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ORIGINAL RESEARCH

Huoxue Jiangtang Decoction Alleviates Type 2 Diabetes Mellitus by Regulating the Oral Microbiota and Food Preferences

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Purpose: As a formula of traditional Chinese medicine (TCM), Huoxue Jiangtang Decoction (HJD) has positive effects on diabetes mellitus (DM) through improving of the metabolism of glycolipid and the function of β-cell. Hence, this research aims to explore the potential therapeutic effects of HJD on diabetes and reveal its underlying mechanisms.

Methods: Diabetic rat models induced by high-fat diet (HFD) and streptozotocin (STZ) were included in this study. Following successful modeling, diabetic rats were treated with HJD, and then its therapeutic effects in eight weeks were evaluated. In addition to biochemical indicators, two-bottle preference tests were carried out to examine the rats' preferences for fat and sugar, and 16S rRNA gene sequencing was performed to disclose the differences of oral microbiota among groups. Finally, Pearson correlation coefficient was used to explore the correlation between oral microbiota and the preferences for fat and sugar.

Results: It was found that HJD significantly improved the levels of fasting blood glucose (FBG), glucose tolerance, and dyslipidemia. Additionally, HJD contributed to decreasing preferences for fat and sugar in diabetic rats, which plays an important role in food intake. Furthermore, HJD regulated the abundance, distribution, and structure of oral microbiota in diabetic rats, serving as one of the underlying mechanisms of its antidiabetic effects.

Conclusion: Taken with other formulas, HJD functions to improve the metabolism of glycolipid and the function of β-cell by inhibiting preferences for fat and sugar, as well as regulating the oral microbiota of diabetic rats. Furthermore, a potential correlation between the oral micro-environment and preferences for fat and sugar in STZ-induced diabetic rats is likely to exist.

Keywords: Huoxue Jiangtang Decoction, diabetes, preferences for fat/sugar, oral microbiota

Introduction

Type 2 diabetes mellitus (T2DM), accounting for approximately 90% to 95% of DM,[1] is mainly characterized by metabolic disorders such as hyperglycemia, hyperlipidemia and insulin resistance (IR).[2] According to the latest data, as of 2021, an estimated 536.6 million people aged 20 to 79 suffered from diabetes worldwide, and this figure is expected to rise to 783.2 million by 2045.[3] Besides, diabetes leads to multiple complications including neuropathy, nephropathy, retinopathy, cardiovascular diseases, and periodontal diseases which seriously influence the life quality of a large population in the world.[4,5] As one of the common complications of DM, periodontal diseases have drawn increasing attention to the oral microbiology of patients with DM. And growing evidences reveal a bidirectional relationship between oral health and DM. Previous studies reported that patients with DM, especially those with poorly controlled conditions, are more susceptible to oral diseases including periodontitis.[6,7] Effective intervention of oral diseases is beneficial to better glycemic control.[7]

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Every day, human mouth produces approximately 1.5 liters of saliva, carrying a large number of bacteria.[8] To date, more than 700 species of microorganisms colonized in human mouth have been identified.[9] Studies have shown that oral microbiota usually maintains inherent stability and resilience, vital in systematic health through pathogen prevention, immune regulation, nutrition absorption and metabolism.[10] Alterations of oral microbial environment have been observed on patients with systemic diseases such as obesity and DM.[11,12] Over the past few years, the role of oral microbiota in the etiology and susceptibility of T2DM has attracted increasing attention. Besides, the easy access to a large quantity of palatable food rich in fat and sugar is a major contributor to the development of obesity and obesity-related disorders like T2DM. In numerous previous studies, obese people were found to have stronger preferences for fat and sweets.[13,14] Such food preferences seem to be more the cause of obesity than the result. Thus, it is reasonable to regard fat and sugar preferences reduction as a potential therapeutic method for metabolic disorders. It is well known that oral cavity is the main place for the perception of taste. Limited studies have described the relationship between oral environment and preferences for fat and sugar, reporting that fat and sugar preferences might be related to taste preference-associated genes and lipid receptors in the cavity.[15,16] Studies also showed that alterations in oral microbial composition are likely to affect the taste of food in obese patients.[17] For example, Besnard et al observed a positive correlation between the abundance of oral Bacteroidetes and the perception thresholds of linoleic acid (LA) in obese subjects.[18]

