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Increasing Dietary Fiber Intake Is Associated with a Distinct Esophageal Microbiome

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Abstract

Introduction: There is increasing evidence that the microbiome contributes to esophageal disease. Diet, especially fiber and fat intake, is a known potent modifier of the colonic microbiome, but its impact on the esophageal microbiome is not well described. We hypothesized that dietary fiber and fat intake would be associated with a distinct esophageal microbiome.

Methods: We collected esophageal samples from 47 ambulatory patients scheduled to undergo endoscopy who completed a validated food frequency questionnaire quantifying dietary fiber and fat intake. Using 16S high-throughput sequencing, we determined composition of the esophageal microbiome and predicted functional capacity of microbiota based on fiber and fat intake.

Results: Among all samples, the most abundant phyla were Firmicutes (54.0%), Proteobacteria (19.0%), Bacteroidetes (17.0%), Actinobacteria (5.2%), and Fusobacteria (4.3%). Increasing fiber intake was significantly associated with increasing relative abundance of Firmicutes ($p = 0.04$) and decreasing relative abundance of Gram-negative bacteria overall ($p = 0.03$). Low fiber intake was associated with increased relative abundance of several Gram-negative bacteria, including *Prevotella*, *Neisseria*, and *Eikenella*. Several predicted metabolic pathways differed between highest and lowest quartile of fiber intake. Fat intake was associated with altered relative abundance of few taxa, with no alterations at the phylum level and no changes in microbiome functional composition.

Conclusions: Dietary fiber, but not fat, intake was associated with a distinct esophageal microbiome. Diet should be considered an important modifier of the esophageal microbiome in future studies. Studies are also needed to elucidate how the effects of dietary fiber on the esophageal microbiome may contribute to esophageal disease.

Introduction

An increasing body of literature links esophageal diseases to the esophageal microbiome. Eosinophilic esophagitis (EOE), gastroesophageal reflux disease (GERD)

and Barrett's esophagus (BE), esophageal adenocarcinoma (EAC), and esophageal squamous cell carcinoma (ESCC) and its precursor lesions have all been associated with altered composition of the esophageal microbiome^{1–5}. Given the proposed role of the microbiome in esophageal pathology, it is important to characterize the host and environmental factors that may shape esophageal microbiota. One such potential factor is diet.

Diet is a potent modulator of the distal gut microbiome, and plays an important role in disease pathogenesis. In

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animal models of the colonic microbiome, diet—especially fat and fiber intake—causes profound alterations of microbial composition. High-fat diet causes significant disruption of colonic microbiota, including inverted proportions of the two dominant phyla, Bacteroidetes to Firmicutes^{6–8}. Such changes are associated with obesity, increased intestinal energy harvest, and intestinal inflammation^{6,7}. Conversely, dietary fiber contributes to different microbial alterations, and is associated with reduced weight gain and improved glycemic and lipid control^{9–11}. The microbiome associated with a fiber-deprived diet may correlate with reduced intestinal mucosal barrier robustness and increased gut permeability¹².

In humans, increased caloric intake and dietary fiber intake are both associated with altered colonic microbial composition. Increased fiber intake and associated changes to the microbiome are correlated with decreased colorectal cancer risk and increased insulin sensitivity^{13–15}. High fiber consumption may confer a lower risk of *Fusobacterium nucleatum*-positive, but not *F. nucleatum*-negative, colon cancer, suggesting that the effect of diet on cancer risk is mediated in part by microbiome composition¹⁶.

The impact of diet on the esophageal microbiome, on the other hand, has not been extensively studied. Dietary intake is associated with risk or management of a variety of esophageal pathologies, including EOE and the reflux-associated esophageal diseases (GERD, BE, and EAC). The mechanism through which diet produces this impact on esophageal health is unknown, raising the possibility that its effects are mediated by the esophageal microbiome.

In this study, we tested the hypothesis that dietary fiber and fat are associated with alterations to the esophageal microbiome. We performed a cross-sectional study using squamous esophagus brushings from patients undergoing upper endoscopy who completed a food frequency questionnaire to quantify dietary fiber and fat intake. Microbiome composition of esophageal samples was determined using 16S rRNA sequencing. We characterized differences in the esophageal microbiome and in predicted functional capacity based on dietary fiber or fat intake.

