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The mechanism of dietary factors regulating intestinal flora in alleviating the defecation dysfunction symptoms after sphincter-saving surgery for rectal cancer: A pilot study

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Abstract

Objectives

This pilot cross-sectional observational study explored how dietary intake affects post-sphincter-preserving surgery defecation dysfunction via regulating intestinal flora. Methods: The study enrolled 81 patients at an East China tertiary hospital (Sep–Dec 2024). General/clinical questionnaires, LARS score, simplified FFQ were used to collect data on demographics, defecation dysfunction and dietary intake; 16S rRNA sequencing analyzed stool samples' gut microbiota. PCA grouped defecation dysfunction into frequency-dominant and incontinence-dominant subtypes, and parallel mediation models examined diet-microbiota-dysfunction mediating effects. Results: The more severe frequency-dominant symptoms were correlated with lower gut microbiota alpha-diversity (Chao1, Faith_pd, Observed_species) and reduced Synergistota enterotype(P = 0.020). Egg-based foods were found to reduce Fusobacteriota abundance, thereby mediating the control of post-surgery defecation dysfunction (especially defecation frequency) within 6 months(-0.019(-0.368,-0.036)). Conclusions: This study clarifies the gut microbiota-mediated mechanism of dietary effects on defecation dysfunction, providing a theoretical basis for scientific dietary management, and suggests further research on postoperative diet-bowel symptom associations and microbiota-dysfunction causality to validate these pilot findings.

1. Introduction

Colorectal cancer is one of the most common malignant tumors of the digestive tract worldwide [1]. According to the Global Cancer Statistics 2022, the incidence and mortality rates of colorectal cancer rank third and second among all malignant tumors globally [2]. In China, with the rapid development of the social economy, the lifestyle, dietary structure and ecological environment of residents have been constantly changing, and the incidence of colorectal cancer has shown a significant upward trend in recent years [3]. In 2022, the incidence of colorectal cancer in China rose to the second place among all malignant tumors [1]. Due to China's large population base, the number of people suffering from and dying of colorectal cancer has reached the first place in the world [1].

At present, radical surgical resection remains the main treatment for rectal cancer [4]. With the continuous update of surgical instruments and techniques, sphincter-saving surgery has gradually become the preferred surgical approach for rectal tumor resection, accounting for more than 70% of all rectal cancer surgeries [5, 6]. This has enabled many rectal cancer patients to retain their anus while receiving radical resection. However, preserving the anus does not mean the complete retention of normal defecation function after surgery. Many patients experience varying degrees of defecation dysfunction after sphincter-saving surgery, including increased frequency of defecation, urgency, frequency, incomplete evacuation, straining during defecation, liquid stools, gas in stools, and fecal incontinence, which is known as low anterior resection syndrome (LARS) [7, 8]. According to research reports, the incidence of defecation disorders after sphincter-preserving surgery for rectal cancer has reached 90%, and about 20–50% of patients experience severe defecation disorders [7, 8, 9, 10]. Moreover, some patients' defecation disorders persist for up to 15 years after surgery [11, 12]. Long-term intestinal symptoms impose a significant burden on patients and seriously affect their quality of life after surgery [13, 12, 14]. Therefore, how to alleviate defecation dysfunction in rectal cancer patients after sphincter-saving surgery has become an urgent issue that needs attention and resolution.

At present, the control of defecation dysfunction of patients after sphincter-saving surgery still mainly relies on the patients' self-management. Diet is closely related to defecation function. Different foods and nutrients can directly affect intestinal motility, regulate intestinal flora, and influence the shape and composition of feces [15]. Previous research indicated that patients after the sphincter-saving surgery felt that consuming certain foods could affect the occurrence of defecation dysfunction. Some patients adjusted their diet after surgery to control bowel symptoms, including changing the time and types of food they took [16, 17–18]. The significant relationship has also been found in previous observational studies conducted by our group, indicating that the average daily intake of livestock and poultry meat, oils and fats, dairy products, vegetables, and lipid nutrients within 6 months after sphincter-saving surgery are the main dietary intake factors affecting the occurrence and alleviation of postoperative defecation dysfunction. These findings reflected that a reasonable and scientific dietary intake by patients after surgery is of great significance for effectively controlling the symptoms of defecation dysfunction. However, there are very few existing studies focusing on dietary management of defecation dysfunction in patients after the sphincter-saving surgery, and most of the dietary managements are based on subjective experiences of patients. Empirical research on personalized and precise dietary intervention for defecation dysfunction in patients with anus preservation is still in its infancy.[19–20]

The foundation of precise and scientific dietary management lies in clarifying the mechanism by which diet affects defecation dysfunction. The intestinal tract contains a large number of microorganisms, which directly participate in various metabolic activities of the body [21, 22]. Diet is an important factor influencing the intestinal flora [23]. Different dietary structures have significant impacts on the composition and diversity of the intestinal flora. Multiple studies have shown that high-fat diets have a significant impact on the gut microbiota. After consuming a high-fat diet, the amount of available carbohydrates in the gut relatively decreases, which inhibits the growth of anaerobic bacteria. At the same time, some by-products of lipid metabolism can damage the large intestine mucosa and lead to dysbiosis [24–25]. As the energy metabolite source of the intestinal flora, dietary components also play a significant role in altering the composition of the intestinal flora and influencing the integrity of the intestinal barrier function. Among them, dietary fiber has been shown in multiple studies to promote the growth of intestinal probiotics and increase the diversity of intestinal microorganisms [26–27]. The intake of different levels of protein and vitamins plays a certain role in altering the abundance and diversity of intestinal microorganisms and intestinal health.[28–29]

Clinical research has found that intestinal flora imbalance is closely related to the intestinal function of patients with colorectal cancer after surgery. A Chinese study compared the differences in intestinal flora between patients with normal postoperative intestinal function and those with diarrhea, and the results showed that the proportion of Bacteroidetes and Bacteroides in the fecal flora of the diarrhea group decreased, while the proportion of Proteobacteria significantly increased. Furthermore, the intestinal flora disorder after colorectal surgery affects the recovery of intestinal function in patients after surgery [30]. Overall, existing research mainly focuses on the relationship between the gut microbiota and diseases such as constipation and inflammatory bowel

disease. However, research focusing on the impact of the structure and composition of the gut microbiota on defecation dysfunction in patients after sphincter-saving surgery remains unclear. According to the literature review, it was found that only a study by Korean scholars Soyoung et al. [31] in 2023 explored the direct association between the gut microbiota and LARS. The results showed that the α-diversity abundance index was significantly negatively correlated with the LARS score in patients with defecation dysfunction mainly characterized by frequency changes, while it was significantly positively correlated with the LARS score in patients with defecation dysfunction mainly characterized by incontinence. More evidences related to the specific influence of gut microbiota on defecation dysfunction among patients after the sphincter-saving surgery needs to be explored.

