Sex, Body Mass Index, and Dietary Fiber Intake Influence the Human Gut Microbiome

Christine Dominianni¹, Rashmi Sinha³, James J. Goedert³, Zhiheng Pei²,⁴,⁵,⁶, Liying Yang⁶, Richard B. Hayes¹,², Jiyoung Ahn¹,²*

¹ Department of Population Health, New York University School of Medicine, New York, New York, United States of America,
² New York University Perlmutter Cancer Center, New York, New York, United States of America,
³ Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, United States of America,
⁴ Department of Pathology and Laboratory Medicine, New York Veterans Affairs Medical Center, New York, New York, United States of America,
⁵ Department of Pathology, New York University School of Medicine, New York, New York, United States of America,
⁶ Department of Medicine, New York University School of Medicine, New York, New York, United States of America

* Jiyoung.Ahn@nyumc.org

Abstract

Increasing evidence suggests that the composition of the human gut microbiome is important in the etiology of human diseases; however, the personal factors that influence the gut microbiome composition are poorly characterized. Animal models point to sex hormone-related differentials in microbiome composition. In this study, we investigated the relationship of sex, body mass index (BMI) and dietary fiber intake with the gut microbiome in 82 humans. We sequenced fecal 16S rRNA genes by 454 FLX technology, then clustered and classified the reads to microbial genomes using the QIIME pipeline. Relationships of sex, BMI, and fiber intake with overall gut microbiome composition and specific taxon abundances were assessed by permutational MANOVA and multivariate logistic regression, respectively. We found that sex was associated with the gut microbiome composition overall (p=0.001). The gut microbiome in women was characterized by a lower abundance of Bacteroidetes (p=0.03). BMI (>25 kg/m² vs. <25 kg/m²) was associated with the gut microbiome composition overall (p=0.05), and this relationship was strong in women (p=0.03) but not in men (p=0.29). Fiber from beans and from fruits and vegetables were associated, respectively, with greater abundance of Actinobacteria (p=0.006 and false discovery rate adjusted q=0.05) and Clostridia (p=0.009 and false discovery rate adjusted q=0.09). Our findings suggest that sex, BMI, and dietary fiber contribute to shaping the gut microbiome in humans. Better understanding of these relationships may have significant implications for gastrointestinal health and disease prevention.

Introduction

The human gastrointestinal tract carries a structured microbial composition consisting of more than ten-fold the number of human cells in the body [1]. The gut microbiota plays an
indispensable role in digesting food components and in host immunological response [1, 2]. Comprehensive genomic-based microbiome assessments have identified significant inter-individual variation in the composition of the human gut microbiome [3]. Increasing evidence suggests that gut microbiome variation is important in the etiology of gastrointestinal diseases [4, 5] including cancer [6, 7], as we recently reported [8]. However, significant knowledge gaps remain in our understanding of personal factors that influence gut microbiome composition. Better understanding of these personal factors and their relationships with the gut microbiome may have important implications for disease prevention.

There is increasing evidence that sex steroid hormone levels are associated with the human gut microbiome [9]; however, the relationships of personal factors associated with these sex steroids in relation to the human gut microbiome has not been studied comprehensively. An animal study recently reported sex-specific differentials in gut microbiome composition [10]. Adiposity, a source of sex steroids [11], may play an additional role in forming a sex-specific microbiome composition, as suggested by animal models and human weight-loss trials [12–15]. Dietary fiber, a major energy source for gut bacterial fermentation [16], and an influence on systemic estrogen levels [17], may also shape the gut microbiome. The fiber hypothesis is supported by short-term dietary interventions [18–22], but there is only sparse data evaluating the impact of long-term dietary fiber on the gut microbiome [23, 24]. Given the findings from these early studies, we hypothesize that sex, adiposity, and dietary fiber operate through similar mechanisms, including hormone mediated pathways, to modulate the human gut microbiome.

To better understand the determinants of the human gut microbiome, we assessed gut microbiome composition in fecal samples from 82 subjects in the control group of a case-control study of colorectal cancer [8]. We investigated the relationship of sex, adiposity, assessed by body mass index (BMI) [25], and dietary fiber, individually and jointly, to gut microbiome composition and distribution of specific taxa and found that sex, BMI, and dietary fiber were associated with differences in the human gut microbiome.

**Methods and Materials**

**Study population**

Samples and data were collected from control group participants in a case-control study of gut microbiome and colorectal cancer, as recently described [8]. Study participants were originally recruited at three Washington, D.C., area hospitals (National Naval Medical Center, Walter Reed Army Medical Center, and George Washington University Hospital) between April 1985 and June 1987, prior to elective surgery for non-oncological, non-gastrointestinal conditions, including orthopedic or neurosurgical procedures, hernia repairs, vascular procedures, and other general surgeries [26, 27].

We included 82 subjects for study among 94 participants in the original investigation with at least 100 mg of lyophilized fecal material available (described below), excluding subjects with less than 1000 microbial sequence reads and with missing or extreme caloric intake (≤500 or ≥5500 kcal/day). None of the eligible subjects reported taking antibiotics during the year prior to being recruited into the study. The study was approved by the institutional review boards from New York University School of Medicine and National Cancer Institute and all participants provided signed written informed consent. All signed written consent forms are documented and stored at the National Cancer Institute.

