	2260	42.3	21.5	44.6	14.5	29.4	853	0	31.6	18.7
Day 13	Pork, croquette, green beans, shallot* sauce	530	2213	38.4	19.1	48.1	13.9	30.7	421	1.9	0.6	3.7
	Jerusalem artichoke* soup	221	640	6.2	11	22.3	6.1	3.3	332	0	12.8	10.5
	Total	751	2853	44.6	30.1	70.4	20	34	753	1.9	13.4	14.2
Day 14	Meatloaf, potatoes, artichoke* salad	472	1127	28.4	23.3	36.9	12.2	24.7	303	0	6.7	9
	Leek* soup	98	408	2.9	0.2	21.1	12.7	8.4	0	0	6.6	1.7
	Total	570	1535	31.3	23.5	58	24.9	33.1	303	0	13.3	10.7

[1]Data are presented as single values per nutrient per day. *Ingredients are sources of ITF.

Afterwards, subjects received an oral load of 16 g of purified ITF (Fibruline Instant, Cosucra) diluted in water and 2 pancakes, as previously described (21). H_2 produced by gut microbiota fermentation was measured every 15 min for 6 h. The level of expired H_2 was measured using a Lactotest 202 breath test monitor (Medical Electronic Construction), expressed in parts per million (ppm) and normalized by CO_2 levels. The AUC was representative of the gut microbiota fermentation activity. The transit time was evaluated as the period of time necessary to reach an increase of 10 ppm of hydrogen excretion above baseline, with this increase being maintained or even increased at least 2 consecutive times.

Food-related behavior and gastrointestinal symptoms

On test days, the subjects were asked to fill out 100-mm visual analog scales (VAS) describing their gastrointestinal symptoms (rumble, burp, bloating, discomfort, nausea, flatulence, and cramp) and appetite-related feelings (satiety, fullness, intention to eat, desire to eat sweet, very sweet, fatty, and salty). The VAS questionnaires were filled out at baseline and every 30 min for 6 h following the ingestion of the inulin load. Each subject completed separate scales, 1 for each symptom and appetite-related feelings. The scales were scored by measuring the distance (in millimeters) from 0 with a ruler. The same VAS questionnaires were filled every day during the nutritional intervention before the lunch, for compliance monitoring. In addition, the subjects filled out a questionnaire on 24-h stool frequency and consistency (Bristol stool scale (BSS); see online **Supplemental Methods**) every day during the intervention and on the 3 test days. On test days, 1 h after a standardized lunch, the subjects filled out questionnaires that assessed psychological variables (perceived stress, mood, (intrapersonal) emotional competence, hedonic attitude, and intention to consume more vegetables in general, and salsify and leek in particular; see online Supplemental Methods). We also determined the sucrose detection threshold following the multiple forced choice presentation method (see online Supplemental Methods) (22).

Analysis of the stool samples for gut microbiota composition

Stool samples were collected (within 2 d before each test day) in tubes labeled with each individual's code number and stored at room temperature with a DNA stabilizer (Stratec Molecular) and then transferred to −80°C for the analysis of the gut microbiota composition following the manufacturer's instructions. Genomic DNA was extracted from feces using a PSP spin stool DNA kit (Stratec Molecular). The V5–V6 region of the 16S rRNA gene was amplified by PCR with modified primers. The amplicons were purified, quantified, and sequenced using an Illumina Miseq to produce 2 × 300-bp sequencing products at the University of Minnesota Genomics Center (23). Subsequent bioinformatics and biostatistics analyses were performed as previously described (24). Initial quality-filtering of the reads was conducted using Illumina Software, yielding a mean of 111,507 pass filter reads per sample. Quality scores were visualized, and reads were trimmed to 220 bp (R1) and 200 bp (R2). The reads were merged with the merge-Illumina-pairs application (25). For all samples but 3, a subset of 30,000 reads was randomly selected using Mothur v.1.25.0 (26) to avoid large disparities in the number of sequences. Subsequently, the UPARSE pipeline implemented in USEARCH v7.0.1001 (27) was used to further process the sequences. Putative chimeras were identified against the Gold reference database and removed. Clustering was performed with a 98% similarity cutoff to designate operational taxonomic units (OTUs). Nonchimeric sequences were also subjected to taxonomic classification using the RDP MultiClassifier 1.1 from the Ribosomal Database Project (28) for phylum to genus characterization of the fecal microbiome. The phylotypes were computed as percentages based on the total number of sequences in each sample. The full protocol, detailed statistical analyses, and accession numbers are provided in the online Supplemental Methods.

Assessment of microbial fermentation in vitro

Six out of the 26 people included in the study were able to provide enough fresh feces before (T0) and after (T1) the nutritional intervention, to perform the in vitro fermentation test. Stool samples were kept under anaerobic conditions at 4°C (see online Supplemental Methods) and then transferred to −80°C before the analysis. For each fecal sample, an inoculum was prepared by diluting the frozen fecal sample (1:40 dilution w/v) in preheated (37°C) buffer solution (29). Subsequently, 15 mL of the inocula was poured into the vials containing the undigested fiber residue recovered after porcine pepsin and pancreatin hydrolysis of vegetables (salsify and Jerusalem artichoke; see online Supplemental Methods) and ITF as substrates, and placed into an airtight container before being incubated in a water bath at a temperature of 37°C. A reading of pressure formed in the flasks following the production of gases during the fermentation was carried out after 2, 5, 8, 12, 16, 20, and 24 h. After 24 h of fermentation, the supernatants were emptied and stored at −20°C until the short-chain fatty acid (SCFA) pattern was measured. The fermentation kinetics parameters were determined according to the model of Groot et al. (30).

