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

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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 uncultivated 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...
(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 H2 producers. The candidates were screened for H2 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 H2 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 B4032016272725). 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 (Megzyme), 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 H2, following the method prescribed by the Rome Consensus (20).
| 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          |
| 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      | 13.8      | 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). H₂ produced by gut microbiota fermentation was measured every 15 min for 6 h. The level of expired H₂ was measured using a Lactotest 202 breath test monitor (Medical Electronic Construction), expressed in parts per million (ppm) and normalized by CO₂ 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 bioinformatic 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-Illuminapairs 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 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 min, 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). 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.

**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 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.

**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): **\( P < .01 \)** compared with T0, ***\( P < .001 \)** 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.
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 savoriness consumption was recorded at T2 compared with T0 (P = .051) (see online Supplemental Figure 4A). 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 interpersonal emotional competence at T2 than at T0 (P < .05; see online Supplemental Figure 4B). 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 2A). 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, 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 OTUs with a feature stability above 0.6 on components 1 and 2; see online Supplemental Table 3B. It is worth noting that there was a decrease in the Prevotellaceae family at T1 compared with T0 (P < .01). Additional taxa that were significantly modified are presented in Supplemental Table 3B. At the OTU level, only levels of Bifidobacterium longum subsp. longum (OTU 69) significantly increased after the intervention (ρ = 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 H2 during the 6 h after an oral load of 16 g of inulin (Figure 3A). Interestingly, on the last day of the intervention (T1), we measured higher H2 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 3B, P < .05). These individuals also showed higher levels of H2 separately from the biological variation between biological samples.)
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 < .01 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.
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 4A). 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 4B). 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 Sutterellaceae 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.
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 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.

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 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

|                        | Inulin-type fructans | Salsify | Jerusalem artichoke | P value | P value | P value |
|------------------------|----------------------|---------|----------------------|---------|---------|---------|
|                        | T0  | T1   | T0  | T1   | T0  | T1   | time | substrate | time × substrate |
| 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 |

1Data 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.
coefficients between fecal bacteria (taxa and OTUs) and gastrointestinal symptoms (AUC during the 6 h after an ITF load), fructans intake, baseline expired H2, fermentation assessed by exhaled H2, despite the modification of which is rich in fiber, for 1 week has no effect on colonic study also showed that the consumption of oatmeal porridge, significant changes in basal or post-ITF load expired H2. Another study reported that 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, 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, 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, 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, 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, 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, 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, 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, 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, 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, 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, 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, except increased flatulence, which returned to the basal level after 3 wk of reversion.

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 H2, 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 expired H2. 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 expired H2, 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 Ruminococcus callidus were both negatively correlated with intestinal symptoms, and especially discomfort. However, another study reported that Ruminococcus 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
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