**Demographic, lifestyle, and dietary fiber assessment**

Demographic information on age, sex, height, weight, and race (White, Black, Hispanic, Asian or Pacific Islander) were ascertained by structured demographic questionnaires completed.
prior to hospitalization and treatment. BMI was calculated by dividing weight in kilograms by squared height in meters. Study participants were then classified as normal weight (<25 kg/m²) or overweight or obese (≥25 kg/m²) based on the WHO definition [28]. Diet intake was assessed using a self-administered 100 item food frequency questionnaire. Long term intake frequency and portion size were queried. Nutrient intakes were derived from frequency and portion-size responses from the food frequency questionnaire, in which nutrient values per portion were multiplied by the daily frequency of intake and summed across all relevant food items, using the US Department of Agriculture pyramid food group serving database [29]. Diet data was standardized by total calorie intake [30]. Total fiber (5.7–11.3, 11.3–14.5, 14.5–17.6, 18.0–38.7 g/day), fruit and vegetable fiber (1.6–6.5, 6.6–8.8, 8.9–11.7, 11.8–21.9 g/day), bean fiber (0–0.9, 0.9–1.3, 1.4–2.3, 2.3–10.7 g/day), and grain fiber (1.0–2.5, 2.8–3.5, 3.6–5.1, 5.1–9.7 g/day) were grouped into quartiles. For taxa-specific analyses, total and specific sources of fiber were treated as continuous values.

**Fecal sample collection**

Prior to hospitalization and treatment, subjects were asked to collect fecal samples at home, over a two day period, and store the material in a plastic container, in a Styrofoam chest containing dry ice. Fecal samples were shipped to a USDA laboratory, where the two-day samples were pooled, lyophilized and stored at a minimum of -40°C in sealed, air-tight containers.

**16s rRNA gene sequencing microbiome assay**

We extracted DNA from the stored fecal samples using the Mobio PowerSoil DNA Isolation Kit (Carlsbad, CA) with bead-beating. As we reported previously [8], 16S rRNA amplicons covering variable regions V3 to V4 were generated using primers (347F-5'GGAGGCAGCAGTRRGGAATT-3' and 803R 5'-CTACCRGGGTATCTAATCC-3') incorporating Roche 454 FLX Titanium adapters (Branford, CT) and a sample barcode sequence [31]. Amplicons were sequenced with the 454 Roche FLX Titanium pyrosequencing system following the manufacturer’s specifications. Laboratory personnel were blinded to personal factor status.

**Sequence data processing and taxonomic assignment**

Multiplexed, barcoded sequencing data was deconvoluted. Poor-quality sequences were filtered based on sequences less than 200 or greater than 1000 base pairs, missing or mean quality score <25, or mismatched barcode and primer sequences. Chimeric sequences were removed with ChimeraSlayer [32]. Filtered sequences were binned into operational taxonomic units with 97% identity and aligned to fully-sequenced microbial genomes (IMG/GG GreenGenes), using the QIIME pipeline [33]. Blinded quality control specimens in all sequencing batches (38 aliquots from 9 unmatched parent study control subjects) had good reproducibility, with intraclass correlation coefficients of 0.90 for principal coordinate analysis, 0.84 for Shannon diversity index, and 0.43 to 0.59 for relative abundances of major phyla [8].

**Statistical analysis**

The relationship of overall gut microbiome composition with personal factors (sex, BMI, and dietary fiber intake) was assessed by principal coordinate analysis (PCoA), based on the unweighted (qualitative) phylogenetic UniFrac distance matrix [34]. PCoA plots were generated using the first two principal coordinates (PCs), according to categories of personal factors. Adonis [35], which uses permutational multivariate analysis of variance (PERMANOVA), was used to test statistical significances of association of overall composition with personal factors.
To control for confounding, we used Generalized Linear Regression where each PC was treated as the outcome and the personal factors were mutually adjusted. Richness and evenness were assessed by Shannon’s diversity index and evenness index [36], respectively, and Monte Carlo permutations were used for assessment of statistical significance [33].

Relationships relating specific taxa abundance to sex and BMI were assessed by Mann-Whitney-Wilcoxon test. To visualize relationships of specific sources of fiber (bean, fruit and vegetable, and grain) with gut microbiome taxa (genus), we developed a heatmap based on unsupervised classification of Spearman correlation coefficients (R package, gplots; R version 2.15.3). To control for confounding in these analyses, we used logistic regression, considering high vs. low taxon abundance (median value cut-off) as the outcome and fiber types as the predictors.

False discovery rate (FDR) adjusted q-values were calculated for comparisons of taxa [37]. To minimize the number of null hypotheses that had to be corrected for, we limited these analyses to the three major phyla, Actinobacteria, Bacteroidetes, and Firmicutes (at least 50% of samples positive, with at least 1% median abundance). All analyses were carried out using SAS, version 9.3 (SAS Institute, Cary, NC) unless otherwise specified.

**Results**

Among the 82 subjects included for study, 62.2% were men and 85.4% were white (Table 1). Their age ranged from 30–83 years (58.4±13.2 [mean ± standard deviation]). This study population included 46.3% former smokers and 9.8% current smokers. Mean intake of total dietary fiber was 14.1 grams per day. From the 82 immediately frozen fecal samples, gut

