Association analysis of dietary habits with gut microbiota of a native Chinese community

LEIMIN QIAN1,2, RENYUAN GAO3,4, LEIMING HONG3,4, CHENG PAN3,4, HAO LI3,4, JIANMING HUANG2 and HUANLONG QIN1,3,4

1Department of General Surgery, The Affiliated Shanghai No.10 People's Hospital of Nanjing Medical University, Shanghai 200072; 2Department of Gastrointestinal Surgery, Jiangyin People's Hospital, Jiangyin, Jiangsu 214400; 3The Tenth People's Hospital Affiliated to Tongji University, Shanghai 200072; 4Research Institute of Intestinal Diseases, School of Medicine Tongji University, Shanghai 200092, P.R. China

Received November 20, 2017; Accepted May 22, 2018

DOI: 10.3892/etm.2018.6249

Abstract. Environmental exposure, including a high-fat diet (HFD), contributes to the high prevalence of colorectal cancer by changing the composition of the intestinal microbiota. However, data examining the interaction between dietary habits and intestinal microbiota of the Chinese population is sparse. We assessed dietary habits using a food frequency questionnaire (FFQ) in native Chinese community volunteers. Based on the dietary fat content determined using the FFQ, the volunteers were divided into HFD group (≥40% of dietary calories came from fat) or low-fat diet (LFD) group (<40%). Fecal and colonic mucosal microbiota composition was determined using 16S rDNA based methods. In stool matter of HFD group, Prevotella and Abiotrophia showed significantly higher abundance, whereas unclassified genus of S24-7 (family level) of Bacteroidetes, Gemmiger, Akkermansia and Rothia were less abundant. On colonic mucosal tissue testing, unclassified genus of S24-7 showed significantly higher abundance while Bacteroides, Coprobacter, Abiotrophia, and Astereolplasma were less abundant in HFD group. A high fat and low fiber diet in a native Chinese community may partially contribute to changes of intestinal microbiota composition that may potentially favor the onset and progression of gastrointestinal disorders including inflammatory, hyperplastic and neoplasic diseases.

Introduction

Colorectal cancer (CRC) is the second leading cause of gastrointestinal tumors with an estimated 380,000 newly diagnosed cases in China in 2011 (1). In addition to genetics, environmental factors, such as lifestyle and dietary habits, are associated with CRC development (2,3). Colonic epithelium is exposed to digested food substances, which may initiate cellular transformation and tumor progression through a variety of direct or indirect processes. It has been suggested that in these processes, the intestinal microbiota plays an important role. With the aid of genetic 16S ribosomal RNA polymerase technique, intestinal dysbiosis has been detected in CRC-associated gut diseases, such as irritable bowel syndrome (IBS) (4,5), inflammatory bowel diseases (IBD) (6-8) and Clostridium difficile associated diarrhea (CDAD) (9). However, it is interesting to note that most of the studies on intestinal micro biota have been conducted on CRC patients with sparse knowledge regarding microbiota in healthy human populations (10).

Dietary fat is the third major source of energy in humans. Ingested fat is conjugated to bile acids, which transform fat into bioactive metabolites absorbed by the intestinal epithelial cells. Primary bile acids that escape intestinal absorption undergo microbe-mediated enzymatic deconjugation in the terminal ileum or colon and hence produce secondary bile acids, which are potentially carcinogenic and have been implicated in the etiology of CRC and other GI diseases (11,12). Key microbiota responsible for deconjugation of primary bile acids from taurine and glycine have been identified as Bifidobacterium, Lactobacillus and Bacteroides (13). Numerous lipids with...
biological activity are also produced by bacteria, including lipopolysaccharide (LPS), a component of the cell wall of gram negative bacteria, which can result in tissue inflammation in the gut and other organs, chronic low grade systemic inflammation in particular, and initiate the development of many metabolic diseases. Consequently, dietary fat can influence the composition and metabolic activity of the gut microbiota and an imbalance in microbiota can adversely impact the digestion and absorption of fat.

Consumption of a high-fat, low-fiber diet has been shown to be associated with various pathologies, including obesity, diabetes, hypertension, cardio-cerebrovascular disease and cancer. A high-fat diet (HFD) affects the colonic environment by increasing exposure to mutagens such as heterocyclic amines (HCAs) formed during the cooking and processing of meat (14,15), and by affecting the composition and diversity of gut microbiota in CRC populations (2,16-18). Two studies in inbred mice and human have shown that the shift of dietary macronutrients could significantly alter the gut microbiota within a single day (19,20). However, it remains unclear how the gut microbiota respond to a HFD and low-fat diet (LFD) in healthy individuals. In this study, we investigated the changes of gut microbiota composition upon the differences in the fat content of the diet in a Chinese community.

