Probiotics and dietary intervention modulate the colonic mucosa-associated microbiota in high-fat diet populations

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ABSTRACT

Background/Aims: *Alterations in the gut microbiota due to a high-fat diet and diet-induced illness have been found in both mouse models and humans. Observational studies suggest that probiotic administration and diet shifts may treat diet-related diseases. However, the effect of these interventions on the colonic mucosa has not yet been elucidated. This study investigated the efficacy of probiotic supplementation and dietary intervention as prophylactic tools under high-fat diet conditions.*

Materials and Methods: *A total of 36 volunteers that normally consumed a high-fat diet were enrolled and treated with either a control diet, a low-fat dietary intervention, Bifidobacterium triple viable capsule therapy, or a combination of a low-fat diet and Bifidobacterium triple viable capsule therapy. Pyrosequencing of the V3 and V4 regions of the 16S rRNA genes was conducted to determine the extent to which probiotics and dietary intervention altered the mucosal microbiota.*

Results: *This study demonstrated that interventional treatment with probiotics and a low-fat diet increased the diversity of the mucosal microbes, dietary intervention alone produced the most significant effect, whereas the combined intervention exhibited no synergetic improvement. Pyrosequencing demonstrated that probiotics and dietary intervention significantly elevated the abundance of some bacterial taxa assigned to the phylum Firmicutes and the beneficial genera Prevotella, Gemmiger, Coprococcus, and Faecalibacterium and reduced some harmful bacterial taxa assigned to the phylum Proteobacteria and genus Streptophyta.*

Conclusion: *The results of this study suggested that the addition of probiotics and dietary intervention could improve the composition of the colonic mucosal microbiota in high-fat diet populations.*

Keywords: *High-fat diet, probiotics, dietary intervention, mucosa-associated microbiota, 16S rRNA*

INTRODUCTION

A healthy gut barrier requires symbiosis between the intestinal epithelium and gut microbiota and is of paramount importance to our well-being. Generally, the gut microbiota is comprised of lumen microbiota and mucosa-associated microbiota (1). Mucosa-associated microbiota is resident bacteria colonized on the surface of the intestinal mucosa or temporarily suspended in mucus, and closer to the intestinal epithelial cells than the luminal microbiota. In theory, alterations in the mucosa-associated microbiota community and metabolites may lead to a direct effect on the intestinal epithelial cells resulting in chronic disease such as inflammatory bowel disease (IBD) (2, 3), colorectal cancer (CRC) (4), as well as metabolic syndrome (adiposity and insulin resistance). Fecal samples are typically used to study intestinal lumen microbiota, and therefore a large amount of data has been accumulated on this subject. However, little information is available regarding intestinal mucosa-associated microbiota, which could be due to fewer available mucosa specimens or the fact that the success rates of culture-dependent methods were lower in the past. Fortunately, with the development of culture-independent next-generation sequencing, the acquisition of bigdata for mucosa-associated microbiota will soon be attainable.

Natural intestinal probiotics primarily consist of *Bifidobacteria* and *Lactobacilli*, which play important roles in maintaining the intestinal micro-ecological balance such as promoting the growth of other intestinal symbiotic bacteria, protecting against pathogenic bacteria, enhancing digestion and nutrient absorption, lowering blood lipids and blood glucose, and preventing cancer (5). Many digestive tract diseases are associated with reduced or even depleted natural probiotics in the gut. Accordingly, exogenous probiotics, when administered in adequate amounts, have been demonstrated to confer a health benefit to the host. In addition, a balanced diet, including prebiotic components of food, can assist with modification of the composition and function of the gut micro-

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biota. Thus, a promising way to beneficially affect host health is to alter the gut microbiome using dietary intervention. However, it has not yet been explicated whether interventional probiotics and diet shift adjust the intestinal mucosa-associated microbiota or inhibit specific detrimental pathogens. This study investigates the efficacy of probiotic supplementation and dietary intervention as prophylactic tools under high-fat diet conditions ahead of the development of GI diseases. Moreover, we assessed whether probiotic treatment and dietary intervention produce a synergistic reaction or play an independent role in colonic mucosa-associated microbiota in the high-fat diet population.

