Dietary Quality and Microbiome Profiles among Rectal Cancer Patients: A Cross-Sectional Pilot Study

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Objective: Examining whether gut microbial taxa abundances and predicted functional pathways correlate with dietary quality scores at the end of neoadjuvant chemoradiotherapy (nCRT) for rectal cancer (RC); identifying differentially abundant bacterial species from the pantothenate and acetyl-coenzyme A biosynthesis pathways that differ among dietary quality groups in a subset of participants.

Methods: RC patients (n = 30) provided stool samples for 16S rRNA gene sequencing. To validate pathway predictions from the 16S rRNA gene data, stool samples from a subset of 17 participants underwent shallow shotgun metagenomics sequencing (SMS). Dietary quality was calculated using the Prime Diet Quality Score (PDQS; 24-hour recall). 16S rRNA gene data were analyzed using QIIME2, and SMS data were analyzed using HUMAnN2.

Results: At the genus level, *Parvimonas*, *Caproiciproducens*, and uncultured Eggerthellaceae abundances positively correlated (Spearman's rho = 0.36 to 0.50) with PDQS scores, whereas abundances of *Prevotella*, *Rothia*, *Peptostreptococcus*, *Paeniclostridium*, *Enterococcus*, and *Howardella* correlated negatively (Spearman's rho = –0.43 to 0.36). Predicted pathways, including those related to B-vitamin biosynthesis and enzyme cofactor biosynthesis (e.g., B5/pantothenate [phosphopantothenate biosynthesis I]), were correlated with higher PDQS scores. Mean abundances of species predicted to encode the vitamin B5–CoA pathway were greater in the high- diet-quality group.

Conclusion: Findings suggest important associations between the taxa abundances of gut bacteria and the abundances of predicted B-vitamin biosynthesis pathways and dietary quality at the end of nCRT. Three bacterial species encoding vitamin B5–CoA biosynthesis pathways were prominent in high-dietary-quality participants.

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Locally advanced rectal cancer (RC) is one of the most frequently diagnosed cancers and a leading cause of death in the United States (1). Rectal cancer treatment may include bi-modal therapy consisting of neoadjuvant chemoradiotherapy (nCRT) followed by additional chemotherapy, which together may allow some patients to avoid surgery (2). However, patients may experience diet-related side effects from cancer treatment (e.g., changes in taste and smell, dry mouth, nausea, diarrhea, or constipation) (3) that may lead to patterns of reduced dietary quality. Reduced dietary quality in cancer patients undergoing active treatment is associated with weight loss, lower energy intake, cancer-related cognitive decline, and lower quality of life (QOL) (4).

Pre-defined diet quality indexes, derived from scoring systems based on national dietary guidelines, guidelines for cancer patients, or both, provide effective measures for evaluating dietary patterns, which reflect the complexity of overall dietary intake (5). For example, a higher Prime Diet Quality Score (PDQS) indicates better diet quality and/or healthier eating patterns (6-7). Among breast cancer survivors, lower Healthy Eating Index (HEI)-2015 (which measures concordance with healthy patterns relative to the 2015-2020 Dietary Guidelines for Americans) (7) scores has been observed. Importantly, high diet quality (associated with diets that are more anti-inflammatory) may play a role in promoting a favorable metabolic and anti-inflammatory environment, which may lower the risk of tumor progression (8).

The effect of cancer treatment for RC on nutrition and nutritionally related physiological systems is incompletely understood but is considered multifactorial, with the gut

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microbiome thought to play a role. Evidence suggests that alterations in diet (exposure) as a result of undergoing cancer treatment can influence the composition and function of the gut microbiota (9). For example, among Asian breast cancer patients, higher diet-quality scores (measured by the Chinese version of the HEI) were significantly correlated with higher alpha diversity (i.e., Shannon index) and inversely associated with depression scores (10). Further research suggests that targeted long-term dietary patterns or changes before or during cancer treatments are critical factors that may positively influence the gut microbiome (9), which in turn may aid in modulating cancer progression and/or improving response to cancer therapy (9).

