Effects of Litchi on Systemic Low-Grade Inflammation and the Structure and Function of Gut Microbiota in HFA Mice

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Abstract

Background: Certain foods are known as “heating” foods in Chinese medicine as over consumption of them can lead to symptoms known as “heating up”, which have been shown to be symptoms of systemic low-grade inflammation. However, the mechanism by which these foods cause inflammation is not clear. In this study, we investigated dysbacteriosis of gut microbiota as a possible cause of inflammation by litchi, a typical “heating” food. Human flora-associated (HFA) mice model was constructed by first sterilizing their guts by oral administration of antibiotics, and then transplantation of fecal microbial suspension from a healthy human adult. After gavaging the mice with litchi powder suspension at low, medium and high doses (400, 800, 1600 mg/kg·d⁻¹ respectively) for 7 days, the serum level of inflammatory cytokines, gut microbiota and the integrity of intestinal mucosal barrier were measured.

Result: The intervention of litchi significantly increased the diversity of mice gut flora. The ratio of Bacteroidetes/Firmicutes and the abundance of Actinobacteria increased significantly. At the genus level, the abundance of *Phascolarctobacterium*, *Akkermansia*, *Megasomonas* and *Lactobacillus* generally decreased, while the abundance of *Prevotella* and *Bacteroides* increased significantly, especially in the high litchi dose group. The abundance of *Bilophila* increased significantly only in high dose group. litchi intervention caused serum TNF-α level to increase by more than two times and LPS level to double, but a decrease in IL-1β and IL-6 levels. Medium and high dose litchi intervention caused a widening of intestinal epithelial cell junction complex, and general weakening of the intestinal mucosal barrier as well as reduced efficiency of gut microbiota in energy conversion.

Conclusion: This study confirmed the notion in Chinese medicine that as a “heating” food, litchi when consumed excessively can lead to low degree systematic inflammation and demonstrated that this is linked to its ability to cause dysbacteriosis of the gut microbiota and weakening of the intestinal mucosal tissues.

Introduction

Certain foods, such as litchi, longan, mango, durian, orange, chili, pepper, etc., are known as “heating” food in traditional Chinese medicine. Excessive consumption of “heating” foods by some people can cause a number of disorders such as red and swollen eyes, acne, sores and ulcers in the mouth and tongue, swollen gums, sore throat, yellow urine, constipation and other symptoms, which are known as “shanghuo” (heating-up) in Chinese medicine [1-3]. Studies have shown that “heating” foods such as litchi and citrus cause “shanghuo” mainly through systemic low-grade inflammatory reactions caused by some macromolecular substances in food, but the exact mechanism is not clear [4-6]. Systemic low-grade inflammation is a non-specific and persistent pathological state of inflammation in the body. It is mainly manifested as significantly elevated levels of inflammatory markers such as local inflammation or TNF-α in the blood that lasts for a long period of time [7]. In recent years, a large number of studies have shown that systemic low-grade inflammation has a direct causal relationship with the occurrence and

Loading [MathJax]/jax/output/CommonHTML/jax.js eases in human such as cancer, diabetes, liver diseases,

inflammatory bowel disease, irritable bowel syndrome, allergies, asthma, autism, depression, alzheimer's disease and aging^[7-9]. It has become one of the focal issues in the field of medicine, health and nutrition.

There are over 1,000 species of microorganisms in gut flora, which contained more than 100 times as many genes as the human body^[10]. Gut microbiota are thus dubbed as "the body's second gene pool", and believed to be vital to human health^[7]. In recent years, owing to the breakthrough in molecular biology techniques for studying gut microbiota, research into the relationship between gut microbiota and systemic low-grade inflammatory chronic diseases has received increasing attention. Previous etiological studies showed that gut microbiota disorders are one of the most important driving forces to induce many chronic low-grade inflammatory diseases such as obesity, diabetes, inflammatory bowel disease, depression and alzheimer's disease^[7, 8, 9, 11, 12]. These findings have pointed to the hypothesis that gut microbiota might be the missing link between "heating" foods and "shanghuo", and modification of gut microbiota by "heating" foods could be the underlying mechanism for such foods to cause systemic low-grade inflammatory symptoms. In this study we tested this hypothesis by investigating the effect of litchi, a typical "heating" food, on human gut microflora and low-grade inflammation indicators in human flora-associated (HFA) mice model. litchi is a delicious fruit widely grown and consumed in many parts of America, Africa and Asia, It is known as a typical "heating" food in Chinese medicine, and thus a good representative of "heating" foods. The overall aim of this study is to understand the relationship between "heating" foods such as litchi and gut microbiota, whereby elucidating the mechanism by which such foods can cause low-grade inflammation symptoms or "shanghuo". The findings of the study could provide a theoretical basis for the prevention and control of such disorders.

