Supplementary Methods

Participants

IBS patients between the ages of 18 and 69 years in our research database were screened consecutively for possible inclusion in this study. The majority of patients in the research database were recruited through community advertisements for physiologic or treatment-related clinical studies by the University of California Los Angeles G. Oppenheimer Center for Neurobiology of Stress and Resilience, and a lesser proportion of patients were recruited for research studies directly through the general GI clinics at University of California Los Angeles. All participants were recruited during the time periods between July 2013 and June 2019. Patient data from previous physiologic studies was used if patients met the inclusion criteria for this study. Of note, microbiome data from only a small subset of HCs (n = 25) was published previously in studies assessing obesity, food addiction, and the brain–gut axis.1,2 No microbiome data from IBS patients had been published previously, and no previous studies in our research database had focused on the effect of diet on the gut microbiota. This study was approved by the Institutional Review Board at the University of California Los Angeles, which allowed for the repurposing of previously collected data.

All participants underwent a medical history and physical examination. IBS participants were included in our study if they met Rome III or Rome IV criteria, depending on the year of recruitment, and were subclassified as having IBS-C, IBS-D, IBS-M, or IBS unclassified. IBS participants were excluded if they had any current organic disease that also could contribute to chronic abdominal pain (ie, inflammatory bowel disease, active peptic ulcer disease, diverticulitis, and so forth), had an overlapping dominant functional disorder such as functional dyspepsia, had any active psychiatric disease, or were on chronic opioid medications. HCs and IBS patients who submitted stool samples for microbiota analysis were excluded if they had received antibiotics within the previous 3 months. Only a very small percentage of IBS participants (2.6%) and HCs (1.18%) reported the use of probiotics. Analyses were performed both with including and excluding these participants on probiotics, and there was no significant effect of probiotic use on our overall findings. Therefore, the participants on probiotics were included in the final analysis.

Diet Checklist

If participants did not believe that the diet they consumed most frequently was reflected by the choices of a standard American diet, modified American diet, Mediterranean diet, Paleo diet, vegan diet, vegetarian diets, gluten-free diet, dairy-free diet, or the low FODMAP diet, the option was given to select “other,” and describe the components of their individual diet in regard to consumption of meat, dairy, eggs, fruits, vegetables, and grains. If a participant selected “other,” their comments regarding intake of food components were reviewed individually, as was that participant’s 24-hour diet diary that reflected every food component consumed in the 24-hour period before stool submission for microbiota analysis. After review of the dietary comments and 24-hour diet diaries, participants who selected “other” then were reclassified into the diet category that was most reflective of their individual diet. This then was verified against the participant’s responses on the DHQ-II, a food frequency questionnaire.

If a participant selected 2 diets on the Diet Checklist, one of which fell into the standard category and one of which fell into the exclusion category, that participant ultimately was characterized as being on an exclusion diet. When available, 24-hour diet diaries were examined for participants on an exclusion diet to confirm compliance. If a participant’s 24-hour diet diary was not consistent with their self-reported exclusion diet from the Diet Checklist, that participant’s responses to the DHQ-II were reviewed and the diet was reclassified as appropriate.

Our institution’s Diet Checklist also was validated internally against the standardized DHQ-II and each patient’s 24-hour diet recall of all foods consumed in a 24-hour period before submitting a stool sample for microbiota analysis. The Food Patterns Equivalents Database from the DHQ-II, which converts foods and beverages in the Food and Nutrient Database for Dietary Studies to 37 United States Department of Agriculture Food Pattern components, was used for validation. Compliance with an exclusion diet on the Diet Checklist (dairy-free, Paleo, vegetarian, or vegan) was defined as 3 or fewer dietary indiscretions per month. This cut-off value was determined to be applicable to real-life compliance scenarios after consultation with a registered GI dietician at the University of California Los Angeles. For example, if a patient stated on their Diet Checklist that they consumed a dairy-free diet, but review of their DHQ-II showed 4 episodes of dairy intake in a 1-month period, this person was determined to not actually follow a dairy-free diet.