Methods

Study population

Patient data and samples used for the current study had been previously collected for a case-control study of the esophageal microbiome in adult patients with and without BE, described elsewhere in detail¹⁷. Briefly, patients ≥ 18 years old who were scheduled to undergo upper endoscopy for clinical indications were included. Demographics and clinical data collected prior to endoscopy included height, weight, waist and hip circumference, smoking history, history of reflux symptoms (using a

modified version of the Mayo Gastro-Esophageal Reflux Questionnaire¹⁸), and medications. Patients were excluded if they had a history of gastric or esophageal cancer, gastric or esophageal surgery, endoscopic therapy for BE or esophageal cancer, immunosuppressed state, or use of antibiotics, steroids, or other immunosuppressants within the previous 3 months. All participants provided written informed consent. This study was approved by the Columbia University Medical Center Institutional Review Board.

Dietary intake assessment

On the day of endoscopy, each patient completed a food frequency questionnaire (FFQ) comprised of 17 questions regarding dietary intake over the preceding 4 weeks. This FFQ was derived from the National Health Interview Survey and validated for assessment of dietary fiber and fat^{19,20}. Results were used to calculate daily fiber intake (grams per day) and fat intake (percentage of calories per day) for each patient. While this FFQ is not specifically validated for fruit and vegetable intake, Pyramid servings per day of fruit/vegetable intake were also calculated²¹.

Sample collection

All subjects were fasting at the time of endoscopy. Saliva and oral swabs were collected prior to endoscopy. During the endoscopy and prior to tissue sampling, the endoscope channel was flushed with 20 mL sterile water. Each microbiome sample was collected by passing a brush 10 times back and forth in four quadrants (Endoscopy Cytology Brush, model G22174; Cook Medical, Bloomington, IN). Two brushings were taken from the following sites: squamous esophagus (3 cm proximal to the squamo-columnar junction), gastric cardia (within 1 cm of the top of the gastric folds), and mid-BE segment in patients with BE. Brush tips were cut using sterile wire cutters and samples were stored in sterile Eppendorf tubes at -80°C . For purposes of this study, only esophageal squamous brushings were analyzed.

Bacterial DNA isolation and microbiome analyses

DNA was extracted from the study samples using the Mo Bio DNeasy PowerSoil kit (Qiagen, Valencia, CA). Bacterial DNA was isolated by PCR targeting the V4 hypervariable region of the 16S rRNA gene using 515F/806R primers, as described²². The V4 region for all samples was sequenced using the Illumina HiSeq 2500 in Rapid Mode with 2×250 bp read length (Illumina, San Diego, CA). The Greengenes reference database was used²³. Taxonomic units were clustered at 97% sequence similarity using USEARCH and taxonomic assignments were made using mothur. A phylogenetic tree of the contigs was generated with FastTree version 2.1.7²⁴, and

diversity indices and unweighted and weighted UniFrac distances were calculated²⁵. Sequence data were uploaded to the NCBI Sequence Read Archive (SRP141237).

For analyses of relative abundance of Gram-negative bacteria, Gram-negative genera and species were identified using a reference list assembled by our group (Supplementary Table 1). The relative abundances of these taxa were summed for each sample.

For analyses of relative abundance of short-chain fatty acid-producing bacteria, relative abundance of genera from Clostridial clusters IV and XIVa were summed for each sample.

Statistical analysis

Continuous variables were summarized using means/medians, and categorical variables were summarized using proportions. Continuous variables were compared using *t* tests or rank-sum tests where appropriate, and categorical variables were compared using Fisher's exact tests. Analyses were performed separately according to fiber or fat intake. Spearman correlation coefficients were calculated to assess for associations between dietary fiber or fat intake and relative abundance of individual phyla. Patients were also grouped into quartiles based on daily fiber or fat intake, with the first quartile (Q1) denoting the lowest daily intake and the fourth quartile (Q4) denoting the highest daily intake. Alpha diversity was assessed by calculating Shannon diversity indices. Average linkage (UPGMA) method was used to cluster patients based on unweighted and weighted UniFrac metrics. Rank-sum tests were used to compare pairwise distances within and between the lowest and highest quartiles of fiber or fat intake. Taxon-level differences between groups were assessed via linear discriminant analysis effect size (LEfSe)^{26,27}. Functional composition of the esophageal microbiome was assessed using predicted metabolic pathways derived by phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) analysis²⁸.

Multivariable linear regression was performed to assess effect of age, sex, smoking, PPI use, aspirin use, BE status, waist:hip ratio, or body-mass index (BMI) on the association between dietary fiber or fat intake and phylum relative abundance. A reduced model was generated by sequentially removing variables with the highest *p*-value that was also > 0.15. As acid suppression was felt to represent a potential effect modifier, a priori secondary analyses were performed stratified by PPI use. Statistical significance was defined as *p* < 0.05. Bonferroni correction was performed in instances of multiple comparisons, with adjusted *p*-values reported. Analyses were performed using Stata 14.1 (StataCorp, College Station, TX) and SPSS Statistics v23.0 (IBM, Armonk, NY).