HJD, a formula of traditional Chinese medicine, is composed of Astragalus Membranaceus (Huangqi), Rehmanniae Radix (Sheng Dihuang), Flos Carthami (Honghua), Radix Ophiopogonis (Maidong), Radix et Rhizoma Rhei (Dahuang), peach kernel (Taoren), Chinese Yam (Shanyao), and Radix Pseudostellariae (Taizi Shen). In previous studies, HJD proved to improve the metabolism of glucose and lipid.[19] Furthermore, it was found that Hydroxysafflor Yellow A, one of the main active components of HJD,[20] could reduce the level of fasting blood glucose and IR, and suppress the apoptosis of β-cell.[21] Even so, more studies exploring the mechanisms of anti-diabetic effects of HJD need to be conducted due to the features of multi-route and multi-target of formulas in TCM. Therefore, this study is dedicated to investigating the therapeutic effect of HJD on T2DM by regulating oral microbiota and food preferences. HFD/STZ-induced T2DM rats model and 16S rDNA gene sequencing are used in this study to provide potential insights into oral microbiota-related mechanism of HJD on T2DM.

Materials and Methods

Drugs

Huoxue Jiangtang Decoction (HJD) is composed of eight herbs (see Table 1). The granule products of those eight herbs which are fully suspended in water before use, were obtained from E-Fong Pharmaceutical Co., Ltd (Foshan, China). Moreover, STZ was purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA), and Metformin from Shiguibao Co., Ltd. (Shanghai, China).

Animals and Treatment

Eight-week-old Sprague Dawley (SD) rats (male, n=32) were obtained from Hunan SJA Laboratory Animal Co., Ltd (Changsha, China, registration number: SCXK 2019–0004), and kept in a specific pathogen-free (SPF) laboratory with

Table 1 Components of Huoxue Jiangtang Decoction (HJD)

Chinese Name	Latin name of Plants	Family	Medical Part	Weight	Batch Number
Huang Qi	Astragali Radix	Fabaceae	Roots	30g	1039321-20
Di Huang	Rehmanniae Radix	Scrophulariaceae	Root tubers	30g	1011601-20
Hong Hua	Carthami Flos	Asteraceae	Flowers	15g	1041181-20
Mai Dong	Ophiopogonis Radix	Liliaceae	Root tubers	20g	1033911-20
Da Huang	Rhei Radix et Rhizoma	Polygonaceae	Root and Rhizome	6g	1070741-20
Tao Ren	Persicae Semen	Rosaceae	Seeds	10g	1060411-20
Shan Yao	Dioscoreae Rhizoma	Dioscoreaceae	Rhizomes	10g	1032761-20
Tai Zishen	Pseudostellariae Radix	Caryophyllaceae	Root tubers	30g	1030251-20

a regular cycle of 12-hour light-dark (lights from 8:00 am to 8:00 pm), average temperature of 22–25°C and relative humidity condition of 60 ± 10%. After one-week accommodation, all the rats were randomly divided into two groups with 8 in the control (CON) group and 24 in the high-fat diet (HFD) group. Rats in the two groups were given normal food and HFD (fat: 60%), respectively. Six weeks later, rats in the HFD group were given STZ (35 mg/kg, ip) to establish T2DM models. In two weeks following the injection of STZ, blood glucose was measured after 12-hour fasting. Only those with FBG ≥11.1 mmol/L were identified as qualified T2DM models. Then, they were randomly divided into the HJD group (n=8), metformin (MET) group (n=8) and model (MOD) group (n=8). One week after modeling, all the rats were given daily gastric gavage with HJD (15.86 g/kg/day), or metformin (0.158 g/kg/day), or 2 mL of saline (0.9%) for 8 weeks. All the animals were anesthetized with pentobarbital sodium (55 mg/kg, ip), and the blood samples were obtained from aorta abdominalis after 8-week treatment. The serum was gained by centrifugation at 3500 rpm for 15 minutes, and then kept at −80°C until analysis.