Using this methodology, only 2 (7.4%) subjects who indicated that they were on a Paleo diet on the Diet Checklist were nonadherent based on their DHQ-II responses, 0 (0.0%) subjects who indicated that they were on a vegan diet were nonadherent based on their DHQ-II responses, 1 subject (4.5%) who reported being on a standard vegetarian diet was nonadherent based on his or her DHQ-II responses, 0 (0.0%) subjects who indicated that they were on an ovovegetarian, lactovegetarian, or ovolactovegetarian diet were...
nonadherent based on their DHQ-II responses, and 1 subject who reported consuming a dairy-free diet was nonadherent based on his or her DHQ-II responses. These subjects were reclassified into the diet category that best matched their eating patterns based on their DHQ-II responses and 24-hour diet recall. Validation was unable to be performed for the gluten-free diet and low FODMAP diet because the DHQ-II does not specifically assess consumption of gluten or distinguish between foods that are low or high in FODMAPs. For the 4 subjects who were not compliant with their indicated diet on the Diet Checklist, we then reviewed their individual Diet Checklist, DHQ responses, and 24-hour diet diary if available. Subjects’ diets were reclassified into the diet category that best reflected the diet they consumed.
Supplementary Figure 1. (A) β-diversity in irritable bowel syndrome (IBS) participants vs healthy controls (HCs) irrespective of diet. (B) α-diversity in IBS participants compared with HCs irrespective of diet. (C) Taxonomic plots in IBS participants compared with HCs irrespective of diet.

Supplementary Figure 2. (A) β-diversity in irritable bowel syndrome (IBS) participants on a standard diet vs not on a standard diet. (B) α-diversity in IBS participants on a standard diet compared with healthy controls (HCs) on a standard diet. (C) Taxonomic plots in IBS participants and HCs on a standard diet.
**Supplementary Figure 3.**

(A) β-diversity in irritable bowel syndrome (IBS) participants on a standard diet vs not on a standard diet. (B) Relative fecal abundances in IBS participants on a standard diet compared with a nonstandard (exclusion) diet.

---

**Supplementary Table 1. Diet Checklist Categories**

| Diet category          | Components                                                                                   |
|------------------------|-----------------------------------------------------------------------------------------------|
| Standard American      | High consumption of processed foods, pastas, and breads                                      |
|                        | Meats, including red meat, fish, eggs, and dairy products consumed                           |
|                        | Vegetables and fruits consumed, but not in large quantities                                  |
| Modified American      | High consumption of processed foods, pastas, and breads (mainly whole grain)                |
|                        | Poultry, fish, eggs, and dairy products consumed                                             |
|                        | Red meat consumed in limited quantities                                                      |
|                        | Vegetables and fruits consumed, but not in large quantities                                  |
| Mediterranean          | High consumption of fruits, vegetables, bread and other cereals, beans, nuts, and seeds     |
|                        | Olive oil is the key monounsaturated fat source                                               |
|                        | Dairy products, fish, and poultry are consumed in low to moderate amounts                    |
|                        | Little red meat is consumed                                                                  |
|                        | Eggs are eaten 0 to 4 times a week and wine is consumed in moderate (or low) amounts          |
| Paleo                  | Consumption of basic foods such as plain meat, fish, shellfish, eggs, nuts, vegetables, fruits, berries, and mushrooms |
|                        | Minimally processed oils, such as avocado, olive, or coconut oil, for cooking               |
|                        | Excludes dairy products, legumes, dry beans, grains, coffee, alcohol, sugar, and processed foods |
| Vegan                  | Focus is on plant-based foods                                                                |
|                        | Includes fruits, vegetables, dried beans and peas, grains, seeds, and nuts                   |
|                        | Excludes all meat and animal products                                                       |
| Vegetarian (6 categories) | Focus is on plant-based foods                                                              |
| Vegetarian             | Includes fruits, vegetables, dried beans and peas, grains, seeds, and nuts                   |
|                        | Excludes all meat but will allow animal-derived ingredients (i.e., honey and gelatin)       |
| Lactovegetarian        | Includes plant foods plus dairy products, no eggs                                             |
| Ovovegetarian          | Includes plant foods plus eggs, no dairy                                                     |
| Lacto-ovovegetarian    | Includes both dairy products and eggs                                                        |
| Pescatarian            | Includes fruits, vegetables, dried beans and peas, grains, seeds, and nuts                   |
|                        | Excludes all meat except fish                                                                |
| Raw vegan/raw food     | Unprocessed vegan foods that have not been heated to higher than 115°F (46°C)               |
| Gluten-free            | Diet includes most foods but avoids the protein gluten, which is found in wheat, barley, and rye |
| Dairy-free             | Diet includes most foods but avoids dairy                                                    |
| Low FODMAP             | Diet limits foods high in sugar and carbohydrates (fructose, lactose, fructans, galactans, and polyols) |