Analysis of SCFAs

Fermentation supernatants of the feces ($n = 6$) used in the fermentation study were analyzed for lactate and SCFA contents with a Waters 2690 HPLC system fitted with an Aminex HPX 87 H column (300 mm × 78 mm; Bio-Rad Laboratories) combined with a UV absorbance detector (Waters 486 tunable absorbance detector) set at 210 nm. The sterile bottle containing 15 mL of fermentation supernatants was mixed on a vortex for 1 minute, and 2 mL was sampled and centrifuged at 13,000 rcf for 15 min; 1.5 mL of the supernatants was transferred to a vial, and pH was adjusted between 1 and 3 using 0.1 M HCl. The SCFAs were eluted as described by Murugesan et al. (31) using SIGMA standard. Fermentation values were corrected for the content of the blanks as well as the inocula.

Statistical analysis

Microbiota analysis.

Significantly affected taxa and OTUs were identified using a Friedman test in R, followed by Dunn's post hoc test using GraphPad (version 7.00). The P value of the Friedman test was adjusted (q value) to control for the false discovery rate (FDR)

for multiple tests according to the Benjamini and Hochberg procedure (32). Multilevel principal component analysis (PCA) and a sparse multilevel partial least squares discriminant analysis (PLS-DA) model were built based on the centered log-ratio transformation of the OTU table, and the performance of the model was computed using mixOmics version 6.3.0 (33).

Overall comparisons.

Results are presented as mean ± SEM. Values of SD and CI for all results can be found in **Supplemental Table 1**. Statistical significance was assessed by GraphPad. Data were analyzed using a repeated measures 1-factor ANOVA with Tukey post hoc tests if the data were parametric, and Friedman's test with Dunn's post hoc tests if the data were nonparametric. Normality was assessed by Shapiro–Wilk test. For in vitro kinetics parameters and SCFA production, an ANOVA mixed model using SAS software (version 9.4), followed by Tukey post hoc tests, was applied with time and type of substrate as fixed effects and subject as a random effect. Each fermentation flask was considered as an experimental unit ($n = 3$).

Correlations between gut microbiota and other variables were assessed by Spearman's correlation tests with FDR correction. A significance level of $P < .05$ was adopted for all analyses. The sample size (number of volunteers) was determined with PASS software (NCSS statistical software, version 14) based on a study previously performed in obese women supplemented with purified inulin (34). In that study, we included 15 obese volunteers to achieve a significant bifidogenic effect of purified inulin (34). On that basis, a total number of 12 individuals are needed to observe an effect size of 1.7 for the relative abundance of *Bifidobacterium* genus taking into account an alpha of 0.05 and a power of 80%. Therefore, we included 26 healthy volunteers in this study taking into account the variability in ITF uptake due to the meals, the possible dropout, losses to follow-up, and exclusion of certain participants.

Results

Subjects

Twenty-six subjects were initially included in the study. Twenty-five subjects completed the study; only 1 volunteer dropped out on the last test day (no reason was given). The baseline characteristics of participants measured on the first test day of the intervention are presented in **Table 2**. The flow diagram of the study (adapted from CONSORT 2010) is presented in **Supplemental Figure 2**. All volunteers ($n = 25$) were included in the analysis. However, for gut microbiota analysis, 1 volunteer did not provide enough sampling material on the last test day and was excluded from the gut microbiota analysis ($n = 24$). Moreover, for behavior data concerning salsify, we took into consideration only the data of participants who knew about salsify before entering the study ($n = 16$). The study was conducted from March to April 2016.

TABLE 2 Subjects' baseline characteristics[1]

Participants	25
Males, %	44
Age, y	21.84 ± 0.39
BMI, kg/m^2	22.29 ± 0.32
Waist circumference, cm	80.68 ± 1.19

[1]Data are expressed as means ± SEMs.

Dietary energy and nutrient intake

The analysis of the food diaries showed that the nutritional intervention led to a 5-fold increase in fructan intake and a 2-fold increase in total fiber intake (**Table 3**). Total energy, lipid, protein, and carbohydrate intake were not affected by the dietary intervention. Only starch consumption was higher 3 wk after the end of the intervention (Table 3).

Appetite-related feelings

After 2 wk of nutritional intervention, the subjects reported higher levels of satiety and a decreased desire to eat sweet and salty food (**Figure 1**). These results persisted 3 wk after the end of the study, and the desire to eat very sweet and fatty food decreased significantly at T2. Neither the sucrose detection threshold (see online **Supplemental Figure 3**) nor body weight (data not shown) changed during the intervention.