Patients and methods

Target population. We enrolled volunteers in the age group of 45-65 years (high risk group for cancer as per World Health Organization guidelines) (21) with a BMI of 18.5-30 kg/m², and able to provide written consent and information regarding basic demographic data as well as dietary history. We excluded subjects who had congenital intestinal defects and or previous gastrointestinal operation(s); chronic gastrointestinal diseases such as inflammatory bowel disease; acute gastrointestinal diseases including acute gastroenteritis and/or ulcers; a prior diagnosis of any cancers; history of hospitalization or medical treatment by increasing exposure to mutagens such as heterocyclic amines (HCAs) formed during the cooking and processing of meat (14,15), and by affecting the composition and diversity of gut microbiota in CRC populations (2,16-18). Two studies in inbred mice and human have shown that the shift of dietary macronutrients could significantly alter the gut microbiota within a single day (19,20). However, it remains unclear how the gut microbiota respond to a HFD and low-fat diet (LFD) in healthy individuals. In this study, we investigated the changes of gut microbiota composition upon the differences in the fat content of the diet in a Chinese community.

Stool collection and colonic mucosal tissue sampling. After filling out the questionnaires, a freshly voided stool sample was collected from volunteers. Out of 14 volunteers in LFD group, 3 subjects have no stool specimens but perform biopsies of intestinal mucosa which are used for analysis of mucosa-associated microbiota. Finally, 15 stool samples from HFD group and 11 from LFD group were obtained. All sample kits were immediately placed in an incubator containing several ice packs and then transported to the laboratory within two hours. Upon arrival in the laboratory, each sample was immediately homogenized in a number of microtubes and frozen at -80°C waiting for microbiota analysis.

In order to visualize the intestinal mucosa and collect colonic mucosal tissue, colonoscopic examination and biopsy were performed. Before the procedure, the subjects were provided with comprehensive health education including procedural information and related potential hazards, and written informed consent was obtained. Final only 24 subjects (13 and 11 for HFD and LFD groups, respectively) gave consent for biopsy. Colonoscopy data showed higher incidence of abnormalities in HFD group including polyps mainly, diverticula and colitis. Most polyps were found under 0.5 cm in diameter and pathologically classified as mixed polyps and low-grade intraepithelial neoplasia (data not shown). Biopsies were obtained from the ascending colon with biopsy forceps. The normal colonic tissue samples were placed into sterile microtubes and then promptly transported to a Thermos bottle containing liquid nitrogen. Finally, the tissue samples were frozen -80°C together with stool samples for microbiota analysis.

Microbiota analysis

DNA extractions. DNA from stool samples and normal colonic mucosal tissue samples, most of which were collected from the same volunteers, was extracted using the Micro Elute Genomic DNA kit (D3096-01; Omega Bio-Tek, Inc., Norcross, GA, USA) according to the manufacturer's instructions. The reagent used to isolate DNA from trace amounts of sample was effective for the preparation of DNA of the majority of bacteria. Sample blanks consisted of unused swabs processed through DNA extraction and were tested to contain no 16S amplicons. The total DNA was eluted in 50 µl of elution buffer by modification of the procedure described by manufacturer (Qiagen, Hilden, Germany) and stored at -80°C until measurement in the PCR by LC-Bio (Hangzhou, China).

PCR amplification and 16S rDNA sequencing. Using total DNA from the samples as a template and the primer (319 forward, 5'-ACTCCTACGGGAGGCAGCAG-3'; 806 reverse, 5'-GGACTACHVGGGTWTCTAAT-3'), we amplified the V3-V4 region of the bacterial 16S rRNA. All reactions were carried out 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 to adjust the volume. PCR reactions were performed in a MasterCycler Gradient Thermal Cycler (Eppendorf, Hamburg, Germany) set to the following conditions: initial denaturation at 98°C for 30 sec; 35 cycles of denaturation at 98°C for 10 sec, annealing at 54/52°C for 30 sec, extension at 72°C for 45 sec; and then final extension at 72°C for 10 min. 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. PCR products were normalized by AxyPrep™ Mag PCR Normalizer (Axygen Biosciences, Union City, CA, USA), which allowed us to skip 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) and with the Library Quantification kit for Illumina (Kapa Biosciences, Woburn, MA, USA), respectively. PhiX Control library (v3) (Illumina, San Diego, CA, USA) was combined with the amplicon library (expected at 30%). The library was clustered to a density of ~570 K/mm². 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 (300 PEs) were overlapped by ~90 bp. The assembled sequences were clustered by using the CD-hit-est based clustering method (24). Software PyNAST (http://qiime.org/pynast/) was used to analyze and calculate the numbers of sequence and operational taxonomic units (OTUs) for each sample. Subsequently, the species abundance and distribution were analyzed followed by cluster analysis. After cluster analysis, the sequences were grouped into various OTUs using Felsentein-corrected similarity matrices such that the sequences within an OTU share at least 97% similarity. The Ribosomal Database Project (RDP) classifier was used to classify the 16S rDNA into distinct taxonomic category by aligning sequences to a curated database of taxonomically annotated sequences. All 16S rDNA sequences were mapped to the RDP database using BLASTN in order to achieve taxonomic assignments. Sequences >97% identity were used to associate a group of OTUs to specific species, while those with <97% identity were considered novel reads. The rank abundance curve was made to roughly assess species abundance and species uniformity. The microbial α diversity in individual stool and colonic mucosal tissue samples was estimated using rarefaction analysis, including four indices: Observed_species, Shannon, Simpson and Chao1 indexes. We used three different algorithms to calculate the distance matrices between the samples: Euclidean, unweighted_unifrac, and weighted_unifrac. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) for clustering of samples was also carried out on Euclidean, unweighted_unifrac and weighted_unifrac matrices. Correspondingly, principal coordinates were
computed for above three distance matrices and used to generate Principal Coordinate Analysis (PCoA) plots. A metagenomic biomarker discovery approach was employed with LEfSe [linear discriminant analysis (LDA) coupled with effect size measurement] which performed 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.