| Characteristic                | Total (N = 82) | Men (N = 51) | Women (N = 31) | p-value<sup>1</sup> |
|------------------------------|---------------|--------------|----------------|---------------------|
| Age (Years, Mean±SD)         | 58.4±13.18    | 58.0±13.58   | 59.2±12.67     | 0.71                |
| BMI (Kg/M², Mean±SD)         | 25.0±4.06     | 25.6±3.49    | 23.8±4.69      | 0.03                |
| Race (%)<sup>2</sup>          |               |              |                | 0.35                |
| White                        | 85.4          | 88.2         | 80.6           |                     |
| Black                        | 12.2          | 7.8          | 19.3           |                     |
| Hispanic                     | 1.2           | 2.0          | –              |                     |
| Asian or Pacific Islander    | 1.2           | 2.0          | –              |                     |
| Smoking Status (%)           |               |              |                | 0.04                |
| Never                        | 43.9          | 35.3         | 58.1           |                     |
| Former                       | 46.3          | 56.9         | 29.0           |                     |
| Current                      | 9.8           | 7.8          | 12.9           |                     |
| Sequence Batch (%)           |               |              |                | 0.50                |
| 1                            | 34.1          | 57.1         | 64.8           |                     |
| 2                            | 65.9          | 42.9         | 35.2           |                     |
| Sources of Fiber (Estimated Daily, Mean±SD)| | | | |
| Total Dietary Fiber (grams)  | 14.1±5.11     | 14.3±5.24    | 13.8±4.95      | 0.07                |
| Bean(grams)                  | 1.8±1.62      | 2.1±1.79     | 1.3±1.15       | **0.003**           |
| Fruit and Vegetables(grams)  | 9.4±4.22      | 8.6±3.89     | 10.7±4.46      | **0.01**            |
| Grain(grams)                 | 3.9±1.63      | 3.8±1.62     | 4.0±1.69       | 0.82                |

<sup>1</sup>All characteristics were compared by sex using either Chi square or Mann-Whitney-Wilcoxon tests. All analyses were carried out using SAS software (version 9.3).

<sup>2</sup>Race was grouped as White and Other for Chi square test.

doi:10.1371/journal.pone.0124599.t001
microbiome was assessed by multiplexed 16S rRNA gene sequencing with amplification of the hyper-variable V3 to V4 region. In total, 331,288 high-quality 16S rRNA sequence reads (4,040±1,843 [mean ± standard deviation] sequences per sample) were binned to operational taxonomic units and subsequently assigned taxonomy.

Sex was significantly associated with gut microbiome composition overall (unweighted UniFrac p = 0.001, Fig 1A, Table 2); sex was specifically associated with the first principal coordinate, after we adjusted for other confounding factors (Table 3, p = 0.0001). Women tended to have a lower abundance of Bacteroidetes (5.5% in women [n = 31] vs. 11.4% in men [n = 51], FDR corrected q = 0.07) (Fig 1B).

BMI (≥25 kg/m² [n = 32] vs. <25 kg/m² [n = 50]) was associated with gut microbiome community composition with marginal statistical significance (Fig 2A, p = 0.05, Table 2). When we stratified by sex, the relationship of BMI with overall gut microbiome composition was significant in women (p = 0.03, Fig 3A, Table 2) but not in men (Fig 3B, p = 0.29, Table 2). In women, Shannon diversity indices were lower in the overweight and obese subjects (n = 10) compared to normal weight subjects (n = 18) (Fig 3C, p = 0.03). In women, there was also a tendency for Bacteroidetes abundance to be lower in overweight and obese subjects, compared to normal weight subjects (Fig 3E).

Sources of dietary fiber intake tended to be associated with the gut microbiome. Fiber from fruits and vegetables (p = 0.06) and from beans (p = 0.06) were marginally associated with overall gut microbiome composition (Fig 4A, Table 2). The relationships of total fiber, fiber from fruits and vegetables, and fiber from grains were particularly stronger in women (p = 0.005, p = 0.05, p = 0.01, respectively, Table 2). Fiber from beans was strongly related to

---

**Fig 1. Gut microbiome according to sex.** (A) Unweighted principal coordinate analysis plot of the first two principal coordinates categorized by sex. Ellipses were added to plots using the R package, latticeExtra (R version 2.15.3). (B) Relative abundance of the three major phyla. Mann-Whitney-Wilcoxon test was used to test for overall differences using SAS software (version 9.3). Nominal p-values are listed below each phylum.

doi:10.1371/journal.pone.0124599.g001
Unsupervised classification showed that intake of fiber from fruits and vegetables clustered notably with class Clostridia (Cluster 1, Fig 4B, S1 Table). Multivariate logistic regression, adjusting for confounding, further supports this

Table 2. PERMANOVA1 analysis of personal factors with the unweighted UniFrac distance matrix.

| Factor                  | F statistic | P-value |
|-------------------------|-------------|---------|
| **Main Analysis**       |             |         |
| Sex                     | 1.49        | 0.001   |
| BMI2                    | 1.12        | 0.05    |
| Total Fiber3            | 1.08        | 0.14    |
| Fruit & Vegetable Fiber3| 1.13        | 0.06    |
| Bean Fiber3             | 1.13        | 0.06    |
| Grain Fiber3            | 1.08        | 0.15    |
| **Men**                 |             |         |
| BMI2                    | 1.04        | 0.29    |
| Total Fiber3            | 1.01        | 0.41    |
| Fruit & Vegetable Fiber3| 1.08        | 0.10    |
| Bean Fiber3             | 1.17        | 0.01    |
| Grain Fiber3            | 0.99        | 0.48    |
| **Women**               |             |         |
| BMI2                    | 1.17        | 0.03    |
| Total Fiber3            | 1.26        | 0.005   |
| Fruit & Vegetable Fiber3| 1.13        | 0.05    |
| Bean Fiber3             | 0.97        | 0.64    |
| Grain Fiber3            | 1.19        | 0.01    |

1Adonis, which uses permutational multivariate analysis of variance (PERMANOVA), was used to test statistical significances of association of overall composition with personal factors. All analyses were carried out using the QIIME pipeline.