The relationship among diet, intestinal flora and intestinal symptoms is also closely related. Previous studies have explored how diet regulates intestinal flora and subsequently affects the occurrence and development of rectal cancer. [32]. However, no relevant research has been conducted on how diet regulates intestinal flora and subsequently affects the occurrence of defecation disorders after sphincter-saving surgery.

The primary objective of this study was to explore the impact of dietary intake on the occurrence and development of defecation dysfunction after sphincter-saving surgery by regulating the intestinal flora. The work also aims to provide a scientific theoretical basis for the implementation of scientific dietary management measures to control defecation dysfunction among rectal cancer patients after a sphincter-saving surgery, and ultimately improve the quality of life of these patients after surgery.

2. Materials and Methods

2.1 Study design

A cross-sectional observational design was applied in this pilot study.

2.2 Setting and participants

Participant recruitment was conducted at a tertiary hospital in East China from July to October 2024. Inclusion criteria were as follows: (1)primary rectal cancer by pathology;(2)completing the sphincter-saving surgery between 2 months and 2 years before recruitment; and (3)voluntary participation with informed consent. The exclusion criteria were as follows: (1) hearing or cognitive disorders; (2) complications (anastomotic fistula, rectovaginal fistula, etc.) after surgery; (3) a temporary stoma has been left in place after the operation, and (4) with cognitive impairment. In total, 81 participants were included in this pilot study, and the demographic data are shown in Table 1.

Table 1
The participant's characteristics(N = 81)

Age (Mean ± SD)		**□□(N)**	**□□□(%)**
	58.81 ± 11.65		
Gender	Male	45	55.6
	Female	36	44.4
Occupation	Staff member	10	12.3
	farmer	7	8.6
	worker	6	7.4
	Freelance work	8	9.9
	Retired	50	61.7
Timing(months)	(0,3]	39	48.1
	(4,6]	11	13.6
	(7,12]	19	23.5
	(13,24]	12	14.8
Diabetes	No	69	85.2
	Yes	12	14.8
Hypertension	No	58	71.6
	Yes	23	28.4
Cardiac disease	No	74	91.4
	Yes	7	8.6
Smoke status before surgery	No	60	69.1
	Yes	21	30.9
Drinking status before surgery	No	72	88.9
	Yes	9	11.1
Chemotherapy status	Haven't received	32	39.5
	Have received	49	60.5
Radiotherapy status	Haven't received	60	74.1
	Have received	21	25.8
Distance between margin of anal and anastomosis (cm)	(0–5]	22	27.2
	(5–10]	48	59.3
	(11–15]	11	13.5
Lymphatic metastasis	No	49	64.5
	Yes	27	35.5
Differentiated degree	Poorly	8	9.9
	Poorly to Moderately	18	22.2
	Moderately	52	64.2
	Highly	3	3.7

2.3 Data collection and measurements

2.3.1 Questionnaires

Brief, but adequately validated, questionnaires were administered to collect data.

(1)General information questionnaire: Basic personal and demographic information was collected through a questionnaire developed by the research team. This questionnaire consisted of 10 items, including age, sex, employment status, education level, tobacco and alcohol use.

(2) Clinical information questionnaire: Basic information regarding diseases and related treatments was gathered using a questionnaire created by the research team. This included data on tumor size and location observed during examinations, conditions of preoperative and postoperative radiotherapy and chemotherapy (specific regimens and treatment courses), the anastomosis site (distance from the anastomosis to the anal verge), the portion of the intestinal tube affected by the tumor, the surgery date, the surgical technique employed (either robotic Dixon surgery or laparoscopic Dixon surgery), the postoperative clinicopathological stage, and the patient's recovery status after surgery.

(3) Low Anterior Resection Syndrome Score (LARS Score) Chinese Version: The questionnaire created by Emmertsen et al. evaluates postoperative defecation dysfunction after sphincter-saving surgery. It consists of five items: exhaustion incontinence, unformed stool incontinence, defecation frequency, control over defecation, and urgency of defecation. Each item provides 3 to 4 options with scores reflecting their impact on quality of life. The total score, which can range from 0 to 42 points, is the sum of the scores from these five items. Scores of 0 to 20 indicate "no LARS," 21 to 29 denote "mild LARS," and 30 to 42 signify "severe LARS." Initially developed in Danish, this scoring system has been translated into Swedish, Spanish, and German, and its reliability and validity have been confirmed in several centers. Research has shown that the Cronbach's coefficient of the Chinese version ranges from 0.602 to 0.856[33].

(4) Simplified Food Frequency Questionnaire(FFQ): The food frequency questionnaire, created by Professor Gao Jian, comprises 25 different food items. It assesses the average consumption frequency through nine intake categories over a specified period. The food intake is generally classified into six groups. By multiplying the frequency of consumption by the portion size, the average daily intake is calculated in grams. Participants' energy and nutritional intake is evaluated based on their frequency ratings and portion sizes. Nutritional analysis was conducted using specialized Nutrition Calculation Software designed to determine the intake of various food categories, as well as the energy and nutrient content.

2.3.2 Stool sample collection and preservation

Stool samples were collected at the time of dietary data collection using the Stool collection kit (Personlabio, China). This all-in-one kit enables fast, hygienic self-collection and contains 2.5 mL of a proprietary protective liquid that preserves native microbial community structure. Every patient received both written and verbal instructions about hygienic self-collection procedures. Once the stool sample was collected as instructions, it was stored at room temperature (15–25°C) for up to 48 hours, and then it would be transferred and stored at -80°C in Zhongshan hospital.

