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An exploratory to analysis the effects of the dirrerent roles of mathca on lipid metabolism and intestinal flora regulation between normal and diabetic mice fed a high-fat diet

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Abstract

Fast food is becoming increasing popular as a social phenomenon, and it usually contains high fat contents. Matcha is one versatile tea, and its application in food brings lots of new consumers. Herein, a high-fat diet containing matcha was prepared, and in this study we investigated the effects of such a diet on lipid metabolism and intestinal flora of normal and diabetic mice. Results showed that diabetes had significant weight loss, hyperphagia, hyperlipidemia and intestinal flora disturbance, with particularly significantly increased *Alistipes*, *Prevotella*, *Helicobacter*, *Acetatifactor* and *Bacteroides*, and decreased *Alloprevotella*, *Lactobacillus*, *Allobaculum* and *Akkermansia*. In diabetes, matcha decreased serum triglyceride and LDL-C, increased HDL-C, reversed those bacteria trends besides *Alistipes*, *Prevotella* and *Akkermansia*. In normal mice, matcha decreased serum LDL-C, increased *Parabacteroides*, *Bacteroidales_unclassified*, *Erysipelotrichaceae_unclassified* and *Barnesiella*, *Lachnospiraceae_unclassified*, and decreased *Helicobacter* and *Clostridium XlVa*. Most importantly, matcha increased *Porphyromonadaceae_unclassified*, *Lactobacillus*, *Alloprevotella*, *Prevotella* and *Allobaculum*; and decreased *Bacteroides* and *Enterobacteriaceae_unclassified* in diabetes, however these changed bacteria in normal mice showed an opposite trend from diabetes. Intestinal flora balance is vital important to host, matcha helps to improve the balance of lipid metabolism and intestinal flora according to different character of host, and is a valuable addition to develop functional food.

Keywords: diabetes; matcha; 16S rDNA sequencing; intestinal flora.

Practical Application: Matha regulates the ecological balance of intestinal flora to support the normal people and diabetes patients against potential threat brought by the high-fat diet.

1 Introduction

Diabetes is a serious worldwide problems and is an incurable disease. Chronic hyperglycemia will damage to their body functions (Chanchamroen et al., 2009; Alba-Loureiro et al., 2007). Furthermore, long-term hyperglycemia will result in obesity, glucose and lipid metabolism disorders. And current studies have found that the incidence of type II diabetes increased rapidly in the last decades (Mengual et al., 2010; Cani, 2012). Besides the genetic factors, the rapidly changes of environmental factors are the major causes of it (Willett, 2002). In the past time, people's daily life has been changed greatly, with particularly in the changes of people's diet. As a kind of social phenomenon, fast food is becoming increasing popular. Fast food mainly include fries, pizza, chips and other. As we know, fast food usually contains high-fat contents, and excessive intake of fast food will cause serious health problems and make you put on weight. These diseases, however, many have as much to do with our way of life and our high-fat diets. Diabetic patients has hyperglycemia and hyperlipidemia. Unlike health people, high-fat diet is a more serious threat to diabetes patients.

Intestinal flora is very important to people, and is closely related to our metabolism and health. Intestinal flora disturbance will cause many diseases like cardiovascular disease (Khan et al., 2014; Tang & Hazen, 2014), intestinal inflammatory (Weingarden & Vaughn, 2017), obesity (Blaut & Klaus, 2012), metabolic diseases (Clavel et al., 2014), cancer (Dapito et al., 2012) etc. And even, the dysbiosis of intestinal flora is closely related to diabetes (Qin et al., 2012). Hence, regulating intestinal fora balance may provide a potential way to improve diabetes, and it has been received widespread attention.

Studies have shown that diabetes had serious intestinal flora disturbance, with markedly decreased some butyrate-producing bacteria levels, and increased various opportunistic pathogens (Qin et al., 2012) levels. Besides, when compared to non-diabetes, diabetes showed significantly decreased phylum *Firmicutes* and class *Clostridia* levels, and increased class *Bataproteobacteria* (Larsen et al., 2010) level. In addition, when compared to health controls, diabetes showed significantly decreased levels of *Actinobacteria*, *Firmicutes*, and the *Firmicutes* to *Bacteroidetes* ratio, increased *Bacteroidetes* at phyla levels. In genera levels, diabetes showed significantly increased *Clostridium*, *Bacteroides* and *Veillonella* levels, and decreased *Lactobacillus* and *Bifidobacterium* (Murri et al., 2013) levels.

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Tea is one kind of non-alcoholic beverage and has been widely consumed. It has many kinds of functional active ingredients like tea polyphenols, which is good for human health (Kwon et al., 2008; He et al., 2007). Functional ingredients in tea has many function actions, including reducing resistance to insulin (Lin and Lin, 2008) inhibiting lipid absorption (Nakai et al., 2005), and enhancing fat oxidation (Sae-tan et al., 2011) . Most importantly, studies have shown that tea could positively modify the intestinal flora (Thomas et al., 2010), and it play a role in protecting against diabetes (Ribaldo et al., 2009) by reversing and promoting the ecological balance of intestinal flora (Zhang et al., 2020; Liu et al., 2022). Matcha is one kind of tea resources, and is loved by young consumers because of its application in food. As we know, matcha (green tea) ice cream, cake, cookie, etc. is becoming highly fashionable. Although matcha is becoming more and more important in our life, the differentiated actions of matcha on biological functions between health and diabetic people still remains unknown, with particularly in these people also eating a high-fat diet at the same time. Therefore, a kind of high-fat diet was developed, and in this study we investigated the effects of such a diet supplemented with matcha on serum lipid metabolism between normal and diabetic mice. On this basis, 16S rDNA sequencing technology was used to deeply elucidate the regulatory mechanism of matcha on intestinal flora, aimed at providing some basic information for functional food development.