All of the animal study protocols were approved by the Ethics Committee of Guangzhou Yongnuo Medical Laboratory Center of Animals (No.: IACUC- AEWC-F2107021) in accordance with the National Institute of Health ethical guidelines. In the process, efforts were made to minimize animal suffering.

Oral Glucose Tolerance Test (OGTT)

After 8-week treatment, all the rats received oral administration of glucose solution (3 g/kg, Sigma, St. Louis, MO, USA) after 12-hour fasting. Then the level of blood glucose was measured with an Accu-Check Compact glucometer (Roche) via sampling from the tail at 0, 0.5, 1, and 2 hours respectively after glucose intake.

Measurements of Blood Glucose and Plasma Lipid

The levels of fasting blood glucose (FBG) were recorded each week following the treatment. The serum levels of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and free fatty acid (FFA) were measured by commercial enzymatic kits (Solarbio, Beijing, China).

Tests of Preferences for Fat and Sweet

Two-bottle preference tests were conducted to detect fat and sugar preferences of all rats after intervention, based on the method of previous studies. Briefly, all the rats were trained to drink the same amount of water from each bottle during the week before tests, followed by two-bottle preference tests. Rats were housed separately in different cages, free to drink from two bottles for 48 hours. In terms of the preference test for fat, linoleic acid (99%, Sigma, St. Louis, MO, USA) was dissolved in the solution with 0.3% of gum xanthan (Sigma, St. Louis, MO, USA) to make the solution with 2% of linoleic acid, and the solution with 0.3% of gum xanthan was applied as the medium for this test.[22] As for the preference test for sweet, two bottles with solution containing 10% of glucose or water without glucose inside were used.[23] Weight changes of the two bottles were measured and recorded accurately at 0.5 and 48 hours, respectively. To minimize the possibility of side bias, positions of the bottles were switched after 24 hours. Having been exposed to linoleic acid solution for 48 hours, the rats went through a washout period for about 48–72 hours before the preference test for sweet, with deionized water in both bottles. The total intake of the linoleic acid solution or solution of sugar was weighted, and the preference ratios were calculated as follows: fat (sugar) intake/(fat (sugar) intake + non-fat (sugar) intake) × 100%.

Collection of Saliva

After 12-hour fasting, rats were anesthetized with 1% of sodium pentobarbital (55 mg/kg, ip) and maintained at supine position with their mouths open. Next, they were subcutaneously injected with pilocarpine-HCl (Sanpilo: 1%, 5 mg/kg; Santen Pharmaceutical Co. Ltd., Osaka, Japan) to promote salivation,[24] and their saliva was carefully collected with pipettes. After that, those samples were kept at −80 °C for further study.

16S rRNA Gene Sequencing

Extraction of DNA and Amplification of PCR

Genomic DNA of microbiota was extracted with E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.). After PCR amplification, the PCR product was extracted from 2% of agarose gel, purified with AxyPrep DNA Gel Extraction

Kit (Axygen Biosciences, Union City, CA, USA) in accordance with the manufacturer's instructions, and quantified using Quantus™ Fluorometer (Promega, USA).

Sequencing of Illumina MiSeq

Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, USA) according to the standard protocols of Wefind Biotechnology Co., Ltd. (Wuhan, China). The raw reads were deposited into a bioinformatics database, named Sequence Read Archive (SRA) established by NCBI (Accession Number: SRR20645831-20645862).