2.3.3 DNA extraction and PCR Amplification

Whole DNA in fecal samples was extracted using the magnetic bead method nucleic acid extraction kit (Personlabio, China). Extracted DNA was eluted in 22 uL of nuclease-free water and stored at -80°C until further analysis.

For the 16S rRNA gene targeting the V3–V4 region, primers F(ACTCCTACGGGAGGCAGCA) and R(GGACTACHVGGGTWTCTAAT) were utilized for amplification. The amplification program was as follows: in the denaturation phase, the temperature is heated to 94–98°C for 30 seconds to 1 minute to separate double-stranded DNA. Next, it is lowered to 50–65°C for 30 seconds to 1 minute to allow primers to bind to the single-stranded DNA. The temperature is then raised to 72°C for DNA polymerase to synthesize DNA strands, typically for 1 minute per thousand base pairs. These steps are repeated 30–40 times for sufficient amplification. Finally, the amplified products are verified using agarose gel electrophoresis to check their size and quality.

2.3.4 Library construction and sequencing

The Illumina NovaSeq 6000 platform was applied for paired-end sequencing of community DNA fragments. During sequencing, the Phred quality score was monitored (Q30 ≥ 85%), and the base detection error rate was tracked in real-time. Raw sequences that passed the initial quality screening were categorized into libraries and samples based on index and barcode information, after which the barcode sequences were removed. Following quality control, reads were assembled, and sequence denoising or OTU clustering was conducted according to the QIIME2 dada2 analysis pipeline or the V search software analysis pipeline[35].

2.4 Statistical analysis

SPSSau software was used for the statistical analysis, and the statistical significance was set at $P < 0.05$ (2-tailed). Patients were excluded from the analysis if more than 30% of the data were missing. The results of FFQ were first recorded into the Nutrition Calculation Software and then the consumption of different food components was calculated by this software. Continuous variables were described using the mean and standard deviation, and categorical variables were described using frequencies and percentages. The Shapiro-Wilk test was used to assess the normality of the distribution for continuous variables. Depending on the distribution, comparisons between two means were conducted using either a t-test (for normally distributed data) or the non-parametric Mann-Whitney U test (for data that did not meet the assumption of normality). Pearson or Spearman correlation analysis was used to analyze the correlations among gut microbiota diversity, food and nutrient intake, and LARS scores.

Defecation dysfunction patterns were grouped using an approach with principal component analysis (PCA) performed on the five LARS questionnaire items. The parallel mediation model in mediation analysis was applied to explore the mediating effect among diet, alpha-diversity of gut microbiota, and defecation dysfunction.

2.5 Ethical considerations

The study design was approved by the Institutional Review Board (IRB) of the Zhong Shan Hospital (IRB no.2024-019R). We followed the principles outlined in the Declaration of Helsinki. Informed consents were obtained from the study participants through the IRB approved.

3. Results

3.1. Participant characteristics

A total of 87 patients were invited to participate in the pilot study. If stool samples were not collected and preserved as required, they should be excluded. Incomplete questionnaires or all entries with the same answer were excluded. Finally, a total of 81 participants were included. Their mean age was 58.81 years (SD = 11.65), and the majority of them were male (55.46%), aged over 55 years old (65.8%), and had retired (61.7%). The length of time since surgery was from 6 weeks to 15 months. Most participants did not have the habit of smoking tobacco (69.1%) or consuming alcohol (88.89%) in the past 1 year. A total of 60.5% of them received chemotherapy, and 25.8% of them received radiotherapy after surgery before the time of this investigation. The average distance between the margin of the anal and anastomosis ranged from 2 to 15 centimeters. Most of the patients' tumors were moderately differentiated(64.2%). 35.5% of patients' tumors developed lymph node metastasis. The participant characteristics are shown in Table 1.

3.2. The status of defecation dysfunction

3.2.1 The grading of defecation dysfunction

Among the 81 subjects, the median LARSS score was 19.0, with the interquartile range being 7.0–28.5. There were 44 individuals (54.3%) without LARS, 21 (25.9%) with mild LARS, and 16 (19.8%) with severe LARS.(Table 2)

Table 2
The grading of defecation dysfunction(n = 81)

Occurrence status of LARS	N(%)/ M(IQR)	χ2χF/χ^2	P
Non-occurrence of LARS n(%)	44(54.3)	125.404.	< 0.001
Mild degree of LARS n(%)	21(25.9)		
Major degree of LARS n(%)	16(19.8)		
LARSS Score S(D)	19.0(7.0-28.5)	48.890	<0.001

3.2 2 Principal component analysis of defecation dysfunction

For the symptom clusters extracted from the five items of the LARSS questionnaire, the principal component analysis (PCA) combined with the maximum variance orthogonal rotation was used for the symptom cluster extraction. Through conducting the KMO test and Bartlett's sphericity test, the results were KMO = 0.621 and Bartlett's sphericity test P < 0.01, which proved that the conditions for conducting principal component analysis were met. The results showed that there were two symptom clusters. The total variance contribution rate was 58.316%.The questionnaire items for liquid stool incontinence, flatus incontinence, clustering, and urgency were grouped and contributed to the first PC variable (PC1)in the PCA, with the variance contribution rate of 37.266%. The questionnaire item for frequency accounted for a greater proportion of the second PC variable (PC2), with the variance contribution rate of 21.050%. The component loading matrix is shown in Table 3. The Factor component diagram was shown in Fig. 1.