2 Material and methods

2.1 Materials

Female Kunming mice (body weight 20 ± 3 g) were provided by Experimental Animal Holding of Jilin University (Changchun, China). Matcha were made in our laboratory, containing 14% tea polyphenol, 4.5% EGCG, 1.0% ECG and 2.3% caffeine.

2.2 Experimental design

A mouse model with diabetes mellitus was established by alloxan (45 mg/kg body weight), and then was randomly allotted into two treatments (n = 8 or 10), named group D-C and D-M. Normal mice was treated with stroke-physiological saline solution, and then was randomly allotted into two treatments (n = 10), named groups N-C and N-M. Groups D-C and N-C fed a high-fat diet, groups D-M and N-M fed a high-fat diet supplemented with 0.25% matcha, respectively. The high-fat diet were consisted with 22.1% fat, 40.94% carbohydrate, and 20.93% protein, and with total calorific value of 19.18 kJ/g.

All the mice were raised under the same conditions, and housed in a temperature-controlled room at 50% ± 5% relative humidity and 24 °C ± 1 °C on a 12-h light/dark cycle. This study was approved by the ethics committee of Zhejiang University (Hangzhou, China), amd all the experimental procedures were approved by Zhejiang University Institutional Animal Care and Use Committee.

Intestinal flora play a significant role in health and disease, and faeces reflect the ultimate result of interaction of entire intestinal flora and host. When simultaneously subjected to a 4-week experimental diet, the fecal samples from N-C, N-M, D-C and D-M were collected, and the changes of intestinal flora were analyzed by 16S rDNA sequencing.

2.3 Fecal sample collection and DNA extraction

Four weeks later, the fecal samples were harvested, collected and dipped in liquid nitrogen immediately. And finally the samples were transported and stored at -80 °C in a deep freezer until used. DNA was extracted from fecal with the E.Z.N.A. ®Stool DNA Kit (D4015-02, Omega, Inc., USA) according to the manufacture's instructions. The total DNA was eluted in 50 μL of Elution buffer and stored at -80 °C until measurement.

DNA extraction, 16S rDNA gene sequencing and data quality control and data analysis

DNA was extracted using an E.Z.N.A. Stool DNA Kit (D4015-02, Omega Inc., USA) according to the manufacturer's instructions. The V4 region of the gene encoding the prokaryotic small (16S) rRNA subunit was amplified with slightly modified versions of primers 515f (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACNVGGGTWTCTAAT-3') (Huang et al., 2013). The 5' ends of the primers were tagged with specific barcodes and universal sequencing primers. PCR amplification was performed with 25 μL reaction-mixture aliquots containing 50 ng of template DNA, 12.5 μL pusion host start flex 2X master mix (M0536L, Shanghai Yitao Biological Instrument co., LTD, China), 2.5 μL of forward primer, 2.5 μL of reverse primer, and PCR-grade water to adjust the volume. PCR amplification procedure were carried out with PCR Amplifier (A200, Hangzhou Langji Scientific Instrument Co. LTD, China), and the specific PCR conditions were as follows: an initial denaturation at 98 °C for 30 s, followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 54 °C for 30 s, and extension at 72 °C for 45 s, and a final extension at 72 °C for 10 min. PCR products were confirmed by 2% agarose gel electrophoresis, purified using AMPure XT breads (Beckman Coulter Genomics, Danvers, MA, USA), and quantified by Qubit (Invitrogen, USA).

Amplicon pools were prepared for sequencing, and library size and quality were assessed using an Agilent 2100 Bioanalyzer (Agilent, USA) and a Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA), respectively. The PhiX control library v3 (Illumina) and the amplicon library were combined except for a 30% PhiX spike-in. The libraries were sequenced using 2500 MiSeq runs. Additionally, one library was sequenced by both this method and using standard Illumina sequencing primers, eliminating the need for a third (or fourth) index read.

Samples were sequenced on an Illumina MiSeq platform according to the manufacturer's recommendations. Paired-end reads was assigned to samples based on their unique barcode and truncated by removing the barcode and primer sequence. Paired-end reads were merged using FLASH software (Magoc & Salzberg, 2011). Quality filtering of raw tags was performed under specific filtering conditions to obtain high-quality clean tags according to FastQC (V0.10.0) (Babraham Bioinformatics, Zerbino & Birney, 2008). Chimeric sequences were filtered using Vsearch software (v2.3.4) (Rognes et al., 2016). Sequences with ≥ 97% similarity were assigned to the same operational taxonomic

units (OTUs) using Vsearch (v2.3.4). Representative sequences were chosen for each OTU, and taxonomic data were then assigned to each representative sequence using Ribosomal Database Project (RDP) classifier (Wang et al., 2007). Determination of differences between dominant species in different groups and multiple sequence alignment were conducted using PyNAST software (Caporaso et al., 2010) to study phylogenetic relationships of different OTUs. The levels of OTUs was normalised relative to the sample with the fewest sequences.

2.4 Statistical analysis

Figures were made by GraphPad Prism (version 6), and the differences between treatments were analyzed by using Nonparametric Kruskal-wallis's test in SPSS (version 24.0). p-values < 0.05 were considered significant.

3 Results and discussion

3.1 Analysis of feed intake and body weight changes

As Figure 1 shows, the body weight levels in group D-C were the lowest, but they consumed the highest feed intake level. From the highest to lowest performance, the body weights in this study were ranked in the following order, N-M > N-C > D-M > D-C. The feed intakes were: D-C > D-M > N-C, N-M. Results shows that diabetic mice had significant weight loss and hyperphagia. Matcha supplementation had no significant influence on feed intake, but improved body weights in normal mice group. A more interesting thing is that matcha supplementation obviously decreased feed intake level, but improved body weights level in diabetic mice group. The results suggesting that matcha may play an important role in improving health status, with particularly in diabetic mice.

