Research Article

Effect of Duyun Compound Green Tea on Gut Microbiota Diversity in High-Fat-Diet-Induced Mice Revealed by Illumina High-Throughput Sequencing

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Intake of a high-fat diet (HFD) is closely related to disorders of the intestinal microbiota, which plays a key role in the pathogenesis of obesity. Duyun compound green tea, an ancient Chinese drink, is widely consumed to reduce weight, although the mechanism is not clear. In this study, 50 mice were randomly divided into 5 groups: normal control group (CK), HFD model control group (NK), positive control group with medicine (YK), low-dose compound tea group (DL), and high-dose compound tea group (DH). After 4 weeks of intervention, the feces of mice were taken under sterile conditions and evaluated using Illumina high-throughput sequencing technology. The results showed that the diversity of intestinal microbiota was the highest in the CK group, the lowest in the NK group, and relatively increased in the compound tea treatment group. Second, there were differences in intestinal microbiota among each group, among which the beneficial bacteri in the intestinal tract of the CK group were higher than those in the other groups, while the beneficial bacteria in each compound tea treatment group were more abundant than those in the NK group, in which harmful bacteria in the intestinal tract were found to be the highest. These results suggest that compounds in tea may be able to attenuate imbalances of intestinal microbiota induced by poor diet, acting as a therapeutic agent in obesity or other diseases associated with gut dysbiosis.

1. Introduction

Intestinal microbiota function in a symbiotic microbial ecosystem with the human body, affecting multiple systems in the host, particularly digestion and absorption of food. Regardless of whether the host is in a state of health or disease, multiple physiological and metabolic characteristics are inevitably affected by the gut microbial network [1]. Improvements to the overall intestinal microbial status can be obtained through means such as reducing harmful bacteria such as Enterobacteriaceae, activating certain host genes, regulating metabolism, increasing intestinal butyrate and other short-chain fatty acids (SCFA), reducing abnormal production and absorption of propionate, and reducing the accumulation of protein fermentation products such as hydrogen sulfide and ammonia [2]. These improvements can effectively regulate appetite, aid in food digestion, promote intestinal immunity, increase insulin resistance [3], increase resistance to pathogenic bacteria, train the immune system, strengthen cancer treatment [4–8], mitigate fatty liver, reduce hyperlipidemia, and attenuate metabolic disorder [9–11].

Tea is rich in tea polyphenols, amino acids, tea polysaccharides, and other substances exhibiting characteristics such as antioxidant, antitumor, antiradiation,
2. Materials and Methods

2.1. Materials and Reagents. The compound tea materials utilized for research consist of 30% Duyun ancient tea (Camellia sinensis) leaves, 14% Duyun broadleaf Holly (Ilex latifolia Thunb.), 14% Duyun original tree sweet tea (Rubus suavissimus), 14% leaf of lotus (Nelumbo SP.), 14% wild mint (Menthae Haplocalycis Herba), and 14% honeysuckle (Lonicerae japonicae flos), which were purchased from Guizhou Bishu Technology Service Co., Ltd. (Guizhou, China). Xuezhikang capsules were provided by Beijing Beida Weixin Biotechnology Co., Ltd. (Beijing, China). SPF (specific pathogen free) male Kunming mice were provided by Hunan SJA Laboratory Animal Co., Ltd. (Hunan, China). The high-fat diet was composed of the following ingredients: 1.5% cholesterol, 10.0% lard, 5.0% yolk powder, 0.5% bile salt, and 83.0% conventional feed.

2.2. Sample Preparation. Tea leaves were infused in boiling water with the ratio of 1:10 for 30 minutes; then filtered with two layers of industrial gauze when the tea had cooled slightly. The tea dregs were extracted again for 20 minutes with the ratio of 1:8 using vacuum filtration. Two filtrates were mixed and brought to a certain concentration using a rotary evaporator (R201B; Shanghai Shensheng Technology Co., Ltd.), precooled at −80°C for 12 hours, vacuum freeze-dried for 28 hours, collected as dry powder, then sealed, and stored in a −80°C refrigerator until further use. The extract compounds from the tea leaves were listed in the supplemental data (Table S1).