Table 3
Cluster analysis of defecation dysfunction

items	Factor loadings	
	PC1	PC2
Incontinence for liquid stool	0.767	− .0148
Urgency	0.724	
Clustering	0.626	0.492
Incontinence for flatus	0.462	0.257
Frequency		0.931

3.3. The relationship between dietary intake and microbial diversity

Spearman correlation analysis was conducted between the diversity indices of the in-testinal microbiota and the average daily intake of different foods and nutrients. The results showed that the average daily intake of rice was significantly negatively correlated with diversity indices of the microbiota,including Chao1(r=-0.258,P = 021), Faith_pd（r=-0.263,P = 018）, Observed_species（r=-0.284,P = 011）, Pielou_e（r=-0.286,P = 010）, Shannon（r=-0.332,P = 003）, and Simpson（r=-0.309,P = 005）; the average daily intake of eggs was significantly negatively correlated with Goods_coverage (r=-0.233,P = 038）; and the average daily intake of carbohydrates was significantly negatively correlated with Pielou_e (r=-0.239,P = 033）.(Table 4)

Table 4
the relationship between microbial diversity and diet intake

Chao1	Rice intake	Porridge intake	Noodles	desserts	fried food	Stuffed food	coarse grains	potatoes	milk	eggs	red meat
	-0.258*	**0.049**	**0.018**	**0.014**	**0.022**	**0.081**	**0.037**	**0.083**	**-0.018**	**0.168**	**0.059**
Faith_pd	-0.263*	0.050	0.002	0.056	0.035	0.089	0.063	0.098	-0.035	0.135	0.027
Goods_coverage	0.105	-0.093	0.109	0.013	-0.022	-0.025	0.008	-0.056	-0.033	-0.233*	0.036
Observed_species	-0.284*	0.053	0.019	0.029	0.015	0.071	0.078	0.098	-0.034	0.158	0.064
Pielou_e	-0.286*	0.009	-0.066	0.133	-0.097	0.059	0.078	0.007	0.012	0.065	-0.153
Shannon	-0.332**	0.061	-0.036	0.136	-0.009	0.111	0.115	0.065	-0.002	0.110	-0.075
Simpson	-0.309**	0.081	-0.036	0.118	-0.050	0.056	0.119	0.012	-0.001	0.109	-0.097
	light vegetables	mushrooms	fruits	beverages	energy	protein	fat	carbohydrate	fiber	cholesterol	
Chao1	-0.084	0.061	0.067	0.020	0.069	0.126	0.197	-0.056	0.092	0.085	
Faith_pd	-0.067	0.025	0.079	0.011	0.047	0.091	0.150	-0.049	0.111	0.043	
Goods_coverage	0.092	-0.097	0.041	-0.104	0.005	0.045	-0.056	0.008	-0.024	0.100	
Observed_species	-0.065	0.046	0.098	0.008	0.067	0.134	0.197	-0.065	0.108	0.099	
Pielou_e	0.038	0.103	-0.028	-0.040	-0.177	-0.102	-0.089	-0.239*	-0.042	-0.016	
Shannon	0.011	0.065	0.048	-0.011	-0.072	0.000	0.049	-0.170	0.061	0.021	
Simpson	0.010	0.063	-0.026	-0.087	-0.106	-0.033	-0.002	-0.184	0.019	0.019	

*:P<0.05; **: P<0.01

Spearman correlation analysis was conducted between the taxonomic composition of microbial and the average daily intake of different foods and nutrients. The results showed that the average daily intake of rice was significantly negatively correlated with Bacteroidota (r=-0.280,P = 012)and Verrucomicrobiota（r=-0.25,P = 045）. The average daily intake of eggs was significantly negatively correlated with desserts（r=-0.260,P = 020）.The average daily intake of Stuffed food was significantly correlated with Firmicutes_D（r = 0.292,P = 009）、Actinobacteriota（r=-0.259,P = 021） and Firmicutes_C（r=-0.369,P = 001）. The average daily intake of coarse grains was significantly correlated with Firmicutes_C(r=-0.224,P = 046). The average daily intake of potatoes was significantly correlated with Bacteroidota（r=-0.243,P = 030）. The average daily intake of eggs was significantly correlated with Verrucomicrobiota（r = 0.284,P = 011）. The average daily intake of freshwater fish was significantly correlated with Bacteroidota（r = 0.276,P = 013）and Actinobacteriota（r=-0.360,P = 001）. The average daily intake of nuts was significantly correlated with Firmicutes_C （r = 0.269,P = 016）. The average daily intake of fruits was significantly correlated with Firmicutes_D（r = 0.221,P = 048）. The average daily intake of carbohydrate was significantly correlated with Synergistota（r = 0.-0.227,P = 043）. The average daily intake of cholosterol was significantly correlated with Actinobacteriota（r=-0.262,P = 019）.(Table 5)

Table 5
the relationship between the taxonomic composition of microbial and die

Firmicutes_A	Rice intake	Porridge intake	Noodles	desserts	fried food	Stuffed food	coarse grains	potatoes	milk	eggs	red meat
	-0.162	**0.109**	**-0.056**	**0.133**	**-0.081**	**0.076**	**0.011**	**0.003**	**-0.079**	**0.005**	**-0.12**
Bacteroidota	-0.280*	0.019	0.064	0.058	-0.052	-0.067	0.002	-0.243*	-0.027	-0.050	-0.04
Actinobacteriota	0.086	0.110	-0.163	0.134	0.050	0.258*	-0.036	-0.044	0.080	-0.001	0.060
Proteobacteria	-0.085	-0.168	0.117	-0.181	0.069	-0.033	-0.132	0.100	-0.012	0.056	-0.00
Firmicutes_D	0.187	0.150	-0.039	0.040	-0.003	0.292**	-0.011	0.113	0.005	0.019	0.052
Firmicutes_C	-0.005	-0.046	0.047	-0.260*	-0.117	-0.369*	0.224*	0.035	0.060	-0.132	0.166
Verrucomicrobiota	-0.225*	-0.057	0.139	-0.045	-0.138	0.031	0.047	0.103	-0.128	0.284*	-0.09
Desulfobacterota_I	-0.203	0.037	-0.052	-0.107	-0.123	-0.171	0.132	0.006	-0.018	-0.122	0.124
Synergistota	-0.145	0.063	-0.048	-0.080	-0.212	-0.097	-0.107	-0.153	-0.051	0.075	-0.10
Fusobacteriota	-0.018	0.027	0.082	-0.001	-0.132	0.071	0.141	-0.139	-0.109	-0.207	0.084
Others	-0.087	-0.009	-0.004	-0.125	0.037	0.115	0.057	0.127	0.048	0.213	0.155
	light vegetables	mushrooms	fruits	beverages	energy	protein	fat	carbohydrate	fiber	cholesterol	
Firmicutes_A	-0.023	0.019	-0.156	-0.001	-0.084	-0.051	-0.055	-0.117	-0.106	0.024	
Bacteroidota	0.061	0.020	0.068	-0.076	-0.091	0.024	-0.004	-0.203	-0.085	0.152	
Actinobacteriota	-0.032	-0.044	0.074	0.120	-0.064	-0.155	-0.058	0.067	0.006	-0.262*	
Proteobacteria	-0.030	0.119	-0.188	-0.014	0.061	-0.022	0.028	0.076	-0.043	-0.051	
Firmicutes_D	0.043	-0.162	0.221*	0.153	0.120	0.033	0.068	0.162	0.174	-0.065	
Firmicutes_C	0.016	0.011	0.084	-0.078	0.115	0.151	0.132	0.059	0.171	0.174	
Verrucomicrobiota	0.179	-0.088	-0.129	-0.177	0.015	0.073	0.017	-0.062	-0.001	0.010	
Desulfobacterota_I	0.019	-0.008	0.076	-0.027	-0.028	0.050	0.023	-0.142	-0.081	0.158	
Synergistota	0.161	0.050	0.018	0.096	-0.173	-0.127	-0.097	-0.227*	-0.117	-0.121	
Fusobacteriota	-0.070	-0.054	-0.047	-0.029	0.129	0.129	0.092	0.106	-0.016	0.159	
Others	0.022	0.058	0.050	0.008	0.109	0.149	0.209	0.025	0.145	0.056	