Results

Comparison of richness and diversity of gut microbiota sequencing. To characterize the pattern of microbiota, we performed 16S ribosomal DNA gene sequencing on 50 samples including 26 stool samples and 24 colonic mucosal tissues collected from the HFD and LFD groups. On a whole, 2,799,033 high-quality sequences were generated from usable raw data after optimization process. Eighty-four percent of the sequences were distributed in the interval of 400-500 bp, which corresponded to the V3-V4 region of 16S rDNA gene (Fig. 1A). After CD-HIT clustering, the numbers of OTUs from all samples at 97% similarity level was 486. The Venn diagram shows the similarity of OTU distribution (Fig. 1B). The same OTUs accounted for 76.13%. The most OTUs were included in the colonic mucosal tissues of HFD group. According to species accumulation curve (Fig. 1C), when the number of samples reached more than 20, the number of OTUs tended to be stable at 500, indicating that the 16S rDNA sequences in four types of samples (S-HFD for stools of HFD group, S-LFD for stools of LFD group, T-HFD for colon mucosa tissues of HFD group and T-LFD for colon mucosa tissues of HFD group and T-LFD for colon mucosa tissues of
LFD group) represent the majority of bacteria in the samples of this study.

We then analyzed the microbial diversity within each subject (α diversity) and the difference between each subject's gut microbiota (β diversity). The estimators of 4 α diversity indices (Shannon, Chao1, Simpson and observed_species) are shown in Table II. There were no significant differences in these indices between the groups S-HFD and S-LFD, or between the groups T-HFD and T-LFD. However, as shown in Table II, there were statistically significant differences in classification number of OTUs, observed_species and Chao1 indices between the stool samples and colonic mucosal tissue samples in both groups, demonstrating significantly higher diversity found in colonic mucosal tissues compared to stools. Furthermore, according to observed_species and Chao1 indices, no rarefaction curve plateaued with the current sequences, suggesting more phylotypes would be expected with additional sequencing (Fig. 2). However, two other indices indicated that most diversity had already been captured. To compare the overall microbiota structure in HFD and LFD subjects, β diversity was analyzed using sample clustering and PCoA based on three distance matrices including euclidean, unweighted_uniFrac and weighted_uniFrac (Fig. 3). The results of OTU clustering and PCoA also revealed no significant difference in bacterial structure in stools or colonic mucosal tissues, respectively, but a segregation of stools and colonic mucosal tissues especially on the basis of unweighted UniFrac matrix, as demonstrated by the first three principal component scores which accounted for 15.97, 11.15 and 8.49% of total variations. In addition, LEfSe was performed to obtain the cladogram representation and the predominant bacteria of the microbiota within stool and colonic mucosal tissue,
Table III. List of stool and tissue genera significantly different between HFD and LFD groups.

