Changes in intestinal flora in patients with type 2 diabetes on a low-fat diet during 6 months of follow-up

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Received January 16, 2020; Accepted June 24, 2020

DOI: 10.3892/etm.2020.9167

Abstract. Type 2 diabetes mellitus (T2DM) is closely associated with changes in the composition of the gut microbiota. To date, studies on the gut microbiota have focused on the genus-level composition and microbial gene sets, whereas changes in the microbiota after clinical treatment have remained largely elusive. In the present study, 16 subjects with T2DM were enrolled and treated long-term with a low-fat diet. Stool samples were collected at the initial diagnosis and after 1, 3 and 6 months of treatment, and named as group T0, T1, T2 and T3, respectively. Simultaneously, stool samples from 16 healthy individuals were collected as a control (group C). In addition, 16S ribosomal RNA sequencing was performed to detect differences in the microbiota between the groups. Following the low-fat diet treatment, the patients' fasting plasma glucose, plasma glucose 2 h after challenge, glycosylated haemoglobin A1c and body mass index (BMI) decreased significantly. The composition of the phylum in patients with type 2 diabetes mellitus was similar to that in healthy individuals. A total of 23 genera from four phyla, namely Firmicutes, Proteobacteria, Bacteroidetes and Actinobacteria, were determined to be different between group T0 and group C, while only 8 genera were different between group T3 and group C. Repeated analysis of variance suggested a complex change during the low-fat diet treatment. The butyrate-producing bacteria *Anaerotruncus* exhibited a slight increase, while *Roseburia* was significantly increased at the T1 stage but then gradually decreased at the later stage. In summary, a low-fat diet was effective for patients with T2DM in reducing blood glucose and the BMI, and, to a certain extent, improving the intestinal flora to reach a normal composition. The study was registered in the Chinese Clinical Trial Registry (ChiCTR; registration no. ChiCTR1900028663).

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Key words: type 2 diabetes mellitus, low-fat diet, stool samples, intestinal flora, *Anaerotruncus, Roseburia*

Introduction

Type 2 diabetes mellitus (T2DM) is a type of metabolic syndrome that accounts for 90-95% of cases of diabetes. Metabolic disorders of proteins, lipids and carbohydrates may lead to obesity, hyperglycaemia, hypertension and dyslipidaemia, as well as to a series of pathological conditions and damage in multiple tissues (1). Due to genetic inheritance and multiple exogenous causes, including lifestyle changes, accelerated pace of life and poor eating habits, current data suggest an ongoing increase in the prevalence of T2DM (2-4).

A notable causative factor for T2DM is overnutrition through poor diet, leading to the onset of an overweight status (4). A high-fat diet may impair the function of Paneth cells, a type of intestinal epithelial cell capable of secreting antimicrobial peptides to resist microbial invasion (5). Loss of intestinal epithelial integrity and reduced expression of certain antimicrobial peptides leads to a significant increase in the levels of lipopolysaccharides, which may result in metabolic inflammation in the liver and serum, promoting the development of metabolic syndrome. A low-fat diet involves reducing the intake of foods with a high lipid content (triglycerides and cholesterol) and increasing the intake of nondigestible but fermentable carbohydrates (dietary fibres), which is an effective strategy for alleviating the disease phenotypes of T2DM (6). A previous study explored the effects of a low-fat diet on inflammation, insulin resistance and hepatic steatosis, and suggested a reduction in monocyte chemoattractant protein-1, F4/80 antibody and tumour necrosis factor-α mRNA following a 12-week low-fat diet in high-fat diet-induced obese mice, highlighting the vital role of a low-fat diet in the prophylaxis of obesity-associated metabolic disorders (7).

Microscopically, intestinal microbes have a critical role in human metabolic activity have a mutual association with human health (8). *Prevotella copri* and *Bacteroides vulgatus* are able to promote the biosynthesis of branched-chain amino acids (BCAAs), but they lack the transport system to

transport the produced BCAAs into bacterial cells, resulting in increased levels of serum BCAAs and insulin resistance (9). *Roseburia* spp. exhibits a negative association with blood glucose levels and has a significantly lower abundance in patients with diabetes than in healthy individuals (10,11). After receiving treatment intervention, a characteristic rearrangement of the composition and diversity of the intestinal microbiota was detected. Wirth *et al* (12) previously analysed the distribution of the microbiota in the duodenum, ileum and intestine and found a rearrangement in the microbial composition in diabetic mice treated with insulin, comparing with those in the streptozocin-induced diabetic and healthy mice. In diabetic mice treated with insulin, the relative abundance of *Bifidobacteriales* and *Clostridiales* increased whereas that of *Lactobacillales* and Proteobacteria were decreased. However, to the best of our knowledge, only a few long-term studies on the effect of low-fat diets on the intestinal flora of patients with T2DM have been performed (13,14).

An in-depth understanding of how the changes in the intestinal flora upon exposure to new environmental conditions caused by the low-fat diet treatment arise is of high importance. In the present study, a low-fat diet was provided to patients with an initial diagnosis of T2DM, followed by regular and irregular follow-up over 6 months combined with 16S ribosomal (r)RNA sequencing technology, and the characteristic changes in the intestinal flora during this period were analysed.