There is a need to better understand the relationship between overall dietary quality scores and stool gut microbial abundance in RC patients undergoing nCRT. In this study, we refer to the vitamin B group generally as "VB" (e.g., VB1 = vitamin B1, VB2 = vitamin B2, VB5 = vitamin B5, VB6 = vitamin B6, VB9 = vitamin B9, VB12 = vitamin B12). This pilot study aimed to explore whether gut microbial taxa abundances and functional pathways (predicted from 16S rRNA sequencing) correlate with dietary quality scores at the end of nCRT for RC. It also aimed to identify differentially abundant bacterial species from the pantothenate (VB5) and acetyl-coenzyme A (VB5–CoA) biosynthesis pathways that differ by dietary quality groups in a subset of participants (using shallow shotgun metagenomics sequencing [SMS]). We anticipate that our findings will inform future RC studies and enable them to determine the longitudinal impact of cancer treatment on dietary patterns, stool VB5–CoA–synthesizing bacteria, and factors affecting microbiome temporal stability and their relationship with treatment outcomes.

Methods

The analyses presented herein are part of a longitudinal parent study that examined gut microbiome and metabolomic correlates of cancer-related symptoms during nCRT for RC. Data were used from 30 participants at the end of nCRT (the time point at which our data suggest that suboptimal dietary quality and the greatest differences in bacterial abundances may occur) who provided a 1-day dietary record and stool samples. Male and female patients (aged ≥ 18 years) from the parent study were included if they had a diagnosis of RC, were scheduled to receive nCRT, American Joint Committee on Cancer Stage II or III, and were able to provide written informed consent. Subjects were excluded if they had a history of alcohol or drug abuse within the past 5 years; an inflammatory or infectious condition (e.g., active COVID-19); a major psychiatric disorder; or exposure to calcium pantothenate, antibiotics, prebiotics, probiotics, steroids, or an extreme diet within 1 month before stool-sample collection. Data collection was conducted from July 2017 through April 2019. The study protocol was approved by the institutional review board of the Southeastern Academic Medical Center prior to data collection.

In tandem with stool-sample collection, each patient completed a 24-hour dietary recall (interview assisted), a demographic questionnaire, and a clinical form. The participants were provided with visual aids on serving and portion sizes to assist with the 24-hour dietary recalls. We avoided collecting data on fasting days or days when the diet was not typical. Diet quality was calculated using the PDQS, 24-hour recall version (6,11). We employed a dichotomous scoring approach in which healthy components were coded as 0 (did not eat) or 1 (ate 1 or more times), with reverse coding for the unhealthy food groups and 1 neutral component (eggs, not coded). Raw scores range from 0 to 21; high scores correspond to high diet quality (11). We grouped individual food items based on the PDQS, which lists 14 healthy foods (e.g., dark green vegetables) and 7 unhealthy foods (e.g., processed meat). The PDQS, 24-hour recall version, has been widely applied as a low-burden, short-form diet-quality screener implemented for global application and shows acceptable correlations with the composite HEI-2015 score (6,11).

16S rRNA Gene Sequencing

At the end of nCRT, total DNA was extracted from 250 mg aliquots of stool from 30 participants using MO BIO's PowerSoil DNA Isolation Kit, per the manufacturer's protocol. The V3–V4 regions of the 16S rRNA gene were amplified using Illumina's 16S rRNA gene library preparation protocol and sequenced on the MiSeq 2×300 bp sequencing platform. Amplicon sequence data were analyzed using the QIIME2 v2020.6 pipeline described by Gonzalez-Mercado et al. (12). Briefly, raw reads were denoised into amplicon sequence variants (ASVs) using the DADA2 plugin. The ASVs were taxonomically classified using the SILVA v138 reference database. The ASV count table was rarefied to 4,594 sequences per sample and used for alpha diversity and beta diversity calculations. The unrarefied ASV count table was used to predict MetaCyc pathway abundances and collapsed at the genus level to calculate genus abundances (13).

SMS and Bioinformatics Analyses

DNA from the stool samples of 17 selected participants was isolated and subsequently sequenced at the NYU Langone Genome Technology Center. The metagenomic libraries were constructed utilizing the Nextera DNA Flex Library Preparation Kit (Illumina, San Diego, CA), and their concentrations were determined using fluorometric methods and a microfluidic chip. The sequencing process was carried out on an Illumina NovaSeq platform and generated between 80 and 110 million reads per sample, with a PhiX spike-in included as a control. To reduce variance from batch processing, library samples were combined across different participant groups for each sequencing run.