2BMI was categorized as normal weight (<25 kg/m²) versus overweight or obese (≥25 kg/m²).

3Total and specific sources of dietary fiber were categorized as low (quartiles 1–3) versus high (quartile 4) intake.

Overall gut microbiome in men (p = 0.01, Table 2). Unsupervised classification showed that intake of fiber from fruits and vegetables clustered notably with class Clostridia (Cluster 1, Fig 4B, S1 Table). Multivariate logistic regression, adjusting for confounding, further supports this

Table 3. Univariate and multivariate linear regressions1 of personal factors and unweighted principal coordinates (PC).

| Individual Factors | PC 1 Estimate±SD | P-Value   | PC 2 Estimate±SD | P-Value |
|--------------------|------------------|-----------|------------------|---------|
| Sex                | -0.11±0.02       | 4.81 x 10^-5 | 0.01±0.02       | 0.76    |
| BMI                | -0.03±0.03       | 0.27      | 0.004±0.02      | 0.86    |
| Fruit & Vegetable  | -0.01±0.01       | 0.21      | -0.01±0.01      | 0.36    |
| Bean               | 0.01±0.01        | 0.45      | 0.004±0.01      | 0.72    |
| Grain              | 0.01±0.01        | 0.39      | -0.004±0.01     | 0.69    |
| **Combined Factors** | **0.003±0.01** | **0.82** | **-0.02±0.01** | **0.11** |
| Fruit & Vegetable  | -0.10±0.02       | 0.0001    | 0.02±0.02       | 0.44    |

1All estimates were computed using generalized linear regression models where principal coordinates were treated as outcomes and all personal factors treated as predictor variables. All analyses were carried out using SAS software (version 9.3).

2Fiber from fruits and vegetables, sex, race, and age were included jointly in multivariate regression models.

This table presents the results of univariate and multivariate linear regressions for personal factors and unweighted principal coordinates (PC). The table includes estimates and p-values for sex, BMI, fruit & vegetable intake, and bean and grain fiber intake. Multivariate logistic regression further supports the association between dietary fiber and gut microbiome composition. The p-values are calculated using SAS software (version 9.3) and indicate the statistical significance of each factor in relation to the unweighted UniFrac distance matrix.
relationship (Table 4, OR for high vs. low Clostridia abundance = 1.24, p = 0.009 and FDR adjusted q = 0.09). We noted a second cluster of fiber from bean intake with members of Actinobacteria phylum (Table 4, OR = 2.24, p = 0.006 and FDR adjusted q = 0.05), and Bifidobacteriales order (OR = 1.65, p = 0.04 and FDR adjusted q = 0.43). No clear associations were observed between intake from grain fiber and microbial taxa (Table 2). Results of fiber intakes with specific microbial taxa were consistent in men and in women.

Discussion

In this study, we found that women had significantly different gut microbiome composition overall and particularly lower Bacteroidetes abundance, compared with men. BMI was associated with altered microbiome community composition. Greater intake of fiber from fruits and vegetables and from beans was associated with greater Clostridia and Actinobacteria abundances, respectively.

We identified significant sex differentials in gut microbiome composition and abundance of specific taxa. Our findings are consistent with an earlier targeted study of fluorescent in situ hybridization, reporting lower abundance of Bacteroidetes in women than men [38]. Furthermore, studies in mice showed that Bacteroidetes abundance is lower in female rodents [39], with sex differences in gut microbiome not appearing until after puberty [10, 39], supporting a role for hormonal and other host factors related to sexual maturity in shaping the gut microbiome. In addition to hormone-microbiome relationships, sex may impact the gut microbiome...
Fig 3. Gut microbiome according to BMI in women and men separately. Unweighted principal coordinate analysis plot of the first two principal coordinates categorized by BMI (<25 kg/m², ≥25 kg/m²) in (A) women and (B) men. Ellipses were added to plots using the R package, latticeExtra (R version 2.15.3). Alpha rarefaction plots of Shannon diversity indices grouped by normal weight (<25 kg/m²; open circles) and overweight/obese (≥25 kg/m²; red circles) status for women (C) and for men (D). Statistical significance was assessed by non-parametric Monte Carlo permutations (QIIME). (E) Relative abundance of Firmicutes and Bacteroidetes. Mann-Whitney-Wilcoxon test was used to test for overall differences using SAS software (version 9.3).

doi:10.1371/journal.pone.0124599.g003
through alterations in gut transit time, which is reported to be greater for women than men [40, 41].

We found significant differences in gut microbiome composition by BMI particularly in women. Ley et al., reported that genetically modified obese mice carried less Bacteroidetes than lean mice [12] and later in humans, that weight-loss diets in obese subjects increased Bacteroidetes abundance [13]. Consistent with this, Turnbaugh et al. reported significantly reduced Bacteroidetes in obese compared to normal weight female twins [14]. Although mechanisms are unclear, adipose tissue is a source of estrogen production in obese individuals [11], and estrogen-microbiome associations have been suggested [9]; consistent with our findings of inter-relationships between sex, BMI, and gut microbiota composition. Our findings provide further support for a role of BMI in shaping the gut microbiome and points to potential sex-specific effects. Further study with larger subject numbers will help to elucidate the role of sex and BMI in influencing the gut microbiome composition. Furthermore, since obesity is a significant risk factor of many chronic diseases [52,53], including colorectal cancer [54], these findings may also point to potentially harmful microbes.