Materials and methods

Patient recruitment. The present study was approved by the Medical Ethics Committee of Zhoushan Putuo District People's Hospital (Zhoushan, China; no. KY2015006) and registered in the Chinese Clinical Trial Registry (ChiCTR; registration no. ChiCTR1900028663). Patients with T2DM were recruited as subjects from our hospital inpatient departments and clinics at The Zhoushan Putuo District People's Hospital from July 21, 2015 to December 24, 2017 in accordance with the World Health Organization standards (1999) (15). The inclusion criteria were as follows: i) Age of >18 years; ii) initial treatment for T2DM without insulin treatment; iii) no major gastrointestinal surgery within five years, such as gastric resection; iv) no inflammatory bowel disease; v) no antibiotic use within the last three months; vi) BMI ≥24.0; and v) current daily eating habits featuring the preference of a high-fat diet, including fried food, fatty meat and cream. All patients must demonstrate good compliance with adhering to the low-fat diet, follow-up schedule and provided stool samples at the specified timepoints, and provide written informed consent. A total of 140 patients were initially diagnosed with T2DM. Of these, 13 were lost to follow-up, 77 had insufficient compliance with the dietary instructions during the study period and 17 patients unable to continue to participate in the study due to aggravation or other illnesses, and were therefore excluded. Finally, 16 patients with T2DM were included in the present study. Furthermore, 16 healthy individuals were included in the study. Basic information of the participants is provided in Tables I and SI.

Study group and sample collection. All patients enrolled in the present study were treated long-term with a low-fat diet. The patients were regularly followed up at 1, 3 and 6 months after the initial diagnosis and had weekly or biweekly irregular follow-ups during the treatment. At the time of initial diagnosis and at each subsequent regular follow-up, ~3 g of stool sample was collected and placed into covered sterile plastic tubes. At the same time, the patients' blood glucose data [fasting plasma glucose (FPG), plasma glucose 2 h after challenge (2hPG) and glycated haemoglobin (HbA1c)], as well as body height and weight, were recorded. Samples were divided into four groups according to sampling time: Group T0 (initial diagnosis), group T1 (1 month of treatment), group T2 (3 months of treatment) and group T3 (6 months of treatment). Stool samples of healthy individuals were also collected by using the same method as the control (group C). A flow chart displaying the movement of the participants in the present study is provided in Fig. 1.

Low-fat diet treatment options. Based on the Mediterranean diet model, combined with local dietary habits, low-fat diet treatment options were developed by the nutritional specialist and varied from person to person (16). Daily energy intake was based on each patient's body weight and intensity of daily activity. In general, the total number of calories required per day for females was 1,800-2,100 kcal and that for males was 2,100-2,400 kcal (17). Patients were encouraged to strictly follow a pre-set ratio of the three major nutrients in their daily energy intake, i.e. 50-60% of carbohydrates and 15-20% of protein per day, and it was important to maintain the intake of fat at a level of <25% by increasing the intake of cellulose and cereals and replacing the intake of lipids with unsaturated fatty acids (18). Irregular follow-ups were performed to ensure that the diet was in line with the requirements ≥6 days per week.

Nucleic acid extraction and library construction. Nucleic acid extraction was performed using a QIAamp DNA Stool Mini kit (Qiagen GmbH). The DNA concentration was measured using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Inc.) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.) was used to evaluate DNA integrity. By comparing the sequences of 16S rRNA V3-V4 regions of a number of bacterial species (accession nos. NC_005296.1, NC_003888.3, NC_001318.1, NC_021046.1, NC_021487.1 and NC_021030.1), the sequences of conserved regions were found. Corresponding forward and reverse primers were designed based on this conserved region using Primer Premier 5.0 (Premier Biosoft International). The sequence of the upstream primer in the V3 region-356 was 5'-TCGTCG GCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGN GGCWGCAG-3' and the sequence of the downstream primer in the V4 region-803 was 5'-GTCTCGTGGGCTCGGAGA TGTGTATAAGAGACAGGACTACHVGGGTATCTAAT CC-3'. The first step of the PCR amplification was performed using Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Inc.) and deionized water (Biomed, Inc.) using thermocycling conditions previously described (19) and the PCR products were purified using Agencourt AMPure XP beads (Beckman Coulter, Inc.). Barcodes and sequencing adapters based on sequences in a Nextera XT Index kit (Illumina, Inc.) were added to the primers aforementioned for the second

Table I. Baseline blood glucose levels and basic information of the healthy individuals and patients with type 2 diabetes.

Group/parameter	Males/females (n)	Age (years)	FPG (mmol/l)	2hPG (mmol/l)	HbA1c (%)	BMI (kg/m^2)
Healthy individuals (n=16)	8/8	51.55±6.23	4.86±0.33	6.5±0.5	5.6±0.4	22.5±1.3
T2DM patients (n=16)	9/7	50.25±4.95	8.05±0.47	12.5±0.5	6.8±0.3	26.4±0.8
t or χ2	0.723[a]	0.602[b]	22.321[b]	35.200[b]	13.804[b]	10.130[b]
P-value	0.478	0.553	<0.001	<0.001	<0.001	<0.001

[a]The χ^2 value was obtained by the χ^2 test; [b]the t-value was obtained by the t-test. FPG, fasting plasma glucose; 2hPG, 2-h postprandial blood sugar; HbA1c, glycosylated hemoglobin A1c; BMI, body mass index; T2DM, type 2 diabetes mellitus.