For each sample, forward and reverse paired-end reads were merged for SMS data analysis. Initial quality control was performed using fastp to identify and trim adapters and low-quality sequences. Following this, KneadData v0.7.4 was employed for additional quality control and to remove any human-related sequences. The sequences were then processed using Trimmomatic v0.39 to remove adapters and low-quality regions, followed by alignment with Bowtie2 v2.3.5.1 against the human genome to filter out any remaining human DNA.

After the cleaning steps, quality was assessed with FastQC, and the non-human reads were analyzed for taxonomic content using MetaPhlAn2. The functional profiling of the microbial

communities was performed with HUMAnN2 v2.8.1, which utilized the Uniref90 reference database. Finally, the resulting UniProt annotations were mapped onto KEGG pathways using the utility mapping conversion tool available in HUMAnN2, providing a comprehensive view of the metabolic pathways present in the microbial communities.

Statistical Analysis

Descriptive statistics, including percentages, mean values, and standard deviations, were calculated for demographic and clinical features, as well as for the PDQS scores. For the 16S rRNA sequencing data, associations between PDQS scores and the abundances of both bacterial genera and pathways were analyzed using Spearman's correlation test. In a separate analysis using SMS data from a subset of 17 participants categorized by dietary quality (high PDQS scores [>6; n = 9] versus low PDQS scores [≤6; n = 8]), we examined associations between end-of-treatment PDQS scores and differentially abundant bacterial species from the VB5–CoA biosynthesis pathways using the t test. For the SMS analysis, P values were corrected for multiple testing using the Benjamini–Hochberg approach. A P value cutoff of .05 was used for all statistical tests.

Results

Stool samples were collected from 30 individuals with RC (50% of whom were males) who received the same nCRT regimen of standard radiotherapy, delivered at doses of 1.8 Gy 5 days a week, to a total of 50.4 Gy, with concurrent fluorouracil (5-FU) infusion or oral capecitabine for 5 weeks, and completed the study. Overall, the study participants (n = 30) had a mean age of 58.2 years ± 10.6 and had a mean PDQS score of 6.5 ±2.5 at the end of nCRT. At the end of nCRT and in a subset of 17 participants, 8 participants (47%) reported low PDQS scores. Participants who reported lower PDQS scores did not significantly differ by age, number of radiotherapy days, or body mass index from participants with high PDQS scores ($P > .05$). The demographic data and clinical characteristics for the overall group of participants are presented in Table 1.

Bacterial Genera Are Associated With Dietary Quality Scores

In this pilot study, we observed no significant associations between alpha diversity (which reflects the within-sample taxonomic diversity) and PDQS scores at the end of nCRT (data not shown). The relative abundances of stool bacteria at the genus level and their correlations with PDQS scores are shown in Figure 1. At the genus level, we found that the relative abundances of Bacillota (syn. Firmicutes) genera, including *Caproiciproducens*, *Parvimonas*, as well as Eggerthellaceae uncultured (phylum: *Actinomycetota*), were positively correlated with PDQS scores (Spearman's rho = 0.36 to 0.50, P < .05). Conversely, negative correlations between PDQS scores and the abundances of genera *Peptostreptococcus*, *Paenniclostridium*, *Howardella*, *Enterococcus*, and *Rothia* (phylum: Actinomycetota) were observed (Spearman's rho = –0.36 to –0.49, $P < .05$) (Figure 1).

Table 1. Demographic and Clinical characteristics of Study participants (n = 30)

Characteristic	Participants (n = 30)
Sex	
Male	15 (50%)
Cancer Stage	
II	6 (20%)
III	21 (70%)
IV	3 (10%)
Chemotherapy	
5-FU 225 mg/m^2 over 24 hours	10 (33%)
Oral capecitabine, 825 mg/m^2 2x/day, 5 days/week	20 (67%)
Race	
African American	2 (07%)
Hispanic White	6 (20%)
Non-Hispanic White	22 (73%)
	Mean (SD)
Age, years	58.2 (10.6) years
Number of radiotherapy days	28.6 (3.4)
BMI, kg/m^2	28.2 (7.2) kg/m^2
PDQS-Total Score	6.5 (2.5)

Abbreviations: BMI, body mass index; PDQS, Prime Diet Quality Score

Vitamin B's Pathways Positively Correlate With Dietary Quality Scores

From the ASV abundance data, 13 predicted MetaCyc pathways, including 3 pathways related to VB biosynthesis (i.e., thiazole component of thiamine diphosphate, flavin, and phosphopantothenate), were significantly correlated with PDQS scores at the end of nCRT (Figure 1).