MATERIALS AND METHODS

Subjects, study design, and intervention

A total of 36 healthy volunteers from Zhouzhuang Town, Jiangyin City, Jiangsu Province, China, that typically consumed a high-fat diet were enrolled in this study. The experimental design was approved by the Ethics Committee of the Institutional Review Board at Shanghai Tenth People's Hospital and Jiangyin People's Hospital. Written informed consent was obtained from all subjects. The inclusion and exclusion criteria are listed in Table 1. All subjects consumed a high-fat diet (HFD) in which dietary fat accounted for over 40% of the total energy at the beginning of the study. These subjects were randomly assigned to four groups the HFD group received a regular HFD as a control group (n=9); the DI group received a low-fat diet daily in which dietary fat accounted for below 40% of the total energy instead of a HFD (n=9); the HFD+Probiotic group received HFD and 2 g of an encapsulated probiotics powder daily (Shanghai Xinyi Pharmaceutical Co., Ltd., Shanghai, China) containing live combined *Bifidobacterium longum* (≥1.0×10^7 CFU/g), *Lactobacillus acidophilus* (≥1.0×10^7 CFU/g) and *Enterococcus faecalis* (≥1.0×10^7 CFU/g) (n=9). The DI+Probiotic group received a low-fat diet and the same probiotic powder as above (n=9). Prior to intervention, there were no significant differences in the following age, gender ratio, the percentage of calories from fat, daily intake of dietary fiber, valid sequencing reads for colonic mucosal microbiota, or the four alpha diversity indices among the four groups which indicated a similar microbiota structure (Table 2). Probiotic treatment and dietary intervention were continued for 4 months. During the intervention period, daily intake of dietary fiber, was similar among the four groups (HFD 10.00 g/d, DI 8.30 g/d, HFD+Probiotic 9.08 g/d, DI+Probiotic 10.63 g/d), and the percentage of calories from fat was different between the DI and DI+Probiotic groups (34.40% and 32.09%, respectively) and the HFD and HFD+Probiotic groups (43.18% and 47.43%, respectively). All volunteers completed the study under strict quality control including daily dietary records, dietary survey feedback at the beginning of each month and drug recovery and distribution verification at the end of each month.

Table 1. Inclusion and exclusion criteria for enrolment of the volunteers that participated in the present study.

Inclusion criteria	Exclusion criteria
Age 45-65 years	BMI<18.5 or >30 kg/m^2
BMI 18.5-30 kg/m^2	Pregnancy
Similar dietary pattern in which	Clinically significant immunodeficiency
dietary fat accounted for over 40%	Intestinal deficiency and gastrointestinal anatomical changes after operation
of total energy	Chronic gastrointestinal diseases (e.g., gastrointestinal dysfunction or dysentery)
	Acute gastrointestinal diseases which affect gastrointestinal function and still require drug treatment(e.g., acute gastroenteritis or acute peptic ulcer)
	Evidence of infection
	History or presence of any tumors
	Usage of antibiotics within 6 weeks
	Probiotics or prebiotics intake within 2 weeks
	Received insulin, steroid or non-steroidal anti-inflammatory drugs
	Unable or unwilling to change diet patterns, or unwilling to take probiotics orally, or allergic to probiotics

BMI: body mass index

Table 2. The comparison of gender ratio, age, BMI, calorie intake from dietary fat, daily intake of dietary fiber, sequences analyzed, and the four alpha diversity indices prior to the intervention.

	HFD	DI	HFD+Probiotic	DI+Probiotic
Male/Female	5/4	3/6	4/5	4/5
Age	51.7±5.0	52.3±7.9	55.8±8.8	52.3±5.8
BMI	25.0±2.8	23.3±3.0	25.7±3.7	24.4±3.2
Fat	40.90%	43.50%	46.10%	42.80%
Dietary fiber	10.19	7.83	8.39	10.59
Valid sequences	49546±7870	43700±8835	46269±9465	45837±4838
Observed species	153.22±46.33	155.00±26.95	158.22±17.68	155.67±9.85
Shannon	4.78±0.41	4.57±0.83	5.01±0.52	4.86±0.38
Simpson	0.92±0.03	0.88±0.07	0.92±0.04	0.93±0.03
Chao1	177.23±60.64	185.47±37.99	193.91±24.09	197.19±35.07

BMI: body mass index

No Significant differences between groups were determined using analysis of variance or the Kruskal–Wallis H-test.

Sample collection and DNA extraction

Colonic mucosal tissue samples were collected from the ascending colon of each subject via colonoscopy. Prior to this procedure, all volunteers were provided with comprehensive health education, including information about the procedure and related potential hazards, and written informed consent was obtained. They received preoperative bowel preparation with sodium phosphate oral solution (27.9 g/800 ml, Hainan Sanfengyou Pharmaceutical Co., China). All samples were placed in liquid nitrogen and transported to the laboratory within 30 min of collection. Finally, the tissue samples were frozen at -80°C for DNA extraction. Metagenomic DNA was extracted from the mucosal samples using MicroElute Genomic DNA extraction kits (D3096-01, Omega, Inc., USA) according to the manufacturer's instructions. The reagent used to isolate DNA from trace amounts of the sample was effective for the preparation of DNA from most of the bacteria. Sample blanks consisted of unused swabs processed through DNA extraction and contained no 16S amplicons. Total DNA was eluted in 50 µl of elution buffer by the procedure described by the manufacturer (Qiagen, Düsseldorf, Germany) and stored at -80°C for one month and then subjected to PCR (LC-Bio, Hang Zhou, P.R. China).