Materials And Methods

1.1 Material

1.1.1 Experimental animals

Specific pathogen free (SPF), 6-week old female C57BL/6J mice were purchased from Beijing Huafukang Biotechnology co., LTD. (Beijing, China) and the license batch number of the animals was SCXK (Beijing) 2016-0002. The animal experiments were conducted in strict accordance with the regulations on animal experimentation of Guangdong Ocean University and the experimental procedures were approved by the University's Ethics Committee on Experimentation with Animals (GDOU-20190724). The experimental animals were raised in Guangdong Ocean University's Laboratory Animal Center (License No. SYXK 2014-0053). The mice were fed with adequate supply of Co60 irradiation-sterilized feed with the nutrient composition according to the Chinese standard GB 14924.3-2001 and sterile water under controlled environment: temperature 20-25°C, relative humidity 40-70%, pressure gradient 20-50 Pa, one-way flow of fresh air, and 12 h light-dark cycle. Water bottle, cage and pad materials used were sterilised by high-pressure steam. Water bottles and pad materials were changed three times a week.

1.1.2 Materials, chemicals and reagents

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Food grade litchi extract powder was purchased from Shenzhen Antai Biotechnology Co., Ltd (Shenzhen, China). Metronidazole (purity $\geq 99\%$) was from Dalian Meilun Biotechnology co., LTD (Dalian, Liaoning Province, China); neomycin sulfate from Beijing Jintai Hongda Biotechnology co., LTD (Beijing, China); vancomycin (purity $\geq 98\%$) from Hefei Bomei Biotechnology co., LTD (Hefei, Anhui Province, China); microplate quantitative chromogenic matrix limulus kit from Xiamen Limulus Reagent Biotechnology co., LTD (Xiamen, Fujian Province, China); mouse TNF- α , IFN- γ , IL-1 β and IL-6 ELISA kits from Neobioscience Technology co., LTD (Shenzhen, China).

1.2 Treatment of experimental animals

1.2.1 Raising and grouping of human fecal associated mice

The raising of human fecal associated (HFA) mice followed the procedure of Hirayama K, et al (1999) with minor modifications^[13]. Briefly, the experimental mice were firstly acclimated for one week under the feeding conditions described above to ensure that they adapted to the feeding environment and reached the same base line of gut microbiota, After acclimation, the mice were gavaged a mixture of antibiotics (vancomycin 400 mg/kg·d⁻¹, neomycin 400mg/kg·d⁻¹, and metronidazole 400 mg/kg·d⁻¹) for 3 days to obtain germ free mice.

Fresh feces were collected from a healthy volunteer (male, 19 years old, without digestive tract or metabolic diseases and had not taken antibiotics in the previous 3 months) for the first bowel movement in the morning. Under the condition of anaerobic asepsis, the mass was measured, and 0.1m PBS buffer was added to dilute the content in a mass/water ratio of 1:9. The mixture was stirred to break up the fecal mass and then vortexed for 2 min to obtain a homogenous suspension, which was stood for 10 min, and the supernatant was collected as the human fecal microbial suspension. Twelve of the germ free mice, obtained as described above, were gavaged with 0.3 ml of the fecal suspension once every other day for three weeks to allow the microbiota to colonize the intestinal tract of the mice. The mice obtained were regarded as human fecal associated mice (HFA)^[13].

The 12 HFA mice were randomly divided into four groups with three mice in each group. The first three groups were gavaged with litchi powder solutions at the concentration of 400 mg/kg·d⁻¹, 800 mg/kg·d⁻¹ or 1600 mg/kg·d⁻¹ daily alongside normal feeding for 7 days, and were regarded as low, medium and high dose groups, respectively. The fourth group was gavaged with sterile water instead of litchi solution as control.

1.2.2 Collection of mice blood and fecal samples

Mice blood (about 1 ml) collected by eyeball extirpating was centrifuged at 1200×g and 4°C for 5 min, the supernatant taken, and the serum samples were stored at -80°C until use. For collection of fecal sample, mice were massaged in the abdomen, and fresh fecal particles were collected into sterile centrifuge tubes which were immediately covered with ice bath before being stored at -80°C until use.

1.3 Measurement of inflammatory markers

Mice TNF- α , IFN- γ , IL-1 β and IL-6 in serum samples were used as inflammatory markers and were measured by respective ELISA kits according to the manufacturer's instructions. Concentration of lipopolysaccharides (LPS) was determined by the microplate quantitative chromogenic matrix limulus kit according to manufacturer's instructions.

1.4 Gut microbiota DNA extraction

Total gut bacterial genomic DNAs were extracted from fecal samples using the PowerMax (stool/soil) DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA), following the manufacturer's instructions, and stored at -20°C prior to further analysis. The quantity and quality of extracted DNAs were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively.