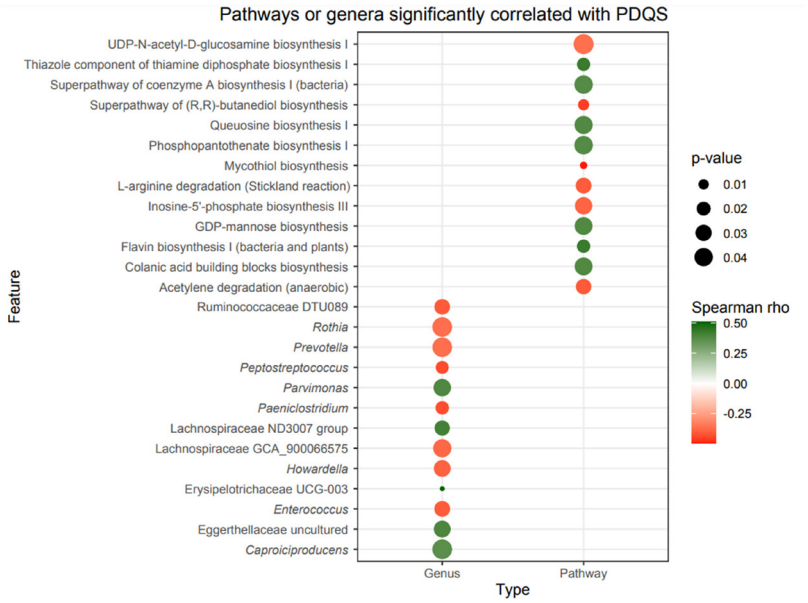
Differentially Abundant Bacterial Species From the VB5–CoA Biosynthesis Pathways Differ by Dietary Quality Group

To validate MetaCyc pathway predictions, we analyzed the SMS data for taxa associated with VB5 synthesis. Bacterial abundances of taxa potentially associated with VB5 biosynthesis or possessing the B5 pathway (e.g., *Alistipes putredinis* and *Odoribacter splanchnicus* from phylum Bacteroidetes, and *Lachnospiraceae bacterium* 5_1 from phylum Bacillota) were significantly more abundant in the high-dietary-quality group (adjusted $P < .05$).

Discussion

With respect to dietary quality, at the end of nCRT, the participants had a mean PDQS score (6.5 ± 2.5) that was lower than the midpoint of the scale (< 10.5), suggesting that their diet quality needed improvement. Although few studies have evaluated diet quality at the end of cancer treatment, 1 study reported low diet quality (HEI-2015 total score of 55.0 of a possible 100

Figure 1. Pathways or Genera significantly correlated with Prime Diet Quality Score (PDQS)



Abbreviation: PDQS, Prime Diet Quality Score.

points) among women with mixed solid cancer diagnoses (mean time since completion of treatment, 4.25 years) compared to the United States HEI-2015 national average of 59 points (14). Nonetheless, the harmful impact of lower diet quality during cancer treatment might contribute to an increased risk of adverse clinical outcomes (e.g., infection, malnutrition, and overall or cancer-specific mortality) and patient-reported aspects of health and functioning (e.g., reduced physical functioning, lower self-rated health, lower QOL, depression, cognitive impairment) (4,8,10,16). These findings highlight the imperative need for routine nutritional screenings and dietary counseling during the cancer-survivorship trajectory.