*:P<0.05; **: P<0.01

3.4 The relationship between alpha-diversity of gut microbiota and defecation dysfunction

Non-parametric tests were used for analysis for the differences in alpha-diversity indicators between two groups of symptom clusters. The result showed that the difference in the α-diversity richness index Goods_coverage between PC1 and PC2 group is significant(p = 0.027). The difference in taxonomic composition of Synergistota between PC1 and PC2 group is significant(p = 0.020). There are no significant differences between two groups in other indices of α-diversity richness and taxonomic composition of gut microbiota(P>0.05).(Table 6–7).

Table 6
The relationship between alpha-diversity richness of gut microbiota and defecation dysfunction

Mann-Whitney U	Chao1	Faith_pd	Goods_coverage	Observed_species	Pielou_e	Shannon	Simpson
	679.000	**713.000**	**581.500**	**703.000**	**736.000**	**813.000**	**774.000**
Z	-1.280	-0.958	-2.204	-1.052	-0.074	-0.009	-0.379
P	0.201	0.338	0.027*	0.293	0.460	0.992	0.705

Table 7
The relationship between taxonomic composition of gut microbiota and defecation dysfunction

Mann-Whitney U	Firmicutes_A	Bacteroidota	Actinobacteriota	Proteobacteria	Firmicutes_D	Firmicutes_C	Verrucomicrobiota	Desulfobacterot
	716.000	**800.000**	**768.000**	**773.000**	**757.000**	**754.000**	**759.000**	**740.000**
Z	-0.502	-0.133	-0.436	-0.389	-0.540	-0.569	-0.541	-0.703
P	0.615	0.894	0.663	0.697	0.589	0.569	0.589	0.482

*:P<0.05

3.5 The mediating effect of dietary intake on regulating gut microbiota influencing defecation dysfunction

Taking two different groups of bowel symptoms as dependent variables, poultry, light-colored vegetables, eggs, and carbohydrates in the diet that are respectively related to defecation categories and intestinal flora as independent variables, and the taxonomic composition index Synergistota and the alpha-diversity index Goods_coverage of gut microbiota as mediating variables. Conduct mediation analysis in SPSSau. Set the number of repeated samplings to 5,000 times and use the default 95% confidence interval simultaneously. The specific paths, effect sizes of each path, and the proportion of the total effect were shown in Table 8. The path of average daily egg coverage = > Goods_coverage = > groups of bowel symptoms has a significant mediating effect (the direct effect is not significant), and the total effect is completely provided by the mediating effect.

Table 8
Serial Mediation Effects of alpha-diversity richness of gut microbiota

	Effect size	Standard error	95%CI		Effect proportion
			LLCL	ULCL	
Overall effect	-0.001	0.002	-0.005	0.003	100%
direct effect	-0.000	0.002	0.004	0.004	0%
Total indirect effect(The average daily coverage of eggs = > Goods_coverage = > different group of bowel symptoms)	-0.001	0.045	-0.169	0.000	100%
The average daily coverage of eggs = > Goods_coverage	-0.000	0.000	-0.000	-0.000	0%
Goods_coverage = > different group of bowel symptoms	82.140	40.034	2.352	161.927	0%

Based on the test results of the Bootstrap mediating effect, two graphs were finally drawn to specifically present the mediating effect model(Fig. 2–3). As to the effect of average daily intake of eggs on the type of defecation disorders after anus-preserving surgery for rectal cancer, the total effect size was − 0.001, and its 95% confidence interval was (-0.005, 0.003). The direct effect size of daily egg intake on defecation disorders after anus-preserving surgery for rectal cancer was − 0.000 for 0% of the total effect size, and the 95% confidence interval was (0.004, 0.004). In terms of mediating effects, the total indirect effect size is -0.001, accounting for 100% of the total effect, and the 95% confidence interval is (-0.169, 0.000), indicating the existence of significant indirect effects. The average daily intake of eggs can negatively predict the microbiota diversity index of Goods_coverage(-2.352,0.021), and the microbiota diversity index of Goods_coverage can positively predict the group of bowel symptoms(2.052,0.044).

The serial Mediation Effects of the taxonomic composition of gut microbiota was showed in Table 9. Among the participants within the first 6 months after the sphincter-saving surgery, as to the effect of average daily intake of eggs on the LARSS scores of the frequency-dominant symptoms group, the total effect size was − 0.012, and its 95% confidence interval was (-0.048, 0.024). The direct effect size of daily egg intake on the LARSS scores of the frequency-dominant symptoms group was 0.007 for 0% of the total effect size, and the 95% confidence interval was (0.025, 0.040). In terms of mediating effects, the total indirect effect size is -0.019, accounting for 100% of the total effect, and the 95% confidence interval is (-0.358, -0.036), indicating the existence of significant indirect effects. The average daily intake of eggs can negatively predict the richness of Fusobacteriota (-0.000,0.036), and the richness of Fusobacteriota can positively predict the LARSS scores of the frequency-dominant symptoms group(277.75,0.001).