Fig 4. Gut microbiome according to dietary fiber intake. (A) Unweighted principal coordinate analysis plot of the first two principal coordinates categorized by fruit and vegetable fiber intake (Low: 1.6–11.7 g/day [equivalent to quartile 1–3], High: 11.7–21.9 g/day [quartile 4]). Ellipses were added to plots using the R package, latticeExtra (R version 2.15.3). (B) A heatmap based on unsupervised classification of Spearman correlations between the relative abundance of taxa (genus level) and three dietary fiber sources using the R package, gplots, (R version 2.15.3). For this analysis, only genera that were present in ≥15% of samples were included. Taxa belonging to Clostridia (Cluster 1, *) and Bifidobacteriales (Cluster 2, °) are marked.

doi:10.1371/journal.pone.0124599.g004
We found that specific sources of dietary fiber were differentially associated with the gut microbiome. Dietary fiber is a heterogeneous and complex mixture of different combinations of monosaccharides [42]. Fiber from fruit and vegetable intake was related to the gut microbiome composition, noted by an increased abundance of the Clostridia class; these findings are supported by two studies of long-term dietary fiber intake [23, 24]. Fiber may be enhancing gram-positive bacteria, including Clostridia [43], by reducing the gut transit time and pH [44, 45]. Clostridia are also an important class of dietary fiber fermenters where the major products are the potentially anti-carcinogenic short chain fatty acids [46, 47]. We recently reported significantly lower abundances of Clostridia in colorectal cancer patients compared to control subjects from this same study population [8]. Our observation here of an association between bean fiber

### Table 4. Odds Ratio$^1$ of having higher relative abundances of taxa by total and specific fiber intake.

|                | Total (95% CI) | p     | q$^2$ | Fruit & Vegetable (95% CI) | p     | q$^2$ | Bean (95% CI) | p     | q$^2$ | Grain (95% CI) | p     | q$^2$ |
|----------------|----------------|-------|-------|-----------------------------|-------|-------|---------------|-------|-------|---------------|-------|-------|
| **Firmicutes** |                |       |       |                             |       |       |               |       |       |               |       |       |
|                | 1.19           | 0.46  | 0.46  | 1.09                        | 0.20  | 0.60  | 1.14          | 0.42  | 0.66  | 0.90          | 0.45  | 0.66 |
|                | (0.75–1.90)    |       |       | (0.96–1.24)                 |       |       | (0.83–1.57)   |       |       | (0.68–1.19)   |       |       |
| **Bacilli**    | 1.01           | 0.87  | 0.87  | 1.00                        | 0.97  | 0.97  | 0.97          | 0.85  | 0.97  | 1.05          | 0.72  | 0.97 |
|                | (0.90–1.10)    |       |       | (0.90–1.13)                 |       |       | (0.70–1.31)   |       |       | (0.80–1.39)   |       |       |
| **Clostridia** | 1.15           | 0.03  | 0.12  | 1.24                        | 0.09  | 0.09  | 1.09          | 0.58  | 0.97  | 1.06          | 0.68  | 0.97 |
|                | (1.01–1.20)    |       |       | (1.05–1.47)                 |       |       | (0.08–1.49)   |       |       | (0.79–1.42)   |       |       |
|                | 1.12           | 0.12  | 0.92  | 1.19                        | 0.07  | 0.60  | 1.12          | 0.89  | 0.95  | 1.03          | 0.86  | 0.95 |
|                | (0.97–1.30)    |       |       | (0.98–1.43)                 |       |       | (0.69–1.82)   |       |       | (0.73–1.45)   |       |       |
| **Blautia**    | 1.04           | 0.49  | 0.92  | 1.02                        | 0.83  | 0.95  | 1.56          | 0.02  | 0.51  | 0.88          | 0.40  | 0.85 |
|                | (0.86–1.04)    |       |       | (0.78–1.02)                 |       |       | (0.98–2.07)   |       |       | (0.59–1.07)   |       |       |
| **Clostridium$^3$** | 0.95   | 0.28  | 0.92  | 0.89                        | 0.09  | 0.64  | 1.43          | 0.06  | 0.60  | 0.80          | 0.13  | 0.74 |
|                | (0.86–1.04)    |       |       | (0.87–1.14)                 |       |       | (1.07–2.27)   |       |       | (0.65–1.19)   |       |       |
| **Coprooccus** | 1.16           | 0.01  | 0.17  | 1.23                        | 0.009 | 0.46  | 1.15          | 0.36  | 0.83  | 1.15          | 0.33  | 0.83 |
|                | (1.03–1.31)    |       |       | (1.05–1.43)                 |       |       | (0.85–1.58)   |       |       | (0.86–1.54)   |       |       |
| **Bacteroidetes** | 0.76   | 0.26  | 0.39  | 0.87                        | 0.06  | 0.27  | 0.90          | 0.53  | 0.66  | 1.08          | 0.59  | 0.66 |
|                | (0.48–1.22)    |       |       | (0.76–1.01)                 |       |       | (0.66–1.23)   |       |       | (0.81–1.43)   |       |       |
| **Bacteroidaceae** | 0.95  | 0.28  | 0.70  | 0.93                        | 0.26  | 0.87  | 0.89          | 0.48  | 0.91  | 1.10          | 0.50  | 0.91 |
|                | (0.86–1.04)    |       |       | (0.82–1.06)                 |       |       | (0.65–1.23)   |       |       | (0.83–1.46)   |       |       |
| **Porphyromonadaceae** | 0.89   | 0.04  | 0.40  | 0.89                        | 0.08  | 0.60  | 0.58          | 0.02  | 0.57  | 0.96          | 0.77  | 0.91 |
|                | (0.80–0.99)    |       |       | (0.78–1.02)                 |       |       | (0.37–0.91)   |       |       | (0.73–1.27)   |       |       |
| **Actinobacteria** | 1.36  | 0.22  | 0.39  | 0.97                        | 0.74  | 0.74  | 2.24          | 0.006 | 0.05  | 1.17          | 0.29  | 0.65 |
|                | (0.83–2.25)    |       |       | (0.86–1.11)                 |       |       | (1.27–3.96)   |       |       | (0.87–1.59)   |       |       |
| **Bifidobacteriales** | 1.01  | 0.82  | 0.84  | 0.97                        | 0.62  | 0.92  | 1.65          | 0.04  | 0.43  | 1.12          | 0.46  | 0.86 |
|                | (0.92–1.12)    |       |       | (0.84–1.11)                 |       |       | (1.02–2.68)   |       |       | (0.83–1.52)   |       |       |
| **Coriobacteriales** | 0.93  | 0.17  | 0.32  | 0.88                        | 0.08  | 0.43  | 0.98          | 0.92  | 0.92  | 1.01          | 0.92  | 0.92 |
|                | (0.83–1.03)    |       |       | (0.76–1.01)                 |       |       | (0.72–1.35)   |       |       | (0.76–1.36)   |       |       |