PCR Amplification and 16S rDNA Sequencing

Using the total DNA from the samples as a template and the primers 319F and 806R (Supplementary Table 1), the V3–V4 region of the bacterial 16S rRNA gene was amplified. All reactions were conducted in 25 µL (total volume) mixtures containing approximately 25 ng of genomic DNA extract, 12.5 µL PCR Premix, 2.5 µL of each primer, and PCR-grade water was used to adjust the volume. PCRs were performed in a Mastercycler Gradient Thermal Cycler (Eppendorf, Hamburg, Germany) set to the following conditions initial denaturation at 98°C for 30 seconds; 35 cycles of denaturation at 98°C for 10 seconds, annealing at 54°C/52°C for 30 seconds, and extension at 72°C for 45 seconds; and then a final extension at 72°C for 10 minutes. The PCR products were identified using 2% agarose gel electrophoresis. Throughout the DNA extraction process, ultrapure water, instead of the sample solution, was used to exclude the possibility of false-positive PCR results as a negative control. The PCR products were normalized by AxyPrep TM Mag PCR Normalizer (Axygen Biosciences, Union City, CA, USA); this facilitated skipping of the quantification step regardless of the PCR volume submitted for sequencing. The amplicon pools were prepared for sequencing with AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and the size and quantity of the amplicon library were assessed on the LabChip GX (Perkin Elmer, Waltham, MA, USA) using the Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA). The PhiX control library (v3) (Illumina) was combined with the amplicon library (expected at 30%). The library was clustered to a density of approximately 570 K/mm^2. The libraries were sequenced on 300PE MiSeq runs, and one library was sequenced with both protocols using the standard Illumina sequencing primers, eliminating the need for a third (or fourth) index read.

Bioinformatic analysis

High-quality 300 bp paired-end reads were overlapped by approximately 90 bp. The assembled sequences were

clustered by using the CD-hit-est based clustering method (6). Online software PyNAST (Supplementary Table 1) was used to analyze and calculate the number of sequences and operational taxonomic units (OTUs) for each sample. Subsequently, the species abundance and distribution were analyzed followed by cluster analysis. Then the sequences were grouped into various OTUs using Felsentein-corrected similarity matrices such that the sequences within an OTU shared at least 97% similarity. The Ribosomal Database Project (RDP) classifier was used to classify the 16S rDNA into distinct taxonomic categories by aligning sequences to a curated database of taxonomically annotated sequences. All 16S rDNA sequences were mapped to the RDP database using BLASTn to achieve taxonomic assignments. Sequences greater than 97% identity were used to associate a group of OTUs with specific species, while those with less than 97% identity were considered novel reads. A Venn diagram was generated to describe the common and unique OTUs among the four groups using online software (http://bioinfogp.cnb.csic.es/tools/venny/). The microbial alpha diversity for the individual samples was estimated using the observed species, Shannon, Simpson, and Chao1 indices. Unweighted UniFrac distance metrics analysis was performed using the OTUs for each sample, and unweighted UniFrac clustering of OTU abundance was constructed using the hclust function of the R gplots package. Principal coordinate analysis was conducted according to the matrix of distance. A metagenomic biomarker discovery approach was employed with linear discriminant analysis (LDA) coupled with effect size measurement (LEfSe), which was used to perform a non-parametric Wilcoxon sum-rank test followed by LDA analysis using online software (http://huttenhower.sph.harvard.edu/galaxy/) to assess the effect size of each differentially abundant taxon (7).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 21.0 (IBM Corp.; Armonk, NY, USA) and GraphPad Prism 5.01 (GraphPad Software Inc., San Diego, CA, USA). Data are presented as the mean±standard error of the mean or as a percentage for relative abundance. Analysis of variance or the Kruskal-Wallis test with Bonferroni correction control was used to analyse intergroup differences for multiple comparisons, as appropriate. All values were considered statistically significant when $p<0.05$.

RESULTS

Microbial diversity

In total, 1,680,484 high-quality reads for colonic mucosal microbiota were analyzed with a mean of 45,799, 51,401, 42,996, and 46,522 reads for the HFD, DI, HFD+Probiotic and DI+Probiotic groups, respectively. As shown in Figure 1a, 371, 427, 426, and 428 OTUs in the HFD, DI, HFD+Probiotic, and DI+Probiotic groups were detected in colonic mucosal tissue samples, respectively. Likewise,