1.5 16S rDNA amplicon pyrosequencing

PCR amplification of the bacterial 16S rRNA genes V4 region was performed using the forward primer 515F (5'- GTGCCAGCMGCCGCGGTAA -3') and the reverse primer 806R (5'- GGACTACHVGGGTWTCTAAT -3'). Sample-specific paired-end 6-bp barcodes were incorporated into the TrueSeq adaptors for multiplex sequencing. The PCR components contained 25 μ l of Phusion High-Fidelity PCR Master Mix, 3 μ l (10 μ M) of each forward and reverse primer, 10 μ l of DNA template, 3 μ l of DMSO, and 6 μ l of ddH₂O. Thermal cycling consisted of initial denaturation at 98 °C for 30 s, followed by 25 cycles consisting of denaturation at 98 °C for 15 s, annealing at 58 °C for 15 s, and extension at 72 °C for 15 s, with a final extension of 1 min at 72 °C. PCR amplicons were purified with Agencourt AMPure XP Beads (Beckman Coulter, Indianapolis, IN) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, amplicons were pooled in equal amounts, and pair-end 2×150 bp sequencing was performed using the Illumina NovoSeq6000 platform at GUHE Info technology Co., Ltd (Hangzhou, China).

1.6 Sequence analysis

The Quantitative Insights Into Microbial Ecology (QIIME, v1.9.0) pipeline was employed to process the sequencing data, as previously described ^[14]. Briefly, raw sequencing reads with exact matches to the barcodes were assigned to respective samples and identified as valid sequences. The low-quality sequences were filtered through the following criteria ^[15]: sequences that had a length of <150 bp, sequences that had average Phred scores of <20, sequences that contained ambiguous bases, and sequences that contained mononucleotide repeats of >8 bp. Paired-end reads were assembled using Vsearch V2.4.4 (-fastq_mergepairs-fastq_minovlen 5). Operational taxonomic unit (OTU) picking using Vsearch V2.4.4 included Dereplication (-derep_full length), cluster (-cluster_fast,-id 0.97), detection of chimeras (-uchime_ref) ^[16]. A representative sequence was selected from each OTU using default

parameters. OTU taxonomic classification was conducted by VSEARCH searching the representative sequences set against the greengenes database.

An OTU table was further generated to record the abundance of each OTU in each sample and the taxonomy of the OTUs. OTUs containing less than 0.001% of total sequences across all samples were discarded. To minimize the difference of sequencing depth across samples, an averaged, rounded rarefied OTU table was generated by averaging 100 evenly resampled OTU subsets under the 90% of the minimum sequencing depth for further analysis.

1.7 Bioinformatics analysis

Sequence data analyses were mainly performed using QIIME and R packages (v3.2.0). OTU-level alpha diversity indices, ACE metric (abundance-based coverage estimator), PD whole_tree, Shannon diversity index, and Simpson index, were calculated using the OTU table in QIIME.

OTU-level ranked abundance curves were generated to compare the richness and evenness of OTUs among samples. Beta diversity analysis was performed to investigate the structural variation of microbial communities across samples using UniFrac distance metrics^[17, 18] and visualized via principal coordinate analysis (PCoA), nonmetric multidimensional scaling (NMDS)^[19].

Principal co-ordinates analysis (PCoA) was conducted based on the genus-level compositional profiles^[19]. Venn diagram was generated to visualize the shared and unique OTUs among samples or groups using R package “VennDiagram”, based on the occurrence of OTUs across samples/groups regardless of their relative abundance^[20]. Taxa abundances at the phylum, class, order, family, genus and species levels were statistically compared among samples or groups by Kruskal test from R stats package. Microbial functions were predicted by PICRUSt (Phylogenetic investigation of communities by reconstruction of unobserved states), based on high-quality sequences^[21]. The output file was further analyzed using Statistical Analysis of Metagenomic Profiles (STAMP) software package v2.1.3^[22]. FAPROTAX is a database that maps prokaryotic clades (e.g. genera or species) to established metabolic or other ecologically relevant functions^[23].

1.8 Hematoxylin and eosin (H&E) staining of intestine tissues

At the end of the experimental period, the mice were sacrificed and dissected. The last 5 cm of the intestine was cut off and then sliced longitudinally. The specimen was immediately immersed in 10% formalin, and then stained with H&E. Sections of the specimen were examined under a light microscope and assessed for histological damage. At least 3 sections from each animal were examined.

1.9 Statistical analysis

Results were presented as means with standard error of the mean (SEM), analyzed by SPSS 21.0 (SPSS, Chicago, IL, USA). Comparisons between groups were made by one-way analysis of variance (ANOVA)

followed by LSD test, with $p < 0.05$ considered significant.