$^1$All estimated OR’s and 95% CI’s were computed using multivariate logistic regression models where low and high relative abundances were treated as outcome and fiber intake treated as continuous variables of interest. Low and high groups were based on median value. Age, sex, race, and smoking status were controlled for. All analyses were carried out using SAS software (version 9.3).

$^2$False discovery rate adjusted q-value.

$^3$Taxa treated as absent/present because they were <80% present across all 82 samples.

doi:10.1371/journal.pone.0124599.t004
intake and Actinobacteria abundance, particularly Bifidobacteriales, is supported by a short-term randomized trial which showed that fiber found in legumes, including beans [48, 49], increased the number of Bifidobacteria [19]. However, additional randomized, crossover studies have shown that concentrations of Bifidobacteria increase with other types of fiber treatments [18, 20, 21]. While the majority of studies are based on short-term randomized trials of high fiber supplements, our study adds to the growing body of evidence [23, 24] that usual long-term fiber intake influences the gut microbiome.

We also observed sex-specific effects on the relationship between dietary fiber intake and the gut microbiome. While mechanisms are not well understood, dietary fiber has been shown to influence the systemic levels of estrogen [17]. Dietary fiber is considered beneficial for human health [55] and is also an important energy source for gut bacterial fermentation [56]. Dietary fiber may be acting through sex hormone mediated pathways to influence the human gut microbiome.

Several limitations in our study should be considered. The study subjects are not representative of the general population, as they were awaiting elective surgeries for non-oncological and non-gastrointestinal conditions [8]. Measurement error is an inherent limitation in dietary intake assessment and may have attenuated the true relationship. We controlled in the statistical analysis for possible confounding factors, although other unmeasured factors may have influenced the observed relationships. For example, dietary fiber intake may mark for intake of foods that are rich in vitamins, minerals, and phytochemicals, or may be a marker of a healthy lifestyle. Stool samples, as used here, are not entirely representative of whole gut microbial composition [50], but obtaining alternative gut tissue biospecimens in healthy subjects is not feasible. Furthermore, archived stool samples may not be optimal compared to freshly collected samples, although our blinded quality control assay indicated good reliability (intraclass correlation coefficients = 0.84–0.90 for overall gut microbiome composition and 0.43–0.59 for major taxa) [8] and the identified major taxon distributions were within ranges of other published studies [51, 52]. We also cannot exclude the possibility that our findings may be due to chance because of the small sample sizes, particularly in our subset analysis.

This study has several strengths. The assessments on demographic, lifestyle, and dietary factors provide the ability to assess the relationship of these factors with the gut microbiome, while controlling for multiple comparisons. Complementing previous short-term intervention studies of high fiber supplement intake [18–22], this study provides useful information on associations of usual long-term daily fiber intake with differences in the gut microbiome in free-living people. Finally, our analysis of specific sources of fiber helps to better understand the fiber and gut microbiome relationship.

We found that sex, BMI, and source of dietary fiber are associated with differences in the gut microbiota. There is increasing evidence of a relationship between gut microbiota and gastrointestinal diseases [4, 5], including colorectal cancer [6–8], and other diseases, such as diabetes [53, 54]. These personal factors are important in an overlapping spectrum of chronic diseases [55–58] and may partly operate through common microbial pathways influencing hormone profiles [11, 17] and gut transit [44]; our findings may have implications for disease prevention.

**Supporting Information**

S1 Table. Genera and spearman correlation coefficients corresponding to Fig 4.

(DOCX)
Author Contributions
Conceived and designed the experiments: CD RBH JA. Performed the experiments: LY. Analyzed the data: CD JA. Contributed reagents/materials/analysis tools: RS JJG. Wrote the paper: CD RS JJG ZP RBH JA.