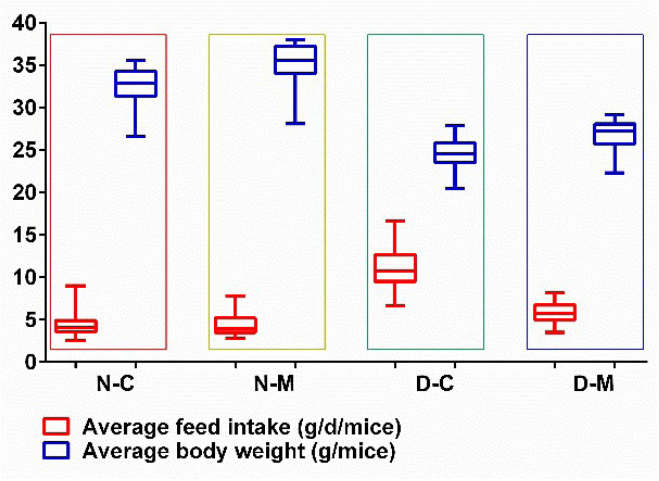

Figure 1. Effects of matcha on feed intake and body weights in normal and diabetic mice fed a high-fat diet. Note: Box parameters, the "- " symbol represents median value, and the upper and lower ranges of the box represent the 75% and 25% quartiles, respectively. Mice in diabetic group D-C fed a high-fat diet, D-M fed a high-fat diet supplemented with matcha, respectively (n = 8 or 10). Mice in normal group N-C fed a high-fat diet, N-M fed a high-fat diet supplemented with matcha, respectively (n = 10).

3.2 Analysis of serum lipid index changes

As shown in Figure 2, no significant difference ($P > 0.05$) were found in the levels of serum triglyceride, cholesterol and HDL-C between groups N-C and group N-M. The levels of LDL-C in group N-M were significantly ($P < 0.05$) decreased compared to group N-C.

When compared to group N-C, the levels of serum triglyceride, HDL-C, LDL-C and cholesterol in group D-C were all obviously increased, except for HDL-C all reached significant levels ($P < 0.05$). Moreover, when compared with group D-C, the levels of serum HDL-C in group D-M were significantly increased ($P < 0.05$), but serum triglyceride and LDL-C were all obviously decreased, but not reached the significant level ($P > 0.05$).

Results shows that diabetic mice had significant hyperlipidemia. Matcha could regulate and promote the balance of blood lipid, with particularly in diabetic mice. LDL-C, is usually regarded as a "bad" cholesterol which delivering fat and cholesterol to the cells. HDL-C, is usually regarded as a good cholesterol which reversing cholesterol transport and mobilizing cholesterol from the periphery to the liver (Stokić & Marinkov, 2007). In this study, high-fat diet were used, and it contains 22.1% fat, 40.94% carbohydrate, and 20.93% protein. Matcha used in this study contains 14% tea polyphenols, 4.5% EGCG, 1.0% ECG and 2.3% caffeine. As we know, tea polyphenols is one major functional ingredient enriched in tea, and it has many active functions. Most importantly, EGCG is one key catechin of tea polyphenols and it has been reached many attentions. Through comprehensive analysis, these functional ingredients in tea may undertake an important role in prompting lipid metabolism balance, and it has been confirmed from many studies. For example, functional ingredients in tea has some different strategy to intervene and regulate lipid metabolism, such as increasing serum HDL-C contents (Bin et al., 2009), interfering lipids emulsification, digestion, and micellar solubilisation (Koo and Noh, 2007) inhibiting lipase activity (Nakai et al., 2005), reducing absorption of lipid via intestinal tract (Klaus et al., 2005) and promoting faecal lipid excretion (Hsu et al., 2006).

3.3 Bacterial taxonomic differences analysis

Analysis of flora changes at the bacterial phylum levels

As Figure 3a-3b shows, analysis of the flora in fecal samples confirmed changes involving 10 phyla, with *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and *Actinobacteria* being highly abundant and prevalent. These four phyla account for 98.36% of the reads in group D-C, 98.87% in D-M, 98.66% in N-C and 97.78% in N-M, respectively.

As Figure 3c illustrates, compared with group N-C, the levels of *Proteobacteria*, *Candidatus Saccharibacteria*, *Cyanobacteria*, *Tenericutes, Bacteria_unclassified* and *Cyanobacteria* in group D-C were all significantly increased ($p < 0.05$).

More importantly, the levels of *Bacteroidetes* and *Actinobacteria* in group D-M were significantly ($p < 0.05$) increased by 23.64% and 141.17% compared to group D-C, respectively; *Firmicutes, Tenericutes* and *Proteobacteria* were decreased by 20.99%,