| Sample  | Phylum    | Family             | Genus       | Relative abundance (%)<sup>a</sup> | HFD     | LFD     | \(\kappa^2\)-value<sup>b</sup> | P-value | Tendency in HFD compared to LFD group |
|---------|-----------|--------------------|-------------|----------------------------------|---------|---------|---------------------------------|---------|--------------------------------------|
| Stool   | Bacteroidetes | Prevotellaceae    | *Prevotella*| 19.05                            | 4.97    | 4.433   | 0.035                           |         | †                                    |
|         | Firmicutes | Aerococcaceae     | *Abiotrophia*| 1.30 \times 10^{-3}             | 0       | 4.302   | 0.038                           |         | †                                    |
|         | Bacteroidetes | S24-7 unclassified |             | 0.19                            | 0.29    | 3.965   | 0.046                           |         | †                                    |
|         | Firmicutes | Ruminococcaceae   | *Gemmiger*   | 0.11                            | 0.52    | 5.616   | 0.018                           |         | †                                    |
|         | Verrucomicrobia | Verrucomicrobiaceae | *Akkermansia*| 0.03                            | 0.19    | 4.447   | 0.035                           |         | †                                    |
|         | Actinobacteria | Micrococcaceae | *Rotia*     | 6.48 \times 10^{-4}        | 1.63 \times 10^{-3} | 4.299   | 0.038                           |         | †                                    |
| Tissue  | Bacteroidetes | S24-7 unclassified |             | 0.1                             | 0       | 5.033   | 0.025                           |         | †                                    |
|         | Bacteroidetes | Bacteroidaceae    | *Bacteroides*| 12.96                           | 29.03   | 5.237   | 0.022                           |         | †                                    |
|         | Bacteroidetes | Porphyromonadaceae | *Coprobacter*| 0                               | 0.05    | 3.863   | 0.049                           |         | †                                    |
|         | Firmicutes | Aerococcaceae     | *Abiotrophia*| 2.48 \times 10^{-4}        | 0.02    | 4.387   | 0.036                           |         | †                                    |
|         | Tenericutes | Anaeroplasmataceae | *Asteroleplasma*| 0                               | 0.72    | 3.863   | 0.049                           |         | †                                    |

<sup>a</sup>Relative contribution of a genus in a group was calculated as percentage of the sequences of this genus to all sequences in this group.  
<sup>b</sup>The \(\kappa^2\)-values were obtained by Mann-Whitney test. HDF, high-fat diet; LFD, low-fat diet.

Figure 3. Two \(\beta\) diversity plots based on three distance matrices OTU clustering on the left and PCoA on the right. OTU, operational taxonomic unit.
which is shown in Fig. 4. The differences in taxa between the HFD and LFD groups are depicted in Fig. 5. *Paraprevotella* was enriched in stool of HFD group, whereas *Alistipes* and *Rikenellaceae* were enriched in the stool of LFD group. *Bacteroidaceae*, *Bacteroides* and *Clostridium XVIII* were enriched in colon mucosa tissue of LFD group.

**Microbial structures of gut in HFD and LFD volunteers.**

We analyzed the stool and colonic mucosal tissue bacterial communities of HFD and LFD volunteers, and assessed the amounts of different phyla and genera using taxonomic assignment of all sequences with RDP Classifier. Including all the groups, there were 11 phyla and 129 genera. The overall microbiota structure for each group at the phylum level is shown in Fig. 6, indicating that the tissue bacteria of LFD subjects covered all phyla. The constituent ratios of stool bacteria at phylum level were significantly different from those of tissue bacteria regardless of HFD group or LFD group (P<0.001), which was consistent with the results of OTU clustering and PCoA.

At the genus level, there were 111 genera in stool microbiota and 117 genera in colonic mucosal tissue microbiota of HFD group, as well as 96 and 117 genera in stool and colonic mucosal tissue microbiota of LFD group, respectively. On comparison of stool genera, HFD group exhibited significantly higher abundance of *Prevotella* and *Abiotrophia* and lower abundance of unclassified genus of *Bacteroidetes*, *Gemmiger*, *Akkermansia* and *Rothia*, while in colonic mucosa, HFD group showed significantly higher abundance of unclassified genus of *S24-7* and a lower abundance of *Bacteroides*, *Coprobacter*, *Abiotrophia*, and *Asteroleplasma* compared to LFD group (Table III).