2.3. Animal Experiments. Fifty 5-week-old male KM mice of SPF grade were housed in a room with a temperature range of 20–26°C and relative humidity of 50%–60%. After 7 days of adaptive feeding, they were randomly divided into 5 groups, with 10 mice in a single cage for each group. Normal group (CK) was fed normal diet and purified water; model group (NK) was fed a high-fat diet (HFD) and purified water; positive group (YK) was fed a HFD along with a lipid-lowering medication called “Xuezhikang” at a dose of 90 mg kg⁻¹·d⁻¹; low-dose group (DL) was fed a HFD and 210 mg kg⁻¹·d⁻¹ of compound green tea extract; and high-dose group (DH) was fed a HFD and 840 mg kg⁻¹·d⁻¹ of compound green tea extract. The doses were chosen based on safety evaluation in preliminary experiment. The medicine and compound green tea extract were fed by gavage to mice. All the food and water were sterile. During the experiment, the activity of mice was observed and body weights were recorded. At the end of the 28-day test, the feces of each mouse were collected aseptically and independently and put into a 1.5 mL sterilized centrifuge tube, labeled, and frozen at −80°C for standby.

2.4. Genomic DNA Extraction and PCR Amplification of Fecal Microbiota. One hundred milligram stool samples with 1.4 mL stool sample lysis buffer (ASL) were prepared after centrifugation. The total DNA of the sample
microorganisms was extracted using the GENEWIZ fecal DNA extraction kit, and the detailed procedure was carried out in accordance with the instructions.

Using 30–50 ng DNA as a template, V3 and V4 regions were amplified with the upstream primer including “CCTACGGGRBGCASCAGKVRVAAKT” sequence and the downstream primer including “GGAC-TACNVGGGTWTCTAAATCC” sequence. Additionally, the PCR product end of 16S rDNA was added with the connector with index by PCR for sequencing by the next generation sequencing [30]. The raw data have been deposited in the NCBI Sequence Read Archive database with the BioProject accession number PRJNA633980.

2.5. Bioinformatics Analysis. The quality of the library was detected using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), and the library concentration was detected using a qubit2.0 fluorometer (Invitrogen, Carlsbad, CA). After the DNA library was mixed, PE250 double-terminal sequencing was carried out according to the instruction manual of Illumina MiSeq (Illumina, San Diego, CA, USA), and the sequence information was read by MiSeq’s own MiSeq control software (MCS). Bioinformatics analysis was performed on the GENEWIZ platform (https://www.genewiz.com.cn/). Briefly, OTUs (operational taxonomic units) and their diversities were recognized by Qiime (1.9.1) and Vsearch (1.9.6); PCoA (principal co-ordinates analysis) and other figures were drawn by R (3.6.3). LDA (linear discriminant analysis) effect size was calculated on LEfSe Online tools.

3. Results

3.1. Sample Sequencing and OTU Cluster Analysis. High-fat diet induced the weight gain of mice (NK group), and high dose of compound tea (DH group) could restore it as the Xuezhikang (YK) did (Table S2 in supplemental data). After high-throughput sequencing based on the Illumina MiSeq platform, a total of 911,515 effective 16S rRNA gene sequences (raw reads) were obtained from 15 samples in 5 groups. Furthermore, three groupings, those of the CK, NK, and DH groups, the samples in each group could not be completely distinguished from one another. However, the effect of the three treatments was not entirely distinguishable.

3.2. Alpha Diversity Analysis of Intestinal Microbial Community. The species diversity of the microbial community is usually expressed by the richness index and diversity index. The larger the value is, the greater the richness will be, while the Shannon index increases with greater species diversity. By analyzing the alpha diversity index, we can measure the species diversity of the samples and draw the box graph of each sample’s Chaol index and Shannon index (Figure 3). Compared with the CK group, the number of OTUs in the other four groups was significantly less. The fecal microbiota richness of tea treatment group indicated that tea was able to significantly change the fecal microbiota richness of mice.

3.3. Principal Coordinate Analysis of Intestinal Microbial Community. Through PCoA analysis (Figure 4), we could see that the samples of the CK group were distinguishable from the other four groups, indicating that the species composition of the non-HFD group was quite different from the other four groups. For the CK and NK groups, the mice samples gathered to form an independent area, and the difference between the groups was greater than differences within the group. For the samples of DL, DH, and YK groups, the samples in each group could not be completely gathered to form an independent area based on sampling difference, but there was a significant distance from the NK group. These data suggest that consumption of Xuezhikang medicine and both doses of tea were able to attenuate HFD-induced alterations in intestinal microbial communities; however, the effect of the three treatments was not entirely distinguishable from one another.