Results

Clinical data

A total of 47 patients met inclusion criteria, completed the FFQ, and underwent endoscopy with squamous esophagus sample collection. Baseline characteristics of the study population are shown in Table 1. No subjects had visible esophagitis on endoscopy. Median dietary fat intake was 33.6% daily calories from fat (IQR 32.4–35.6), with mean 30.9% daily calories from fat in the lowest quartile of fat intake (Q1) and mean 37.7% daily calories from fat in the highest quartile of fat intake (Q4). Median dietary fiber intake was 16.0 g/day of fiber (IQR 13.1–18.4), with mean 11.2 g/day in the lowest quartile of fiber intake (Q1) and mean 24.3 daily calories from fat in the highest quartile of fiber intake (Q4). Within individuals, there was no significant correlation between dietary intakes of fat and fiber. There was a strong positive correlation between daily servings of fruit/vegetable intake and dietary fiber intake ($\rho = 0.76$, $p < 0.0001$).

Squamous esophageal microbiome

The most abundant bacterial phyla among all samples were Firmicutes (mean 54.0%, SD 21.1%), Proteobacteria (mean 19.0%, SD 15.5%), Bacteroidetes (mean 17.0%, SD 12.1%), Actinobacteria (mean 5.2%, SD 5.9%), and Fusobacteria (mean 4.3%, SD 4.7%). All other phyla had relative abundance <1%. At the genus level, *Streptococcus* was

Table 1 Characteristics of study population (n = 47 patients)

Age (years), median (IQR)	65.0 (54.0–71.5)
Male sex, no. (%)	37 (78.7)
Body mass index, median (IQR) ^a	28.4 (25.7–32.5)
Waist:hip ratio, median (IQR)	0.96 (0.94–1.00)
Ever smoker, no. (%) ^b	27 (57.4)
PPI use, no. (%)	37 (78.7)
Daily aspirin use, no. (%) ^b	14 (29.8)
History of reflux symptoms, no. (%) ^c	39 (83.0)
Indication for endoscopy, no. (%)	
GERD	5 (10.6)
Barrett's esophagus	31 (66.0)
Other	11 (23.4)
Dietary intake, median (IQR)	
Grams per day of fiber	16.0 (13.1–18.4)
Percent daily calories from fat	33.6% (32.4–35.6%)

IQR interquartile range, PPI proton pump inhibitor, GERD gastroesophageal reflux disease

^aData missing for three patients

^bData missing for one patient

^cPer symptom questionnaire

most abundant, with a mean relative abundance of 37.9% (SD 21.5%). Gram-negative bacteria comprised a mean of 49.0% (SD 22.6%) of bacteria in the esophagus.

There was a non-significant inverse association between fiber intake and alpha diversity by Shannon index ($\rho = -0.26$, $p = 0.07$), and no association between fat intake and alpha diversity ($\rho = -0.15$, $p = 0.32$). When stratified by PPI use, the trend toward inverse correlation between fiber intake and alpha diversity neared significance among PPI users ($\rho = -0.34$, adjusted $p = 0.08$).

Beta diversity analyses were performed comparing the lowest (Q1) and highest (Q4) quartiles of dietary fat or fiber intake. In the unweighted analysis of dietary fiber, there were significantly lower UniFrac distances within Q1 than within Q4 (adjusted $p = 0.005$) or than within Q1 versus Q4 (adjusted $p = 0.03$; Supplementary Figure 1). There were no significant differences in the weighted analysis, or in comparisons of lowest and highest dietary fat quartiles.

Dietary fat intake and the esophageal microbiome

There were no significant correlations between fat intake and relative abundance of any phyla (Supplementary Figure 2A–E) or relative abundance of Gram-negative bacteria overall. When stratified by PPI use, there remained no association between fat intake and relative abundance of any phyla. To identify differentially abundant taxa, comparisons were made of lowest (Q1) versus highest (Q4) quartile of fat intake. There were few differences in relative abundance of specific taxa in the two groups. Patients in Q1 had increased relative abundance of *Leptotrichia* and of unclassified genera within families Streptococcaceae and Neisseriaceae (Supplementary Figure 3).