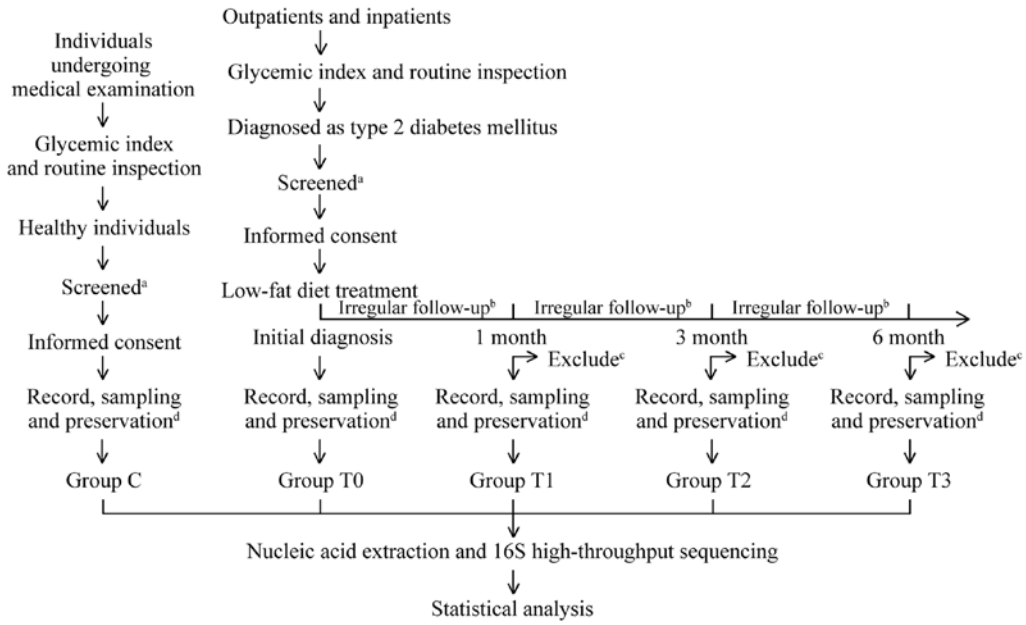

Figure 1. Flow chart of the movement of the patients throughout the study. [a]Patient recruitment based on screening criteria. [b]At weekly or biweekly follow-ups, participants were requested to provide their dietary records. [c]Participants who were unable to follow the dietary instructions during the study period, who had withdrawn from the course and those who were unable to continue to participate in the study due to aggravation or other illnesses were excluded. [d]Values of fasting plasma glucose, plasma glucose 2 h after challenge and glycated haemoglobin were recorded. Groups: C, healthy control; T0, T1, T2 and T3, patients with type 2 diabetes mellitus at the initial diagnosis and after 1, 3 and 6 months of treatment, respectively.

PCR step. The second PCR was performed using the purified products from the first PCR as the template (19), with the same reaction system as that for the first step aforementioned. The final PCR product was purified using Agencourt AMPure XP beads (Beckman Coulter, Inc.). Quality control for all libraries was performed using an Agilent 2100 Bioanalyzer (Thermo Fisher Scientific, Inc.) and a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Inc.). The purified products (~630 bp) were quantified to a final concentration of 24 nM and sequenced from 5' to 3'using the Miseq Reagent Kit V3 (cat. no. MS-102-3003; Illumina, Inc.) for 600 cycle runs. The effective reads of the paired-end reading sequence could reach 2x300 bases. The raw data generated by an Illumina MiSeq sequencer (Illumina, Inc.) were used in the further analyses.

High-throughput sequencing and data analysis. The FastQC (v0.10.1; http://www.bioinfor matics.bbsrc.ac.uk/projects/fastqc) was employed to evaluate the base quality of the test data. The raw test sequence was subjected to an error rate check, joint processing and other steps to obtain clean reads. QIIME software (v1.7; http://qiime.org/) was used to filter low-quality original tags. The USEARCH tool (v10.0.240) (20) was applied to compare the filtered sequences with the database and remove the embedded sequence (chimaera sequence) to obtain the final valid tag. Operational taxonomic unit clustering was performed at a 97% similarity level using the UCLUST method in QIIME software.

Statistical analysis. The data were analysed using SPSS 19.0 (IBM Corp.). χ^2 tests were used to determine differences between rates and percentages. Two-tailed Student's t-tests were used to compare the two groups of continuous variables. Comparison of intestinal flora composition was performed using one-way analysis of variance (ANOVA). Repeated-measures ANOVA modified by Greenhouse-Geisser parameters was used for analysis of repeated measurement data and then the Bonferroni correction were used for pairwise comparisons. The significance level was set at $P<0.05$.