The genera abundances of *Prevotella*, *Peptostreptococcus*, *Rothia*, family Ruminococcaceae (i.e., Ruminococcaceae DTU089) and family Lachnospiraceae (i.e., Lachnospiraceae GCA_900066575) were negatively correlated with PDQS scores. In support of our finding, some *Prevotella* species have been linked to discretionary food items such as sweet drinks, sweet desserts, and animal fats (17), while *Peptostreptococcus* was associated with a lower long-term plant-based diet index (18), which may align with lower dietary quality. In contrast to our findings, higher abundances of taxa in the families Lachnospiraceae and Ruminococcaceae have been linked to high HEI-2015 total scores, as well as to high vegetable, fruit, and grain consumption (19), suggesting better diet quality. However, species belonging to *Peptostreptococcus* (e.g., *P. anaerobius*) have also been linked to colorectal cancer (CRC) (20), while the Actinobacteria genus *Rothia* has been associated with intestinal oxidative stress damage and lipopolysaccharide-induced liver injury in rats (21).

Higher abundances of the genera *Caproiciproducens* (*Bacillota* genera), Lachnospiraceae ND3007 group, and an uncultured genus from the family Eggerthellaceae were positively associated with PDQS scores. Similarly, among breast cancer patients (n = 55), higher HEI scores (reflecting higher diet quality) were significantly correlated with the abundance of Firmicutes and with higher fruit component scores (10). Further, evidence supports a higher abundance of taxa in the family Lachnospiraceae (*Lachnospiraceae* ND3007) associated with higher HEI-2015 scores in a large longitudinal multiethnic cohort study (n = 5936) (22). Similar associations have been reported in patients consuming healthy, fiber-rich, plant-based diets (23). A possible beneficial link between higher dietary quality and increased Lachnospiraceae ND3007 abundance may be related to the members of *Lachnospiraceae* family being potential producers of the short-chain fatty acid butyric acid (24). Butyric acid serves as a source of energy for intestinal epithelial cells and strengthens the mucosal barrier (24). Similarly, *Caproiciproducens* is an acid-producing bacterium in which the end products of anaerobic fermentation may include caproic acid. Caproic acid is linked to improved immunity, enhanced gut microbiota, and the inhibition of pathogenic bacteria (25). Higher levels of family *Lachnospiraceae* and family *Eggerthellaceae* were also observed in individuals with a lower intake of bioactive compounds and xenobiotics derived from food processing, which may reflect a more favorable gut microbial profile (26). The *Eggerthellaceae* family has also been known to metabolize (poly)phenols, polyphenols, and phenolic metabolites, which may enhance antioxidant, anti-inflammatory, and gut barrier function (27-28). Importantly, higher abundances of the genera *Caproiciproducens*, and Eggerthellaceae family may have implications for future studies, as a diet supplemented with polyphenol-rich aronia fruit juice resulted in a significantly increased abundance of genus within the Eggerthellaceae family and protection against high-fat-diet-induced metabolic changes in humanized mice (28). Similarly, there is some evidence that Naoxintong capsule, a Chinese medicine, inhibits the development of cardiovascular diseases by ameliorating high-fat-diet-induced metabolic disorders, partly through improving the gut microbiota abundance of *Caproiciproducens* (25).

Interestingly, analysis of the bacterial functional pathways predicted from the 16S rRNA gene data indicates a positive correlation between the abundances of pathways related to VB and enzyme-cofactor biosynthesis, including B1/thiamine (thiazole component of thiamine diphosphate), B2/riboflavin (flavin biosynthesis I), B5/pantothenate (phosphopantothenate biosynthesis I), and CoA biosynthesis and PDQS scores. Importantly, sources of VBs for humans are mostly various

foods (e.g., liver, eggs, and chicken for VB5) but B vitamins can also be synthesized by bacteria in the gastrointestinal tract (29,30). The importance of VBs in human health has been the subject of extensive review (29,30). For example, VB5 (pantothenic acid) plays a role as a precursor of CoA, which is a cofactor for the tricarboxylic acid (TCA) cycle and fatty acid oxidation, all of which are related to energy metabolism. Recent evidence suggests that the indirect role of VB in human health may be mediated by gut microbiota contributions to the maintenance of immune homeostasis (29,30).