Processing of Sequencing Data

The raw reads of 16S rRNA gene sequencing were demultiplexed and quality-filtered by fastp (version 0.20.0), and merged by FLASH (version 1.2.7.). Operational taxonomic units (OTUs) at the threshold of 97% sequence identity were clustered with UPARSE (version 7.1), and chimeric sequences were recognized and eliminated. The taxonomy of every representative sequence of OTU was analyzed using RDP Classifier (version 2.2) against the 16S rRNA database (Silva v138) with a confidence threshold at 0.7.

Statistical Analysis

Data in this study were presented as mean ± standard deviation (SD). Independent t tests of samples were conducted to compare the means between the two groups. One-way ANOVA was used to analyse the significance of the differences among three or more groups. P-values of 0.05 or less in two-tailed tests were considered to be statistically significant. GraphPad Prism 9.3.1 was applied to analyze the results.

Results

HJD Improves the Levels of FBG and Glucose Tolerance in STZ-Induced Diabetic Rats

As shown in Figure 1A, the levels of FBG≥11.1 mmol/L were observed in all rats in model groups within two weeks after the injection of STZ. After eight-week treatment, FBG of rats in the MET and HJD groups exhibited a notably

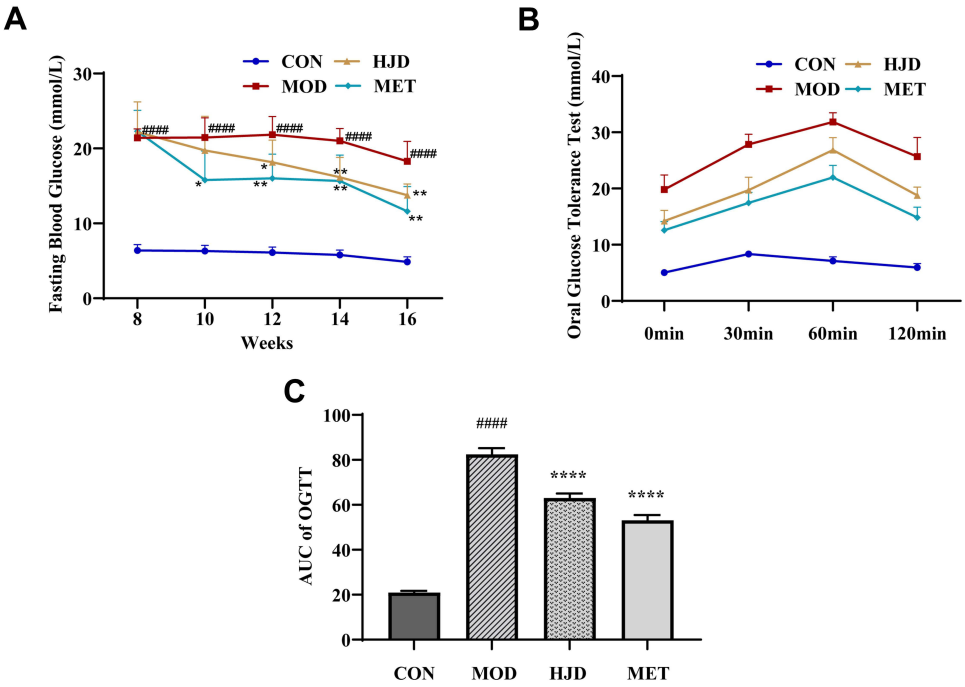

Figure 1 HJD improves the levels of fasting blood-glucose and glucose tolerance in T2DM rats. (**A**) Fasting blood-glucose level. ####P<0.0001, vs CON; **P< 0.01, *P<0.05, vs MOD. (**B**) Oral glucose tolerance test (OGTT). (**C**) Area under curve of OGTT. ####P<0.0001, vs CON; ****P<0.0001, vs MOD.

decreasing trend compared with that in the MOD group (P<0.01). Besides, the results of OGTT showed that AUC-OGTT in the MOD group (82.47) were obviously higher than those in the HJD group (63.03) and MET group (53.13) (P<0.0001) (see Figure 1B and C), indicating that HJD could partially improve the function of pancreatic β-cell in STZ-induced diabetic rats.