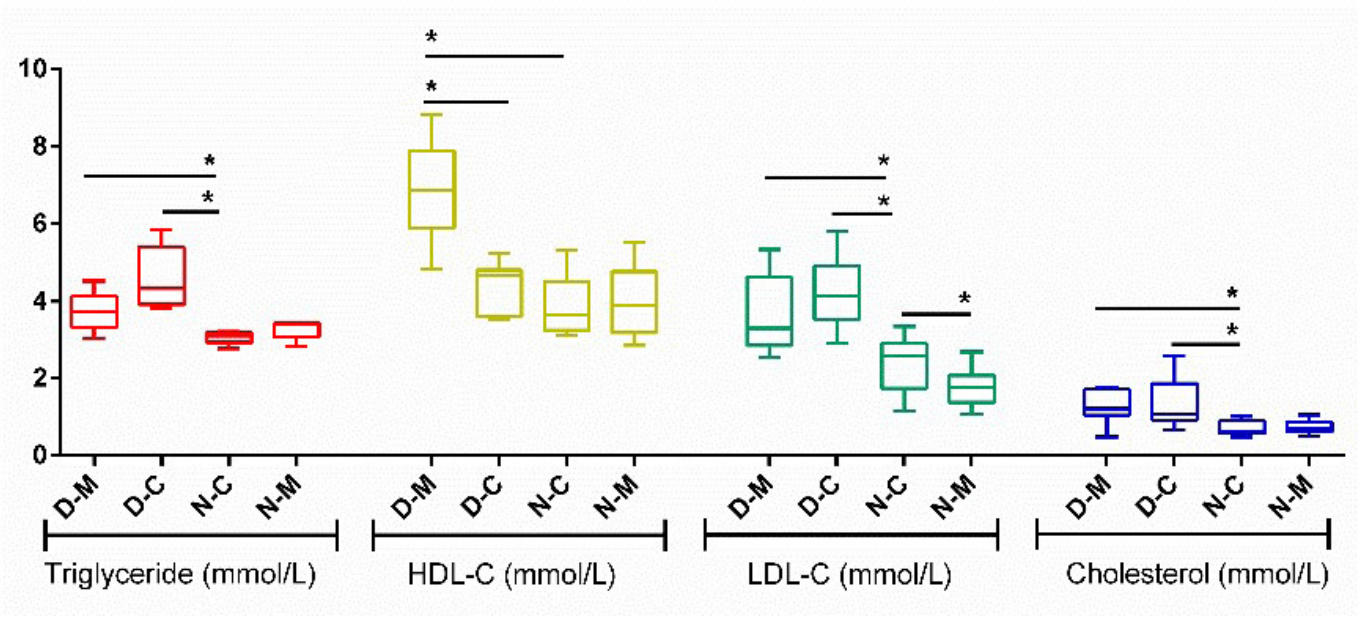

Figure 2. Effects of matcha on serum lipid index in normal and diabetic mice fed a high-fat diet. Note: Box parameters, the "- " symbol represents median value, and the upper and lower ranges of the box represent the 75% and 25% quartiles, respectively. "*" represent significant difference were identified between groups. Mice in diabetic group D-C fed a high-fat diet, D-M fed a high-fat diet supplemented with matcha, respectively (n = 8 or 10). Mice in normal group N-C fed a high-fat diet, N-M fed a high-fat diet supplemented with matcha, respectively (n = 10).

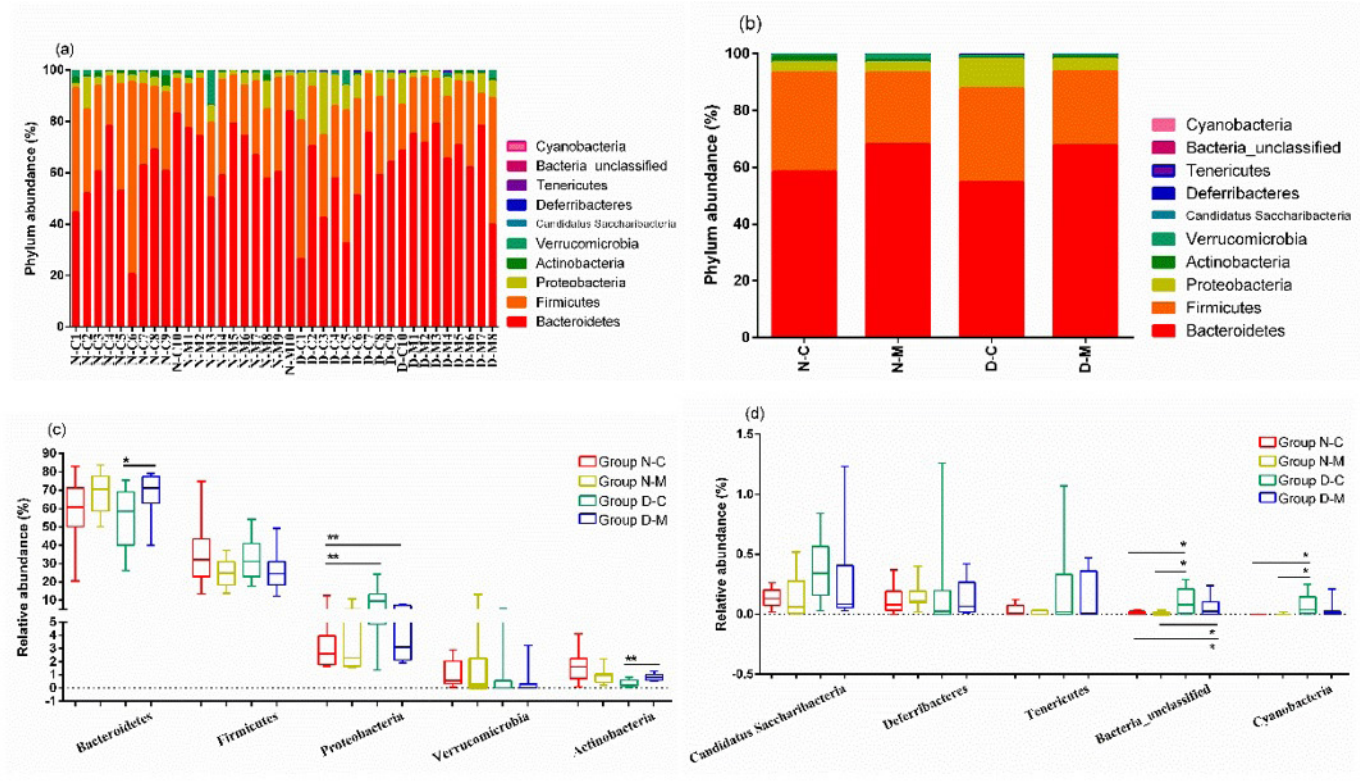

Figure 3. Effects of matcha on intestinal flora at the phyla levels in normal and diabetic mice fed a high-fat diet. a. Comparison of the abundances of bacterial phyla of each sample; b. Comparison of the average abundance of each bacterial phyla in treatment groups, respectively; c. Differences among the abundances of discriminatory phyla among four treatments. Values were express as Median with interquartile, each symbol represents a sample, and three horizontal line, respectively represent 3/4, 1/2, 1/4 quantile from the top to bottom. *p*-values were calculated using the non-parametric Kruskal-Wallis test. "*" "**""***" represent significant difference were identified between groups and the differences levels reached as $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. Mice in diabetic group D-C fed a high-fat diet, D-M fed a high-fat diet supplemented with matcha, respectively (n = 8 or 10). Mice in normal group N-C fed a high-fat diet, N-M fed a high-fat diet supplemented with matcha, respectively (n = 10).