---

**Figure 4.** Different structures of microbiota between stool and colon mucosa tissue. (A) Taxonomic representation of statistically and biologically consistent differences between stool and colonic mucosal tissue. Differences are represented by the color of the most abundant class (red indicating stool, green representing colonic mucosal tissue and yellow meaning non-significant). Each circle’s diameter is proportional to the taxon’s abundance. (B) Histogram of the LDA scores for differential abundance. Cladogram was calculated by LEfSe, and displayed according to effect size.
The findings of the present study prove that diet has a measurable effect on the composition of gut microbiota. Although the concept of enterotypes as robust clustering of human gut community compositions was largely driven by the abundance of key bacterial genera (25), the enterotypes of HFD populations remains to be elucidated. It is well-known that more than 90% of all the phylotypes at adulthood belong to Bacteroidetes and Firmicutes (26). Each of these enterotypes is identifiable by the variation in the levels of one of three genera: Bacteroides (enterotype 1), Prevotella (enterotype 2) and Ruminococcus (enterotype 3) (27). Wu et al (20) further suggested that microbial enterotypes were significantly affected by long-term diets. They found that the higher intake of proteins and animal fats was associated with higher abundance of the Bacteroides dominant enterotype while the consumption of carbohydrate-rich diet facilitated growth of the Prevotella enterotype. Kelder et al (28) demonstrated that changes in Bacteroidetes and Firmicutes levels correlate with changes in carbohydrate and fat oxidation, respectively. Our data showed that Bacteroidetes and Firmicutes together accounted for 80-90% of the bacterial phyla in stool and colonic mucosal tissue irrespective of the diet composition. However, Bacteroides was the most abundant phyla in stool, whereas Firmicutes occupied the first phyla in colonic mucosal tissue, which has been rarely reported. The abundance of Proteobacteria in colonic mucosa
was higher than that in stool. There has been recent interest in
the potential associations between gut microbiota and obesity
which was thought to be the clinical model of a HFD, in
particular, a decreased proportion of Bacteroidetes in obese
individuals (26,29). Contrastingly, our study detected a higher
abundance of Bacteroidetes in HFD group, most likely as a
HFD does not necessarily indicate obesity, or because our
probes and primers admittedly do not cover all Bacteroidetes
species. Furthermore, due to differences in methodology,
results cannot be compared directly to previous 16S rDNA
based studies. Also, recent work from other researchers could
not confirm the proposed association between lower propor-
tions of Bacteroidetes and obesity (30). Combined with a
relatively higher proportion of colonic disorders, including
colorectitis, polyps, adenomas and carcinoid tumors in HFD
group, our observations are consistent with the hypothesis that
diet mediated differences in gut microbiota may contribute to
the observed increased risk of colorectal diseases in HFD
populations (31,32).

It has been previously shown that an increase in the
proportion of carbohydrates in the Western diet is remarkably
associated with a significantly increased representation of
Prevotella (10,33,34). Mozes et al demonstrated, using fluores-
cent in situ hybridization, that Bacteroides/Prevotella (BAC)
prevalence decreased in the jejunum of obese rats consuming
HFD (35). Agrarian diets are associated with greater microbial
diversity and a predominance of Prevotella over Bacteroides.
In contrast, our findings demonstrated that Prevotella consti-
tuted more than 19% of stool microbiota of HFD volunteers.
Similarly, vervets consuming a Western diet showed a rela-
tively higher abundance of Prevotella (36). These findings
provide the first evidence implying a species-specialization of
the gut microbiota. It is also important to note that Western
and Asian human HFDs vary markedly across populations. In
our study, Chinese high fat diet is mostly related to cooking
oil. However, western HFD mostly contains saturated animal
fat. The daily intake of fat, protein, and carbohydrate by HFD
populations in our study resembled that of the control groups in
other studies based on naïve Western and non-Western popula-
tions, or even lower, with no difference in the proportions of
dietary energy from these three sources between Western and
Asian populations (37). Likewise, the data were not generated
using the same methodology. Our study further showed that
Abiotrophia, which has been associated with infective endo-
carditis (38), was of a higher representative proportion in HFD
populations compared to LFD group. This indicates that a high
fat diet may lead to cardiac events through gut dysbiosis. On
the contrary, there was significantly lower representation of genera
Gemmiger, Akkermansia, and Rothia and an unclassified S24-7
in the feces of HFD subjects. The family S24-7 is a member of
Bacteroidetes, belonging to butyrate-producing bacterium,
which has been shown to be beneficial for digestive health (39).
The decrease of family S24-7 due to a HFD is consistent with
findings of other studies (39-41). Some studies suggested that
Gemmiger was found to be highly abundant, or stimulated,
by prebiotics in the microbiota of subjects with a low BMI
suggesting a potential role in weight loss, and positively
associated with blood levels of LDL cholesterol and glucose
levels (42,43). Akkermansia is a member of the Verrucomicrobia
phylum and capable of degrading mucin (44). A growing body
of research including rodent and human models of obesity has
suggested that Akkermansia spp. abundance in stool negatively
 correlates with the onset of inflammation, altered adipose tissue
metabolism and metabolic syndrome, thus reinforcing the
beneficial role of this bacterium on lipid metabolism (45-47).
Moreover, recent findings indicated that the higher abundance
of Akkermansia muciniphila is also associated with improved
glucose metabolism and lower body mass (48), although this
has not yet been demonstrated in humans. Rothia is most
frequently isolated from the oral cavity and may be associated
with periodontal inflammatory disease (49,50). Several studies
have indicated that this bacterium may be associated with the
occurrence of obesity and obesity-related diseases such as
inflammatory bowel disease (IBD) probably by its shift to the
gut from the oral cavity (51,52).