Table 9
Serial Mediation Effects of the taxonomic composition of gut microbiota

	Effect size	Standard error	95%CI		Effect proportion
			LLCL	ULCL	
Overall effect	-0.012	-0.682	-0.048	0.024	100%
direct effect	0.007	0.018	-0.025	0.040	0%
Total indirect effect(The average daily coverage of eggs = > Fusobacteriota = > LARSS scores of the frequency-dominant symptoms group	-0.019	0.086	-0.358	-0.036	100%
The average daily coverage of eggs = > Fusobacteriota	-0.000	0.000	-0.000	-0.000	0%
Fusobacteriota = > LARSS scores of the frequency-dominant symptoms group	277.75	73.71	128.90	426.60	0%
‘					

4. Discussion

This pilot study is the first to reveal the impact of dietary intake on the occurrence and development of defecation dysfunction after sphincter-saving surgery by regulating the gut microbiome. For this purpose, a cross-sectional observational investigation was conducted. The results showed that the bowel symptoms among rectal cancer patients after the sphincter-saving surgery were divided into two symptom groups– frequency-dominant and incontinence-dominant. This finding is consistent with previous research results[36]. In the LARS questionnaire, frequency-dominant symptoms were expressed through questions regarding the frequency of bowel movements, whereas incontinence-dominant symptoms were expressed through clustering, urgency, fecal and flatus incontinence. The specific questions included in each type of bowel symptom are different from those in another previous study [37].These differences may emerge from different baseline information of the samples.

The alpha diversity index Goods_coverage varies significantly among patients with two types of bowel symptoms. The frequency-dominant symptoms group had lower alpha diversity index Goods_coverage compared to the incontinence-dominant symptoms group. Among the frequency-dominant symptoms group, the more severe bowel symptoms of patients are, the lower their alpha diversity will be. Higher scores of LARS are significantly related to a lower index of Chao1、Faith_pd and Observed_species. A healthy gut microbial ecosystem is characterized by high richness and diversity of the gut microbiome, which are indicative of the microbiome's stability and resilience. The relationship in this study between the alpha diversity and the scores of LARS reflected that a healthy intestinal microenvironment is conducive to the control of defecation dysfunction symptoms, which was consistent with the results in previous studies. Soyoung Park et al found that the decline in the alpha diversity is an important sign of the severity of LARS after low anterior resection of rectal cancer, especially more closely associated with "frequency-dominated"bowel symptoms. Gwoncheol et al pointed out that individuals with high Alpha-Diversity have a more stable intestinal microenvironment, which can effectively buffer the impact of dietary changes and thus maintain a stable bowel rhythm[38].A Chinese study showed that Chao, Ace and Shannon indices in the severe DC group were significantly lower than those in the mild DC group among patients of laparoscopic anterior low resection combined with terminal ileum enterostomy, suggesting that the diversity was significantly lower than that in the mild group[39].

This study observed differences in the taxonomic composition of the gut microbiota among different LARS groups and the results showed that the taxonomic composition of Synergistota in the frequent-dominant symptoms group was significantly lower than that in the incontinence-dominant symptoms group. Several previous studies suggest that the composition of the gut microbiota is associated with defecation frequency. Hye Jung et al pointed out that the Firmicutes ratio was higher in the subjects with a small number of defecation than in those with a large number of defecation, and Bifidobacterium was less abundant at the genus level in the subjects with a small number of defecation[40].This finding was consistent with that reported in some other studies, which showed that Bifidobacterium improves bowel movement frequency in adults[[41–43]. Some studies also found a significant relationship between the taxonomic composition of the gut microbiota and the fecal characteristics. Vandeputte et al[44] identified that the Prevotella enterotype is more correlated with loose stool, while the Ruminococcaceae-Bacteroides enterotype dominates the harder stool. In this study, the differences in the taxonomic composition of the gut microbiota among different LARS groups were significant, indicating that the taxonomic composition of the gut microbiota was related to the specific type of defecation dysfunction, some more details about the relationship are needed to be explored.

The relationship between the diet intake and the alpha diversity of the gut microbiota has been found in this study which has been shown in many previous studies. Long-term high-fat diet could lead to a significant decrease in α diversity[45–46] High-quality and diverse diets, which include foods rich in dietary fiber, whole grains, vegetables, and fruits, can significantly increase the alpha diversity index (such as the Shannon index)[47]. In this study,the intake of rice is negatively correlated with several diversity indices, including Shannon,Simpson et al. The discrepancy between this and previous research results may mainly be attributed to the fact that the association between dietary patterns and diversity indices is typically stronger than that between single food intake amounts. Moreover, dietary patterns can more comprehensively reflect the cross-influences among different types of foods. The relationship between the diet intake and the abundance of a specific bacterial genus has also been found in this study which has been shown in many previous studies. Lawrence A. et al reported that the animal-based diet increased the abundance of bile-tolerant microorganisms (Alistipes, Bilophila, and Bacteroides) and decreased the levels of Firmicutes that me-tabolize dietary plant polysaccharides (Roseburia,Eubacterium rectale, and Rumino-coccus bromii)[48]. However, no significant relationship was found between both the intake of fat nutrients and animal-based foods and the taxonomic composition of the gut microbiota in this study. Only one type of food is related to the richness of species of Actinobacteriota and Bacteroidota, and that is the intake of freshwater fish. Bacteroides was reported in previous studies that it experienced a decrease in diversity when consuming a high animal protein diet[49]. The intake of fruits in this study was positively related with the abundance of Firmicutes_D, which is consistent with the results in previous studies, pointing out that consuming high-fiber foods such as vegetables is associated with a higher abundance of probiotics, such as Anaerostipes, Bifidobacterium and Faecalibacterium[47, 50]。