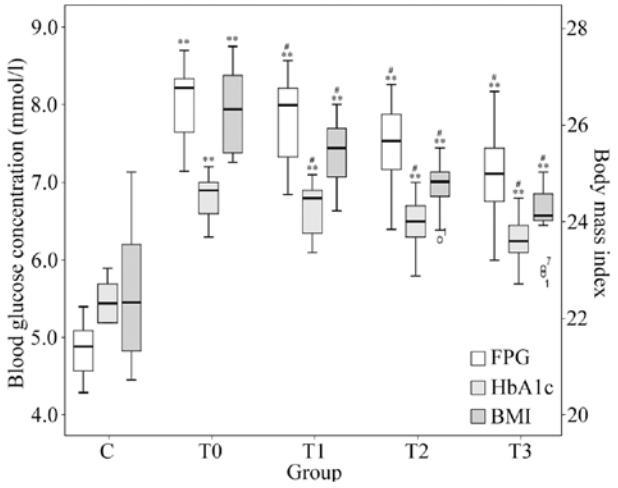

Figure 2. Changes in FPG, HbA1c and BMI. Boxes of different colours represent the value of FPG, HbA1c and BMI, respectively (n=16 in each group). The values for FPG and HbA1c refer to the scale of the ordinate on the left-hand side and the values for the BMI refer to the scale of the ordinate on the right-hand side. $^{\#}$P<0.05 vs. the previous timepoint. **P<0.001 vs. group C. Groups: C, healthy control; T0, T1, T2 and T3, patients with type 2 diabetes mellitus at the initial diagnosis and after 1, 3 and 6 months of treatment, respectively. FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin A1c; BMI, body mass index.

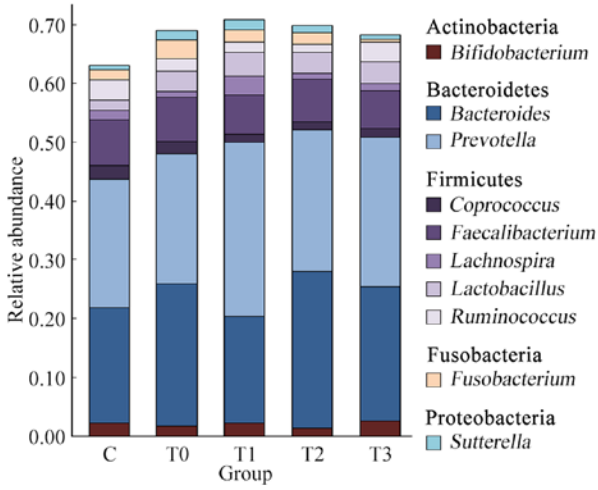

Figure 3. Relative abundance at the genus level, as revealed by 16S ribosomal RNA gene sequencing. Each column represents a group and different colours indicate different genera among the microbiota. The 10 most abundant genera are listed. Groups: C, healthy control; T0, T1, T2 and T3, patients with type 2 diabetes mellitus at the initial diagnosis and after 1, 3 and 6 months of treatment, respectively.

Results

Changes in blood glucose and body mass index (BMI) in patients with T2DM during treatment. A total of 16 patients with T2DM and 16 healthy individuals were included in the present study. There were no significant differences in age and gender between patients with T2DM and healthy individuals. FPG, 2hPG, HbA1c and BMI of patients with T2DM were significantly higher compared with those in healthy individuals (Tables I and SI).

The low-fat diet treatment generally improved blood glucose and the BMI in patients with T2DM. Blood glucose information at different stages of low-fat diet treatment is provided in Fig. 2. One-way repeated-measures ANOVA after Greenhouse-Geisser corrections suggested that the differences in FPG, HbA1c and BMI at different stages of treatment were statistically significant (P<0.001). At each follow-up (1, 3 and 6 months of treatment), the average FPG, HbA1c and BMI were all indicated to be significantly lower compared with that at the previous timepoint (P<0.05).

Comparative analysis of the intestinal flora composition between patients with T2DM and healthy individuals. At the phylum level, the top 5 microorganisms of the gut microbiota were Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria and Proteobacteria. The composition of bacteria at the phylum level exhibited no significant difference between healthy individuals and patients with T2DM. Simultaneously, there was no significant difference in the composition of the intestinal flora at the phylum level at different stages of treatment (Fig. S1).

At the genus level, *Prevotella*, *Bacteroides*, *Faecalibacterium*, *Lactobacillus*, *Ruminococcus*, *Bifidobacterium*, *Fusobacterium*, *Coprococcus*, *Lachnospira* and *Sutterella* were dominant (Fig. 3). These taxa are common in the human intestinal flora (18). The top 3 predominant genera, accounting for ~50% of the total microbial abundance, in both healthy individuals and patients with T2DM were *Prevotella*, *Bacteroides* and *Faecalibacterium*. The relative abundance of the top 3 genera was 21.85, 19.54 and 7.84% in healthy individuals and 22.05, 24.09 and 7.58% in patients with T2DM, without any significant difference between group C and group T0 (P=0.853). *Ruminococcus* and *Lactobacillus* also occupied a large proportion, with a relative abundance of 3.48 and 1.76% in the healthy individuals and 2.06 and 3.48% in patients with T2DM, respectively.

Differences in intestinal genera in patients with T2DM and healthy individuals. ANOVA was used to analyse differences in the intestinal flora between healthy individuals (group C) and patients with T2DM (group T0) and the results are presented in Fig. 4A. A total of 23 genera from four phyla, i.e. Firmicutes, Proteobacteria, Bacteroidetes and Actinobacteria, were identified to be different (P<0.05). Among these 23 genera, the relative abundances of 6 genera, namely *Enterococcus, Gallibacterium, Epulopiscium* and *Neisseria*, as well as undefined genera of Peptostreptococcaceae and Aeromonadaceae, were significantly higher in patients with T2DM than in healthy individuals and the relative abundances of the other 17 genera were significantly lower.