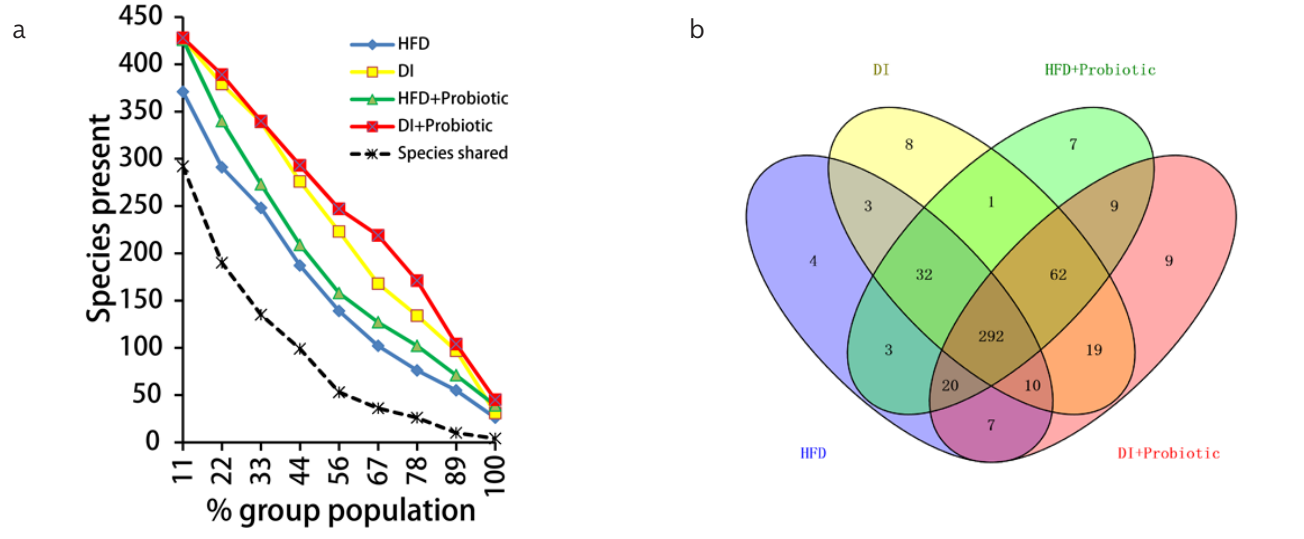

Figure 1. a, b. A line chart (a) and a Venn diagram (b) show presence of shared species in colonic mucosa. The number of bacterial species shared between the groups is dependent on the number of estimated subjects (a). 11 percent (1/9) of the population in each group is compared with one another (b).

when comparing 100%, 89%, 78%, 67%, 56%, and 44% of the population, the number of tissue species shared among all groups was 4, 10, 26, 36, 53, and 99, respectively. A Venn diagram also showed that the number of shared species for colonic mucosal tissue was the highest when the 11% criterion was applied, and 292 species were shared among all four groups (Figure 1b).

According to the observed species, Simpson, Shannon, and Chao1 indices (Figure 2), there were no significant differences between the HFD group and the HFD+Probiotic group or between the DI group and the DI+Probiotic group ($p>0.05$). The DI group exhibited significantly higher observed species ($p<0.001$) and Chao1 ($p<0.001$) indices than the HFD group. All indices mentioned above in

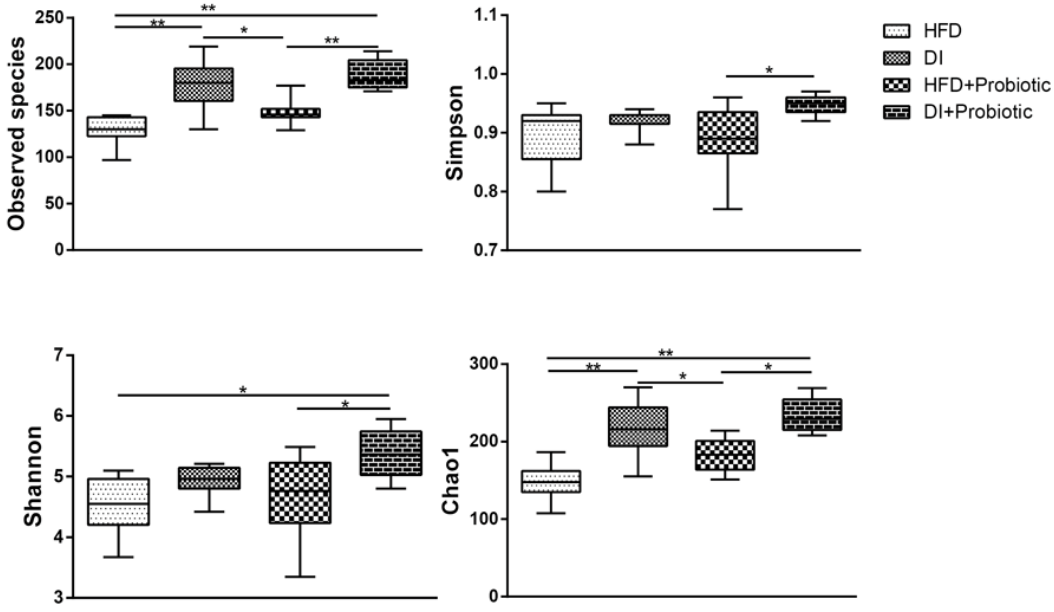

Figure 2. The comparison of four alpha diversity indices at ending time.