Dietary fiber intake and the esophageal microbiome

Shifts in mean relative abundance of major phyla were noted in response to dietary fiber intake (Fig. 1a). Increasing fiber intake was significantly correlated with increasing relative abundance of Firmicutes ($\rho = 0.39$, adjusted $p = 0.04$), and trended toward an inverse correlation with relative abundance of Proteobacteria ($\rho = -0.33$, adjusted $p = 0.10$) (Fig. 1b, c). Fiber intake was not associated with relative abundance of other phyla (Supplementary Figure 2F–H). In multivariate analyses, the associations between fiber intake and relative abundance of Firmicutes and Proteobacteria were not altered by adjustment for patient characteristics, including BMI or history of GERD symptoms, or medication use. Increasing BMI was independently associated with decreasing relative abundance of Firmicutes ($p = 0.03$) and increasing relative abundance of Proteobacteria ($p = 0.05$), the opposite pattern as observed for dietary fiber. Other

patient characteristics, including age, sex, smoking status, history of BE, and aspirin use, were not associated with relative abundance of Firmicutes or Proteobacteria. Similar to fiber, dietary fruit/vegetable intake was positively correlated with relative abundance of Firmicutes ($\rho = 0.48$, adjusted $p = 0.004$) and inversely correlated with relative abundance of Proteobacteria ($\rho = -0.35$, adjusted $p = 0.09$) and Gram-negative bacteria ($\rho = -0.51$, adjusted $p = 0.002$).

Among PPI users, there was a trend toward a positive correlation between fiber intake and Firmicutes ($\rho = 0.35$, adjusted $p = 0.15$), and there was a significant inverse correlation between fiber intake and Proteobacteria ($\rho = -0.40$, adjusted $p = 0.05$). In PPI non-users, the correlations were attenuated and not significant (Firmicutes: $\rho = 0.22$, adjusted $p = 1.00$; Proteobacteria: $\rho = -0.13$, adjusted $p = 1.00$).

There was a significant inverse correlation between fiber intake and relative abundance of Gram-negative bacteria overall ($\rho = -0.39$, adjusted $p = 0.03$). This association was not altered by adjustment for patient characteristics, including BMI or history of GERD symptoms, or medication use. Increased BMI was independently associated with increased relative abundance of Gram-negative bacteria ($p = 0.007$), again with an association opposite that of fiber. When stratified by PPI use, the inverse association between fiber intake and relative abundance of Gram-negative bacteria trended towards significance among PPI users ($\rho = -0.35$, adjusted $p = 0.08$).

When the lowest quartile of fiber intake (Q1) was compared to the highest quartile of fiber intake (Q4), subjects in Q1 had significantly increased relative abundance of numerous taxa (Fig. 2). These increases occurred predominately within class Beta-proteobacteria, including genera *Neisseria*, *Lautropia*, and *Eikenella*. Subjects in Q1 also had increased relative abundance of the genus *Prevotella*, among other taxa. Subjects in Q4 had increased relative abundance of an unspecific genus in family Pasteurellaceae.

Short-chain fatty acid-producing bacteria

We explored the possibility that diet may be associated with relative abundance of short-chain fatty acid (SCFA)-producing bacteria, as has been observed in the colon. SCFA producers were very rare in the esophagus; of the 47 subjects included in the study, only 10 had a specimen containing any SCFA-producing bacteria. The highest combined relative abundance in a single sample was 0.9%. Comparing subjects with and without any SCFA-producing bacteria, there was no difference in intake of fiber ($p = 0.18$) or fat ($p = 0.96$). There was also no association with PPI use ($p = 0.64$).