The intestinal microbiota plays a central "mediator" role in regulating the defecation function, which has been reported in previous studies. The gut microbiota has established a "bridge" between diet and defecation through metabolites and neural-immune interactions. The dietary fiber is fermented by probiotics, generating short-chain fatty acids (SCFA), which subsequently lowers the pH value of the colon, stimulating the contraction of the colon smooth muscle and promoting intestinal peristalsis[51–52]. Consuming a high-fat diet will increase lipopolysaccharides (LPS) and saturated fatty acids in the intestines. By activating the TLR4 (Toll-like receptor 4) signaling pathway in the intestines, it may cause damage to intestinal nerve cells and motor dysfunction, leading to constipation or diarrhea[53–54].)In this study, the mediating effect of dietary fiber and high-fat diet on the regulation of the microbiota and its impact on defecation symptoms was not significant. The only mechanism that has been identified to play a mediating regulatory role is that egg-based foods can reduce the abundance of Fusobacteriota and thereby control the occurrence of defecation disorders, especially the frequency of defecation among patients within six months after the sphincter-saving surgery. This result might because the proteins and choline present in eggs can indirectly affect the frequency and form of defecation by regulating the structure of the intestinal flora, influencing bile acid metabolism, and altering the osmotic pressure of the gastrointestinal tract[48, 55]. It is suggested in this study that ensuring a sufficient daily intake of eggs can reduce the abundance of some harmful bacteria, thereby alleviating the bowel symptoms after the sphincter-saving surgery. Since there are scarce studies focusing on the influence of dietary

factors on the intestinal flora and its impact on defecation dysfunction after the sphincter-saving surgery. Further exploration is needed in future to clarify how dietary intake regulates the intestinal flora and affects defecation dysfunction in patients.

This pilot study has some limitations. First, the sample size of this study is relatively small which is mainly related to the far distance between the patient's home and the hospital, resulting in a decrease in the number of patients for follow-up visits. The limited sample size may lead to a decrease in the representativeness of the research and a reduction in statistical power. Subsequently, a multi-center, large-sample investigation and research will be carried out in order to discover more about the internal mechanisms by which dietary regulation affects the microbiome and subsequently in-fluences defecation dysfunction symptoms. Second, this is a cross-sectional survey study. However, the dietary intake of patients after surgery may change over time, so this study was unable to identify the influencing mechanisms at different stages. Subsequently, further cohort studies based on different severity levels of defecation dysfunction can be conducted to evaluate the association between postoperative diet changes and bowel symptoms and the causality of the gut microbiome on defecation dysfunction.

5. Conclusions

This study provides preliminary data on the impact of dietary intake on the occurrence and development of defecation dysfunction after sphincter-saving surgery by regulating the intestinal flora. The bowel symptoms among rectal cancer patients after the sphincter-saving surgery were divided into two symptom groups– frequency-dominant and incontinence-dominant. Among the frequency-dominant symptoms group, the more severe bowel symptoms of patients are, the lower their alpha diversity will be. Higher scores of LARS are significantly related to a lower index of Chao1、Faith_pd and Observed_species. Synergistota enterotype in the frequent-dominant symptoms group was significantly lower than that in the incontinence-dominant symptoms group. The mechanism that has been identified to play a mediating regulatory role is that egg-based foods can reduce the abundance of Fusobacteriota and thereby control the occurrence of defecation dysfunction, especially the frequency of defecation among patients within six months after the sphincter-saving surgery. Future cohort studies with large samples are needed to evaluate the exact mechanisms of the influence of dietary intake on the disorder of defecation mediated by the intestinal microbiota through exploring the association between postoperative diet changes and bowel symptoms and the causality of the gut microbiome on defecation dysfunction.

Declarations

Ethics approval and consent to participate

This study has undergone full ethical review by the Zhongshan Hospital Medical Ethics Committee and was approved 23rd January 2024 in accordance with the Declaration of Helsinki(NO: (B2024-019R)). Informed consents were obtained from the study participants through the IRB approved.

Consent for publication

Not applicable

Author Contribution

Liuwen and Qibirong conceived and designed the intervention program and the methodology of this trial. Chentingting , LiuWen, Tang wentao and Caoqiujun will carry out the intervention and collect data. LiuWen and Qibirong drafted the manuscript. Jin yinyin and Wang qingling made the data stastical anlalysis and revise the manuscript. ZhuAiYong and XuJianMin all provided a critical review of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during the current study are available in the Genome Sequence Archive(GSA) repository, the relevant accession number to datasets is HRA019702,Please access it from the following link:https://ngdc.cncb.ac.cn/gsa-human/browse/HRA019702