FODMAP, fermentable oligo-, di-, monosaccharides, and polyols.
### Supplementary Table 2. Components of the IBS-SSS and the Relationship With Exclusion and Restrictive Diets

| Question | Exclusion diet | Nonexclusion diet | P value | Restrictive diet | Nonrestrictive diet | P value |
|----------|----------------|-------------------|---------|------------------|---------------------|---------|
| **Question 1a (abdominal pain, yes, %)** | 96 | 94 | .537 | 94 | 95 | .928 |
| **Question 1b (severity of abdominal pain [0–100]; means ± SD)** | 47.18 ± 20.39 | 44.42 ± 20.02 | .230 | 48.53 ± 21.92 | 44.45 ± 20.39 | .108 |
| **Question 1c (number of days of abdominal pain in past 10 days; means ± SD)** | 5.55 ± 2.77 | 4.46 ± 2.60 | <.0001 | 5.88 ± 2.87 | 4.54 ± 2.77 | <.0001 |
| **Question 2a (abdominal distention, yes, %)** | 89 | 82 | .069 | 92 | 82 | .021 |
| **Question 2b (severity of abdominal distention [0–100]; means ± SD)** | 69.15 ± 24.06 | 68.86 ± 23.1 | .054 | 72.05 ± 24.62 | 67.84 ± 24.06 | .088 |
| **Question 3 (bowel habit satisfaction [0–100]; means ± SD)** | 69.15 ± 24.06 | 68.86 ± 23.1 | .914 | 72.05 ± 24.62 | 67.84 ± 24.06 | .143 |
| **Question 4 (IBS affecting quality of life [0–100]; means ± SD)** | 64.19 ± 20.03 | 56.99 ± 22.34 | <.001 | 67.74 ± 18.87 | 57.00 ± 20.03 | <.001 |

**NOTE.** The bolded values indicate statistical significance.

IBS-SSS, Irritable Bowel Syndrome Severity Scoring System; SD, standard deviation.