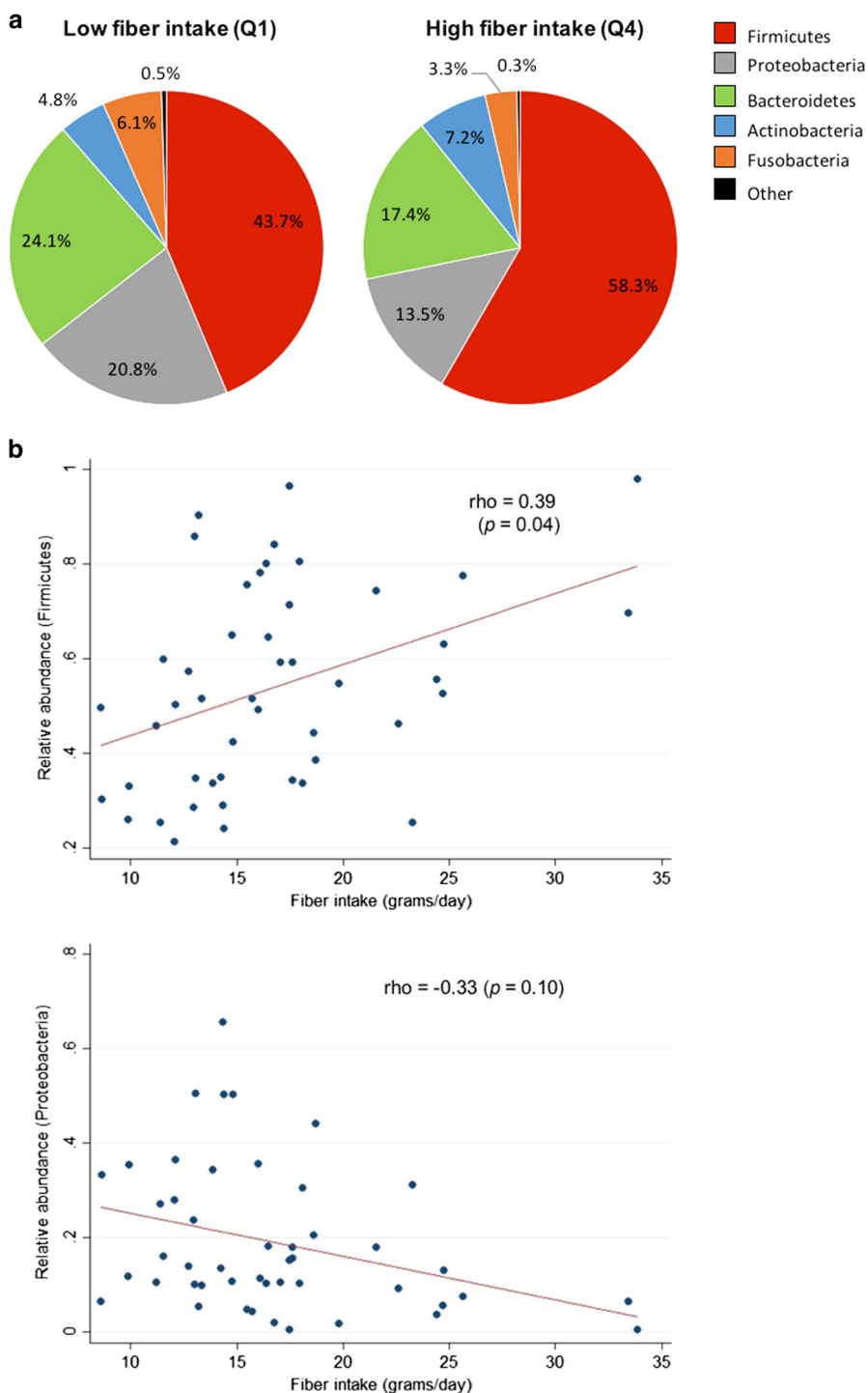


Fig. 1 Relative abundances of esophageal Firmicutes and Proteobacteria were associated with dietary fiber intake. **a** Mean relative abundances of all phyla in samples from patients in the lowest (Q1) and highest (Q4) quartiles of dietary fiber intake. **b** Increasing dietary fiber intake was associated with increasing relative abundance of Firmicutes and decreasing relative abundance of Proteobacteria (all subjects). *p*-values are adjusted for multiple comparisons

Functional composition of the esophageal microbiome

Relative abundances of predicted metabolic pathways were compared between patients in the lowest (Q1)

versus highest (Q4) quartiles of fat or fiber intake. Fat intake was not associated with differential relative abundance of any metabolic pathways. Fiber intake was