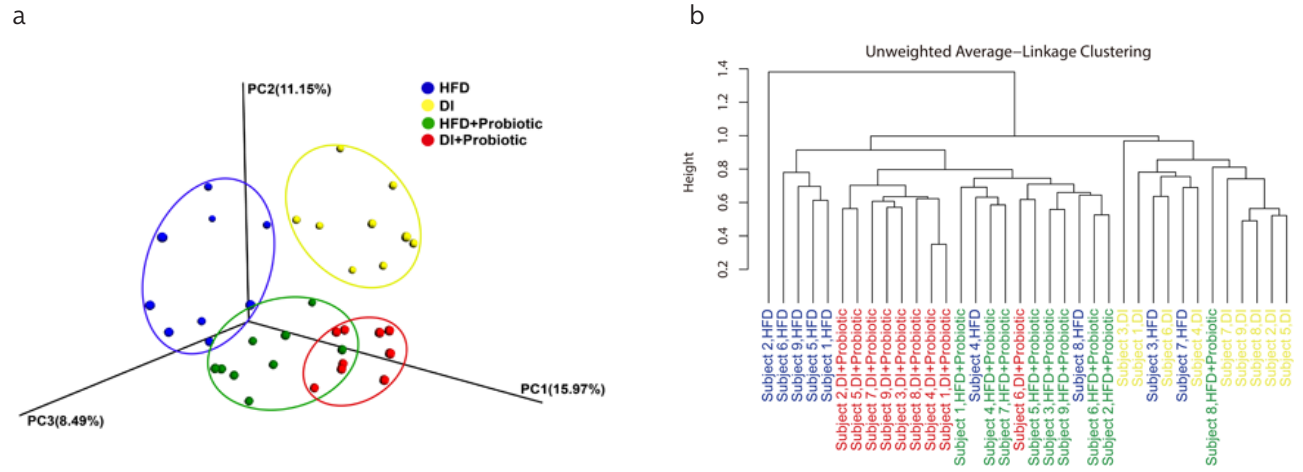

Figure 3. a, b. PCoA (a) and UPGMA (b) show the clustering of samples based on colonic mucosal microbiota communities. PCoA, Principal Coordinates Analysis; UPGMA, Unweighted Pair-Group Method with Arithmetic mean.

the DI+Probiotic group were higher than those in the HFD+Probiotic group ($p<0.05$). These results demonstrated that the addition of probiotics alone had no significant effect on the alpha diversity of the colonic mucosa-associated microbiota. Instead, dietary intervention produced the most beneficial effect, while the combined intervention had no synergetic improvement.

Beta diversity analysis also showed that the colonic mucosal samples were intermingled and did not form dis-

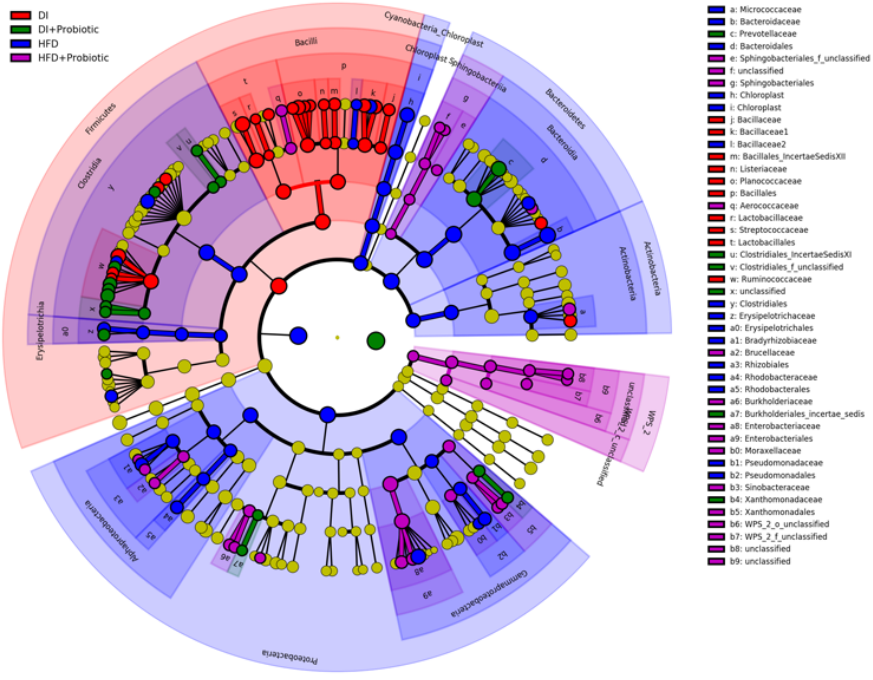

Figure 4. Cladogram representation based on 16S rRNA sequencing analysis of colonic mucosal microbiota in the HFD, DI, HFD+Probiotic, and DI+Probiotic groups. The brightness of each dot is proportional to its effect size. Differences are represented by the color of the most abundant class. The diameter of each circle is proportional to the abundance of the taxon.