After 6 months of low-fat diet treatment (group T3), the number of genera in the intestine of patients with T2DM with different relative abundances from healthy individuals decreased from 23 to 8 species (Fig. 4B). Among these 8 genera, the relative abundances of 3 genera, *Roseburia* and undefined genera of Clostridiales and Helicobacteraceae, exhibited significant differences from those in group C, not only at the baseline but also after 6 months of treatment. The other 5 genera had newly emerged after treatment. The relative abundances of the genera were significantly lower than those of group C, except for the case of *Actinomadura*.

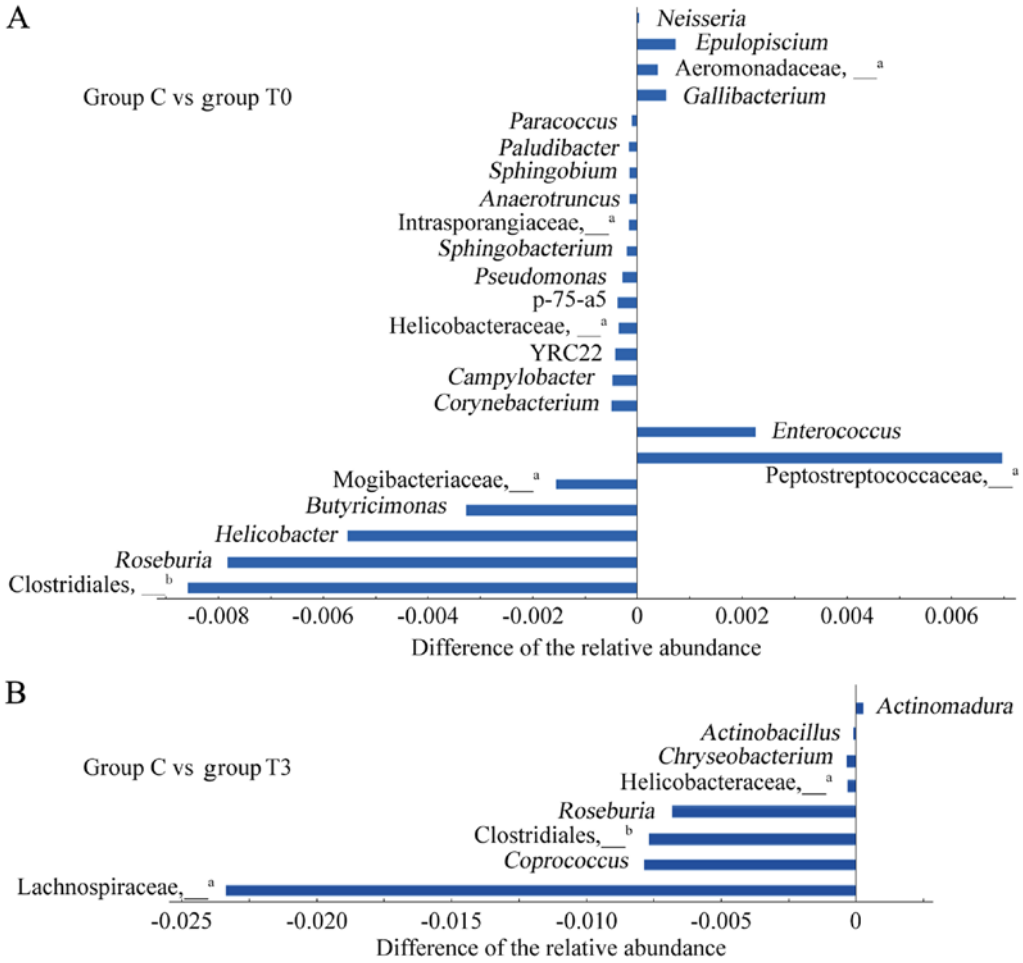

Figure 4. Differences in the intestinal flora between healthy individuals and patients with T2DM. (A) A total of 23 different genera were compared between group C and group T0. (B) A total of 8 different genera were compared between group C and group T3. The abscissa represents the difference in the relative abundance of the differential genera in group T and group C. The abscissa on the right-hand side indicates a significant increase in group T compared to group C, and the abscissa on the left-hand side indicates a significant decrease. The ordinate represents the name of the differential genus. [a]Not possible to be defined at the genus level by OTU clustering, and therefore, it is represented at the family level. Not possible to be defined at the genus level by OTU clustering, and therefore, it is represented at the order level. Groups: C, healthy control; T0 and T3, patients with T2DM at the initial diagnosis and after 6 months of treatment, respectively. T2DM, type 2 diabetes mellitus; OTU, operational taxonomic unit.

Changes in the abundance of genera during treatment with a low-fat diet. Attempts to extract nucleic acids failed in two samples from group T1. In the repeated ANOVA, this was represented using null values and subsequent analysis was performed. Repeated-measures ANOVA suggested that the relative abundance of certain genera changed significantly at a certain stage of the low-fat diet. The relative abundance of *Lachnospira, Dialister* and an undefined genus of Clostridiaceae significantly increased in the T0-T1 phase during treatment with a low-fat diet ($P<0.05$; Fig. 5A). *Acinetobacter* exhibited an upward trend in T0-T2 phase, while *Treponema* and CF231 had an upward trend in the T2-T3 phase ($P<0.05$; Fig. 5B).