### Supplementary Table 3. Diet Categories and Bowel Habit Subtypes in IBS Participants

| Diet | IBS-C, n | IBS-D, n | IBS-U, n | IBS-M, n | \( \chi^2 \) value | P value | FDR-adjusted P value |
|------|----------|----------|----------|----------|---------------------|---------|-----------------------|
| Standard diet | 64 | 77 | 11 | 52 | 6.14 | .105 | .262 |
| American | 65 | 89 | 12 | 48 | 2.38 | .498 | .685 |
| Mediterranean | 16 | 7 | 3 | 14 | 10.90 | .012 | .132 |
| **Exclusion diet** | 40 | 62 | 14 | 24 | 6.14 | .105 | .262 |
| Vegan | 1 | 6 | 1 | 1 | 3.36 | .340 | .534 |
| Vegetarian (all subtypes) | 12 | 19 | 3 | 9 | 0.28 | .963 | .981 |
| Paleo | 9 | 10 | 2 | 6 | 0.18 | .981 | .981 |
| Low FODMAP | 6 | 5 | 2 | 5 | 1.78 | .620 | .758 |
| Gluten-free | 17 | 19 | 7 | 9 | 4.14 | .247 | .453 |
| Dairy-free | 15 | 32 | 8 | 8 | 9.15 | .027 | .148 |
| **Restrictive diet** | 28 | 40 | 11 | 15 | 5.86 | .119 | .262 |
| Low FODMAP | 6 | 5 | 2 | 5 | 1.78 | .620 | .758 |
| Gluten-free | 17 | 19 | 7 | 9 | 4.14 | .247 | .453 |
| Dairy-free | 15 | 32 | 8 | 8 | 9.15 | .027 | .148 |

FDR, false-discovery rate; FODMAP, fermentable oligo-, di-, monosaccharides, and polyols; IBS-C, constipation-predominant irritable bowel syndrome; IBS-D, diarrhea-predominant irritable bowel syndrome; IBS-M, irritable bowel syndrome with mixed symptoms; IBS-U, irritable bowel syndrome unclassified.
### Supplementary Table 4. Association Between DHQ-II Food Variables and IBS

| Food variable            | HC, means (SD) | IBS, means (SD) | Z-value | OR (95% CI), IBS vs HCs | FDR adjusted P value |
|--------------------------|---------------|-----------------|---------|-------------------------|----------------------|
| Energy, kcal/d           | 1995.62 (1589.95) | 1853.51 (926.57) | -2.16   | 1.00 (0.99–1.00)        | .078                 |
| Total protein, g/d       | 84.45 (82.72)  | 76.29 (44.56)    | -2.35   | 0.99 (0.99–1.00)        | .052                 |
| Animal protein, g/d      | 52.58 (63.42)  | 47.13 (34.89)    | -1.97   | 0.99 (0.98–1.00)        | .083                 |
| Vegetable protein, g/d   | 31.85 (24.05)  | 29.13 (16.98)    | -2.80   | 0.97 (0.96–0.99)        | .026                 |
| Total fat, g/d           | 79.49 (70.65)  | 74.29 (42.44)    | -1.80   | 0.99 (0.99–1.00)        | .113                 |
| Total saturated fat, g/d | 24.43 (22.91)  | 22.42 (13.92)    | -1.92   | 0.98 (0.96–1.00)        | .089                 |
| Total monounsaturated fat, g/d | 30.46 (27.23)  | 28.93 (17.44)    | -1.63   | 0.99 (0.97–1.00)        | .145                 |
| Total polyunsaturated fat, g/d | 17.16 (15.17)  | 16.07 (9.55)     | -1.70   | 0.98 (0.95–1.00)        | .132                 |
| Total sugars, g/d        | 100.01 (71.45) | 89.47 (52.45)    | -2.61   | 0.99 (0.98–0.99)        | .038                 |
| Fructose, g/d            | 28.05 (30.07)  | 25.00 (20.86)    | -1.61   | 0.99 (0.98–1.00)        | .145                 |
| Galactose, g/d           | 0.59 (0.66)    | 0.45 (0.38)      | -3.63   | 0.17 (0.06–0.39)        | .005                 |
| Glucose, g/d             | 26.23 (22.89)  | 23.90 (16.41)    | -2.00   | 0.98 (0.96–1.00)        | .083                 |
| Lactose, g/d             | 0.59 (0.66)    | 0.45 (0.38)      | -3.63   | 0.17 (0.06–0.39)        | .005                 |
| Maltose, g/d             | 3.48 (3.21)    | 3.59 (3.85)      | -2.08   | 0.90 (0.82–1.00)        | .078                 |
| Sucrose, g/d             | 35.36 (29.90)  | 31.72 (20.42)    | -2.10   | 0.90 (0.82–1.00)        | .078                 |
| Starch, g/d              | 96.34 (83.94)  | 89.78 (55.85)    | -2.05   | 0.99 (0.99–1.00)        | .078                 |
| Total dietary fiber, g/d | 25.54 (19.67)  | 22.85 (13.98)    | -3.06   | 0.96 (0.94–0.99)        | .017                 |
| Soluble fiber, g/d       | 7.78 (5.05)    | 6.99 (4.35)      | -3.50   | 0.86 (0.79–0.93)        | .005                 |
| Insoluble fiber, g/d     | 17.72 (15.23)  | 15.80 (10.38)    | -2.83   | 0.96 (0.93–0.98)        | .026                 |
| Total grains, g/d        | 4.93 (4.75)    | 4.80 (3.54)      | -0.06   | 0.99 (0.58–1.70)        | .954                 |
| Whole grains, g/d        | 0.60 (0.49)    | 0.70 (0.70)      | -1.58   | 0.93 (0.84–1.01)        | .147                 |
| Refined grains, g/d      | 4.34 (4.47)    | 4.10 (3.14)      | -1.50   | 0.94 (0.86–1.02)        | .166                 |
| Carbohydrates, g/d       | 238.94 (175.10)| 218.89 (109.67)  | -2.49   | 1.00 (0.99–1.00)        | .044                 |
| Total meat, oz/d         | 1.470 (2.88)   | 1.19 (1.87)      | -1.39   | 0.91 (0.77–1.03)        | .198                 |
| Cured meat, oz/d         | 0.69 (1.41)    | 0.52 (0.77)      | -1.21   | 0.84 (0.61–1.10)        | .259                 |
| Organ meat, oz/d         | 0.04 (0.22)    | 0.01 (0.02)      | -0.89   | 0.07 (NA to 1.16)       | .417                 |
| Total vegetables, cup equivalents/d | 3.06 (3.26) | 2.52 (2.02) | -2.58   | 0.83 (0.71–0.95)        | .038                 |
| Total fruits, cup equivalents/d | 1.73 (2.02) | 1.69 (1.82) | -0.73   | 0.93 (0.75–1.12)        | .495                 |
| Total dairy, cup equivalents/d | 1.34 (1.26) | 1.17 (1.01) | -2.44   | 0.65 (0.46–0.90)        | .045                 |
| Alcohol, g/d             | 5.58 (8.17)    | 6.90 (10.78)     | 0.37    | 1.01 (0.97–1.04)        | .734                 |