Table 3. Comparison of the colonic mucosa-associated microbiota structure at the phylum level.

	Relative abundance (%)				
Phylum	HFD	DI	HFD+Probiotic	DI+Probiotic	p
Firmicutes	32.374	49.426	27.252	44.021	0.011
Bacteroidetes	28.898	38.456	31.100	39.054	0.533
Proteobacteria	24.098	6.218	31.645	13.320	0.021
Fusobacteria	5.312	3.832	7.758	0.641	0.233
Actinobacteria	4.014	1.041	1.427	0.984	0.171
Cyanobacteria/Chloroplast	4.611	0.002	0.388	1.850	0.430
Candidatus Saccharibacteria	0.004	0.001	0.144	0.042	0.015
WPS-2	0.034	0.004	0.192	0.028	0.001
Verrucomicrobia	0.151	0.044	0.012	0.056	0.660
Synergistetes	0.064	0	0.083	0.005	0.510
Tenericutes	0.443	0.975	0	0	0.530

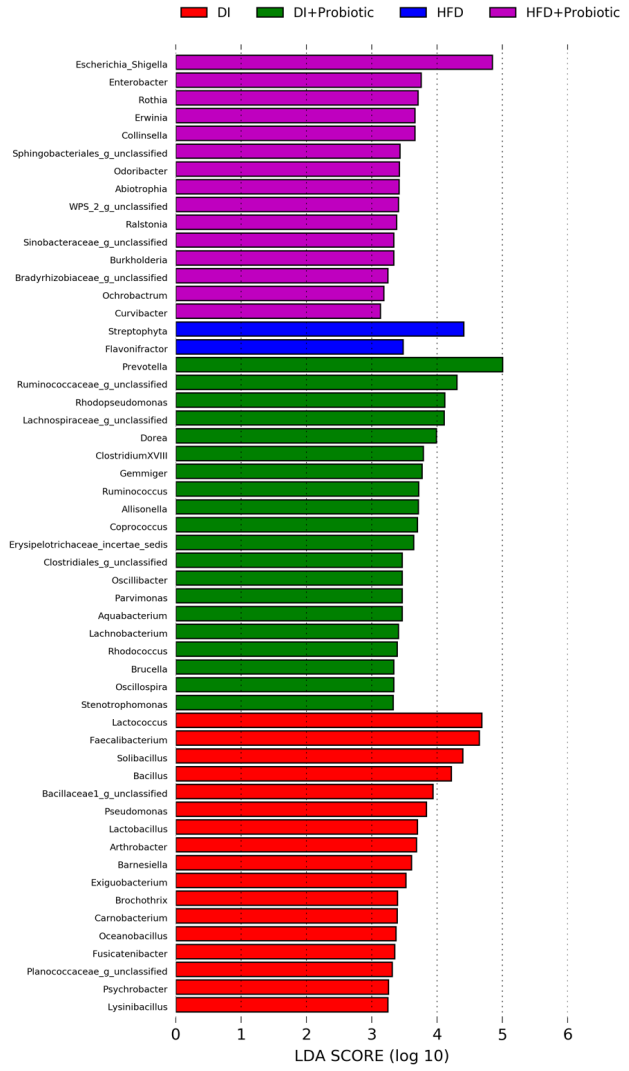

Figure 5. Histogram of the linear discriminant analysis scores for differentially abundant genera in the HFD, DI, HFD+Probiotic, and DI+Probiotic groups. The cladogram was calculated by LDA (linear discriminant analysis) and displayed according to effect size.

tinct non-overlapping clusters (Figure 3), which preliminarily indicated that there were differences in the gut microflora structure among the four groups.

Composition of microbiota

The colonic mucosal microbiota structure was compared at various bacterial levels. At the phylum level, there were no significant differences in classification among the four groups 11 in the HFD group, 10 in the DI group, 10 in the HFD+Probiotic group, and 10 in the DI+Probiotic group. A microbiota whose abundance was >0.1% contributed to 99.18% of the total category, including Firmicutes, Bacteroidetes, Proteobacteria, *Fusobacteria,* and Actinobacteria. According to Table 3, the relative abundance of Firmicutes in the DI group was higher than that of the HFD and HFD+Probiotic groups, while the relative abundance of Proteobacteria in the DI group was significantly lower compared to that of the HFD+Probiotic group. The abundance of dominant bacteria, such as Bacteroidetes, *Fusobacteria,* and Actinobacteria, were not significantly different among the four groups. Candidatus Saccharibacteria, as one of the low-abundance phyla, was significantly higher in the HFD+Probiotic group than in the HFD and DI groups, while another low-abundance phylum, WPS-2, was significantly higher in the HFD+Probiotic group than the other three groups.