References
1. Savage DC. Microbial ecology of the gastrointestinal tract. Annual review of microbiology. 1977; 31:107–33. PMID: 334036
2. Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. Nature. 2011; 474(7351):327–36. doi: 10.1038/nature10213 PMID: 2167749
3. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. Science. 2009; 326(5960):1694–7. doi: 10.1126/science.1177486 PMID: 1998294
4. Salonen A, de Vos WM, Palva A. Gastrointestinal microbiota in irritable bowel syndrome: present state and perspectives. Microbiology. 2010; 156(Pt 11):3205–15. doi: 10.1099/mic.0.043257-0 PMID: 20705664
5. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome biology. 2012; 13(9):R79. doi: 10.1186/gb-2012-13-9-r79 PMID: 23013615
6. Sobhani I, Tap J, Roudot-Thoraval F, Roperch JP, Letulle S, Langella P, et al. Microbial dysbiosis in colorectal cancer (CRC) patients. PloS one. 2011; 6(1):e16393. doi: 10.1371/journal.pone.0016393 PMID: 21297998
7. Kostic AD, Gevers D, Pedamallu CS, Michaud M, Duke F, Earl AM, et al. Genomic analysis identifies association of Fusobacterium with colorectal carcinoma. Genome research. 2012; 22(2):292–8. doi: 10.1101/gr.126573.111 PMID: 22009990
8. Markle JG, Frank DN, Mortin-Toth S, Robertson CE, Feazel LM, Rolle-Kampczyk U, et al. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science. 2013; 339(6123):1084–8. doi: 10.1126/science.1233521 PMID: 232328391
9. Bulun SE, Chen D, Moy I, Brooks DC, Zhao H. Aromatase, breast cancer and obesity: a complex interaction. Trends in endocrinology and metabolism: TEM. 2012; 23(2):83–9. doi: 10.1016/j.tem.2011.10.003 PMID: 22169755
10. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102(31):11070–5. PMID: 16033867
11. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006; 444(7122):1022–3. PMID: 17183309
12. Tuohy KM, Conterno L, Gasperotti M, Viola R. Up-regulating the human intestinal microbiome using whole plant foods, polyphenols, and/or fiber. Journal of agricultural and food chemistry. 2012; 60(36):8776–82. doi: 10.1021/jf3039959 PMID: 22607578
13. Gaskins AJ, Mumford SL, Zhang C, Wactawski-Wende J, Hovey KM, Whitcomb BW, et al. Effect of daily fiber intake on reproductive function: the BioCycle Study. The American journal of clinical nutrition. 2009; 90(4):1061–9. doi: 10.3945/ajcn.2009.27990 PMID: 19692498
14. Gibson GR, Beatty ER, Wang X, Cummings JH. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology. 1995; 108(4):975–82. PMID: 7998613
19. Bouhnik Y, Raskine L, Simonneau G, Vicaud E, Neut C, Flourie B, et al. The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: a double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. The American journal of clinical nutrition. 2004; 80(6):1658–64. PMID: 15585783

20. Whelan K, Judd PA, Preedy VR, Simmering R, Jann A, Taylor MA. Fructooligosaccharides and fiber partially prevent the alterations in fecal microbiota and short-chain fatty acid concentrations caused by standard enteral formula in healthy humans. The Journal of nutrition. 2005; 135(8):1896–902. PMID: 16046714

21. Benus RF, van der Werf TS, Welling GW, Judd PA, Harmsen HJ, et al. Association between Faecalibacterium prausnitzii and dietary fibre in colonic fermentation in healthy human subjects. The British journal of nutrition. 2010; 104(5):693–700. doi: 10.1017/S0007114510001030 PMID: 20346190

22. Hooda S, Boler BM, Brulc JM, Staeger MA, Boileau TW, et al. 454 pyrosequencing reveals a shift in fecal microbiota of healthy adult men consuming polydextrose or soluble corn fiber. The Journal of nutrition. 2012; 142(7):1259–65. doi: 10.3945/jn.112.158766 PMID: 22649263

23. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011; 334(6052):105–8. doi: 10.1126/science.1208344 PMID: 21885731

24. Chen HM, Yu YN, Wang JL, Lin YW, Kong X, Yang CQ, et al. Decreased dietary fiber intake and structural alteration of gut microbiota in patients with advanced colorectal adenoma. The American journal of clinical nutrition. 2013; 97(4):1044–52. doi: 10.3945/ajcn.112.046607 PMID: 23553152

25. Romero-Corral A, Somers VK, Sierra-Johnson J, Thomas RJ, Collazo-Clavell ML, Korinek J, et al. Accuracy of body mass index in diagnosing obesity in the adult general population. Int J Obes (Lond). 2008; 32(6):959–66. doi: 10.1038/jbo.2008.11 PMID: 18283264

26. Schifflman MH, Andrews AW, Van Tassell RL, Smith L, Daniel J, Robinson A, et al. Case-control study of colorectal cancer and fecal mutagenicity. Cancer research. 1989; 49(12):3420–4. PMID: 26655996

27. Schifflman MH, Van Tassell RL, Robinson A, Smith L, Daniel J, Hoover RN, et al. Case-control study of colorectal cancer and fecapentae excretion. Cancer research. 1989; 49(5):1322–6. PMID: 2917361

28. Classification BMI. Global Database on Body Mass Index World Health Organization. 2006.

29. Subar AF, Midthune D, Kulkirmar D, Brown CC, Thompson FE, Kipnis V, et al. Evaluation of alternative approaches to assign nutrient values to food groups in food frequency questionnaires. American journal of epidemiology. 2000; 152(3):279–86. PMID: 10933275

30. Willett W. Nutritional Epidemiology: Oxford University Press; 1998.

31. Nossa CW, Oberdorf WE, Yang L, Aas JA, Paster BJ, Desantis TZ, et al. Design of 16S rRNA gene primers for 454 pyrosequencing of the human foregut microbiome. World journal of gastroenterology: WJG. 2010; 16(33):4135–44. PMID: 20806429

32. Haas BJ, Gevers D, Earl AM, Feldgarden M, Ward DV, Giannoukos G, et al. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genome research. 2011; 21(3):494–504. doi: 10.1101/gr.112730.110 PMID: 21212162

33. Kuczynski J, Stombaugh J, Walters WA, Gonzalez A, Caporaso JG, Knight R. Using QIIME to analyze microbial community diversity in a phylogenetic context. BMC bioinformatics. 2011; 12:296.