The effect of a HFD on the mucosa-associated bacterium
community has rarely been reported. Previous studies have
shown that the mucosa-associated microbiota differs from the
composition of the fecal microbiota, which is in agreement
with our study including data of OTU clustering, PCoA and
LeiSe analysis. The mucosa-associated microbiota may be
particularly important for performing the nutrient exchange
at the epithelial interface by converting glycans into short-
chain fatty acids (SCFA) that provide energy for the nearby
colonocytes and other gut epithelial cells. Such mucosa-associa-
ted microbiota are likely to exert a more disproportional
effect than the luminal microbiota on the development of
the local immune system, and provide resistance against the
colonization of pathogenic bacteria at the mucosal epithelial
cells in the gut. In this study, we found that five genera had
significant differences in prevalence between HFD and LFD
groups. Within colonic mucosa, Bacteroides, a member of
dominant genera, significantly declined in HFD compared
to LFD groups. Most species of Bacteroides scattered
among the microbiota-associated phyla degraded the mucin,
which possibly led to the availability of oligosaccharides
for other bacteria that do not have glycolytic enzymes (53).
Several studies have used high-throughput techniques to
study the mucosa-associated microbiota in IBD and IBS
patients (54,55). Interestingly, there is still no consensus on the
changes in Bacteroides abundance in colonic mucosal tissues
of intestinal-related diseases (56-58). However, we believe that
the decrease of Bacteroides on the mucosal layer due to
a HFD may be involved in the perturbation of intestinal
mucosal barrier, thereby promoting the occurrence of IBD or
CRC. The relative abundance of mucosa-associated unclassi-
fied S24-7 increased and genus Abiotrophia decreased in the
HFD group, which may explain the inverse prevalence in stool
samples. Two genera, Coprobacter and Asterooleplasma, not
detectable in the colonic mucosal tissue of HFD populations,
were present in small proportions in colonic mucosa of LFD
subjects. The genus Coprobacter, first described in 2013, is
classified within the family Porphyromonadaceae, the order
Bacteroidales, and the phylum Bacteroidetes, and comprise of
a single species, Coprobacter fastidiosus (59,60). The genera
Asterooleplasmais also rarely reported. The role of these two
genera on human health, to date, remains unknown.

In conclusion, gut microbiota is significantly affected by
dietary habits and HFD may serve as an etiologic factor of
colorectal disorders by altering the fecal and mucosal
microbiota composition especially at genus level. Whether modifying diet or supplementation with probiotics can prevent such diseases remains to be elucidated. Furthermore, since colonic diseases such as CRC and the aforementioned illnesses can change the intestinal microbiota composition themselves as shown by fecal transfer studies (17), further investigations are required to link microbiota composition with carcinogenesis.

Acknowledgements
Not applicable.

Funding
This study was supported by grants from the National Nature Science Foundation of China (nos. 81472262 and 81230057) and the Emerging Cutting-Edge Technology Joint Research Projects of Shanghai (no. SHDC12012106).

Availability of data and materials
All data generated or analyzed during this study are included within the article.

Authors' contributions
LMQ, RYG and HLQ designed the study. LMQ, RYG and JMH collected and analyzed the data. LMH, CP and HL contributed to sample collection and intellectual input. LMQ drafted and wrote the manuscript. RYG and HLQ revised the manuscript critically for intellectual content. All authors gave intellectual input to the study and approved the final version of the manuscript.

Ethics approval and consent to participate
All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional and National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by the Ethics Committee of the Institutional Review Boards of Shanghai Tenth People's Hospital (Shanghai, China) and Jiangyin People's Hospital (Jiangyin, China). Signed informed consents were obtained from the patients or the guardians.