HJD Attenuates Hyperlipidemia in Diabetic Rats

The plasma levels of TC, TG, LDL-C and FFA in rats increased significantly after modeling. Compared with the MOD group, treatment with HJD could significantly reverse these indicators in diabetic rats (see Figure 2A–D). However, no significant difference in plasma level of HDL-C was observed among the CON group, MOD group, MET group and HJD group (see Figure 2E).

HJD Decreases the Preferences for Fat and Sugar of Diabetic Rats

The results of two-bottle preference tests showed that diabetic rats had stronger preferences for fat and sugar. After eight weeks of treatment, the ratio of preferences for fat and sugar declined markedly in the HJD group (see Figure 3A–D), indicating that HJD significantly decreased the preferences for fat and sugar of STZ-induced diabetic rats.

HJD Regulates the Abundance and Distribution of Oral Microbiota of STZ- Induced Diabetic Rats

To identify whether HJD regulated oral microbiota of diabetic rats, the saliva of those rats was analyzed after eight weeks of treatment. In this research, alpha diversity indices were applied to assess the differences of diversity among groups. Chao1 index was performed to determine the abundance of species, while Shannon and Simpson indexes were employed to examine the diversity of saliva samples of each group. The results demonstrated that Chao1 index increased in the MOD group when compared with that in the CON group. However, no significant difference between them was observed (see Figure 4A). A significantly increasing diversity (Shannon and Simpson indexes, CON vs MOD, P<0.05) of oral microbiota in samples of diabetic rats was observed in contrast to that of the healthy ones (see Figure 4B and C). Those