38.10% and 58.43%, respectively, but these differences were not significant ($p > 0.05$).

Moreover, levels of *Bacteroidetes* in group N-M were increased by 16.82% compared to group N-C; *Firmicutes*, *Proteobacteria* and *Actinobacteria* were decreased by 39.70%, 1.94% and 79.76%, respectively, but all not significantly ($p > 0.05$).

Analysis of flora changes at the bacterial family levels

As Figure 4a-4b shows, twenty families demonstrated flora changes. The 10 most prominent were *Porphyromonadaceae*, *Lachnospiraceae*, *Lactobacillaceae*, *Bacteroidaceae*, *Prevotellaceae*, *Bacteroidales_unclassified*, *Helicobacteraceae*, *Erysipelotrichaceae*, *Ruminococcaceae* and *Enterobacteriaceae* (93.17%, 94.04%, 94.73% and 94.37% of reads in groups D-C, D-M, N-C and N-M, respectively).

Figure 4c also shows that when compared to group N-C, the levels of *Porphyromonadaceae*, *Bacteroidales_unclassified* and *Erysipelotrichaceae* in group N-M were increased by 1.49%, 78.82% and 7.24%; *Lachnospiraceae*, *Helicobacteraceae* and *Ruminococcaceae* were decreased by 40.25%, 39.34% and 22.32%, respectively.

When compared with group N-C, the levels of *Lachnospiraceae*, *Ruminococcaceae* and *Enterobacteriaceae* were all significantly ($p < 0.05$) increased; *Porphyromonadaceae* and *Prevotellaceae* were all significantly ($p < 0.05$) decreased in D-C. Meanwhile, when compared to group D-C, the levels of *Porphyromonadaceae*, *Bacteroidales_unclassified* and *Erysipelotrichaceae* in group D-M were increased by 44.90%, 43.70% and 60.98%, respectively, but these differences were not significant ($p > 0.05$); *Lachnospiraceae*, *Helicobacteraceae* and *Ruminococcaceae* were decreased by 40.17% ($p > 0.05$), 55.68% ($p > 0.05$) and 70.22% ($p < 0.05$), respectively.

For this study, the most interesting is that the regulatory effects of matcha on intestinal flora between normal and diabetic mice is quite different in the family *Lactobacillaceae*, *Prevotellaceae*, *Bacteroidaceae* and *Enterobacteriaceae*. Results showed that after matcha treatment, the levels of *Lactobacillaceae* and *Prevotellaceae* were increased in diabetic mice group by 101.36% and 81.44%, but they were decreased by 26.92% and 5.23% in normal mice, respectively compared to their counterparts. Besides, the levels of *Bacteroidaceae* and *Enterobacteriaceae* were decreased in diabetic mice by 51.50% and 78.00%, but they were obviously increased in normal mice, respectively.