At different stages of low-fat diet therapy, the relative abundances of differential genera exhibited different changes. Among the 23 genera with differences between the T0 and C groups, repeated ANOVA indicated that the relative abundances of *Butyricimonas*, YRC22, p-75-a5 and *Pseudomonas* increased significantly during treatment with a low-fat diet ($P<0.05$; Fig. 5C), while the changes in the other 19 genera were not significant ($P>0.05$). The relative abundance of these 19 genera changed continually and gradually tended to become more similar to that in healthy individuals (Fig. 6). Although these changes were not significant, a certain pattern was observed. After 1 month of treatment, the relative abundance of *Sphingobacterium, Paludibacter, Paracoccus* and an undefined genus of Aeromonadaceae increased and continued to increase during the subsequent treatment (increase in T0-T3; Fig. 6A). The relative abundance of *Helicobacter, Corynebacterium* and *Sphingobium*, as well as undefined genera of Mogibacteriaceae and Helicobacteraceae, increased from T0-T1 and T2-T3, and slightly decreased from T1-T2 (Fig. 6B). In general, the changes in these genera tended towards similarity with healthy individuals after 1 month of low-fat diet treatment. The change in *Roseburia* was the opposite. The relative abundance of *Roseburia* increased in T0-T1 and then gradually decreased in T1-T3 (Fig. 6A). After 3 months of treatment, certain genera changed their original trend and tended towards relative abundances closer to those of healthy individuals. The relative abundance of *Campylobacter* and an undefined genus of Clostridiales increased and the relative abundance of *Epulopiscium* decreased in T1-T3. The relative abundance of *Anaerotruncus* and an undefined genus

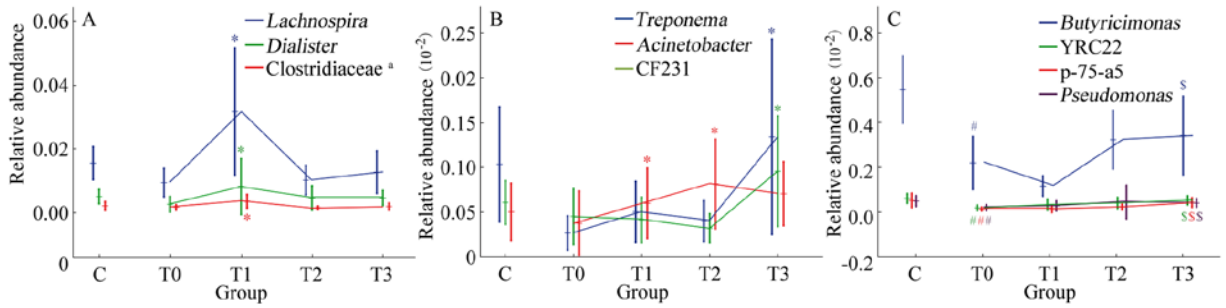

Figure 5. Significant trends in the relative abundance of genera during low-fat diet treatment. (A) Significant trends in the relative abundance of *Lachnospira*, *Dialister* and an undefined genus of Clostridiaceae. (B) Significant trends in the relative abundance of *Treponema, Acinetbacter* and CF231. (C) Significant trends in the relative abundance of *Butyricimonas*, YRC22, p-75-a5 and *Pseudomonas*, which were presented in Fig. 4A (genera that differed between group C and group T0). $^{\#}$P<0.05 vs. group C; $^{\$}$P<0.05 vs. group T0. *P<0.05 vs. the previous stage. ^{a}Not possible to be defined at the genus level by operational taxonomic unit clustering. Groups: C, healthy control; T0, T1, T2 and T3, patients with type 2 diabetes mellitus at the initial diagnosis and after 1, 3 and 6 months of treatment, respectively.

of Intrasporangiaceae substantially increased in stage T1-T2 and exhibited a slight decrease in T2-T3 (Fig. 6C). The relative abundance of *Enterococcus, Gallibacterium, Neisseria* and an undefined genus of Peptostreptococcaceae gradually increased over the first 3 months and then decreased between 3 and 6 months of treatment (T2-T3; Fig. 6D).

Discussion

The microbial community of the intestine interacts with the human body in various ways. Imbalances in metabolism cause imbalances in the intestinal flora, resulting in shifts in the abundance of certain pathogenic bacteria or an increase in conditional bacteria (21). Patients with T2DM frequently have abnormal glucose and lipid metabolism, which leads to obesity, hypertension and insulin resistance. Low-fat diets, which are able to control blood sugar and body weight by increasing dietary fiber and reducing lipid intake, are an effective dietary treatment for patients with T2DM. In the present study, patients with T2DM consumed a low-fat diet and participated in long-term follow-up. Stool samples were collected at different stages of treatment and high-throughput sequencing technology was utilized to analyse changes in the distribution of microbial communities and the association between a low-fat diet and the microbial flora in the intestine.

In the present study, the patients were newly diagnosed with T2DM, the FPG was 8.05±0.47 mmol/l, the Hba1c was 6.8±0.3%, and the BMI was 26.4±0.8. The blood glucose index were higher compared with that of the reference values, synonymous with the early stages of the development of T2DM. After 6 months of low-fat diet treatment, the patients' FPG and HbA1c levels had decreased and the BMI was also decreased significantly. This demonstrated that a low-fat diet is effective for patients with T2DM in certain aspects.