3.4. Cluster Analysis of Intestinal Microbial Community by UPGMA Tree. Through UPGMA tree cluster analysis (Figure 5), it was clear that mice in the CK group were significantly different than mice in all HFD treatment groups. Furthermore, three groupings, those of the CK, NK,
and YK/DH groups, all maintained their own unique cluster branches, indicating that there were significant differences among them. The YK and DH groups overlapped with each other, however, did not overlap with the CK and NK groups, while the DL group overlapped with YK/DH in addition to the NK group, suggesting that the low-dose tea group was less effective attenuator of HFD-induced microbial alterations than YK and high-dose tea treatments.

3.5. The Structure of Microbial Community and Abundance Changes. According to the histogram of microbial community composition at the phylum level, it was found that Firmicutes and Bacteroidetes were the main dominant phyla of fecal microbiota in all mice, although other important phyla such as actinomycetes, proteus, soft wall phylum, and verruca were also detected. The results of comparative analysis of Firmicutes and Bacteroidetes in each group are shown in Figure 6. In the NK group, the content of Firmicutes increased, Bacteroidetes decreased, Proteobacteria increased, and epsilon bacteria decreased compared with all other groups, indicating that HFD-induced obesity caused a change in intestinal microbiota composition. Following low-dose tea treatment, Firmicutes and Bacteroidetes remained the dominant phyla, and the Firmicutes/Bacteroidetes ratio was attenuated to a level insignificantly different from that of the CK group, indicating that low-dose tea treatment could effectively regulate intestinal microbiota communities, restoring microbial imbalances induced by a high-fat diet to a level similar to a non-HFD group. Both the DH and YK groups showed attenuations in the Firmicutes/Bacteroidetes ratio at a level beyond that of the CK group, suggesting not only a reversal of harmful imbalances brought about by HFD but also a net improvement to microbial communities beyond the status quo of HFD-aversion. The microbial community compositions at other categorical levels were shown in supplemental data (Figures S1–S5).

At the species level, the LDA difference analysis of fecal microbiota in CK, NK, YK, DL, and DH mice was compared to the strains with scores greater than 3. The column distribution of LDA values is shown in Figure 7. The cladogram could be found in supplemental data (Figure S6). Only dominant species with significant difference were found in the CK, NK, and DL groups, and the numbers of dominant species with significant difference were 5, 7, and 1, respectively. The dominant species with significant difference in CK were Bacilli, Lactobacillales, Lactobacillaceae, Lactobacillus, and Prevotellaceae; the dominant species with significant difference in NK were Proteobacteria, Deltaproteobacteria, Desulfovibrionaceae, Lachnospiraceae, Ruminiclostridium, and Desulfovibrio; the dominant species with significant difference in DL was Proteobacteria.

4. Discussion

Intestinal microbiota are able to form symbiotic relationships with the host and play a key role in host homeostasis. In turn, homeostatic stability has an important

| Sample  | Raw reads | Final reads | OTUs |
|---------|-----------|-------------|------|
| CK1-FB  | 41105     | 31931       | 197  |
| CK2-FB  | 53507     | 43568       | 214  |
| CK3-FB  | 66881     | 51034       | 223  |
| DH1-FB  | 56185     | 42320       | 187  |
| DH2-FB  | 59501     | 46491       | 192  |
| DH3-FB  | 59414     | 49647       | 189  |
| DL1-FB  | 43549     | 39312       | 186  |
| DL2-FB  | 75001     | 61896       | 184  |
| DL3-FB  | 76483     | 65921       | 180  |
| NK1-FB  | 61254     | 49908       | 169  |
| NK2-FB  | 52906     | 44321       | 188  |
| NK3-FB  | 60807     | 55289       | 189  |
| YK1-FB  | 50510     | 42059       | 180  |
| YK2-FB  | 76126     | 62163       | 201  |
| YK3-FB  | 78286     | 61479       | 199  |
impact on key physiological functions of the body. In this study, the microbial community and structure in the feces of mice were detected by the high-throughput sequencing technology of Illumina. It was found that there were some differences in species richness and diversity among different treatment groups.

![Rarefaction curves](image)

**Figure 2:** Rarefaction curves in the figure show the number of sequences per sample and the ordinate is the number of OTUs. The results showed that the increasing speed and trend of new species were observed with the increase of sequencing depth.

![Box plots](image)

**Figure 3:** Chao1 index and Shannon index indicate species diversity of the samples. The box chart represents the minimum value, the lower quartile, the median, the upper quartile, and the maximum value from the bottom to the top.