Results

2.1 Effect of litchi on inflammatory factors in HFA mice

Gavaging HFA mice with litchi caused significant changes on the four inflammatory markers, TNF- α , IFN- γ , IL-1 β and IL-6, but the effect on the markers were quite different. Litchi exhibited a dose-related effect on TNF- α . The level of TNF- α in the low litchi dose group was not significantly different from that of the control, but the level in the medium and high dose mice was significantly higher than the control ($p < 0.05$) (Fig. 1a). The level of TNF- α in the high litchi dose group was more than 2 times higher than that in the control mice group. Gavaging litchi to HFA mice at all three doses caused a significant ($p < 0.05$) decrease in the IL-1 β concentration compared with the control, but the three dose groups did not show a significant difference among themselves ($p > 0.05$) (Figure 1c). The IL-6 level in the high litchi doses group was significantly lower than that in the control, as well as those in the low and medium dose groups ($p < 0.05$) but the latter two groups showed no significant difference between themselves ($p < 0.05$) (Figure 1d). No significant difference ($p > 0.05$) in the IFN- γ level was observed between the litchi gavaged HFA mice and the control (Figure 1b).

The plasma LPS levels in the different group of mice are shown in Fig. 2. The LPS levels in the low-dose and medium-dose litchi groups were higher than the control, but the differences were not statistically significant, while plasma LPS concentration of the litchi high-dose group was 106.12% higher than the control significantly ($p < 0.05$).

2.2 Effect of litchi intervention on the diversity of mice gut microbiota

2.2.1 Effect of litchi intervention on alpha diversity

Table 1 shows that goods coverage values in all the experimental groups were above 99.00%, and value in the control group was above 99.88%, which met the requirements. This demonstrates that the data volume of the sequencing was sufficient, and the sequencing results can appropriately represent the real situation of the sample. As shown in Table 1, both the Shannon and Simpson indices in the low, medium and high-dose litchi groups were significantly ($p < 0.05$) higher than those in the control group ($p < 0.05$). Furthermore, Chao1 and Ace indices for the three litchi groups were about 39-80% and 32-82% higher than the control, respectively. These results show that both the abundance and diversity of the gut flora in the litchi groups were significantly higher than the control mice.

Tab. 1
Effect of litchi on alpha diversity of gut microbiota in mice

Litchi dose	Shannon	Simpson	Chao1	Ace	Goods coverage
Low	5.475187	0.936068	959.0170	951.4314	0.992366
Medium	5.639477	0.954772	860.4412	812.6894	0.997877
High	5.703059	0.932552	1115.216	1118.703	0.991044
Control	4.181543	0.759437	616.6304	614.5490	0.998882

2.2.2 Effect of litchi intervention on OTU of mice gut microbiota

A Venn diagram was plotted (Fig. 3) which shows the common and unique OUT among the four groups of mice (low, medium and high dose of litchi, and control). Of the >1300 observed OTUs, 243 were common to all groups. The common number of OTUs between the control group and the low, medium and high dose group was 353, 342, 274, respectively. 784 specific OTUs showed in the low-dose group while 64 showed in the control group; 663 specific OTUs showed in the medium-dose group while 75 showed in the control group; 227 specific OTUs showed in the high-dose group while 143 showed in the control group. The results indicated that litchi intervention caused significant increases in the species richness ($p<0.05$) and greater increases occurred with low and medium doses of litchi intervention than high dose.

2.2.3 Effect of litchi intervention on the β -diversity of gut microbiota in mice

β -Diversity estimates of the gut microflora of the different mice groups were calculated by computing unweighted UniFrac and visualized by principal coordinates analysis (PCoA) (Fig. 4). The distances between the control and all three litchi dose groups were very large, while the distances between the three litchi dose groups were quite small. The results indicate that litchi intervention at all three doses caused significant changes in the β -diversity of gut flora of mice compared with the control ($p<0.05$).

2.3 Effect litchi intervention on gut flora of mice at phylum and genus level

The gut microbiota of the mice was analyzed at the phylum level (Fig.5). The gut microbiota of all the mice groups were dominated by four phyla, Bacteroidetes, Firmicutes, Proteobacteria and Verrucomicrobia, which together accounted for more than 98% of the gut microflora. litchi intervention significantly altered the composition of the gut microbiota. The abundance of Bacteroidetes and Proteobacteria increased by about 27-40% and 1-38%, respectively, in the litchi intervention groups compared with the control, with greater increases occurred in the low litchi dose group. On the other hand, litchi intervention caused Firmicutes and Verrucomicrobia to decrease in abundance by 11-47% and 11-71%, respectively compared with the control. The abundance of two minor phyla, Actinomycete and

Fusobacteria also changed significantly as a result of litchi intervention, with the former increased by 4.4-36.4 times while the latter decreased by 8.7-297.9 times, compared with the control.