TABLE 3 Dietary energy and nutrient intake in healthy subjects during intervention[1]

	T0	T1	T2
Energy, kcal/d	1974.56 ± 76.51	1888.29 ± 70.07	1949.35 ± 74.72
Carbohydrates, g/d	254.22 ± 10.45	221.66 ± 9.59	240.99 ± 10.13
Starch, g/d	139.91 ± 8.63	124.25 ± 7.20	153.48$^{\#}$ ± 10.61
Sugars, g/d	87.07 ± 5.79	83.63 ± 4.19	83.69 ± 6.17
Lipid, g/d	67.32 ± 5.08	68.24 ± 3.83	70.63 ± 4.19
Protein, g/d	74.85 ± 3.32	80.64 ± 3.06	74.57 ± 3.90
Fiber, g/d	18.29 ± 1.60	31.46*** ± 1.08	16.62$^{\#\#\#}$ ± 1.45
Fructans, g/d	3.06 ± 0.51	15.67*** ± 0.31	2.68$^{\#\#\#}$ ± 0.26

[1]Data are expressed as means ± SEMs and were analyzed by a repeated-measures 1-factor ANOVA followed by Tukey post hoc tests (if parametric), or Friedman test followed by Dunn's post hoc tests (if nonparametric): $^{***}P < .001$ compared with T0. $^{\#}P < .05$, $^{\#\#\#}P < .001$ compared with T1. $n = 25$. T0 is the first test day, taking place before the nutritional intervention. T1 is the second test day, taking place 14 d after the nutritional intervention. T2 is the third test day, taking place 3 wk after the return to normal eating habits.

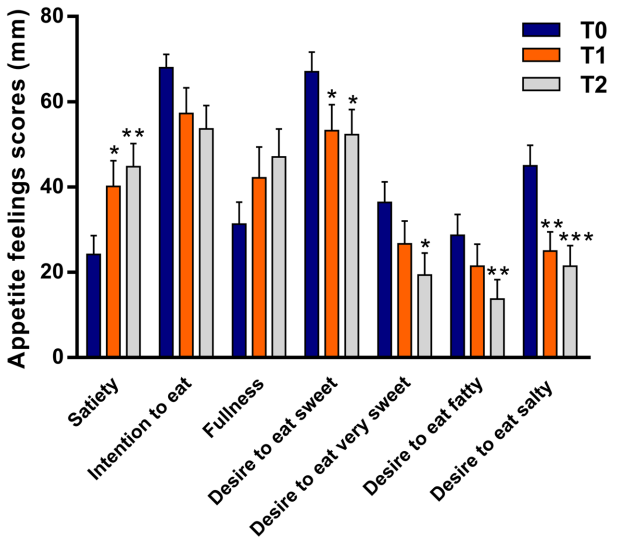

FIGURE 1 Appetite feelings scores on test days before inulin load (fasting). Data are expressed as mean ± SEM and were analyzed by a repeated-measures 1-factor ANOVA followed by Tukey post hoc tests (if parametric) or a Friedman test followed by Dunn's post hoc tests (if nonparametric): $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ compared with T0. $n = 25$. T0 is the first test day, taking place before the nutritional intervention. T1 is the second test day, taking place 14 d after the start of the nutritional intervention. T2 is the third test day, taking place 3 wk after the return to normal eating habits.

Behavioral measures

A trend toward increased hedonic attitude regarding salsify consumption was recorded at T2 compared with T0 ($P = .051$) (see online **Supplemental Figure 4**A). The hedonic attitude for vegetables and leek consumption was high before the study and remained stable throughout the intervention, whereas the intention to eat more vegetables, leek, and salsify did not change (see online Supplemental Figure 4A). The subjects presented a higher level of intrapersonal emotional competence at T2 than at T0 ($P < .05$; see online **Supplemental Figure 4**B). No changes were observed in the perceived stress score (see online Supplemental Figure 4B).

Gut microbiota composition

No major changes in gut microbiota composition were observed during the dietary intervention at the phylum and family levels (**Figure 2**A). Alpha diversity was decreased following the nutritional intervention. In fact, after 2 wk of an ITF-rich diet, there was a reduction in the observed species index of richness, which remained lower after returning to the usual diet. There was no change in the evenness indices (see online **Supplemental Figure 5**).

The β-diversity was not modified by the treatment as shown by the principal coordinate analysis of the Morisita–Horn index and the Weighted Unifrac index (**Figure 2**B, C). The observed distribution of β-diversity was mainly due to interpersonal variability (explaining 83% of the total variation, against 1% for the variable treatment for the Morisita index and 73% compared with 2% for the Weighted Unifrac index) (Figure 2B,C). However, a multilevel principal component analysis (allowing the within-subject treatment effect to be examined separately from the biological variation between biological samples) suggested a subtle separation of the microbial profile observed at T1 compared with T0 and T2 along the first axis (**Figure 2**D). This was confirmed by a multilevel sparse PLS-DA analysis (**Figure 2**E), which highlighted 20 bacterial OTUs able to repeatedly discriminate for the dietary intervention (bacterial OTUs with a feature stability above 0.6 on components 1 and 2; see online **Supplemental Table 2**). Among discriminant OTUs, we can point out several OTUs of interest, assigned to *Bifidobacterium longum* subsp. *longum*, *Bifidobacterium pseudocatenulatum*, *Bifidobacterium bifidum*, *Bifidobacterium adolescentis*, and *Blautia* sp.