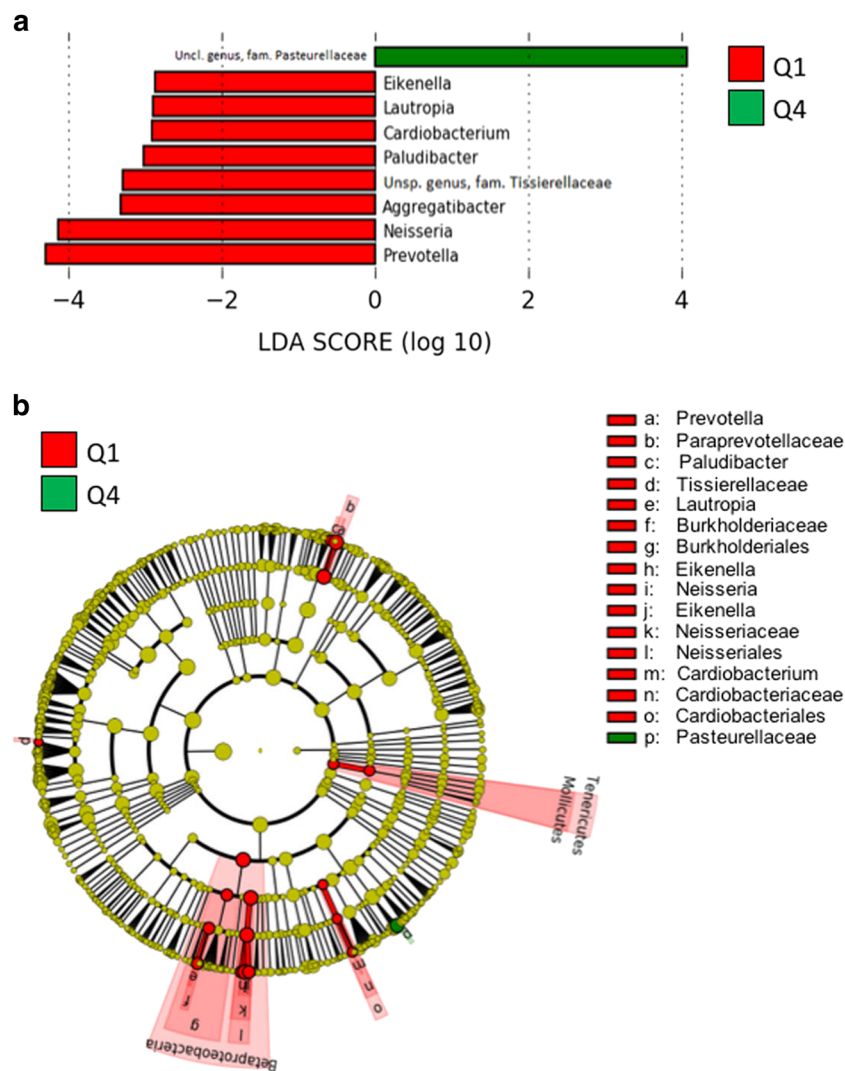


Fig. 2 There were several differentially abundant taxa comparing subjects in the lowest and highest quartiles of fiber intake. **a** Linear discriminant analysis effect size (LEfSe) representation of differentially abundant genera, and **b** cladogram representation of all differentially abundant taxa in esophageal samples between subjects in the lowest quartile (Q1) compared to the highest quartile (Q4) of fiber intake

significantly associated with altered relative abundance of a number of predicted metabolic pathways (Fig. 3a). The majority of pathways affected were involved in cell metabolism, with more of these pathways increased in Q4 than in Q1 (Fig. 3b).

Discussion

In this prospective study of the association between dietary fat and fiber intake and esophageal microbial composition, fiber intake was associated with a distinct microbiome. Increased fiber intake was associated with increased relative abundance of Firmicutes and decreased relative abundance of Gram-negative bacteria overall. Many taxonomic alterations among patients with the lowest quartile of fiber intake were observed within class

Betaproteobacteria. Relative abundance of numerous predicted metabolic pathways varied based on fiber intake. Fat intake was associated with only minor alterations to the esophageal microbiome and no predicted functional changes.

When patients were stratified according to PPI use, the significant associations between fiber intake and microbiome composition were restricted to patients taking PPIs. PPIs are known to impact the upper gastrointestinal microbiome²⁹, so it is possible that this exposure either acts synergistically with dietary fiber to magnify the impact on the microbiome or makes the microbiome more susceptible to effects of fiber. Ultimately, the differential observations between PPI users and non-users must be interpreted with caution due to small sample size.