Figure 6 compares the gut microbiota of mice with different treatments at genus level (Fig.6). *Bacteroides* is the most dominant genus in all the mice groups, but several other genera, including *Akkermansia*, *Bilophila*, *phascolarctobacterium*, were also present in significant proportions. Gavaging of mice with litchi had a significantly impact on the composition of gut microflora of the mice at the genus level. For some genera, litchi intervention resulted in significant increases in their abundance. This included *Bacteroides*, whose proportion in the gut microflora was 61.39%, 42.92% and 49.39% in the low, medium and high dose litchi groups, which were significantly higher than the control group at 26.55%, while the proportion of *Bilophila* was 3.4 times higher in the high-dose litchi group than in the control group. Other genera, such as *Prevotell* also saw major increases in their abundance as a result of litchi intervention. *Prevotell* was undetected in the control mice, but accounted for 0.56%, 1.07% and 0.24% of the gut microflora, respectively, in the low, medium and high dose litchi groups, On the other hand, the abundance of some genera, including *Akkermansia* *Phascolarctobacterium*, *Megamona* and *Lactobacillus* generally decreased as a result of litchi intervention. The abundance of *Akkermansia* was 3.4, 2.9 and 3.3 times lower and the abundance of *Phascolarctobacterium* was 1.5, 2.8 and 3.1 times lower in the low, medium and high dose groups, respectively, than the control group ($p<0.001$). The abundance of *Megamonas* decreased by 99.3 and 7.1 in the low and high-dose groups, (but increased by 72.9% in the medium dose group), while the abundance of *Lactobacillus* decreased by 4.7 and 2.1 in the low and high-dose groups, (but increased by 74.0% in the medium dose group), respectively, compared with the control group ($p<0.001$).

2.4 Functional and metabolic PICRUSt analysis and Faportax analysis of gut microbiota

PICRUSt analysis of the metabolic pathways showed that with low-dose litchi intervention in the mice, the ether lipid metabolism and the fatty acid elongation in mitochondria were down-regulated (Fig. 7). With medium dose litchi intervention, the synthesis of restriction enzymes for the synthesis of secondary metabolic functional proteins as well as lipid metabolism was up-regulated, but ether lipid metabolism was down-regulated. With high-dose litchi intervention, the phosphate transferase system and ether lipid metabolism in lipid metabolism were down-regulated ($p<0.05$).

Fig.8 shows the results of the Farportax functional analysis. Low, medium and high-dose litchi intervention led to significant up-regulation of the pathogenic bacteria related to human diarrhea in the gut microflora of the mice, while medium and high doses of litchi intervention also resulted in significant increases in the total number of human pathogenic bacteria.

The abundance of gram-negative bacteria in gut microflora was also analyzed but no significance differences were observed between the control and the three litchi invention groups of mice ($p>0.05$) (Fig.9).

2.5.1 Changes in the intestinal permeability

Histological analysis of the intestinal tissue cross sections of mice (Fig. 9) shows that litchi intervention had a notable impact on the colon tissue structure. In the control and low-dose litchi groups, the epithelial cells were whole and tightly packed, and the structural gap of the connective complex between the epithelial cells was normal. However, in the mid- and high-dose litchi groups, the colonic mucosal epithelial cells were loose and not tightly packed, and the gap between the epithelial connective complex was widened, and the epithelial cells had sparse microvilli. The mid- and high-dose litchi groups also showed some degrees of pathological lesions including superficial epithelial damage, colonic crypt hyperplasia, goblet cell depletion, and thickening of the muscularis propria (Fig. 10).

Discussion

This study was conducted to investigate the interrelations between foods, systematic low degree inflammation and gut microbiome with the aim of elucidating the mechanism by which foods such as litchi, which is a typical “heating” foods in Chinese medicine, affect human health. To achieve this goal, we constructed a mice model where their gut were first sterilized by administration of antibiotics, and then transplanted with fecal microbial suspension from a healthy human adult. When the mice were given a high-dose of litchi extract, the levels of serum TNF- α , a typical inflammatory cytokine, and LPS were more than two times higher than that in the control mice. It is generally believed that when the serum level of TNF- α increases by 2-4 times of the normal level, systemic low-grade inflammation occurs [24]. Thus, a clear link between excessive consumption of litchi and systematic low degree inflammation was found. However, litchi intervention of the mice caused a significant decrease in serum IL-1 β and IL-6 levels. Increased production of IL-1 β have been reported to be associated with several autoinflammatory disorders [25] while IL-6 is linked with the pathogenesis of a number diseases such as rheumatoid arthritis, cancer, multiple sclerosis, anemia, inflammatory bowel disease, Crohn’s disease, and Alzheimer’s disease [26]. In this regard, the consumption of litchi may also confer a number of health benefits through the lowering of IL-1 β and IL-6 levels in the body. Thus, the relationship between litchi invention and health appears to be complex and may be related to the intervention dosage.