Using univariate analyses, we also found that an ITF-rich diet induced specific changes at the taxa level: 1) an increase in Actinobacteria phylum and class, Actinobacteridae subclass, Bifidobacteriales order, *Bifidobacteriaceae* family, and *Bifidobacterium* genus; 2) a decrease in unclassified Clostridiales; and 3) a trend toward a decrease in *Oxalobacteraceae* family ($P = .052$) (**Figure 2**F and **Supplemental Table 3**A). The transient increase in the *Bifidobacterium* genus, one of our key results related to the gut microbiota, was confirmed using qPCR (**Supplemental Figure 6**). Using this technique, we found that consuming inulin-rich food for 2 wk increased *Bifidobacterium* levels by 3.0-fold. This result is similar to that obtained by sequencing (3.8-fold increase). Accordingly, qPCR and sequencing values were strongly correlated (Supplemental Figure 6), as reported in a previous study (24). Three weeks after the end of the nutritional intervention, the count of these bacteria returned to the baseline value. Interestingly, the fold change for *Bifidobacterium* genus between T0 and T1 was negatively correlated with fiber intake at T0 ($\rho = -0.549$, $P < .01$). Additional taxa that were significantly modified are presented in Supplemental Table 3A. At the OTU level, only levels of *Bifidobacterium longum* subsp. *longum* (OTU 69) significantly increased after the intervention ($q = 3{,}16.10^{-5}$) (Figure 2F), with an additional 74 OTUs showing significant changes, as presented in Supplemental Table 3B. It is worth noting that there was a decrease in the *Alistipes* genus and *Oscillibacter* genus, and an increase in the *Prevotellaceae* family at T1 compared with T0 as a result of the nutritional intervention. At T2, we observed an intermediate level (between the levels obtained at T0 and T1) of fecal *Oscillibacter* genus and *Prevotellaceae* family. A Fisher test also revealed a reduction in an unclassified *Lachnospiraceae* species-like taxa (OTU 805) at T1 compared with T0 (see online Supplemental Table 3B). Interestingly, this decrease persisted 3 wk after the subjects returned to their usual diet.

Microbial fermentation: in vivo and in vitro data

In vivo.

Microbial fermentation assessed in vivo was not modified by the intervention, as shown by the AUC of expired H_2 during the 6 h after an oral load of 16 g of inulin (**Figure 3**A). Interestingly, on the last day of the intervention (T1), we measured higher H_2 levels at baseline in the subgroup of subjects who consumed the soup with the highest ITF content (10.5 g ITF/soup) the evening before the test (as shown with crosses in **Figure 3**B, $P < .05$). These individuals also showed higher levels of H_2