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Figures

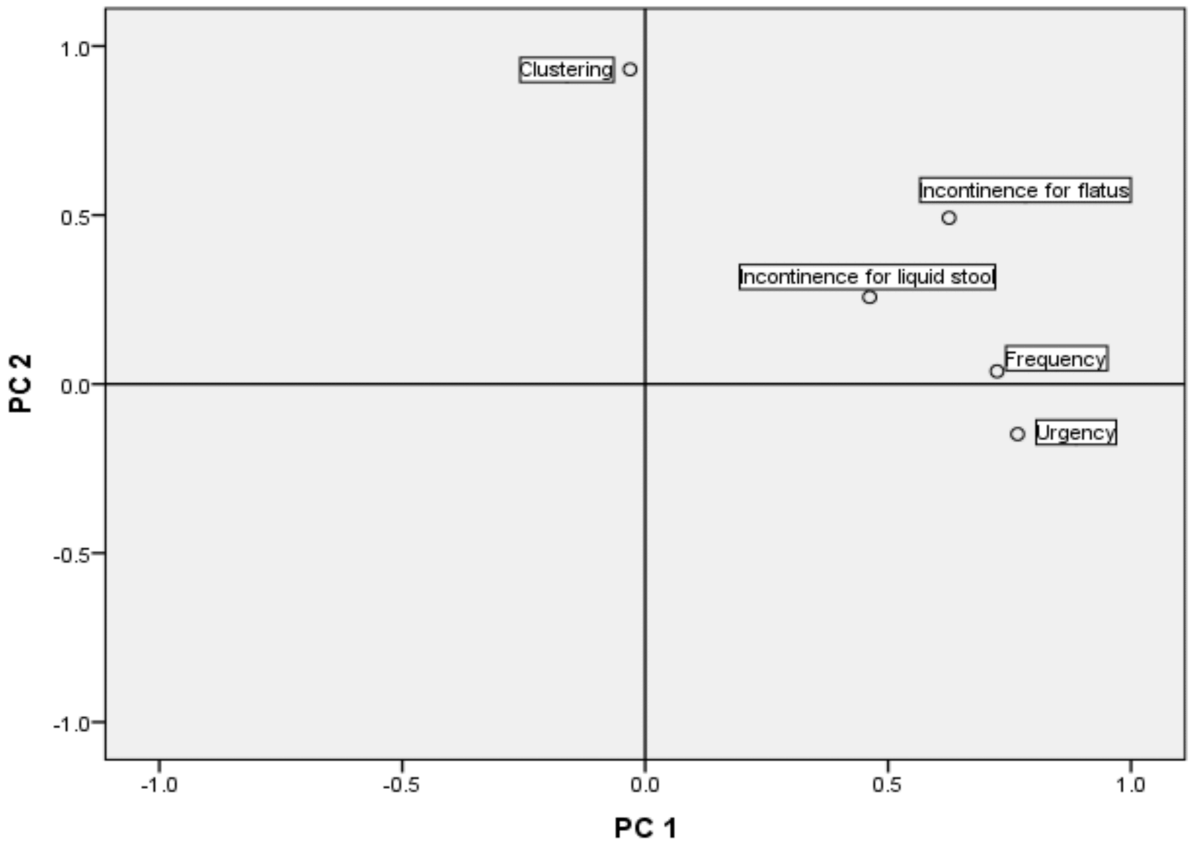


Figure 1 Factor component diagram

Figure 1

See image above for figure legend.

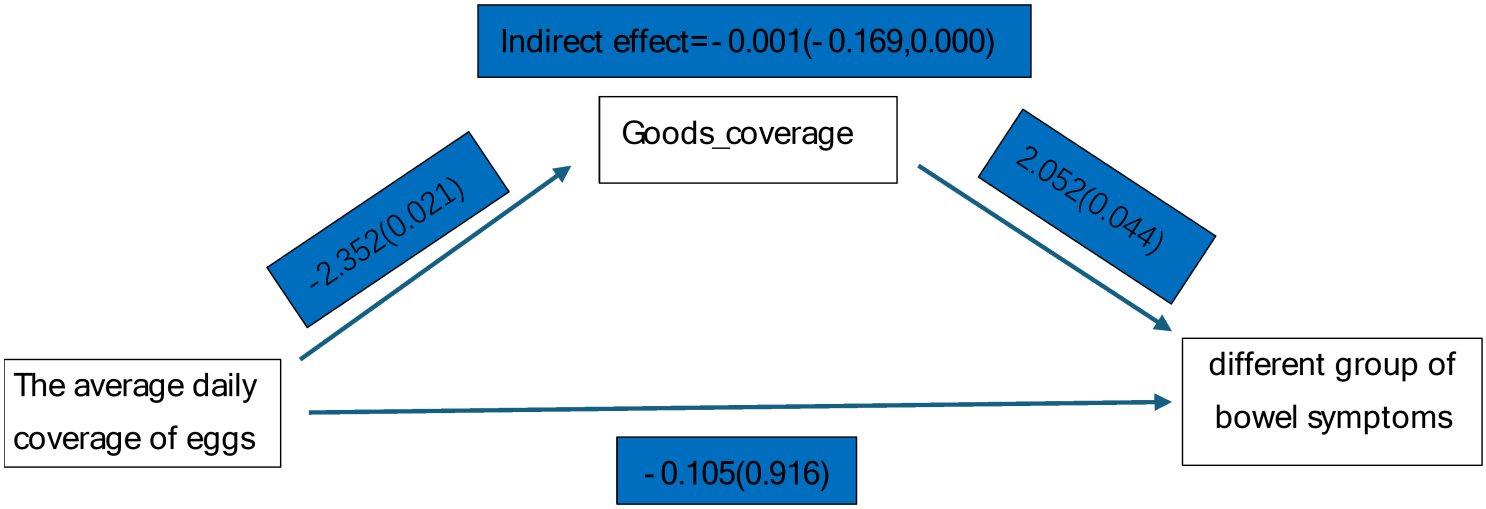


Figure 2 Analysis of the mediating Role of Gut Microbiota between Egg Intake and Defecation Symptoms

Figure 2

See image above for figure legend.

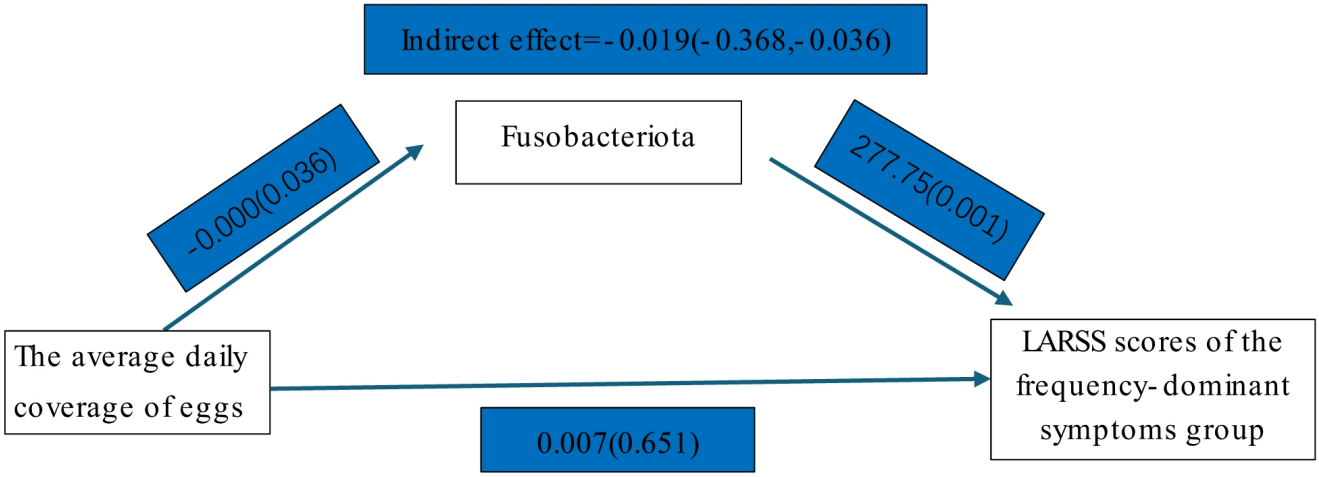


Figure 3 Analysis of the mediating Role of Gut Microbiota between Egg Intake and LARSS scores of the frequency-dominant symptoms group

Figure 3

See image above for figure legend.