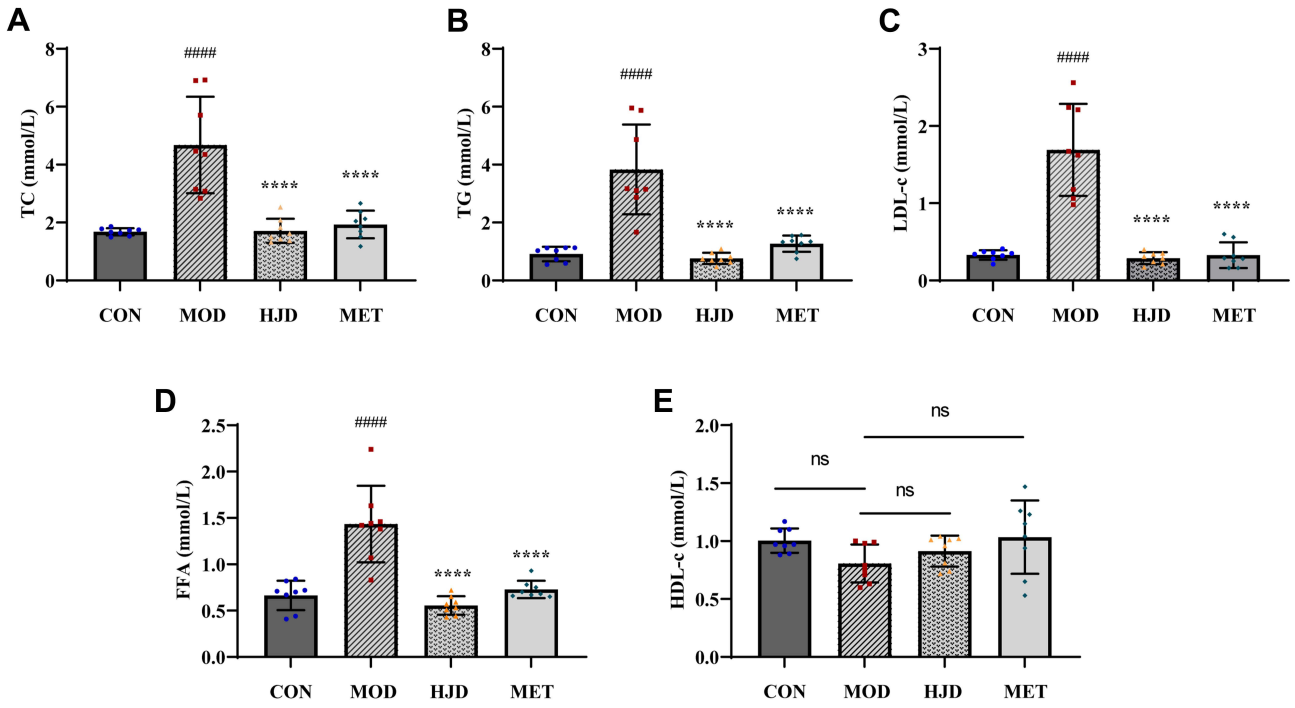

Figure 2 HJD attenuates the levels of blood lipid in T2DM rats. (**A**) Total cholesterol (TC) level; (**B**) Triglycerides (TG) level; (**C**) Low-density lipoprotein cholesterol (LDL-C) level; (**D**) Free fatty acid (FFA) level; (**E**) High-density lipoprotein cholesterol (HDL-C) level. ####P<0.0001, vs CON; ****P<0.0001, vs MOD.

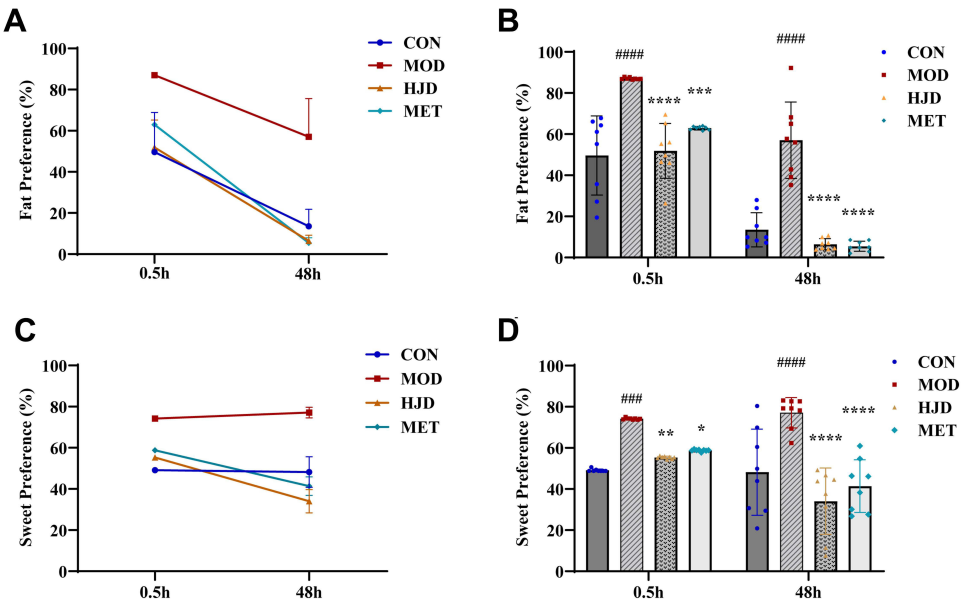

Figure 3 HJD decreases the preferences for fat and sugar of T2DM rats. (**A** and **B**) Levels of preference for fat at 0.5 and 48 hours respectively. #####P<0.0001, vs CON; *****P<0.0001, ***P<0.001, vs MOD. (**C** and **D**) Levels of preference for sugar at 0.5 and 48 hours respectively. #####P<0.0001, ###P<0.001, vs CON; *****P<0.0001, **P<0.01, *P<0.05, vs MOD.

results also revealed that HJD increased alpha diversity indices of oral microbiota in diabetic samples, but no significant difference was observed.