Although we did not evaluate human intake of supplements or other unmeasured dietary components, we cannot exclude the possibility that the higher abundances of VB-synthesizing taxa and pathways may be related to such intake. For example, food sources of VB2 include milk and dairy products, while cereals, meat products, and fruits are sources of VB1—all of which can be part of a patient's diet. Evidence from a systematic review suggests that 64%–81% of cancer survivors use vitamins and/or dietary supplements (31). However, a prior study of gastric cancer patients reported decreased intake of VB1, VB6, and VB12, as assessed using a semi-quantitative food-frequency questionnaire, after 6 months of receiving chemotherapy (32). This is clinically relevant because higher intake of some VBs (VB1 and the 1-carbon metabolism vitamins VB9, VB2, and VB6) has been associated with reduced cancer cell proliferation (33), whereas dietary deficiency in VB6 has been associated with an increased risk of developing CRC (34). The use of VB nutritional supplements and the benefits of healthy dietary patterns combined with a healthy lifestyle are areas of further exploration in cancer prevention and/or survivorship.

Table 2. Differentially abundant Bacterial Species in the Bacterial Pantothenate (Vitamin B5) and Acetyl-Coenzyme A Biosynthesis Pathways Between High- and Low-Dietary-Quality Groups

Species	Mean Abundance High-Dietary-Quality Group (n = 9), Mean Abundance	Low-Dietary-Quality Group (n = 8), Mean Abundance	*P*-adjusted value
Alistipes putredinis	107.98	25.94	.02230*
Clostridium difficile	0	7.55	.04060*
Lachnospiraceae bacterium 5_1_63FAA	69.85	38.54	.04800*
Odoribacter splanchnicus	19.35	0.86	.0016**
Porphyromonas asaccharolytica	0.17	132.45	.04060*
Proteus mirabilis	0	1.84	.04250*

*adjusted P < .05, **adjusted P < .01; t-test with Bonferroni correction

To validate the VB pathways predicted from the 16S rRNA gene data, we conducted shallow SMS, which analyzes the genome of the entire gut microbial population in a sample, on a subset of the samples. We focused our analysis on identifying differentially abundant bacterial species from the VB5 biosynthesis pathways that differ by dietary quality group. Interestingly, a longitudinal study using SMS proposed a link between the VB5–CoA pathway and dietary quality in healthy Chinese adults (35). The study team reported healthy diet scores that were associated with both increased alpha diversity and enrichment for metabolic pathways related to CoA, vitamin biosynthesis, and the TCA cycle. As previously stated, our particular interest in the VB5 pathway lies in its potential to aid in the identification of biological factors, including VB5-producing species, and dietary factors associated with changes during nCRT. If these findings are confirmed in future studies, they may guide targeted preventive measures prior to nCRT (e.g., adjusting the diet, taking oral supplements, and optimizing treatment response). In fact, mean abundances of the species *Alistipes putredinic*, *Odoribacter splanchnicus*, and *Lachnospiraceae bacterium 5_1_63FAA* were greater in the high-diet-quality group, while abundances of *Clostridium difficile*, *Porphyromonas asaccharolytica*, and *Proteus mirabalis* were enriched in the low-dietary-quality group. Evidence of the specific roles of these bacterial species in the metabolism of cofactors and vitamins, for example through the VB5–CoA biosynthesis pathway, is scarce. One study reported that the abundance of genera *Alistipes* (*A. putredinic* species were abundant in our high-dietary-quality group) in colonic mucosa-associated gut microbiota was associated with higher dietary consumption of VBs as well as with cruciferous vegetable intake (e.g., cabbage, cauliflower, broccoli, and kale) (36), which suggests better dietary quality. Similarly, the abundances of species belonging to the families of Lachnospiraceae (*Lachnospiraceae bacterium 5_1_63FAA*, also abundant in our high-dietary-quality group) may be influenced by VB intake (36). However, known bacterial species that possess the pathway or can produce VB5, such as *Bacteroides fragilis* and *Prevotella copri* (Bacteroidetes); some Ruminococcus spp. (*R. lactaris* and *R. torques*) (Firmicutes); and *Escherichia coli*, *Salmonella typhimurium*, *Salmonella enterica*, and *Helicobacter pylori* (Proteobacteria) (29), did not differ significantly between high- and low-dietary-quality groups in our study. In contrast, some strains of *C. difficile* (abundant in the low-dietary-quality group) lack the VB5–CoA biosynthesis pathway, suggesting that they may compete with the host for VB5 (29,30).