34. Lozupone C, Hamady M, Knight R. UniFrac—an online tool for comparing microbial community diversity in a phylogenetic context. BMC bioinformatics. 2006; 7:371. PMID: 16893466

35. Anderson BHMMJ. Fitting multivariate models to community data: a comment on distance based redundancy analysis. Ecology. 2001; 82(1):290–7.

36. Shannon CE. A Mathematical Theory of Communication. At&T Tech J. 1948; 27(4):623–56.

37. Benjamin Y, H. Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. 1995; 57(1):289–300.

38. Mueller S, Saunier K, Hanisch C, Norin E, Alm L, Midveld T, et al. Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study. Applied and environmental microbiology. 2006; 72(2):1027–33. PMID: 16461645

39. Yurkovetskiy L, Burrows M, Khan AA, Graham L, Volchkov P, Becker L, et al. Gender bias in autoimmunity is influenced by microbiota. Immunity. 2013; 39(2):400–12. doi: 10.1016/j.immuni.2013.08.013 PMID: 23973225

40. Lampe JW, Fredstrom SB, Slavin JL, Potter JD. Sex differences in colonic function: a randomised trial. Gut. 1993; 34(4):531–6. PMID: 8387940

41. Degen LP, Phillips SF. Variability of gastrointestinal transit in healthy women and men. Gut. 1996; 39(2):299–305. PMID: 8977347
42. Stipanuk M, Caudill MA. Biochemical, Physiological, and Molecular Aspects of Human Nutrition. 3rd ed: Elsevier Saunders; 2013.

43. Duncan SH, Louis P, Thomson JM, Flint HJ. The role of pH in determining the species composition of the human colonic microbiota. Environmental microbiology. 2009; 11(8):2112–22. doi: 10.1111/j.1462-2920.2009.01931.x PMID: 19397676

44. Probert CS, Emmett PM, Heaton KW. Some determinants of whole-gut transit time - a population-based study. QJM: monthly journal of the Association of Physicians. 1995; 88(5):311–5. PMID: 7796084

45. Lewis SJ, Heaton KW. Increasing butyrate concentration in the distal colon by accelerating intestinal transit. Gut. 1997; 41(2):245–51. PMID: 9301506

46. Louis P, Flint HJ. Diversity, metabolism, and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS microbiology letters. 2009; 294(1):1–8. doi: 10.1111/j.1574-6968.2009.01514.x PMID: 19222573

47. Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost FJ, Brummer RJ. Review article: the role of butyrate on colonic function. Alimentary pharmacology & therapeutics. 2008; 27(2):104–19.

48. Landa-Habana L, Pina-Hernandez A, Agama-Acevedo E, Tovar J, Bello-Perez LA. Effect of cooking procedures and storage on starch bioavailability in common beans (Phaseolus vulgaris L.). Plant foods for human nutrition (Dordrecht, Netherlands). 2004; 59(4):133–6. PMID: 15678742

49. Yadav BS, Sharma A, Yadav RB. Studies on effect of multiple heating/cooling cycles on the resistant starch formation in cereals, legumes and tubers. International journal of food sciences and nutrition. 2009; 60 Suppl 4:258–72. doi: 10.1080/09637480902970975 PMID: 19562607

50. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science. 2005; 308(5728):1635–8. PMID: 15831718

51. Martinez I, Kim J, Duffy PR, Schlegel VL, Walter J. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. PloS one. 2010; 5(11):e15046. doi: 10.1371/journal.pone.0015046 PMID: 21151493

52. Walker AW, Ince J, Duncan SH, Webster LM, Holtrop G, Ze X, et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. The ISME journal. 2011; 5(2):220–30. doi: 10.1038/ismej.2010.118 PMID: 20686513

53. Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PloS one. 2010; 5(2):9985. doi: 10.1371/journal.pone.0009985 PMID: 20140211

54. Nielsen DS, Krych L, Buschard K, Hansen CH, Hansen AK. Beyond genetics. Influence of dietary factors and gut microbiota on type 1 diabetes. FEBS letters. 2014.

55. Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. Frontiers in neuroendocrinology. 2014; 35(3):347–69. doi: 10.1016/j.yfme.2014.04.004 PMID: 24793874

56. Vucenik I, Stains JP. Obesity and cancer risk: evidence, mechanisms, and recommendations. Annals of the New York Academy of Sciences. 2012; 1271:37–43. doi: 10.1111/j.1749-6632.2012.06750.x PMID: 23059062

57. Versini M, Jeandel PY, Rosenthal E, Shoenfeld Y. Obesity in autoimmune diseases: Not a passive bystander. Autoimmunity reviews. 2014.

58. Slavin J. Fiber and prebiotics: mechanisms and health benefits. Nutrients. 2013; 5(4):1417–35. doi: 10.3390/nu5041417 PMID: 23609775