In addition, a better demonstration of bacterial abundance in each group was provided by Venn diagram analysis in this study. The results revealed that 2953 cases of OTUs were shared among the three groups, and a total of 2517 specific OTUs were found in the CON group, 3249 OTUs in the MOD group and 3419 in the HJD group (see Figure 4D). Meanwhile, the Shannon–Wiener curve was used to confirm the authenticity and reasonability of those data. The flat curve exhibited in Figure 4E indicated that the sequence identification has contained the majority of microbial diversities in the samples.

To further illustrate the effects of HJD on oral microbial composition, beta diversity analysis was applied. Besides, the principal component analysis (PCA) was performed to display different composition of oral microbiota among the three groups. According to the analysis results of PCA, there were significant differences and great dispersion in structures of bacterial colony between the CON and MOD groups, indicating that diabetes significantly changed the oral microbial composition in rats. While after eight-week intervention of HJD, the oral microbial composition changed to some extent (see Figure 4F).

Analysis of the Structure of Oral Bacteria

The classification of each sample at the phylum or genus level was presented as stacked columns to compare the structure of bacterial colony in each group. The results of analysis of 16S rDNA revealed that diabetes and HJD remarkably changed the structure of bacterial colony in oral cavities of diabetic rats. At the phylum level, the three groups were predominantly comprised of Firmicutes, Proteobacteria, and Actinobacteria, accounting for roughly 90% of the total abundance (see Figure 5A). The Abundance of Firmicutes in the MOD group were significantly higher in contrast with that in the CON group (P<0.0001), showing a downtrend after the intervention of HJD (P<0.001) (see Figure 5C). In this research, the abundance of Proteobacteria and Actinobacteria decreased in the MOD group (P<0.0001). An increasing abundance of Proteobacteria could be observed in the HJD group compared with that in the MOD group, but there was no remarkable difference (see Figure 5D). Besides, those results indicated that HJD could significantly increase the richness of Actinobacteria (P<0.001) (see Figure 5E). At the genus level, Streptococcus, Lactobacillus, Veillonella, Rothia, and