It is common to estimate the distribution of microbial communities in the gut through the evaluation of the microbial community in stool samples. In the present study, the intestinal microflora of healthy individuals and patients with T2DM consisted almost exclusively of Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria and Proteobacteria, with these phyla accounting for >99% of the intestinal microflora. Regardless, the abundance of *Prevotella, Bacteroides* and *Faecalibacterium* in the intestinal flora was relatively stable. *Ruminococcus*, which has an important role in the degradation of cellulose and hemicellulose (22), was the most dominant bacterium in the intestine of healthy individuals. To a certain extent, *Ruminococcus* is able to promote cellular uptake of sugar and reduce insulin resistance. However, long-term intake of large amounts of sugar may lead to obesity due to the role of *Ruminococcus* in promoting sugar absorption (23). In the present study, the relative abundance of *Ruminococcus* in the intestinal tract of patients with T2DM was relatively low, which may have been due to the diet of these patients being hyperlipidic and high in protein. The low-fat diets were energy-balanced and required patients to increase their intake of carbohydrates and dietary fibre, including vegetables, fruits and grains, and control fat intake to <25% of the total daily energy intake. After 6 months of low-fat diet treatment, the relative abundance of *Ruminococcus* in the intestine increased slightly.

The predominant bacterial taxon in the gut of patients with T2DM is Lactobacillus, which produces lactic acid and is recognized as a probiotic bacterium (24). The higher relative abundance of *Lactobacillus* in the intestine of patients with T2DM was likely due to dysbacteriosis, involving decreased abundance of *Ruminococcus, Coprococcus, Bifidobacterium, Blautia* and others. The present results are consistent with those of Sedighi *et al* (25). There are two conversion pathways of lactate *in vivo*: Conversion to propionate and acetate or conversion to butyrate, which promotes mucin synthesis and maintains intestinal epithelial cell integrity (26). According to the results of Patterson *et al* (27), compared with the control group, the abundance of lactic acid-producing bacteria, as well as lactate and acetate concentrations, increased in diabetic rats, whereas butyrate levels decreased. Therefore, the role of lactic acid bacteria in the intestine is determined not only by the relative abundance but also by the content of butyrate-producing bacteria.

In the present study, the relative abundances of *Roseburia* and *Anaerotruncus* in patients with T2DM, both of which are members of Firmicutes and butyrate-producing bacteria, were significantly lower than those in group C prior to treatment. *Anaerotruncus* is an acetyl-CoA acetyltransferase-based butyrate-producing bacterium that is able to express 3-hydroxyacyl-CoA dehydrogenase and enoyl-CoA hydratase, which are necessary for butyrate production. In the final