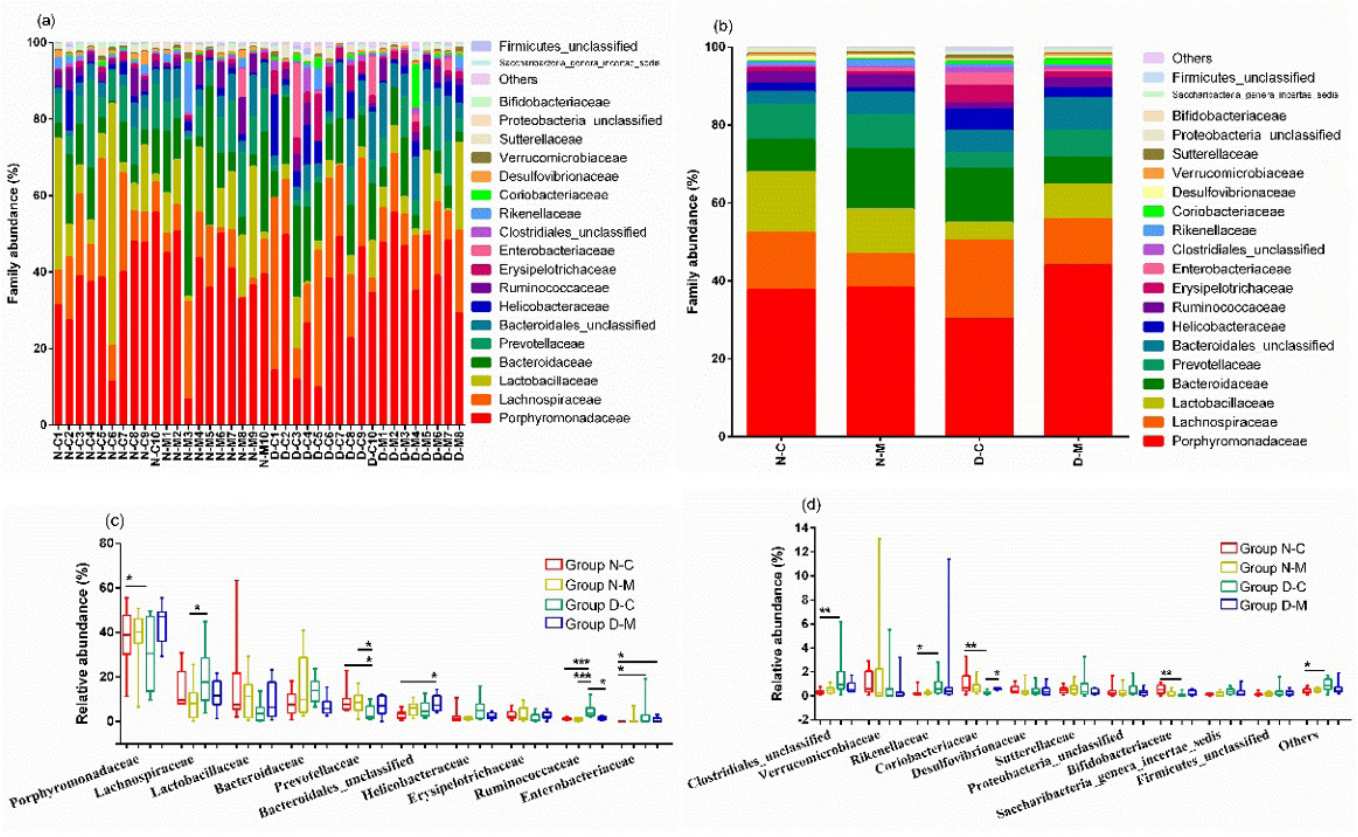

Figure 4. Effects of matcha on intestinal flora at the family levels in normal and diabetic mice fed a high-fat diet. a. Comparison of the abundances of bacterial family of each sample; b. Comparison of the average abundance of each bacterial family in treatment groups, respectively; c. Differences among the abundances of discriminatory family among four treatments. Values were express as Median with interquartile, each symbol represents a sample, and three horizontal line, respectively represent 3/4, 1/2, 1/4 quantile from the top to bottom. *p*-values were calculated using the non-parametric Kruskal-Wallis test. "*" "**""***" represent significant difference were identified between groups and the differences levels reached as $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. Mice in diabetic group D-C fed a high-fat diet, D-M fed a high-fat diet supplemented with matcha, respectively (n = 8 or 10). Mice in normal group N-C fed a high-fat diet, N-M fed a high-fat diet supplemented with matcha, respectively (n = 10).

Analysis of flora changes at the bacterial genus levels

As Figure 5a-5b shows, analysis of the flora in fecal samples confirmed changes involving 20 genera, with *Porphyromonadaceae_unclassified*, *Lachnospiraceae_unclassified*, *Lactobacillus*, *Bacteroides*, *Parabacteroides*, *Alloprevotella*, *Bacteroidales_unclassified*, *Helicobacter*, *Barnesiella*, *Clostridium XlVa*, *Erysipelotrichaceae_unclassified*, *Ruminococcaceae_unclassified*, *Enterobacteriaceae_unclassified*, *Prevotella* and *Allobaculum* being highly abundant and prevalent. These 10 genera account for 89.05% of the reads in group D-C, 90.89% in D-M, 92.88% in N-C and 92.84% in N-M, respectively.

Figure 5c shows that when compared with group N-C, the levels of *Enterobacteriaceae_unclassified*, *Ruminococcaceae_unclassified* and *Clostridiales_unclassified* in group D-C were significantly ($p < 0.05$) increased; *Bacteroides*, *Bacteroidales_unclassified*,