It is also clinically relevant to mention that cancer and/or cancer treatments may be contributing factors to the risk of *C. difficile* colonization and infection. This may occur through the weakening of the immune system or by directly damaging the intestinal lining and altering the gut microbiome composition (37). Alarmingly, *C. difficile* infection (CDI) may be responsible for poorer outcomes in cancer patients with the infection (e.g., increased in-hospital mortality rate, hospital length of stay) compared to cancer patients without CDI (38). Further complicating the risk factors for CDI and related to our study, evidence supports a link between low-quality diet and the risk and severity of CDI, as reviewed in Castro et al., 2025 (39). In fact, in murine CDI models, a Western dietary

pattern (e.g., refined grains, processed meats—both high in fat and sodium) has been associated with enriched *C. difficile* and *Proteus mirabilis* (40) (abundant in our low-dietary-quality group), perhaps due to changes in bile acid composition, which may favor *C. difficile* growth (41). This is nutritionally important because some evidence, also from a murine CDI model, showed that mice fed low-protein diets had better outcomes (e.g., survival, decreased weight loss and disease severity) compared to mice fed a traditional house-chow diet (39,41). In addition, some clinical studies suggest that the prebiotic oligofructose may help prevent recurrent CDI (42), while the probiotic Clostridium butyricum MIYAIRI 588 may reduce the risk of CDI among critically ill patients admitted to the intensive care unit (43); given these findings, clinical studies of cancer patients are warranted.

Porphyromonas asaccharolytica, also abundant in the low-dietary-quality group, has been linked primarily to human periodontal diseases but has also been associated with CRC in multi-ethnic cohorts (44). In addition, ultra-processed foods are associated with lower dietary diversity and poorer micronutrient adequacy, including reduced VB5 (45). Therefore, the roles of dietary quality, VB-synthesizing species, and metabolite levels should be further investigated, particularly with respect to better understanding the mechanisms of cancer-therapy side effects in RC patients.

Our findings should be interpreted with caution in light of several study limitations. While this study presented associations between gut microbial diversity and overall dietary quality scores in RC patients at the end of nCRT, the cross-sectional design prevented us from including relevant covariates (i.e., Bristol Stool Scale scores, sex, age, and body mass indices) in the analysis, thereby precluding us from drawing causal conclusions. In addition, we acknowledge that although we had sufficient biological replicates for statistical analysis, our sample size was moderate and our study's findings are intended to identify preliminary evidence that diet quality patterns are linked to stool microbiota in RC patients at the end of CRT. In the future, we plan to recruit a larger sample and collect more rigorous data on potential confounders, including clinical characteristics (e.g., tumor location, tumor size, clinical staging before nCRT) that can be used in inferential statistics to identify significant and more generalizable correlations between these characteristics, dietary quality scores, and microbial taxa diversity. This study also assessed dietary quality by a single retrospective 24-hour recall. While this approach is less time-consuming and less resource-intensive (46), it may introduce additional study limitations, such as not accounting for day-to-day variability in food choices, patients' long-term dietary habits, recall bias (e.g., memory limitations of foods and beverages consumed in the past 24 hours, reporting bias toward healthier food choices, inaccurate portion-size estimation; 46), and reduced statistical power. These limitations may have led to the inaccurate representation of the typical dietary intake of 1 or more participants. To address these limitations, we are currently collecting dietary-intake data over 3 non-consecutive days (2 weekdays and 1 weekend) before each stool collection to better investigate these associations while accounting for variability in the reported diet intake. In future studies, we will consider additional measures of dietary quality (e.g., the HEI-2020, an alternate Mediterranean diet, the American Cancer Society guidelines) and markers of malnutrition, in addition to continuing to consider VBs. Our findings should be validated in a larger sample that includes different treatment time points and a comprehensive evaluation of dietary intake and quality throughout nCRT.