Inflammation is a double-edged sword for the health of the body. Moderate inflammation is important for the body's own defense, but excessive or persistent systemic inflammation can have adverse effects, leading to a variety of chronic diseases [9-10]. There is growing evidence that the disorder of gut microbiota (dysbacteriosis) may play a key role in the development of chronic inflammatory diseases [7, 9, 12, 27, 28]. For example, Ridaura et al. (2013) and Dao et al. (2016) found that the proportion of Firmicutes to Bacteroides and the abundance of *Oscillibacter*, *Clostridium* and *Akkermansia muciniphila* in the gut microflora were associated with the systemic inflammation induced by obesity [27-28]. Rosen et al. (2017) reported that inflammatory bowel disease was related to the decreases in the abundance of microbes with anti-inflammatory potential (such as *Bifidobacterium* and *Lactobacillus*), and increases in the abundance of pathogenic bacteria (such as *Staphylococcus aureus* and *Clostridium difficile*) [12]. However,

there have been relatively few studies on the role of food in causing the disorder of gut microbiota and its association with systemic low-grade inflammation. Most of the studies are focused on the well-known unhealthy foods or food components such as high-fat diet, white bread, saturated fat, emulsifiers and other ingredients^[10, 28-31]. This study is among the first to examine the possible role of seemingly healthy foods such as litchi, a delicious fruit, in causing dysbacteriosis and inducing low grade systematic inflammation. It was found that litchi intervention significantly increased the diversity and species richness of the gut microbiota of mice, but the effect was much greater with low litchi dose than high dose. Litchi intervention also significantly altered the composition of gut microflora both at the phylum and genus levels. With litchi intervention, the abundance of *Phascolarctobacterium*, *Akkermansia*, *Megasomonas* and *Lactobacillus* generally decreased significantly, while the abundance of *Prevotella* and *Bacteroides* increased significantly. The effect was especially obvious in high-dose group. *Phascolarctobacterium*, *Akkermansia*, *Megasomonas* and *Lactobacillus* have all been reported to reduce inflammatory and play a beneficial role in the control of inflammation^[32]. A significant reduction in the abundance of all four species is likely to increase the risk of inflammation. Furthermore, it has been suggested that an increase in the abundance of *Bilophila* and *Prevotella* may also lead to increased risk of intestinal inflammation^[33-34]. Thus, the decreases in the abundance of *Megasomonas* and the increase in *Bacteroides* and *Bilophila*, especially in the high-dose group, might be linked with the systemic low grade of inflammation caused by the overconsumption of litchi.

There has been considerable research into the mechanisms by which dysbacteriosis of gut microbiota induce systemic low-grade inflammation, and the structural integrity of the intestinal barrier is generally believed to play an important role in the process^[35]. Dysbacteriosis of gut microbiota can induce abnormal apoptosis of intestinal epithelial cells, alter cytoskeletal structure, and affect specific tight junction proteins of the intestinal tract^[36]. This in turn causes changes in the permeability of the intestinal barrier, leading to uncontrolled entry of intestinal bacteria and their products into a sterile internal environment, which activates the inflammatory response and promote the release of inflammatory factors such as TNF- α , with resultant systemic chronic inflammation. In this study, it was found that litchi intervention, especially at the medium and high doses, caused visible increases in the gap of the intestinal epithelial junction complex, and a number of other pathological damages to epithelial tissues, which may allow entry of intestinal bacteria and their products, whereby inducing an inflammatory response. Furthermore, with high litchi dose, diarrhea and related pathogenic bacteria as well as the total number of pathogenic bacteria in the gut microbiota of mice were significantly up-regulated, which could increase the risk of intestinal mucosal inflammation, thus affecting the integrity of intestinal mucosal barrier. These results agree with the increased concentration of inflammatory factors such as TNF- α in the serum in the high dose litchi group. In addition, it was found that with litchi intervention, the phosphatase transferase system and lipid metabolism capacity of the gut microbiota of were significantly downregulated, indicating that litchi intervention reduced the energy conversion efficiency of gut microbiota. As litchi contained a large amount of sugar (mainly glucose and sucrose), the reduced energy conversion efficiency of the intestinal flora would cause an increase in the intestinal absorption of

sugar, which may have health implications, but the current results cannot determine whether this is detrimental or beneficial to health.

Conclusion

This study shows that intervention of HFA mice with litchi, a typical “heating” food in Chinese medicine, can significantly alter the gut microflora, induce inflammation and cause damages to intestinal mucosal tissues of the mice. litchi intervention, especially at high dose, caused the serum level of TNF- α to increase by more than two times and the level of serum LPS to more than double. The intervention led to significant decreases in the abundance of microorganisms that are associated with reducing inflammation and increases in those that are linked to elevated inflammation. It also resulted in significant increases in the total number of pathogenic bacteria related to diarrhea and other diseases. Furthermore, litchi intervention at medium and high doses resulted in a widening of intestinal epithelial cell junction complex, and general weakening of the intestinal mucosal barrier as well reduced efficiency of gut microbiota in energy conversion. Thus, this study confirmed the notion in Chinese medicine that as a “heating” food, litchi when consumed excessively can lead to low degree systematic inflammation or “heating up”. The study also demonstrated that litchi induced inflammation is linked to its ability to cause dysbacteriosis of the gut microbiota and weakening of the intestinal mucosal tissues. However, this study did not examine the components of litchi that are responsible for these changes in mice, which could be a worthy topic for future studies.