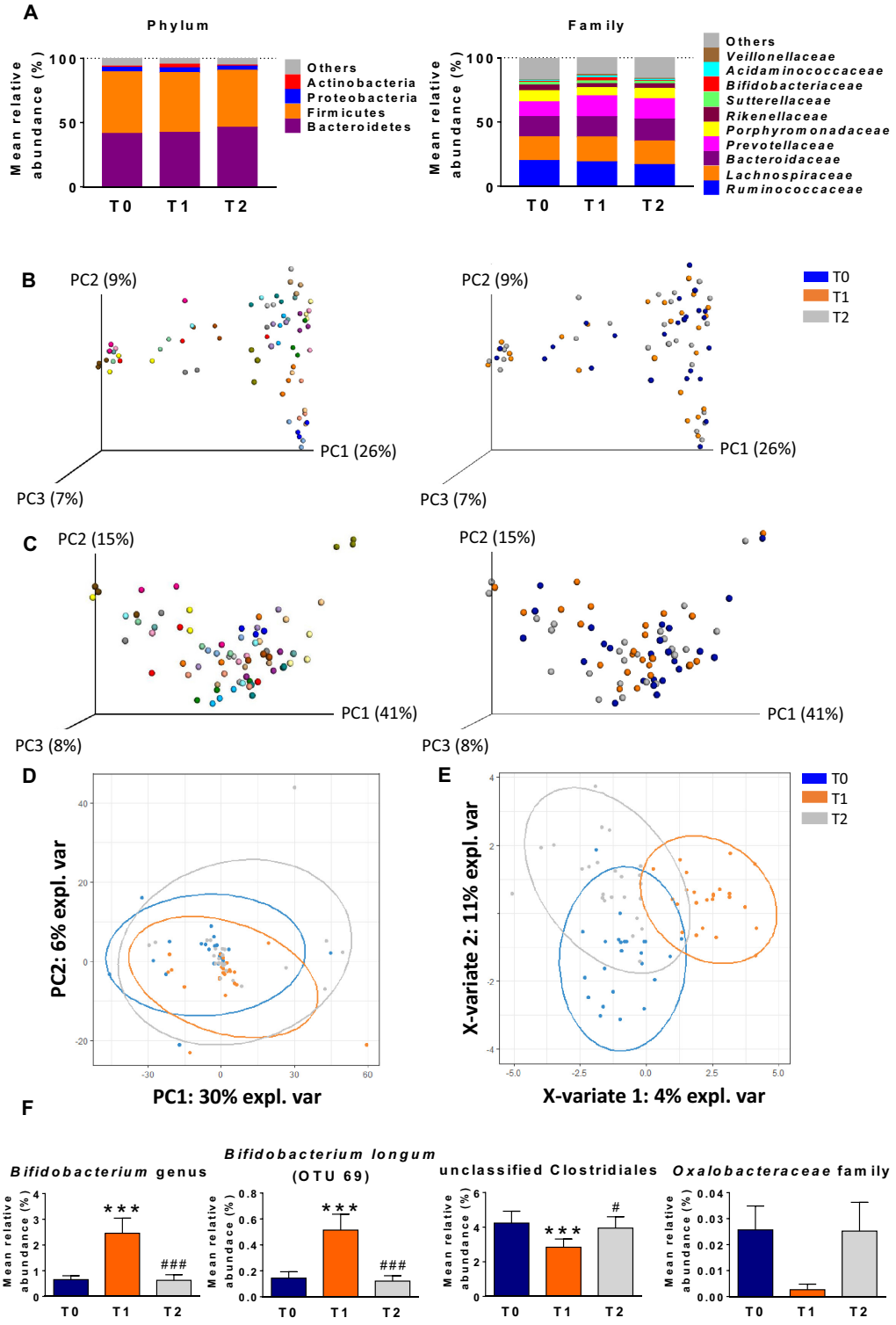

FIGURE 2 Changes in microbial composition upon dietary intervention. (A) Relative abundances of bacterial taxa accounting for more than 1%, at the phylum and family levels. (B, C) Principal coordinates analysis of the β-diversity indexes Morisita–Horn (B) and Weighted UniFrac (C), colored by volunteer (on the left) or by day of intervention (on the right). (D) Multilevel principal component analysis of the OTU relative abundances colored by day of intervention. (E) Multilevel sparse PLS-DA of the OTU relative abundances colored by day of intervention. (F) Relative abundance of *Bifidobacterium* genus, OTU 69 (identified as *Bifidobacterium longum* with an identity score of 1), unclassified Clostridiales, and *Oxalobacteriaceae* family. Data are presented as mean ± SEM and analyzed using Friedman's test followed by Dunn's post hoc tests: $^{***}P < .001$ compared with T0. $^{\#}P < .05$, $^{\#\#\#}P < .001$ compared with T1. $n = 24$. T0 is the first test day, taking place before the nutritional intervention. T1 is the second test day, taking place 14 d after the start of the nutritional intervention. T2 is the third test day, taking place 3 wk after the return to normal eating habits. OTU, operational taxonomic unit.

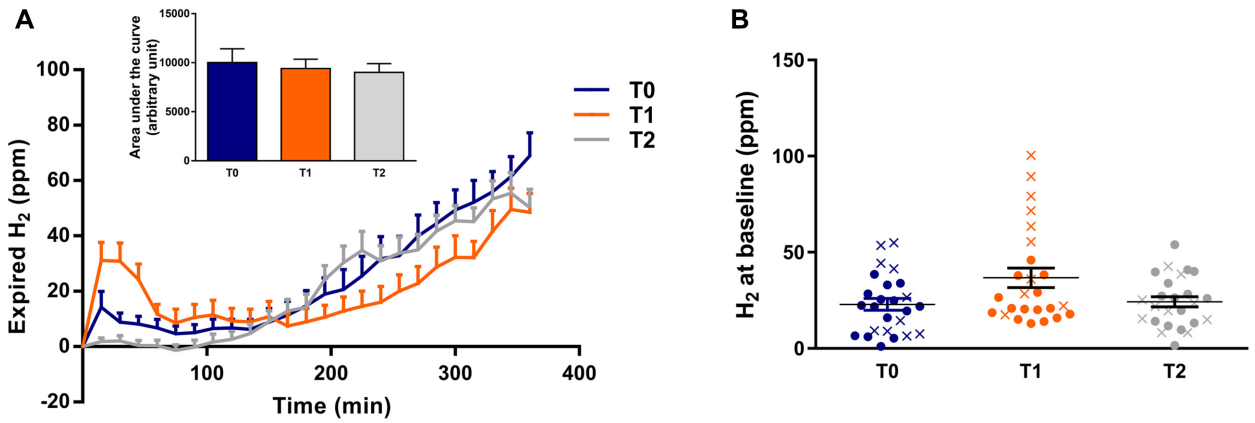

FIGURE 3 Breath-test evaluation of in vivo microbial fermentation. (A) Expired H_2 levels measured every 15 min for 6 h following an oral load of inulin (expressed as change from baseline) and corresponding AUC. (B) Expired H_2 levels (ppm) measured on test days before inulin load (fasting). Crosses highlight subjects who ate the soup containing the highest amount of ITF (10.5 g compared with 1.71 g ITF) the evening before T1**.** Data are presented as mean ± SEM and were analyzed using Friedman's test followed by Dunn's post hoc tests. $n = 25$. T0 is the first test day, taking place before the nutritional intervention. T1 is the second test day, taking place 14 d after the start of the nutritional intervention. T2 is the third test day, taking place 3 wk after the return to normal eating habits. ITF, inulin-type fructan.

production during the first 60 min at T1 compared with T0 and T2 (Figure 3A).

In vitro data allowed evaluation of the putative effect of dietary treatment on the capacity of the gut microbiota to ferment isolated ITF and of 2 ITF-rich vegetables. The statistics (time–substrate interaction by mixed model ANOVA) revealed no significant effect either on the kinetics of gas production or on SCFA production (**Tables 4** and **5**).