In the results of intestinal microbiota diversity analysis, the OTU number of mice in each group was different. The intestinal microbiota diversity of the CK group was the highest, the YK group was second, the DH group was also relatively high, and the NK group was the lowest. These results indicated that a high-fat diet
Figure 4: PCoA diagram of samples at the genus level of intestinal microbiota.

Figure 5: UPGMA tree diagram of samples.
reduced the intestinal microbial diversity of mice, while
the drug Xuezhikang and high-dose tea extract had a
significant effect on the recovery of intestinal microbiota
of mice. In the study of alpha and beta diversity, it was
found that the CK group had the highest abundance and
diversity of intestinal microbiota, the NK group had the
lowest, and DL and DH groups had similar diversity of
intestinal microbiota. The low and high doses for mice
were set to be 5 and 20 times of the recommended amount
for adults, respectively. The recommended amount of
dried compound tea leaves for adults is 9 g/day. We
usually put 3 g of dry tea in a tea pot and infuse it several
times with boiling water, so it is equivalent to making
three pots of the compound tea.

![Figure 6: Structure of the microbial community and abundance changes at phylum level. The abscissa in the figure is the sample component, and the ordinate represents the sample richness. Different bacterial groups are represented by different colors.](image)

![Figure 7: LEfSE diagram of the significant taxonomies between groups. The vertical coordinate is the species groups with significant differences between groups, and the horizontal coordinate is the bar graph to show the LDA difference analysis of each species group. The scores (LDA value is greater than 3) are sorted according to the scores, so as to describe their differences in different groups of samples. The longer the length is, the more significant the difference will be. The different colors of the bar chart indicate the sample groups with higher abundance.](image)
Firmicutes and Bacteroidetes are the two most abundant bacteria in the human intestine [31], which jointly promote host energy absorption and storage. The relative abundance of Firmicutes has a significant positive correlation with obesity, while Bacteroidetes has a significant negative correlation [32–34]. Generally, the ratio of Firmicutes to Bacteroidetes (F/B value) is used as an index to measure obesity, and the F/B value of obese individuals is higher than that of normal individuals [32], and current research suggests that the internal environment of intestinal microbiota is an important factor affecting obesity and fat accumulation [18]. This index is similar in human and mice. For example, researchers detect the effects of Pu-erh tea both in mice and humans and get consistent results [35]. This study found that Firmicutes was highest in the NK group and lowest in the DH group, while Bacteroidetes was lowest in the NK group, and the F/B value was highest, indicating that the high-fat diet caused intestinal microbial imbalance and that supplementation with tea was able to normalize that imbalance to a certain degree.

Intestinal microbiota are also closely related to host metabolism. Butyrate is the most important nutrient for intestinal cell renewal. The increase of butyrate induced by host genes can improve insulin response and reduce the risk of type 2 diabetes [36]. In this study, the trend of relative abundance of Lachnospiraceae was the same as similar studies. In addition, the composition of the intestinal microbiota in patients with spondyloarthropathy is related to the decrease in Dorea in the genus Trichophyton [37]. There is a characteristic bacterial interaction network in intestinal microorganisms. In this study, Ruminococcus was associated with metabolic disorders, and its relative abundance in intestinal tract of obese hosts was high [38, 39]. Relevant studies have shown that the disorder of interaction network between trichosphirillaceae and Ruminococcaceae can cause Crohn’s disease (CD) and ulcerative colitis (UC), while adhesive invasive Escherichia coli (AIEC) can utilize the inflammation caused by Salmonella and colonize stably in the intestine, and AIEC colonization can promote colon fibrosis [40, 41]. Vibrio desulfuricans (Desulfovibrio) is an intestinal pathogenic bacterium that can produce endotoxin. The increase of Proteobacteria will cause intestinal immune disorders and lead to intestinal inflammation, which is consistent with the results of the detection of dominant bacteria in mice intestinal microbiota induced by high-fat diet in this study. It was rarely or not found in other groups.

To sum up, compound tea could increase the abundance and diversity of intestinal microbiota in mice, which had a positive effect on the regulation of intestinal microbiota disorder caused by high-fat diet. Compound tea increased the number of beneficial microbiota to a certain extent and inhibited the growth of the relevant pathogenic bacteria. These results provide useful information for the treatment of hyperlipidemia.

**Data Availability**

The data that support the findings of this study are openly available in the GenBank of NCBI at https://www.ncbi.nlm.nih.gov, reference number: PRJNA633980.
Evidence-Based Complementary and Alternative Medicine

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