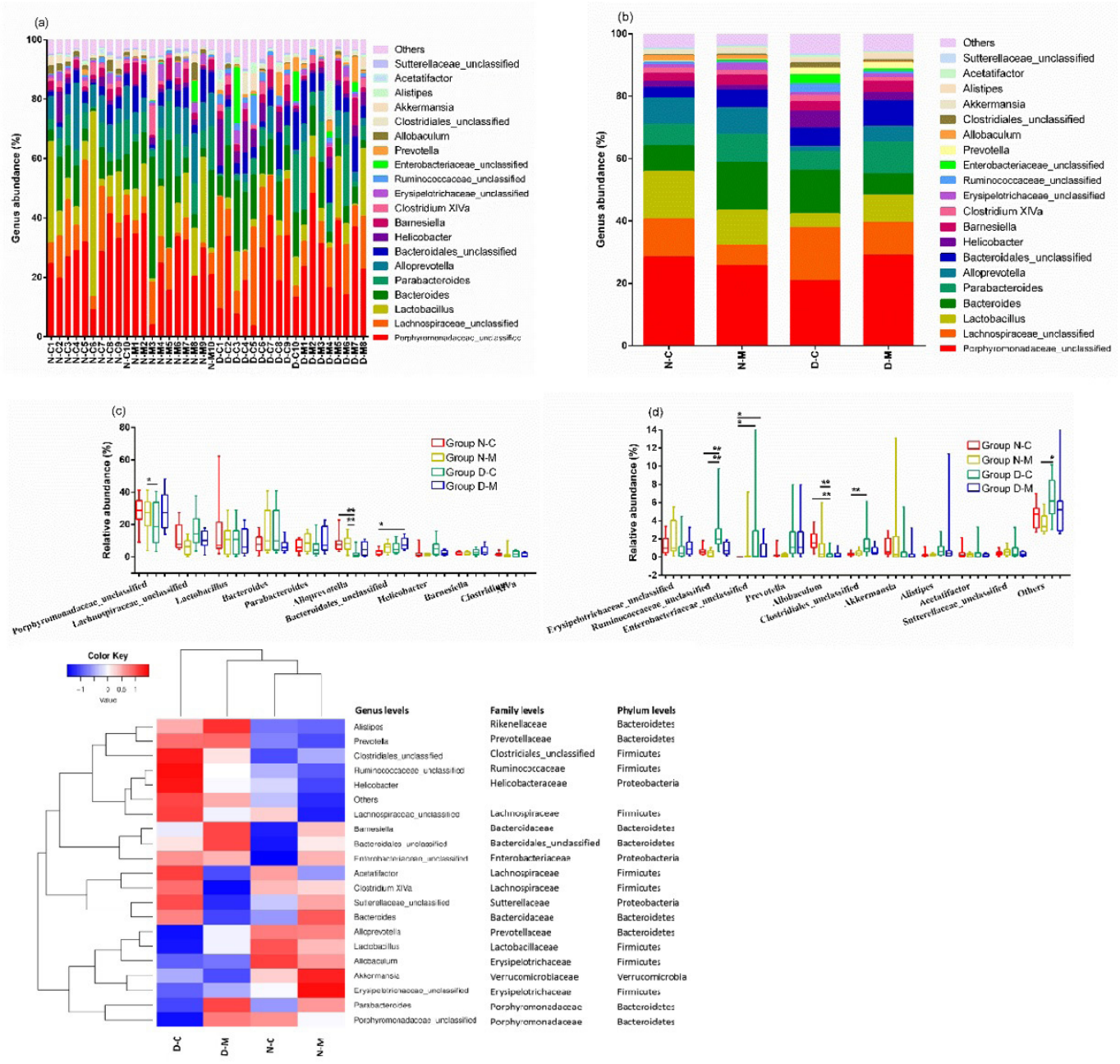

Figure 5. Effects of matcha on intestinal flora at the genera levels in normal and diabetic mice fed a high-fat diet. a. Comparison of the abundances of bacterial genus of each sample; b. Comparison of the average abundance of each bacterial genera in treatment groups, respectively; c. Differences among the abundances of discriminatory genus among four treatments. Values were express as Median with interquartile, each symbol represents a sample, and three horizontal line, respectively represent 3/4, 1/2, 1/4 quantile from the top to bottom. *p*-values were calculated using the non-parametric Kruskal-Wallis test. "*" "**""***" represent significant difference were identified between groups and the differences levels reached as $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. Mice in diabetic group D-C fed a high-fat diet, D-M fed a high-fat diet supplemented with matcha, respectively (n = 8 or 10). Mice in normal group N-C fed a high-fat diet, N-M fed a high-fat diet supplemented with matcha, respectively (n = 10).

Helicobacter, *Alistipes* and *Prevotella* were all increased, but not significantly ($p > 0.05$); *Alloprevotella* and *Allobaculum* were significantly ($p < 0.05$) decreased; *Lactobacillus* was obviously decreased, but not significantly ($p > 0.05$).

When compared with group D-C, the levels of *Parabacteroides*, *Bacteroidales_unclassified*, *Erysipelotrichaceae_unclassified* and *Barnesiella* in group D-M were all increased; *Lachnospiraceae_unclassified*, *Helicobacter* and *Clostridium XlVa* were all decreased, respectively, but not significantly ($p > 0.05$). Meanwhile, when compared to group N-C, the levels of *Parabacteroides*, *Bacteroidales_unclassified*, *Erysipelotrichaceae_unclassified* and *Barnesiella* in group N-M were all increased; *Lachnospiraceae_unclassified*, *Helicobacter* and *Clostridium XlVa* were all obviously decreased, respectively, but again these differences were not significant ($p > 0.05$).

It is also interesting to note that the regulatory effects of matcha on flora changes between normal and diabetic mice is quite different in the genus *Porphyromonadaceae_unclassified*, *Lactobacillus*, *Alloprevotella*, *Prevotella*, *Allobaculum, Bacteroides* and *Enterobacteriaceae_unclassified.* After matcha treatment, levels of *Porphyromonadaceae_unclassified*, *Lactobacillus*, *Alloprevotella*, *Prevotella* and *Allobaculum* were increased in diabetic mice group, but they were decreased in normal mice group when compared to their counterparts. Besides, the levels of *Bacteroides* and *Enterobacteriaceae_unclassified* were decreased in diabetic mice group, but they were increased in normal mice group.

Intestinal flora is closely related to our health, and increasing a lot of total bacteria or certain bacterial groups of it represents the changes of intestinal flora resilience and gut health (Turnbaugh et al., 2009). In this study, significant differences were observed in the changes of intestinal flora at genera, family and phyla levels of diabetes compared to health controls. The results showed that diabetes had significant changes of some specific bacteria, and those significant changes may related to diabetes mellitus. Most importantly, matcha supplementation obviously changed and reversed those changed specific bacteria in diabetes. Therefore, these results, as some supplementary evidences, suggesting that matcha may improve and promote the intestinal microecological balance. Through comprehensive analysis, results also showed the functional actions of matcha on intestinal flora regulation between normal and diabetic mice groups is quite different.

After 16S rDNA sequence analysis, results show here that many genus bacteria like *Alloprevotella*, *Lactobacillus*, *Allobaculum*, *Alistipes*, *Prevotella*, *Ruminococcaceae_unclassified*, *Helicobacter* and *Clostridiales_unclassified* changed greatly among treatment groups. Diabetes had significant intestinal flora disturbance, and showed increased levels of *Alistipes*, *Prevotella*, *Ruminococcaceae_unclassified*, *Helicobacter* and *Clostridiales_unclassified* at the genera levels, which are associated with *Rikenellaceae*, *Prevotellaceae*, *Ruminococcaceae*, *Helicobacteraceae* and *Clostridiales_unclassified* at the family levels, *Bacteroidetes*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria* at phyla levels. Meanwhile, diabetes also showed decreased levels of *Alloprevotella*, *Lactobacillus*, *Allobaculum* and *Akkermansia* at the genera levels, which are associated with *Prevotellaceae*, *Lactobacillaceae*, *Erysipelotrichaceae* and *Verruncomicrobiaceae* at the family levels, *Bacteroidetes*, *Firmicutes*, *Firmicutes* and *Verrcomicrobia* at the phyla levels.

Evidences have shown that the composition of intestinal flora between health and diabetes is quite different. Investigating the changes of intestinal flora is an important way to study the pathogenesis of metabolic diseases (Bäckhed et al., 2007). The differentiated intestinal flora mentioned above represent different group functions, and maybe represent the dysfunction of intestinal flora. Till now, many studies have found that those changed bacteria are closely related to our health. For example, the decreased levels of *Akkermansia* may increase the competitiveness of intestinal flora. Studies have shown that *Akkermansia* were identified as benign microbes (Shang et al., 2017), and also *Akkermansia* does not compete with other flora in getting nutrient from intestine. *Alloprevotella* and *Allobaculum* are closely related to energy metabolizing bacteria. *Alloprevotella* can produce short-chain fatty acids (Shang et al., 2017; Qu et al., 2017). *Allobaculum* is a kind of active glucose utilizers (Herrmann et al., 2017). Hence, the decreased levels of *Allobaculum*, *Akkermansia* and *Alloprevotella* in diabetes represent the growing competitiveness of intestinal flora, with particularly in getting nutrient from host intestine.