Gastrointestinal tolerance

VAS scores for all gastrointestinal symptoms were reported daily during the 2 wk of the intervention. Only flatulence increased, whereas burping, bloating, rumbling, cramp, discomfort, and nausea were globally unaffected (**Figure 4**A). When subjects were fasting on test days, they reported lower ratings of gastrointestinal discomfort and a tendency to decrease bloating at T2 compared with T0 ($P < .05$ and $P = .076$ respectively) (**Figure 4**B). Gastrointestinal symptoms were also recorded during the 6 h after purified ITF loading, but no modifications were observed (data not shown). We observed no effects of nutritional intervention on transit time (assessed by a breath test), stool frequency, or stool consistency (assessed by the Bristol stool scale) (see online **Supplemental Figure 7**).

A correlation analysis was performed on gastrointestinal symptoms and fecal bacteria; **Figure 4C** shows the most significant correlations. Overall, only a few correlations appeared significant. We observed a positive correlation between flatulence and the *Bacteroides* genus and *Bacteroidaceae* family. The *Sutterellacea*e family, which belongs to the Burkholderiales order and Betaproteobacteria class, was positively correlated with nausea. Similarly, the Proteobacteria phylum, *Bacteroides* species (OTU 204), and *Oscillibacter* species (OTU 259) were also positively correlated with burping, whereas unclassified *Coriobacteriaceae* were positively correlated with transit time. Conversely, *Ruminococcus callidus* and *Clostridium* IV species were negatively correlated with intestinal symptoms and especially discomfort, whereas unclassified Burkholderiales were negatively correlated with rumbling (Figure 4C).

Discussion

To ensure adequate intestinal functions, the European Food Safety Authority recommends that healthy adults consume a minimum of 25 g of fiber per day (35). It has been estimated that at least 12 g of ITF should be consumed to maintain adequate intestinal transit function (36). In this study, we demonstrated that it is possible to reach a minimum intake of 9 g/d and a mean intake of 15 g/d of fructans by the consumption of specific vegetables, namely salsify, Jerusalem artichoke, artichoke, leek, onion, garlic, and scorzonera.

Consuming ITF-rich vegetables for 2 wk led to increased satiety and a reduced desire to eat sweet and salty food. Three weeks after subjects returned to their usual diets, these effects were further strengthened, and the desire to eat very sweet and fatty food was significantly decreased. This is in line with recent studies showing that isolated ITF and oligofructose supplementation increases satiety (37) and fullness (38), and decreases reported levels of hunger (10), prospective food consumption (37, 38), and desire to eat fatty, sweet, and salty food (39). Thereby, we show for the first time that an ITF-rich diet has a long-lasting effect on appetite-related sensations. A putative explanation for the persisting effect of an ITF-rich diet on appetite-related sensations could be the modulation of gut hormones (GLP-1, PYY) as previously shown with ITF supplementation in animal models and in humans (10, 39, 40). However, the reversibility of the effect of ITF on gut peptides has never been tested and could constitute an interesting path of investigation. Changes observed in the desire to eat very sweet food were not linked to sucrose detection threshold, which was not modified during the intervention. Moreover, energy intake was not changed throughout the intervention, as has been shown in several other studies (10, 37). It is well known that the gut

TABLE 4 Kinetics of total gas volume produced in vitro from fresh feces samples incubated with ITF, salsify, or Jerusalem artichoke before and after the nutritional intervention[1]

	Inulin-type fructans		Salsify		Jerusalem artichoke		*P* value time	*P* value substrate	*P* value time × substrate
	T0	T1	T0	T1	T0	T1			
Total gas produced, mL/g DM	259 ± 22	286 ± 23	236 ± 11	254 ± 12	232 ± 11	253 ± 13	.0680	.1966	.9628
Time to produce half of total gas volume, h	8.2 ± 0.4	7.1 ± 0.5	7.9 ± 0.3	6.0 ± 0.2	8.2 ± 0.3	5.7 ± 0.2	<.0001	.0817	.1326
Maximum rate of gas production, mL/h × g DM	27 ± 3	30 ± 3	26 ± 2	32 ± 2	25 ± 2	32 ± 2	.002	.9980	.6927
Time to reach the maximum rate of gas production, h	6.4 ± 0.4	4.8 ± 0.5	6.1 ± 0.3	4.0 ± 0.2	6.4 ± 0.3	3.8 ± 0.2	<.0001	.2368	.2999

[1]Data are presented as mean ± SEM and were analyzed by a mixed model ANOVA. $n = 6$. DM, dry matter; T0 is the first test day, taking place before the nutritional intervention; T1 is the second test day, taking place 14 d after the nutritional intervention.

microbiota composition is modulated by diet, but it is still unclear whether the actual microbiota can drive food preferences. Few studies highlight the capacity of the microbiota to change food preferences by altering the expression of taste receptors (41). Thus, prebiotic supplementation could lead to specific microbiota modification, resulting in adapted food choices.

We analyzed whether consumption of an ITF-rich diet could affect the hedonic attitude and the prospective intention to eat more vegetables in general, and specifically leek and salsify, which are 2 ITF-rich vegetables. Interestingly, hedonic attitude towards salsify increased slightly over time, but the difference between baseline and 3 wk after a return to the subject's usual diet was only marginally significant. These results have to be treated with caution because of the low internal reliability of the scale and the small sample size for the psychological variables. We hypothesized that proposing a diet containing vegetables that are rarely consumed in the general population (i.e., salsify: less than once every 3 mo; Broers et al. 2018, unpublished data) may increase positive attitudes and the intention to eat this particular vegetable in the future.