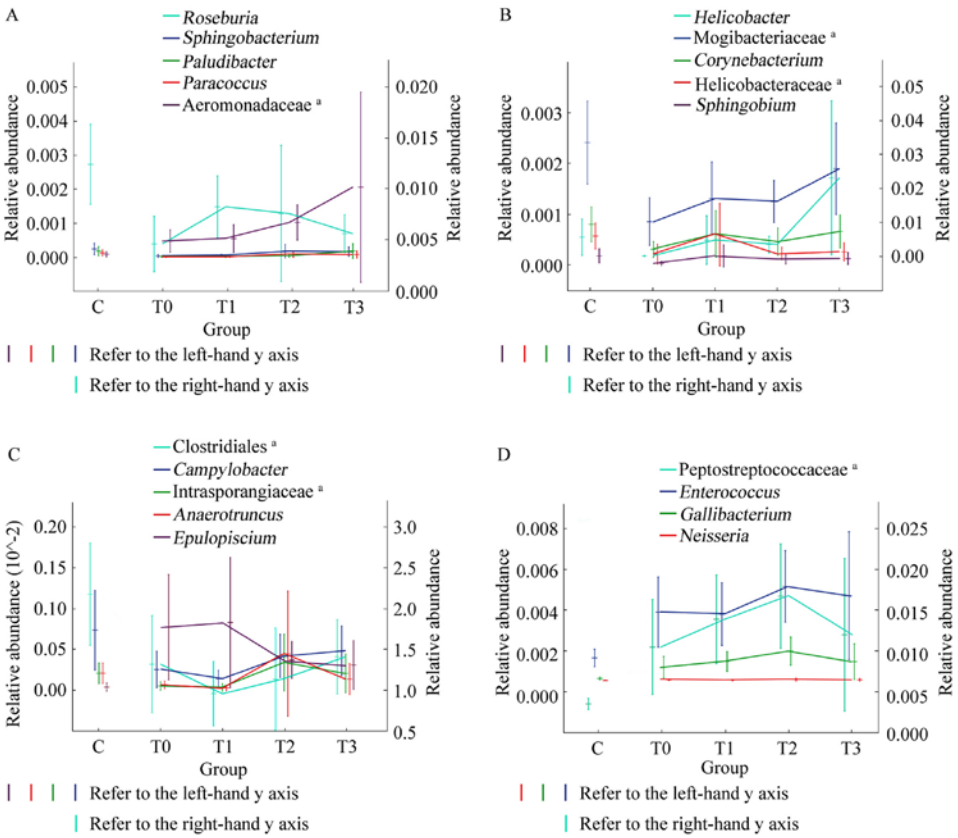

Figure 6. Trends in the relative abundance of differentiated bacteria in patients with type 2 diabetes mellitus and healthy individuals. The x-axis of each graph represents grouping, including groups C, T0, T1, T2 and T3. The y-axis on the left and right sides of each graph represent the relative abundance ranges. The vertical lines in each graph represent the error bars, the horizontal line in the middle of the vertical line is the average value. (A) Changes in the relative abundance of *Roseburia, Sphingobacterium, Paludibacter, Paracoccus*, and an undefined genus of *Aeromonadaceae* during treatment with low-fat diet. (B) Changes in the relative abundance of *Helicobacter, Corynebacterium, Sphingobium* and an undefined genera of *Mogibacteriaceae* and *Helicobacteraceae* during treatment with low-fat diet. (C) Changes in the relative abundance of *Campylobacter, Anaerotruncus, Epulopiscium*, and undefined genera of Clostridiales and Instrasporangiaceae. (D) Changes in the relative abundance of *Enterococcus, Gallibacterium, Neisseria* and an undefined genus of *Peptostreptococcaceae*. [a]Not possible to be defined at the genus level by operational taxonomic unit clustering. Groups: C, healthy control; T0, T1, T2 and T3, patients with type 2 diabetes mellitus at the initial diagnosis and after 1, 3 and 6 months of treatment, respectively.

step of butyrate biosynthesis, a moderate amount of class IV alcohol dehydrogenase may be provided by *Anaerotruncus* to facilitate butyrate production (28). Ijaz *et al* (29) reported on depletion of *Anaerotruncus* species in the intestines of mice fed a high-fat diet, where calories in high-fat foods accounted for 60% total calories, compared with mice fed a low-fat diet, where calories in high-fat foods accounted for 12% total calories. In the present study, the abundance of *Anaerotruncus* in the intestinal tract of patients with T2DM was significantly lower than that in healthy individuals and it increased slightly after 6 months of low-fat diet treatment.

Likewise, *Roseburia* is also a butyrate-producing bacterium abundant in the intestinal mucosa. *Roseburia* is able to break down indigestible carbohydrates and produce short-chain fatty acids, including butyrate, propionate formic acid and acetate, to enhance the response to pro-inflammatory factors and maintain stable intestinal immune mechanisms regulating intestinal physiology and immune homeostasis through anti-inflammatory properties that increase proinflammatory cytokine responses (30). It also gradually saturates excess polyunsaturated fat enriched in the intestine or converts it into a conjugated linoleic acid to promote the growth of beneficial bacteria such as *Lactobacillus* and *Faecalibacterium* and maintain the ecological health and stability of the intestinal microflora precursor (31). A significantly decreased abundance of *Roseburia* was detected in patients with T2DM. Haro *et al* (32) pointed out that the Mediterranean diet may be linked to an increased presence of *Roseburia* due to its high carbohydrate intake. Cantu-Jungles *et al* (33) purified and fermented the polymer of insoluble dietary fibre from *Cookeina speciosa* and observed a specificity increase in the relative abundances of *Butyrogenic, Anaerostipes* and *Roseburia* in an *in vitro* human stool fermentation model. In contrast to these studies, although the relative abundance of *Roseburia* increased slightly at T1, a decreasing trend in the subsequent low-fat diet treatment process was observed in the present study. Therefore, there may be various factors in patients with T2DM that reduce the abundance of *Roseburia*.

After adherence to 6 months of low-fat diet treatment and follow-up, a reconstitution of the intestinal flora was observed. At various stages of low-fat diet therapy, the relative abundance of different microorganisms changed. Although these changes may not have been significant, they exhibited a trend in the alteration of relative abundance of intestinal microbes under the intervention of low-fat diet therapy. Further study is of great significance to elucidate the metabolic pathways of

these microorganisms and analyse how these microorganisms affect human health through these metabolic pathways. In the present study, none of the enrolled patients received insulin therapy and finally only 16 patients improved their blood glucose through a low-fat diet regimen, resulting in a smaller sample size and is a limitation of the present study.

In summary, through treatment with a low-fat diet, the blood glucose and BMI of patients with T2DM were effectively controlled. The changes in intestinal flora were complex. However, the difference in intestinal flora between patients with T2DM and healthy individuals decreased and the structure of the intestinal flora gradually tended to be more similar to that of healthy individuals after 6 months of treatment. The relative abundances of butyrate-producing bacteria, including *Anaerotruncus* and *Roseburia*, were significantly lower in the intestinal tract of patients with T2DM than in healthy individuals. During treatment, the abundance of *Anaerotruncus* increased, while *Roseburia* only significantly increased at the T1 stage and then gradually decreased at the later stages, implying a complex response to treatment.

Acknowledgements

Not applicable.

Funding

The study was financially supported by the Public Technology Application Research of Zhejiang Province Science and Technology Hall (grant no. 2016C33242).

Availability of data and materials

The corresponding datasets were submitted to the public database Sim TK and the URL link of the datasets was https://simtk.org/docman/?group_id=1835.

Authors' contributions

CL and MG conceptualized the study. WS was responsible for patient selection and follow-up during the research process. JL and QG collected samples and analyzed the data. FM performed 16S rDNA sequencing. JJ analyzed the data; CL and JJ wrote the paper. All authors read, revised and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Zhoushan Putuo District People's Hospital (Zhoushan, China; no. KY2015006). All patients had provided written informed consent. This study was retrospectively registered in the ChiCTR (registration no. ChiCTR1900028663).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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