In this study, diabetes showed a decreased levels of *Lactobacillus* at the genera levels. The changes of *Lactobacillus* may result in exacerbation of diabetes. Studies have shown that *Lactobacillus* is closely related to body health. *Lactobacillus* (*Lactobacillus casei*) can regulate reduce plasma glucose level and modify the host immune responses in diabetes (Matsuzaki et al., 1997a). *L*actobacillus (*Lactobacillus casei*) can regulate and reduce the incidence of diabetes (Matsuzaki et al., 1997b). Hence, *Lactobacillus* exhibits great potential against diabetes (Bejar et al., 2013). And studies have found that *Lactobacillus acidophilus* and *Lactobacillus casei* can decrease the accumulation of glycogen in liver, improve glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidative stress (Yadav et al., 2007). Besides, *Lactobacillus* is one kind of energy metabolizing bacteria. *Lactobacillus spp.* administration can increase body weight (Khan et al., 2007). Therefore, the decreased levels of *Lactobacillus* in diabetes may be not a good thing to diabetes patients.

Previous studies showed that positive *Helicobacter* (*Helicobacter pylori*) colonization (Bener et al., 2007), frequency of *Helicobacter* (*Helicobacter pylori*) infection (Oldenburg et al., 1996) are higher than that of health controls, and this kind of findings also can been found in our results. Our results also showed that diabetes exhibited an increased levels of *Helicobacter. Helicobacter* infection will result in an increasing rate of incident diabetes (Jeon et al., 2012). Most importantly, matcha supplementation changed and reversed the changes of some specific intestinal flora. In particular, matcha supplementation resulted in an increased levels of *Alloprevotella*, *Lactobacillus*, *Parabacteroides* and *Allobaculum*; a decreased *Acetatifactor*, *Ruminococcaceae_unclassified*, *Clostridiales_unclassified*, *Helicobacter* and *Bacteroides* when compared with diabetic controls. The levels of *Alistipes* were increased in diabetes compared to health controls. Interestingly enough, matcha supplementation further increase the levels of *Alistipes* in diabetes. Genera *Alistipes* contains three species, *Alistipes finegoldii*, *Alistipes onderdonkii* and *Alistipes shahii*.

And they are the normal members of normal subject intestinal flora (Song et al., 2006; Nagai et al., 2010). The increased levels of *Alistipes* may be a passive regulatory outcomes because of the decreased levels of other bacteria. Therefore, according to above analysis, matcha plays an important role in the relieve diabetes by improving and promoting the recovery from intestinal flora disturbance.

Most importantly it is also interesting to note that the regulatory effects of matcha on flora changes between normal and diabetic mice is quite different, which involving in the genus *Porphyromonadaceae_unclassified*, *Lactobacillus*, *Alloprevotella*, *Prevotella*, *Allobaculum, Bacteroides* and *Enterobacteriaceae_unclassified.* After matcha treatment, levels of *Porphyromonadaceae_unclassified*, *Lactobacillus*, *Alloprevotella*, *Prevotella* and *Allobaculum* were increased in diabetic mice group, but they were decreased in normal mice group. In addition, levels of *Bacteroides* and *Enterobacteriaceae_unclassified* were decreased in diabetic mice group, but they were increased in normal mice group. Throughout comprehensive analysis, intestinal flora haven't different between distingue and nidering except for some pathogenic bacteria, their ecological balance is vital important to host, which suggesting that promoting the ecological balance of intestinal flora have served as a right strategy to improvement diabetes.

4 Conclusion

Mice in diabetes had significant weight loss, hyperlipidemia and intestinal flora disturbance. In particular, diabetes showed increased levels of genus *Alistipes*, *Prevotella*, *Helicobacter*, *Acetatifactor* and *Bacteroides*, decreased *Alloprevotella*, *Lactobacillus*, *Allobaculum* and *Akkermansia*. These differentially expressed intestinal flora are mainly related to energy metabolizing bacteria. Matcha supplementation changed and reversed the those specific bacterial changes besides *Alistipes*, *Prevotella* and *Akkermansia,* suggesting matcha can improve diabetes by regulating and restoring the balance of intestinal flora. Mice in normal groups fed high-fat supplemented with matcha showed increased levels of genus *Parabacteroides*, *Bacteroidales_unclassified*, *Erysipelotrichaceae_unclassified* and *Barnesiella*, *Lachnospiraceae_unclassified*, decreased *Helicobacter* and *Clostridium XlVa* compared to normal mice fed high-fat diet. Throughout comprehensive analysis, the regulatory effects of matcha on flora changes between normal and diabetic mice is quite different at the genera levels, which involving in *genus Porphyromonadaceae_unclassified*, *Lactobacillus*, *Alloprevotella*, *Prevotella*, *Allobaculum, Bacteroides* and *Enterobacteriaceae_unclassified.* After matcha treatment, the levels of *Porphyromonadaceae_unclassified*, *Lactobacillus*, *Alloprevotella*, *Prevotella* and *Allobaculum* were increased in diabetic mice, but they were decreased in normal mice compared to their counterparts. In addition, the levels of *Bacteroides* and *Enterobacteriaceae_unclassified* were decreased in diabetic mice, but they were increased in normal mice. Intestinal microorganisms are vital important to host, and they haven't different between distingue and nidering besides some pathogenic bacteria. Matcha helps to improve the balance of lipid metabolism and intestinal flora according to different character of host, and is a valuable addition to develop functional food.

Conflict of interest

No potential conflict of interest was reported by the authors.

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Author contributions

Conceived and designed the experiments: JL, RT. Performed the experiments: JL. Analyzed the data: JL. Contributed the reagents/materials/analysis tools: JL, QL, RT. Wrote the manuscript: JL.

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