List Of Abbreviations

LP: Litchi powder

qPCR: Quantitative real-time polymerase chain reaction

ELISA: Enzyme-linked immunosorbent assay

LPS: Lipopolysaccharide

TNF: Tumor necrosis factor

IFN: Interferon

IL: Interleukin

Declarations

Availability of data and materials

All the necessary data except sequencing reads are included in the article. Sequencing reads were deposited in the NCBI's sequence read archive under accession number [PRJNA648688](#). Further data will

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be shared by request.

Acknowledgements

Not applicable

Ethics approval and consent to participate

All subjects had given written informed consent. The animal experiment was approved by The Guangdong Ocean University Animal Experimentation Ethics Committee. All animal studies were performed in accordance with guidelines approved by the Animal Experimentation Ethics Committee of The Guangdong Ocean University.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

DS designed the research. DS and CW performed the research. DS and CW conducted the data analyses. DS, CW, LS, LH, YW, YL, ZF, QD, DX and JZ collaboratively interpreted the results and revised the manuscript. All authors have given approval to the final version of the manuscript.

References

1. Huang, Wu CM. Differential effects of foods traditionally regarded as *heat* $\in g'$ and cooling' on prostaglandine E2 production by a macrophage cell line[J]. Neuro-chirurgie, 2002, 31:287-293.
2. Yan H, Ji Q, Chen D, Wu J, Peng S, Ma Z, et al. A novel macromolecular extract screened from satsuma with pro-inflammatory effect[J]. Food & Function, 2014, 5(2):295-302.
3. Ma J, Xiao W, Wang J, et al. Propofol Inhibits NLRP3 Inflammasome and Attenuates Blast-Induced Traumatic Brain Injury in Rats[J]. Inflammation, 2016, 39(6):1-10.
4. Yang AL, Kashyap PC. A clinical primer of the role of gut microbiome in health and disease[J]. Tropical Gastroenterology Official Journal of the Digestive Diseases Foundation, 2015, 36(1):1.

5. Yan H, Ji Q, Chen D, Wu J, Peng S, Ma Z, Deng X. A novel macromolecular extract screened from satsuma with pro-inflammatory effect[J]. *Food & Function*, 2014, 5(2):295-302.
6. Wang H, Eckel RH. Lipoprotein lipase: from gene to obesity[J]. *Am J Physiol Endocrinol Metab*, 2009, 297(2):E271-288.
7. Clemente JC, Manasson J, Scher JU. The role of the gut microbiome in systemic inflammatory disease[J]. *Bmj*, 2018, 360:j5145.
8. Breban M. Gut microbiota and inflammatory joint diseases[J]. *Joint Bone Spine Revue Du Rhumatisme*, 2016, 83(6):645-649.
9. Muller PH, Meij TD, Westedt M, Groot ED, Allaart C, Brinkman D, et al. Disturbance of Microbial Core Species in New-Onset Juvenile Idiopathic Arthritis[J]. *Journal of Pediatric Infectious Diseases*, 2017, 12(02):131-135.
10. Kotsakis GA, Chrepa V, Shivappa N, Wirth M, Hébert J, Koyanagi A, Tyrovolas S. Diet-borne systemic inflammation is associated with prevalent tooth loss[J]. *Clinical Nutrition*, 2017.
11. Belkaid Y, Hand TW. Role of the Microbiota in Immunity and Inflammation[J]. *Cell*, 2014, 157(1):121-141.
12. Rosen CE, Palm NW. Functional Classification of the Gut Microbiota: The Key to Cracking the Microbiota Composition Code: Functional classifications of the gut microbiota reveal previously hidden contributions of indigenous gut bacteria to human health and disease[J]. *Bioessays*, 2017:1700032.
13. Hirayama K, Rafter J. The role of lactic acid bacteria in colon cancer prevention: mechanistic considerations[J]. *Antonie Van Leeuwenhoek*, 1999, 76(1-4):391.
14. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data[J]. *Nature Methods*, 2010.
15. Steven RG, Mihai P, Robert TD, Paul BE, Peter JT, Buck SSamuel, et al. Metagenomic analysis of the human distal gut microbiome.[J]. *Science*, 2006,312(5778):1355-9.
16. Torbjørn R, Tomáš F, Nichols B, et al. VSEARCH: a versatile open source tool for metagenomics[J]. *PeerJ*, 2016, 4(10).
17. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities[J]. *Applied & Environmental Microbiology*, 2005, 71:8228-8235.
18. Lozupone C, Hamady M, Kelley S, Knight R. Quantitative and qualitative diversity measures lead to different insights into factors that structure microbial communities[J]. *Applied and Environmental Microbiology*, 2007.
19. Ramette A, Tiedje JM. Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem[J]. *Proceedings of the National Academy of Sciences of the United States of America*, 2007, 104(8):2761-2766.
20. Zaura E, Keijser BJ, Huse SM, et al. Defining the healthy "core microbiome" of oral microbial communities[J]. *BMC Microbiology*, 2009, 9(1):1-12.