At the genus level, after removing unclassified genera, 102 classified genera were present in the HFD group, 92 in the DI group, 100 in the HFD+Probiotic group, and 96 in the DI+Probiotic group. Bacteria whose abundance was >0.01% contributed 95.04% (including unclassified genera) of the total category in the HFD group, compared with 93.68% in the DI group, 96.59% in the HFD+Probiotic group and 89.82% in the DI+Probiotic group. A cladogram representation of the structure of the mucosal microbiota (Figure 4) and a bar graph showing the predominant bacteria (Figure 5) were generated by LEfSe. The greatest differences in taxa between the four communities were displayed. For example, increased genera were *Lactococcus* and *Faecalibacterium* in the DI group, *Enterobacter* in the HFD+Probiotic group, and *Prevotella*, *Dorea*, *Gemmiger*, *Ruminococcus*, *Coprococcus*, and *Erysipelotrichaceae_incertae_sedis* in the DI+Probiotic group, while two genera, *Streptophyta* and *Flavonifractor*, were reduced following the intervention.

DISCUSSION

Many studies have demonstrated that the gut microbiota could be used as a target for the treatment of high-fat diet-related diseases with the direct addition of probiotics, which have achieved good clinical efficacy (8-10). However, prior to the occurrence of high-fat diet-related diseases, few studies had reported the effects of dietary intervention, or probiotic addition in these populations. Therefore, the present study, by providing probiotics and dietary intervention to a healthy population consuming a HFD, comprehensively analyzed the effect of probiotics on the structure of the colonic mucosa-associated microbiota to provide a scientific basis for the mucosa-related microbiome in the prevention of high-fat diet-related diseases.

Diversity of the intestinal bacteria is of great importance for a healthy intestinal microecosystem. In the present study, the results demonstrated that dietary intervention could significantly increase the diversity of the colonic mucosal microbiota, while the combined intervention with probiotics did not enhance this effect. Our previous study of patients with CRC found that probiotic treatment increased the diversity of mucosal microbes, and inhibited the growth of some mucosa-associated pathogens (11). Ng SC et al. (12) found that inflammatory bowel disease patients after 4 weeks of treatment with probiotic mix VSL#3, exhibited increased diversity in the rectum mucosa-associated microbiota. Furthermore, a mouse study by Karmin's research team in Canada suggested that a HFD for 5 weeks followed by a low-fat control diet for 2 weeks partially restored the alpha- and beta diversity of the mucosal microbiota in the caecum and rectum to the pattern observed in mice fed a control diet for 7 weeks (13).

Although lower diversity is one of the signs of intestinal microbiota disorder, reduction in diversity does not necessarily lead to a microbiota disorder. For example, probiotics inhibit the growth of pathogenic bacteria through nutrient competition and antibacterial activity, and the overall microbiota diversity is reduced as a result. Therefore, a healthy intestinal microbiota structure reflects the diversity of the microbiota but also requires a stable intestinal microecology, including an ideal microbiota structure and good function, in which the microbiota composition and abundance are the basis. Unfortunately, to date, no clear conclusions have been made about the correlation between the ideal composition of the microbiota and good intestinal function.