We also acknowledge that dietary quality scores may frequently be used as exposures in research rather than outcomes. As an example of a health-related finding, a recent systematic review and meta-analysis on cancer prognosis demonstrated that better overall diet quality was associated with improved survival among breast and CRC survivors. The study group found that adherence to the Mediterranean diet was associated with lower risk of mortality in CRC and prostate cancer survivors (45). This suggests that adherence to a high-quality diet through the cancer survivorship trajectory may be highly beneficial. With respect to behavioral outcomes, other studies have found that among women with ovarian cancer, poor diet quality may be linked to post-treatment anxiety or depression (48). Interestingly, a few studies are beginning to investigate the effects of changes on dietary patterns or of an impaired bacterial VB5–CoA–synthesis pathway on outcomes such as the clinical response to anti-cancer therapies or recurrence-free survival in CRC (49) or RC patients (50). Future mechanistic studies are needed to explore potential roles of VB-synthesizing bacteria in inflammation, in cancer progression, and as indicators of dietary VB deficiency or of diet quality. Doing so will provide the foundation for novel trials investigating the effects of VB5-rich diets, supplements, or related metabolites on clinical responses to anti-cancer therapies and eventually on recurrence-free survival.

Conclusion

The adverse consequences of both chemotherapy and pelvic radiation for the treatment of RC on nutrition (i.e., dietary quality) and related physiological systems (i.e., gut microbiome) in patients are not well understood. Our cross-sectional pilot study findings showed that RC patients with lower dietary quality harbored higher levels of the genera *Peptostreptococcus*, *Paenniclostridium*, *Howardella*, *Enterococcus*, and *Rothia* in their stool samples at the end of CRT. In a sub-group of participants, 3 species known to be related to the VB5–CoA pathway (*Alistipes putredinic*, *Lachnospiraceae bacterium 5_1_63FAA*, and *Odoribacter splanchnicus*), were enriched in the higher-dietary-quality group. Based on predicted functional analysis, higher abundances of pathways related to VB5 biosynthesis and cofactors were positively correlated with dietary-quality scores. Our next step in a new prospective cohort is to use quantitative polymerase chain reaction to quantify changes in the abundances of VB5–CoA–synthesizing bacteria and their related metabolites (e.g., pantothenate and CoA), as well as changes in host VB5-related immune markers that may be correlated with changes in diet over the course of treatment. Doing so should facilitate the development of molecular markers indicative of dietary quality and disease progression, which could be helpful in informing clinical interventions. In addition, because nCRT may induce nutritional responses, such as the adoption of lower-quality dietary patterns with considerable interindividual variability, we also aim to define latent subgroups of patients whose diets differ in quality over the course of RC treatment.

Tracking changes in dietary patterns over the course of treatment is important because any such changes that occur may reveal the best timing of dietary assessment or intervention, as some patterns may be more amenable to change while others—often the most healthful—appear more stable and may require intervention sooner.

Resumen

Objetivo: Examinar si abundancia de taxones microbianos intestinales y vías funcionales metabólicas se correlacionan con puntuaciones de calidad de la dieta al final de quimio-radioterapia neoadyuvante concurrente para cáncer de recto. En un subgrupo de participantes, identificar especies bacterianas diferencialmente abundantes de las vías de biosíntesis de pantotenato (vitamina B5-VB5) y acetil-coenzima A (VB5-CoA, por su abreviatura en inglés) que difieren según los grupos de calidad de la dieta. Métodos: Treinta pacientes proporcionaron muestras de heces para la secuenciación del gen 16S rRNA. Muestras de heces de un subgrupo de 17 participantes se sometieron a secuenciación metagenómica superficial (SMS). La calidad de la dieta se calculó utilizando la puntuación de calidad de la dieta principal de 24 horas. Los datos del gen 16S se analizaron utilizando QIIME2 y los datos de SMS utilizando HUMAnN2. Resultados: A nivel de género, abundancias de varios taxones (p.ej., *Parvimonas*, *Caproiciproducens*) se correlacionaron positivamente (rho=0.36-0.50) con las puntuaciones de calidad de dieta, mientras que otros taxones (p.ej., *Prevotella*, *Rothia*) se correlacionaron negativamente (rho=-0.43-0.36). Las vías metabólicas previstas incluyeron aquellas relacionadas con la biosíntesis de vitaminas B (p. ej., fosfopantotenato]), los cuales se correlacionaron con puntuaciones más altas de calidad de dieta. La abundancia media de especies relacionadas con las vías metabólicas previstas de VB5-CoA fueron mayores en el grupo de alta calidad de la dieta. Conclusión: Los resultados sugieren asociaciones importantes entre la abundancia de taxones de bacterias intestinales y la abundancia de vías metabólicas previstas relacionadas con la biosíntesis de VB con la alta calidad de la dieta.

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