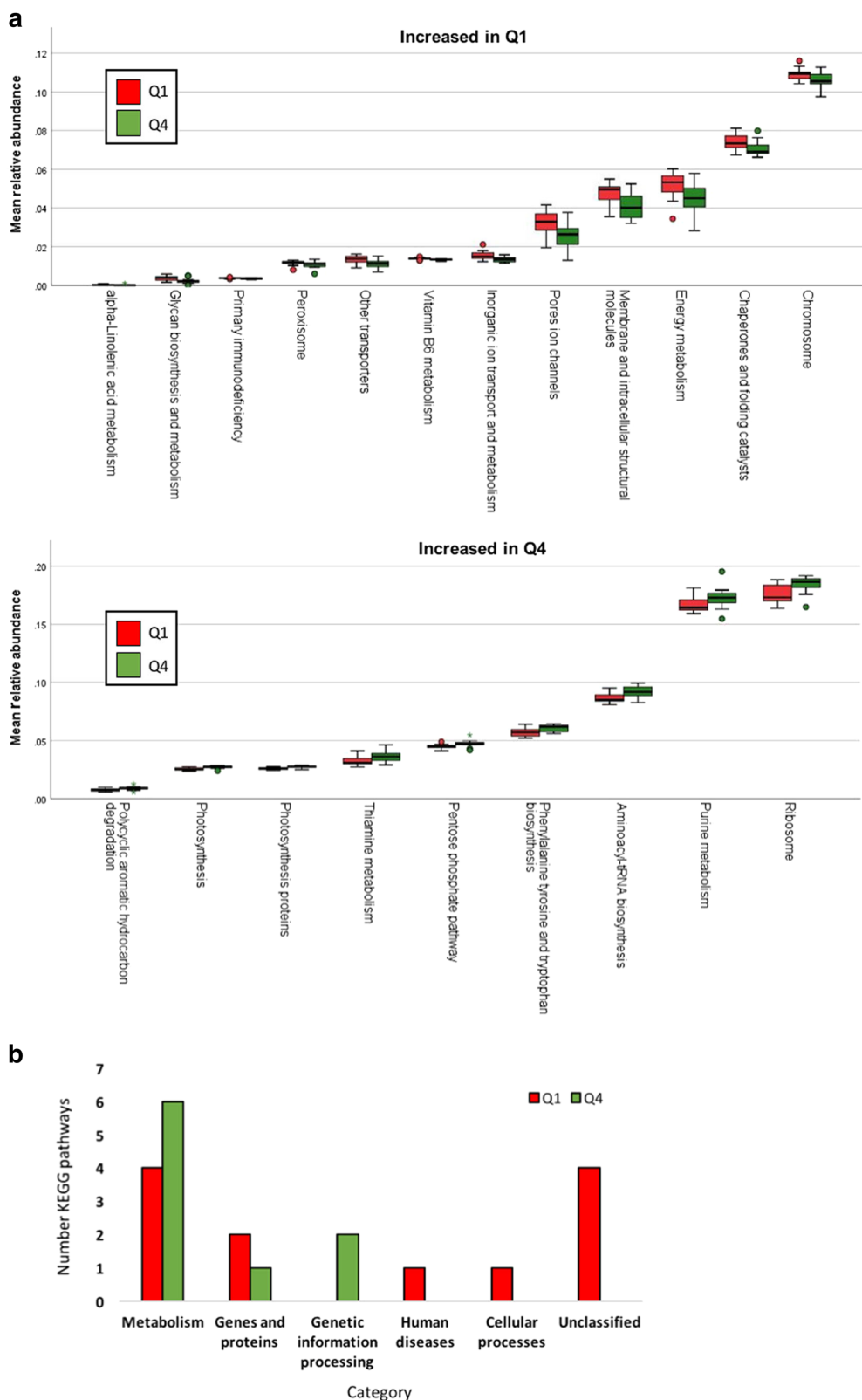


Fig. 3 Functional changes to the esophageal microbiome were associated with fiber intake. a Predicted metabolic pathways increased in lowest quartile (Q1) or highest quartile (Q4) of fiber intake. **b** Number of pathways increased in a given KEGG category in Q1 compared to Q4

Many of the trends observed among PPI non-users were similar to those of PPI users, and the former group may have been underpowered to detect significant alterations.

The mechanisms for the association between dietary intake and esophageal microbiome composition may be multifactorial. Given that food passes only very transiently through the esophagus, it is possible that dietary intake impacts the esophageal microbiome indirectly. One plausible mechanism is through effects on the microbiome of adjacent areas of the gastrointestinal tract. Diet—such as high sugar intake or intake of certain vitamins—is known to affect composition of the oral microbiome^{30,31}. Oral and esophageal microbial compositions are closely related, possibly due to movement of oral microbes to more distal gastrointestinal sites;³² as such, it is possible that the associations between dietary fiber and esophageal microbiota may be mediated by effects of fiber or other related factors on the oral microbiome. Similarly, perhaps through association with obesity, fiber intake could be associated with frequency or severity of acid reflux and reflux-associated disease, another factor with known associations with altered upper gastrointestinal microbiome composition^{3,17,33}. On the other hand, dietary fiber could impact the esophageal microbiome via its effects on host metabolism. In our study, increased BMI had significant independent associations with esophageal microbiota. The phyla affected by increased BMI were the same as those associated with fiber intake, but in the opposite direction; for example, increased relative abundance of Firmicutes was associated with increasing fiber intake but decreasing BMI. It is possible that the impact of fiber on the esophageal microbiome is mediated in part by its impact on the distal GI tract or on systemic metabolism, which in turn influences BMI. Low fiber intake can lead to weight gain and obesity³⁴, and high fiber intake may contribute to increased SCFA production in the colon and improved systemic insulin sensitivity;³⁵ these changes might in turn have effects on the esophageal microbiome.

Changes to the esophageal microbiome associated with dietary fiber may have important implications for esophageal disease. In this study, fiber intake was positively associated with relative abundance of Firmicutes and negatively associated with relative abundance of Gram-negative bacteria, most notably Betaproteobacteria. Some studies of the distal esophagus microbiome have posited the presence of two predominant “types”: one dominated by *Streptococcus*, a species within phylum Firmicutes, and associated with a healthy, phenotypically normal esophagus, and one with high abundance of Gram-negative anaerobes and microaerophiles and associated with abnormal esophagus, including reflux esophagitis and BE^{2,3}. The inverse association between dietary fiber and prevalence of Gram-negative bacteria in this study

provides one mechanism through which increased fiber consumption might protect against inflammation-mediated esophageal disease.