**NOTE.** The bolded values indicate statistical significance.

DHQ-II, Diet History Questionnaire-II; FDR, false-discovery rate; HC, healthy control; IBS, irritable bowel syndrome; OR, odds ratio; SD, standard deviation.

---

### Supplementary Table 5. Differences in the Fecal Microbiota Observed Between HCs and IBS, Adjusted for Race

| Family           | Genus          | Base mean | Relative abundance | Log₂ fold change | lfcSE | Stat  | P value | Adjusted P value | Q value |
|------------------|----------------|-----------|--------------------|------------------|-------|-------|---------|------------------|--------|
| Rikenellaceae    |                | 2682.122  | 0.020              | 0.960            | 0.263 | 3.655 | .000    | .013             | 0.010  |
| Porphyromonadaceae| Parabacteroides| 2934.118  | 0.0221             | 0.708            | 0.236 | 3.001 | .003    | .0446            | 0.0335 |

HC, healthy control; IBS, irritable bowel syndrome; lfcSE, standard error of the log₂FoldChange estimate; Stat, Wald statistic.
Supplementary Table 6. Differences in the Fecal Microbiota Observed Between HCs and IBS, Adjusted for Diet and Race

| Family   | Genus     | Base mean | Relative abundance | $\log_2$ fold change | lfcSE | Stat | $P$ value | Adjusted $P$ value | Q value |
|----------|-----------|-----------|--------------------|----------------------|-------|------|-----------|-------------------|---------|
| Rikenellaceae | 2682.122 | 0.020     | 0.955              | 0.268                | 3.566 | .000 | .018      | 0.014             |