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References
1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J: Cancer statistics in China, 2015. CA Cancer J Clin 66: 115-132, 2016.
2. Jobin C: Colorectal cancer: Looking for answers in the microbiota. Cancer Discov 3: 384-387, 2013.
3. Hold GL: Gastrointestinal microbiota and colon cancer. Dig Dis 34: 244-250, 2016.
4. Rajilic-Stojanovic M, Biagi E, Heilig HG, Kajander K, Kekkonen RA, Timis S and de Vos WM: Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. Gastroenterology 141: 1792-1801, 2011.
5. Ringel Y and Ringel-Kulka T: The Intestinal microbiota and irritable bowel syndrome. J Clin Gastroenterol 49 (Suppl 1): S56-S59, 2015.
6. Sepehr S, Kotlovski R, Bernstein CN and Krause DO: Microbial diversity of inhaled and noninhaled gut biopsy tissues in inflammatory bowel disease. Inflamm Bowel Dis 13: 675-683, 2007.
7. Chang C and Lin H: Dysbiosis in gastrointestinal disorders. Best Pract Res Clin Gastroenterol 30: 3-15, 2016.
8. Bassaganya-Riera J, Viladomiu M, Pedragosa M, De Simone C and Hontecillas R: Immunoregulatory mechanisms underlying prevention of colitis-associated colorectal cancer by probiotic bacteria. PLoS One 7: e34676, 2012.
9. Fukugita MH, Ignacio A, Fernandes MR, Ribeiro Júnior U, Nakano V and Avila-Campos MF: High occurrence of Fusobacterium nucleatum and Clostridium difficile in the intestinal microbiota of colorectal carcinoma patients. Braz J Microbiol 46: 1135-1140, 2015.
10. Conlon MA and Bird AR: The impact of diet and lifestyle on gut microbiota and human health. Nutrients 7: 17-44, 2014.
11. Ridlon JM, Kang DJ and Hylemon PB: Bile salt biotransformations by human intestinal bacteria. J Lipid Res 47: 224-259, 2006.
12. Ou J, DeLany JP, Zhang M, Sharma S and O'Keefe SJ: Association between low colonic short-chain fatty acids and high bile acids in high colon cancer risk populations. Nutr Cancer 64: 34-40, 2012.
13. Nicholson JK, Holmes E, Kinross J, Barcelin R, Gibson G, Jia W and Pettersson S: Host-gut microbiota metabolic interactions. Science 336: 1262-1267, 2012.
14. Shen CL, Purewal M, San Francisco S and Pence BC: Absence of PhIP adducts, p53 and Apc mutations, in rats fed a cooked beef diet containing a high level of heterocyclic amines. Nutr Cancer 30: 227-231, 1998.
15. Butler LM, Sinha R, Millikan RC, Martin CF, Newman B, Gammon MD, Ammerman AS and Sandler RS: Heterocyclic amines, meat intake, and association with colon cancer in a population-based study. Am J Epidemiol 157: 434-445, 2003.
16. Louis P, Hold GL and Flint HJ: The gut microbiota, bacterial metabolites and colorectal cancer. Nat Rev Microbiol 12: 661-672, 2014.
17. Schulz MD, Atay C, Heringer J, Romrig FK, Schwitalla S, Aydin B, Ziegler PK, Varga J, Reindl W, Pommerenke C, et al: High-fat-diet-mediated dysbiosis promotes intestinal carcinogenesis independently of obesity. Nature 514: 508-512, 2014.
18. Yu YN and Fang JY: Gut microbiota and colorectal cancer. Gastrointest Tumors 2: 26-32, 2015.
19. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R and Gordon JI: The effect of diet on the human gut microbiome: A metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med 1: 6ra14, 2009.
20. WuGD, ChenJ, Hoffmann C, Bitterger K, Chen YY, KeilhaufSA, Bewtra M, Knights D, Walters WA, Knight R, et al: Linking long-term dietary patterns with gut microbial enterotypes. Science 334: 105-108, 2011.
21. Williams T: Building health information systems in the context of national strategies for the development of statistics. Bull World Health Organ 83: 564, 2005.
22. Zhao W, Hasegawa K and Chen J: The use of food-frequency questionnaires for various purposes in China. Public Health Nutr 5 (6A): 829-833, 2002.
23. Chiu BC, Ji BT, Dai Q, Gridley G, McLaughlin JK, Gao YT, Fraumeni JF Jr and Chow WH: Dietary factors and risk of colon cancer in Shanghai, China. Cancer Epidemiol Biomarkers Prev 12: 201-208, 2003.
24. Li W and Godzik A: Cd-hit: A fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22: 1658-1659, 2006.
25. Koren O, Knights D, Gonzalez A, Waldron L, Segata N, Knight R, Huttenhower C and Ley RE: A guide to enterotypes of the human microbiome. Science 336: 1262-1267, 2012.
26. Ley RE, Turnbaugh PJ, Klein S and Gordon JL: Microbial ecology: Human gut microbes associated with obesity. Nature 444: 1022-1023, 2006.

27. Amonkar MG, Raes J, Pelletier E, Le Pailler D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM, et al; MetaHIT Consortium: Enterotypes of the human gut microbiome. Nature 473: 174-180, 2011.

28. Kelder T, Stroeve JH, Bijlsma S, Radonjic M and Roesellers G: Correlation network analysis reveals relationships between diet-induced changes in human gut microbiota and metabolic health. Nutr Diabetes 4: e122, 2014.

29. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD and Gordon JL: Obesity alters gut microbial ecology. Proc Natl Acad Sci USA 102: 11070-11075, 2005.

30. Duncan SH, Lobjel GE, Holtop G, Incce J, Johnstone AM, Louis P and Flint HJ: Human colonic microbiota associated with diet, obesity and weight loss. Int J Obes 32: 1720-1724, 2008.

31. Vipperla K and O’Keefe SJ: Diet, microbe, and dysbiosis: A ‘recipe’ for colorectal cancer. Food Funct 7: 1731-1740, 2016.

32. Brown K, DeCoffe D, Molcan E and Gibson DL: Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. Nutrients 4: 1095-1119, 2012.

33. Bleau C, Karelis AD, St-Pierre DH and Lamontagne L: Crossover study on gut microbiota, adipose tissue and skeletal muscle as an early event in systemic low-grade inflammation and the development of obesity and diabetes. Diabetes Metab Res Rev 31: 545-561, 2015.

34. Kovatcheva-Datchary P, Nilsson A, Akrami R, Lee YS, De Vadder F, Arora T, Hallen A, Martens E, Björck I and Bäckhed F: Dietary fiber-induced improvement in glucose metabolism was associated with increased abundance of Prevotella. Cell Metab 22: 971-982, 2015.

35. Mozes S, Bujánková D, Séfčiková Z and Kmet V: Developmental changes of gut microbiota and enzyme activity in rat pups exposed to fat-rich diet. Obesity (Silver Spring) 16: 2610-2615, 2008.

36. Amato KR, Yeomans CM, Cerda G, Schmitt CA, Cramer JD, Miller ME, Gomez A, Turner TR, Wilson BA, Stumpf RM, et al: Variable responses of human and non-human primate gut microbiota to a Western diet. Microbiome 3: 53, 2015.

37. Ou J, Carbonero F, Zootendal EG, DeLany JP, Wang M, Newton K, Gaskins HR and O’Keefe SJ: Diet, microbe, and microbial metabolites in colon cancer risk in rural Africans and African Americans. Am J Clin Nutr 98: 111-120, 2013.

38. Al-Jasser AM, Barczo A and Bassaganya-Riera J: Modeling-enabled systems nutritional immunology. Front Nutr 3: 5, 2016.

39. Yasir M, Angelakis E, Bibi F, Azhar EI, Bachtar D, Lagier JC, Gaborit B, Hassan AM, Jiman-Fatani AA, Alshali KZ, et al: Comparison of the gut microbiota of people in France and Saudi Arabia. Nutr Diabetes 5: e153, 2015.

40. Lee SM, Donaldson GP, Mikulski Z, Boyajian S, Ley K and Mazmanian SK: Bacterial colonization factors control specificity and stability of the gut microbiota. Nature 501: 426-429, 2013.

41. Walker AW, Sanderson JD, Churcher C, Parkes GC, Hudspith BN, Rayment N, Brostoff J, Parkhill J, Dougan G and Petrovska L: High-throughput clone library analysis of the mucosa-associated microbiota reveals dysbiosis and differences between inflamed and non-inflamed regions of the intestine in inflammatory bowel disease. BMC Microbiol 11: 7, 2011.

42. Parkes GC, Rayment NB, Hudspith BN, Petrovska L, Lomer MC, Brostoff J, Whelan K and Sandersen JD: Distinct microbial populations exist in the mucosa-associated microbiota of sub-groups of irritable bowel syndrome. Neurogastroenterol Motil 24: 31-39, 2012.

43. Verma R, Verma AK, Ahuja V and Paul J: Real-time analysis of mucosal flora in patients with inflammatory bowel disease in India. J Clin Microbiol 48: 4279-4282, 2010.

44. Hans W, Schömerich J, Gross V and Falk W: The role of the resident intestinal flora in acute and chronic dextran sulfate sodium-induced colitis in mice. Eur J Gastroenterol Hepatol 12: 267-273, 2000.

45. Yao P, Cui M, Wang H, Gao H, Wang L, Yang T and Cheng Y: Quantitative analysis of intestinal flora of Uygur and Han Ethnic Chinese patients with ulcerative colitis. Gastroenterol Res Pract 2016: 9186322, 2016.

46. Chaplin AV, Efimov BA, Khokhlova EV, Kafarskaia LI, Tupikin AE, Kablov MR and Shkoporov AN: Draft genome sequence of Coprobacter fastidiosus NSB1. Genome Announc 2: 2, 2014.

47. Nakata T, Kyouri D, Takahashi H, Kimura B, Kuda T and Inoue H: Inhibitory effects of laminaran and alginate on production of putrefactive compounds from soy protein by intestinal microbiota in vitro and in rats. Carbohydr Polym 143: 61-69, 2016.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.