Currently, there are a few studies on the intervention of the colonic mucosal microbiota, and this topic requires further research (14). In the current study, following the dietary intervention and probiotic administration, the structure of the colonic mucosal microbiota related to a HFD had changed at the phylum level. For example, the relative abundance of Firmicutes, Candidatus Saccharibacteria, and *WPS-2* increased, and Proteobacteria decreased following the intervention. It has been tentatively implied in our previous study that the Firmicutes/Bacteroidetes (F/B) ratio in the feces may be contrary to that of the intestinal mucosa (15). Therefore, variation trends in the F/B ratio in the mucosa may indirectly reflect the situation in the feces. It is well known that a HFD can lead to dysbiosis, characterized by an increase in the F/B ratio in the feces (16, 17), and treatment with pro-, pre-, and synbiotics could reduce the F/B ratio (18, 19). Our present study demonstrated that an increase in the F/B ratio in the mucosa exhibited a beneficial modulatory effect on the gut microbiota associated with dietary intervention and probiotics. Litvak et al. (20) suggested that the increased abundance of Proteobacteria was a microbiological marker of colonic mucosal epithelial dysfunction, so the decreased relative abundance of Proteobacteria following intervention was conducive to maintaining good colonic epithelial function. However, the changes discovered in other phyla were different from those identified in previous studies. Shang et al. (13) found that after 5 weeks on a HFD, C57BL/6J mice were administered a low-fat diet for 2 weeks, and the abundance of Firmicutes in the caecum and colonic mucosa decreased while the abundance of Bacteroidetes increased compared with the HFD group at 7 weeks, but the abundance of Bacteroidetes did not return to the level of the 7-week low-fat diet control group. Following the intervention of probiotics alone and in combination with a dietary shift, the number and abundance of beneficial bacteria in the colonic mucosa at the genus level increased significantly (*Prevotella*, *Faecalibacterium*, *Gemmiger,* and *Coprococcus*), while the abundance of harmful bacteria (*Streptophyta*) decreased. The increase in *Prevotella* abundance was either related to increased carbohydrate intake (21) or affected by the increase in *Prevotella* abundance in the feces, which was likely migrated from the intestinal cavity to the intestinal mucosa and ultimately promoted colonization. Studies have demonstrated that mucosal-associated *Faecalibacterium* decreased in patients with inflammatory bowel disease and was beneficial to the survival period of patients with colon cancer. This strain is considered to be one of the most important characteristic markers of intestinal microbiota health (22, 23). The addition of *Faecalibacterium* spp. significantly improved inflammatory bowel disease, and this species played a beneficial role by inhibiting Th17 immune cell differentiation and cytokine Il-17 secretion in the lamina of the large intestine mucosa (24, 25). Our previous study reported that there was a low-abundance of colonic mucosa-associated *Coprococcus* in people with a HFD (15), and in this study, *Coprococcus* increased after the combined intervention. Although there are a few studies on *Coprococcus*, it is believed that *Coprococcus* may play a beneficial role in human health (26). *Gemmiger* abundance increased in the colonic mucosa after the intervention, which also showed a beneficial effect (27). Two genera, *Streptophyta* and *Flavonifractor*, have been less frequently identified in mucosa-associated microbiota and were reduced in abundance in our findings. A recent report by Segata et al. (7) indicated that, in healthy humans, the genus *Streptophyta* was higher in the non-mucosal sites compared to the mucosal

body sites. The relative abundance of *Streptophyta* was significantly increased in the intestinal mucosa of juvenile rhesus monkeys with idiopathic chronic diarrhea and returned to the control level following treatment (28). It is well known that *Flavonifractor*, as an important member of the butyrate producers, could be depleted in obese lumen-associated microbiota, and was negatively correlated with BMI (27). *Flavonifractor* is considered a crucial species for a healthy gut (29). Our data showed that mucosa-associated, *Flavonifractor* decreased in abundance following the intervention. Whether it could lead to an increase in *Flavonifractor* in the feces after interventional treatment remains to be verified. Interestingly, the abundance of these probiotic strains in the colonic mucosal tissues did not increase significantly following the oral administration of probiotics, potential causes for this result might include that the dose was insufficient, the intervention time was short, or the exogenous probiotics were not well transplanted into the intestinal mucosa; thus, this subject requires further investigation.

In conclusion, this study has shown that dietary intervention combined with probiotics could effectively change the diversity, structure, and abundance of the colonic mucosa-associated bacteria in people consuming a HFD. Furthermore, this combination, inhibited specific potential pathogens, including Proteobacteria and *Streptophyta*, and increased the abundance of some beneficial microorganisms, such as *Prevotella*, *Faecalibacterium*, *Gemmiger,* and *Coprococcus*. Although the clinical effect was not examined, the findings of the present study provided an important basis for probiotic and dietary intervention-associated prevention and treatment strategies for the prevention or treatment of high-fat diet-related inflammatory bowel disease and colorectal cancer.

This study demonstrates that high-fat diet populations treated with probiotics and a low-fat diet have increased diversity of the colonic mucosal microbes. Moreover, pyrosequencing indicated that the abundance of some beneficial bacterial taxa were elevated and some harmful bacterial taxa were reduced following probiotics treatment and dietary intervention. Our data show that probiotics addition and dietary intervention could improve the composition of the colonic mucosal microbiota in high-fat diet populations.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Shanghai Tenth People's Hospital Affiliated to Tongji University (approval no. SHSY-IEC-3.0/16-53/01).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - L.Q., H.Q.; Design - L.Q., H.Q.; Supervision - H.Q.; Funding - H.Q.; Materials - L.Q., J.H., H.Q.; Data Collection and/or Processing - L.Q.; Analysis and/or Interpretation - L.Q.; Literature Review - L.Q., H.Q.; Writer - L.Q.; Critical Review - H.Q.

Conflict of Interest: The authors have no conflict of interest to declare.

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Supplementary Table 1. Sequences of primers.

Primers	Sequences
319F	ACTCCTACGGGAGGCAGCAG
806R	GGACTACHVGGGTWTCTAAT

The website of software PyNAST is http:/qiime.org/pynast/.