In studies of the distal GI microbiome, the beneficial effects of fiber to colonic health are driven in part by promotion of SCFA production by Clostridial clusters IV and XIVa³⁶. These microbes are thought to contribute to colonic health via SCFA—specifically butyrate—production, which provides energy to colonocytes and contributes to mucosal barrier integrity, thus increasing resistance to opportunistic colonic infections^{37,38}. We explored whether diet may impact esophageal health via effects on SCFA-producing bacteria. In our study, however, SCFA-producing bacteria were only detected in 10 of 47 patients, and the sum relative abundance of SCFA-producing bacteria for all individuals was very low. A significant impact of dietary fiber on esophageal SCFA production is therefore unlikely, or at least difficult to detect due to low abundance.

This study had several strengths. This is one of the first studies to investigate the impact of diet on the upper GI microbiome. Dietary intake in our study population closely mirrored that of US adults, among whom mean dietary fat intake is 33.6% daily calories from fat³⁹ and mean dietary fiber intake is 17.3 g/day⁴⁰. There was a broad spread of fiber intake in the study population. Data about patient medication use, smoking history, and anthropometrics were collected, which allowed for assessment of how these factors affected the association between fat or fiber intake and microbiome composition. Several significant associations between fiber intake and esophageal microbiota were identified.

There were certain limitations. Some analyses, such as stratification by PPI use, were limited by sample size. There was a relatively modest difference in daily dietary fat intake between the highest and lowest quartiles; the interquartile range of median daily percent calories from fat was only 3.1%. As such, it is possible that the effect of dietary fat on the esophageal microbiome was underestimated. While the median 16.0 g/day of dietary fiber intake in our study was representative of average intake in the United States, this amount is substantially lower than the FDA-recommended 25 g/day of fiber. Future studies of populations with more varied dietary intake may reveal a stronger association between fat and fiber and esophageal microbiome composition. As PPIs substantially impact the esophageal microbiome and most patients in our study with a history of reflux symptoms were taking PPIs, meaningful analyses assessing the impact of reflux symptoms in the absence of PPIs could not be performed. Finally, while the FFQ used for this study was validated for fat and fiber intake, a more extensive questionnaire in future studies may provide more granular detail with regard to the impact of specific nutrients or food products

on the esophageal microbiome. The FFQ did allow for calculation of fruit and vegetable intake, which correlated closely with dietary fiber; it is possible that the associations between dietary fiber and esophageal microbiome composition in this study are attributable in part to other aspects of fruit and vegetable consumption.

Dietary fiber intake was associated with distinct esophageal microbiome composition, while fat intake appeared to have a much lesser impact. In the current study, increased fiber intake was associated with increased relative abundance of Firmicutes and decreased relative abundance of Gram-negative bacteria, as well as altered abundance of several specific taxa. These findings may indicate an association between low fiber intake and an esophageal microbiome that promotes chronic inflammation. Dietary fiber may play a powerful role in shaping the esophageal microbiome, and should be considered an important modifier in studies of the esophageal microbiome. Future studies are needed to elucidate how the effects of dietary fiber on the esophageal microbiome contribute to esophageal health and disease.

Study Highlights

What is current knowledge

- Microbiome composition is associated with a variety of esophageal diseases.
- Diet profoundly impacts the colonic microbiome, but its effect on the esophageal microbiome is unknown.

What is new here

- Dietary fiber intake was associated with a distinct human esophageal microbiome.
- Fiber intake was associated with increased *Firmicutes* relative abundance and decreased Gram-negative bacteria relative abundance.
- Dietary fat intake was associated with only minimal changes to the esophageal microbiome.

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Conflict of interest

Guarantor of the article: Julian A. Abrams, MD, MS.

Specific author contributions: Y.R.N.—study concept and design, interpretation of data, manuscript preparation, critical revision of manuscript, approval of final draft submitted. E.J.S.—study conduct, interpretation of data, critical revision of manuscript, approval of final draft submitted. G.C.—study conduct, critical revision of manuscript, approval of final draft submitted. D.E.F.—analysis

and interpretation of data, critical revision of manuscript, approval of final draft submitted. H.K.—analysis and interpretation of data, critical revision of manuscript, approval of final draft submitted. C.J.L.—study conduct, interpretation of data, critical revision of manuscript, approval of final draft submitted. N.C.T.—analysis and interpretation of data, critical revision of manuscript, approval of final draft submitted. J.A.A.—study concept and design, study conduct, analysis and interpretation of data, manuscript preparation, critical revision of manuscript, approval of final draft submitted.

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