HC, healthy control; IBS, irritable bowel syndrome; lfcSE, standard error of the log2FoldChange estimate; Stat, Wald statistic.
### Supplementary Table 7. Summary of Previous Evidence of Dietary-Induced Changes in the Genera Pertinent to this Study

| Genus       | Details                                                                 | Pertinent previous dietary studies in IBS patients                                                                 | Pertinent previous dietary studies in non-IBS patients                                                                 | Findings in this study                                                                                                                                 |
|-------------|-------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|
| **Bifidobacterium** | Gram-positive, nonmotile anaerobic bacteria  
Some bifidobacterial are used as probiotics given positive health benefits | IBS patients on a habitual diet had a greater fecal concentration of *Bifidobacterium* compared with patients on the low FODMAP diet  
Likely related to a greater abundance of fructans  
A paradoxic increase in IBS symptoms was seen with the habitual diet  
IBS patients on a traditional diet had greater fecal *Bifidobacterium* compared with patients on a low FODMAP diet | *Bifidobacterium*-containing probiotics associated with a reduction in IBS symptoms compared with probiotics containing *Lactobacillus* alone | IBS participants on a standard diet had a significantly greater fecal abundance of *Bifidobacterium* and lesser IBS symptom severity compared with IBS participants not on a standard diet |
| **Prevotella** | Gram-negative, predominantly commensal, rod-shaped bacteria             | –                                                                                                                   | *Prevotella* is associated with diets high in carbohydrates and simple sugars  
*Prevotella* ferments dietary fiber into short-chain fatty acids | IBS participants on a standard diet had a significantly greater fecal abundance of *Prevotella* compared with IBS participants not on a standard diet |
| **Lachnospira** | Gram-positive, non-spore-forming, anaerobic, rod-shaped bacteria, involved in the fermentation of pectin, a complex fiber and heteropolysaccharide found in the cell walls of certain fruits and vegetables  
*Lachnospira* also are known to be short-chain fatty acid producers, which can have positive effects on immune function, intestinal barrier integrity, mucus production, and overall gut health | –                                                                                                                   | Vegetable intake was associated positively with an increased relative abundance of *Lachnospira*  
Vegetable-based diet associated with *Lachnospira* | IBS participants on an exclusion diet had a significantly greater abundance of *Lachnospira* compared with those not on an exclusion diet |
| Genus      | Details                                                                 | Pertinent previous dietary studies in IBS patients | Pertinent previous dietary studies in non-IBS patients | Findings in this study                      |
|------------|-------------------------------------------------------------------------|-----------------------------------------------------|-------------------------------------------------------|--------------------------------------------|
| Eubacterium| Heterogenous group of Gram-positive, non-spore-forming, butyrate-producing, anaerobic bacilli | --                                                  | Eight-week trial of a gluten-free diet in healthy individuals associated with a decreased abundance of *Eubacterium hallii*, which may have been partially driven by changes in dietary fiber\[^{10}\] | IBS participants on an exclusion diet had a lower fecal abundance of *Eubacterium* compared with those not on an exclusion diet |
| Lactobacillus | Gram-positive, anaerobic or microaerophilic, rod-shaped bacteria that convert sugars to lactic acid and are considered beneficial to human health | --                                                  | A gluten-free diet over a 1-month period in healthy individuals was associated with a decreased abundance of *Lactobacillus*\[^{11}\] The ratio of *Lactobacillus*:*Blifidobacterium* to *Bacteroides-EScherichia coli* was reduced significantly in celiac patients on a gluten-free diet (with either active or inactive disease) compared with controls\[^{12}\] | IBS participants on a restrictive diet had a lower fecal abundance of *Lactobacillus* compared with *IBS* participants not on a restrictive diet |

FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols; IBS, irritable bowel syndrome.