21. Langille MGI, Zaneveld J, Caporaso JG, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences[J]. *Nature Biotechnology*, 2013, 31(9):814-821.
22. Parks DH, Tyson GW, Philip H, et al. STAMP: statistical analysis of taxonomic and functional profiles[J]. *Bioinformatics*(21):3123.
23. Louca S, Jacques SMS, Pires APF, et al. High taxonomic variability despite stable functional structure across microbial communities[J]. *Nature Ecology & Evolution*, 2016, 1(1):0015.
24. Pietzner M, Kaul A, Henning AK, et al. Comprehensive metabolic profiling of chronic low-grade inflammation among generally healthy individuals[J]. *Bmc Medicine*, 2017, 15(1):210.
25. Masters SL, Simon A, Aksentijevich I, Kastner DL. Horror autoinflammaticus: the molecular pathophysiology of autoinflammatory disease. *Annual Review of Immunology*, 2009, 27: 621–68.
26. Kaur S, Bansal Y, Kumar R, Bansal, G. A panoramic review of IL-6: Structure, pathophysiological roles and inhibitors. *Bioorganic & Medicinal Chemistry*, 2020, 28 (5), 115327.
27. Ridaura VK, Faith JJ, Rey FE, [Cheng J](#), [Duncan AE](#), [Kau AL](#), et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice[J]. *Science*, 2013, 341(6150):1241214.
28. Dao MC, Everard A, Aron-Wisnewsky J, Sokolovska N, Prifti E, Verger EO, et al. *Akkermansia muciniphila* and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology.[J]. *Gut*, 2016, 65(3):426.
29. Arias M, Cobo M, Jaime-Sanchez P, Pastor J, Marijuan P, Pardo, J, et al. Gut microbiota and systemic inflammation changes after bread consumption: The ingredients and the processing influence[J]. *Journal of Functional Foods*, 2017, 32:98-105.
30. Conlon MA, Bird AR. The Impact of Diet and Lifestyle on Gut Microbiota and Human Health[J]. *Nutrients*, 2015, 7(1):17-44.
31. Xiaoling Y, Mei Z. Research progress on the relationship between diet and chronic low-grade inflammation[J]. *China Public Health*, 2017, 8:30-33. (In Chinese)
32. Chu H., Khosravi A., Kusumawardhani IP, Kwon A H, Vasconcelos AC, Cunha LD. Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease. *Science*, 2016,352(6289), 1116.
33. Feng Z, Long W, Hao B, et al. A human stool-derived *Bilophila wadsworthia* strain caused systemic inflammation in specific-pathogen-free mice[J]. *Gut Pathogens*, 2017, 9(1):59.
34. Larsen MJ. The immune response to *Prevotella* bacteria in chronic inflammatory disease[J]. *Immunology*, 2017, 151(4).
35. Shafikhani S. Dysregulated inflammatory responses lead to enhanced infection and impaired healing in diabetic wound[J]. *Journal of Diabetes & Metabolism*, 2018, 09.
36. Drago, L. Probiotics and Colon Cancer[J]. *Microorganisms*, 2019.

Figures

Figure 1

Effects of litchi doses on the four inflammatory markers, TNF- α (a), IFN- γ (b), IL-1 β (c), and IL-6 (d)

Figure 2

LPS concentration in the plasma of mice with different treatments. Low, middle and high denote mice received low middle and high doses of litchi gavaging; Control denotes the mice did not receive litchi gavaging.

Figure 3

Comparison of OTUs of gut flora between litchi gavaged HFA mice and control mice. The Venn diagram showed the common and unique OTUs in the different groups. The number in the core represents the OTUs common to all groups, and the numbers on the non-overlapping areas represent the total OTUs of each group minus the number of shared OTUs.

Figure 4

PCoA analysis of gut microbiota in mice from litchi experimental group and control group

Figure 5

The composition of gut microbiota of mice in the litchi gavaged and control groups at the phylum level

Figure 6

The composition of gut microbiota of mice in the litchi intervention and control groups at the genus level

Figure 7

PICRUSt analysis of the differences between the metabolic pathway of the litchi intervention and control groups of mice

Figure 8

Farportax function analysis of the gut microflora in the litchi intervention and control groups of mice.

Figure 9

Comparison of the abundance of gram-negative bacteria in gut microbiota between the litchi intervention and the control groups of mice

Figure 10

Histological appearance of intestinal tissue cross sections of mice with different treatments. Control (a); low-dose litchi intervention (b); mid-dose litchi intervention (c); high-dose litchi intervention (d).

Figure 11

Ratio of intestinal villi length to of crypt depth (a) and mean number of goblet cells in visual field (b).