We show that changing dietary habits by introducing ITF-rich vegetables slightly increases intrapersonal emotional competences, independent of perceived stress, which was stable among all time points. This result highlights the potential of prebiotic use in the context of emotional impairment. Again, these results have to be treated with caution because of the low internal reliability of the scale and the small sample size for psychological variables.

The overall composition of the gut microbiota remained stable throughout the intervention, but we observed a decrease in richness. Interestingly, a recent study showed that prebiotic supplementation in individuals with type 2 diabetes mellitus decreases gene richness in addition to a significant clinical improvement (42). Moreover, a decrease in alpha diversity was also observed in a clinical intervention performed in obese and overweight children using oligofructose-enriched inulin (9). In the future, more data will help to unravel the clinical significance of decreased microbial richness for health outcomes.

Two weeks of consuming vegetables rich in ITF led to a 3.8-fold increase in the *Bifidobacterium* genus, which has already been reported through the consumption of purified ITF in several papers (9, 34, 37, 43). Moreover, at the species level, we observed an increase in *B. longum* subsp. *longum* and, to a lesser extent, *B. pseudocatenulatum*, *B. bifidum*, and *B. adolescentis*, consistent with a recent study that found elevated levels of *B. longum* subsp. *longum* and *B. adolescentis* after ITF supplementation in overweight and obese children (9). Few studies have described the effect of a diet naturally rich in fiber with prebiotic activity on gut microbiota composition/activity and its capacity to be tolerated at the intestinal level. Vegetable shots containing Jerusalem artichoke puree/juice given to healthy volunteers for 3 wk led to an increase in *Bifidobacterium* (13). Similarly, healthy volunteers consuming snack bars containing Jerusalem artichoke syrup for 1 week had increased counts of *Bifidobacterium* (14). Interestingly, in our study, *Bifidobacterium* genus fold change between T0 and T1 was negatively correlated with the fiber intake

TABLE 5 Production of SCFA in vitro from fresh feces samples incubated with inulin-type fructans, salsify, or Jerusalem artichoke before and after nutritional intervention[1]

	Inulin-type fructans		Salsify		Jerusalem artichoke		*P* value time	*P* value substrate	*P* value time × substrate
	T0	T1	T0	T1	T0	T1			
Total SCFA, mg/g DM	520 ± 25	507 ± 24	431 ± 9	428 ± 11	429 ± 13	433 ± 10	.9144	<.0001	.8591
Molar ratio, %									
Acetate	0.626 ± 0.028	0.602 ± 0.022	0.713 ± 0.016	0.701 ± 0.013	0.737 ± 0.017	0.683 ± 0.012	.0651	<.0001	.9553
Propionate	0.178 ± 0.021	0.162 ± 0.026	0.154 ± 0.011	0.157 ± 0.010	0.171 ± 0.010	0.161 ± 0.009	.6257	.5030	.7541
Butyrate	0.177 ± 0.017	0.211 ± 0.022	0.102 ± 0.011	0.141 ± 0.006	0.113 ± 0.012	0.154 ± 0.006	<.0001	<.0001	.9637
BCFA	0.008 ± 0.004	0.025 ± 0.014	0.007 ± 0.002	0.001 ± 0.001	0.004 ± 0.003	0.002 ± 0.001	.6601	.0012	.0093

[1]Data are presented as mean ± SEM and were analyzed by a mixed model ANOVA. $n = 6$. DM, dry matter; BCFA, branched-chain fatty acids regrouping isobutyrate, valerate, and isovalerate; T0 is the first test day, taking place before the nutritional intervention. T1 is the second test day, taking place 14 d after the nutritional intervention.

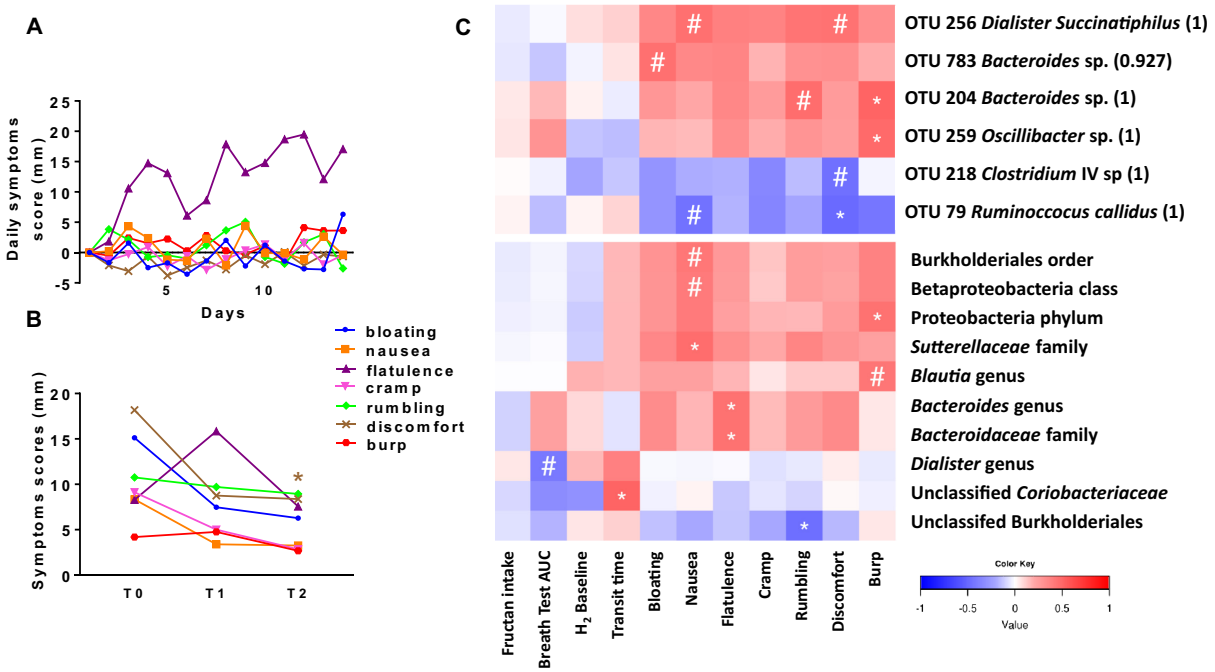

FIGURE 4 Gastrointestinal tolerance assessed by visual analog scale and correlation with fecal microbiota. (A) Daily gastrointestinal symptoms scores (mm) reported during the 2-wk nutritional intervention ($n = 25$). (B) Symptoms scores on test days, before inulin load (fasting). Data are means and were analyzed by Friedman's test followed by Dunn's post hoc tests: $^{*}P < .05$ compared with T0 for discomfort ($n = 25$). (C) Heatmap of Spearman correlation coefficients between fecal bacteria (taxa and OTUs) and gastrointestinal symptoms (AUC during the 6 h after an ITF load), fructans intake, baseline expired H_2, expired H_2 during the 6 h after an ITF load (AUC) and transit time. $^{*}q < 0.05$ and $^{\#}q < 0.1$. $n = 24$. T0 is the first test day, taking place before the nutritional intervention. T1 is the second test day, taking place 14 d after the start of the nutritional intervention. T2 is the third test day, taking place 3 wk after the return to normal eating habits.

at T0. Thus, we can infer that the individual bifidogenic response to an ITF-rich diet is dependent on dietary habits at baseline and will be more important in the case of low fiber intake. In parallel with increased bifidobacteria levels, we observed that the nutritional intervention led to reduced levels of unclassified Clostridiales and a trend toward fewer *Oxalobacteraceae*. A few studies have reported beneficial health effects for *Oxalobacter formigenes*, which reduces the risk of kidney stone formation with its ability to degrade oxalates (44). Clostridiales order has been shown to increase following a high-fat diet in rats (45). Therefore, its decrease may be related to greater consumption of vegetables. However, further investigation is needed to prove a causal relation between health improvement and the change in abundance of these bacterial taxa.

Three weeks after the end of the intervention, the *Bifidobacterium* genus, *Oxalobacteraceae* family, and unclassified Clostridiales returned to preintervention levels. These results underline the importance of continuing to consume a fiber-rich diet in order to maintain the beneficial effects on gut microbiota composition.

The modifications in the composition of the gut microbiota induced by the nutritional intervention were not associated with significant changes in basal or post-ITF load exhaled H_2. Another study also showed that the consumption of oatmeal porridge, which is rich in fiber, for 1 week has no effect on colonic fermentation assessed by exhaled H_2, despite the modification of gut microbiota activity (46).

By performing an in vitro fermentation with ITF and ITF-rich vegetables, we observed that the fecal inoculum after the nutritional intervention did not exhibit significant changes in the kinetics of gas or SCFA production depending on the nature of the substrate. Increasing the amount of dietary fiber is often proposed in view of its positive effect on transit time. Here, we observed no modification of transit time, stool frequency, or consistency, as previously reported for purified ITF in healthy subjects (47).

The gastrointestinal intolerance of fermentable nutrients is a matter of debate, since the discovery of the fact that avoiding FODMAPs (including fructo-oligosaccharides) improves symptoms associated with inflammatory bowel disease (48). Overall, the consumption of a diet naturally rich in ITF was well tolerated in our study, as we observed no effect on intestinal symptoms except increased flatulence, which returned to the basal level after 3 wk of reversion. In our study, we observed a positive correlation between *Bacteroides* genus and flatulence production, as previously reported (49).

When fasting, subjects reported a reduced discomfort score following the nutritional intervention, which decreased further after 3 wk of dietary reversion. *Clostridium* cluster IV and its member *Rumminococcus callidus* were both negatively correlated with intestinal symptoms, and especially discomfort. However, another study reported that *Rumminococcus callidus* was positively correlated with abdominal pain and bloating in healthy adults (50).

Our trial had some limitations, including the absence of a control group (each individual being their own control in our study), the limited number of subjects, their young age (leading to extrapolation to the aging population), and the decision to focus on hydrogen-producing individuals as an inclusion

criterion. However, we can clearly conclude that, by increasing the consumption of selected vegetables, it is possible to boost the intake of fructans and thereby create a shift in the gut microbiota composition. The high intake of fructans in the diet was well tolerated and even contributed to appetite regulation and a reduction in the desire to eat unhealthy food (i.e., sweet, fatty, and salty food). Interestingly, changes in gut microbiota function may occur without a significant effect on hydrogen production, one of the contributors to gastrointestinal dysfunction. Our data support the fact that vegetables rich in inulin-type fiber might be promoted in healthy individuals as part of a healthy diet, which takes into account the importance of the gut microbial ecosystem as a "partner" in health maintenance.

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