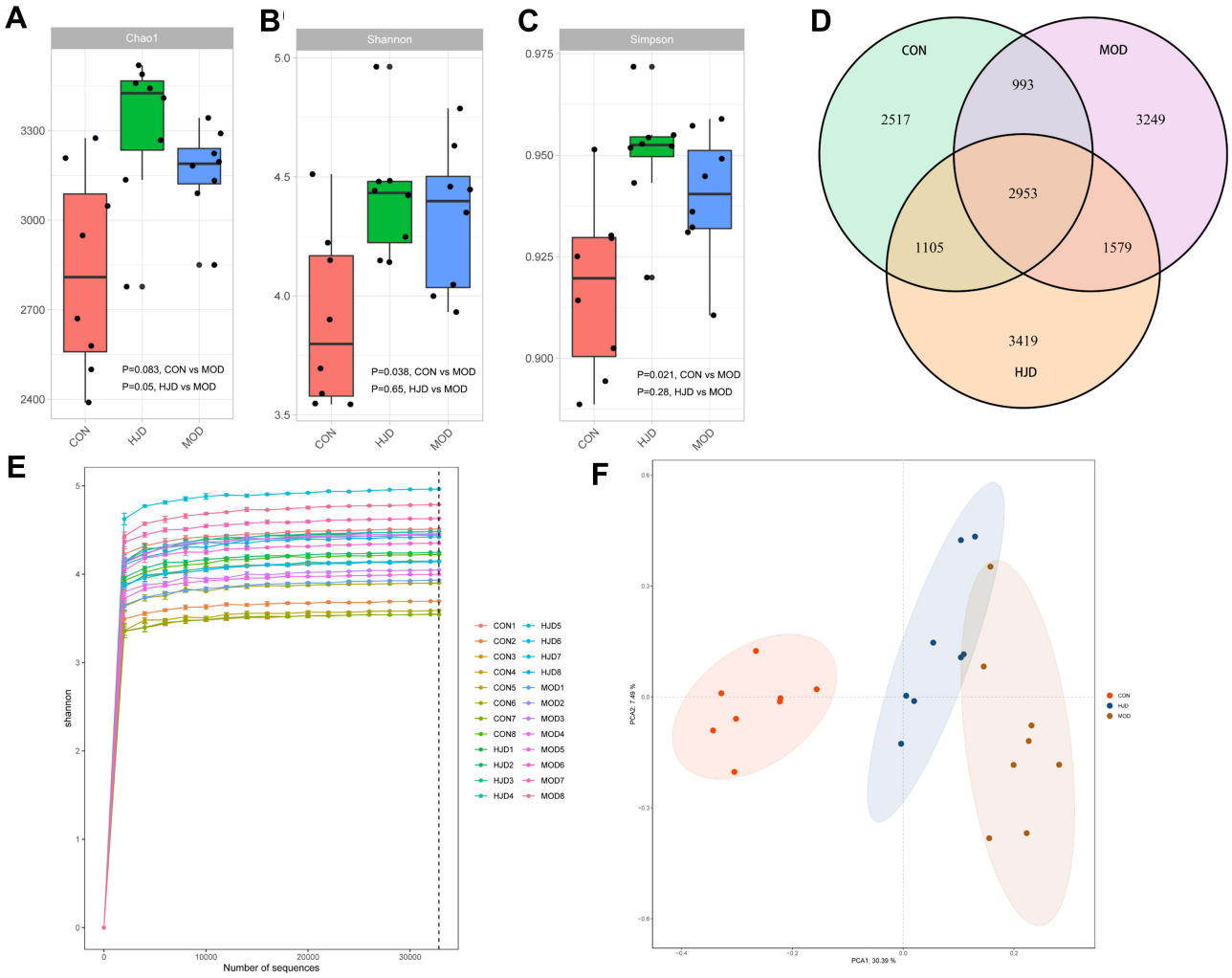

Figure 4 HJD regulates the abundance and distribution of oral microbiota of T2DM rats. (**A**) Chao1 index; (**B**) Shannon's index; (**C**) Simpson's index; (**D**) Venn diagram; (**E**) Shannon–Wiener curve; (**F**) The principal component analysis (PCA).

Pseudomonas were the most abundant genera (see Figure 5B). Among the abundant genera, the relative abundance of Streptococcus, Lactobacillus and Veillonella revealed a remarkable higher tendency in the MOD group in contrast to the CON group (Streptococcus: $p<0.0001$; Lactobacillus: $p<0.0001$; and Veillonella: $p<0.01$). To conclude, HJD effectively lowered the abundance of Streptococcus, Lactobacillus and Veillonella compared with that in the MOD group (see Figure 5F–H). Moreover, the abundance of Rothia in saliva of diabetic rats significantly decreased in contrast to that of non-diabetic rats ($p<0.0001$). And the levels of Rothia in rats exposed to HJD were increased ($P < 0.01$, see Figure 5I).

Difference Analysis of Oral Microbiota

Analysis of LDA Effect Size (LEfSe) was applied to reveal the differences of oral microbiota among groups and evaluate the impact on them. LDA (LDA >4), combined with measurements of effective size, was used to identify bacterial taxa whose sequences were of significant difference in abundance of each group. As shown in Figure 6A, three discriminative features were of notably different abundance at the genus level in each group. Specifically, Streptococcus in the MOD group, Bifidobacterium in the HJD group and Rothia in the CON group were of great abundance. A similar conclusion could be drawn from the cladogram which also showed that the class Negativicutes and the order Selenomonadales were more abundant in the MOD group (see Figure 6B).

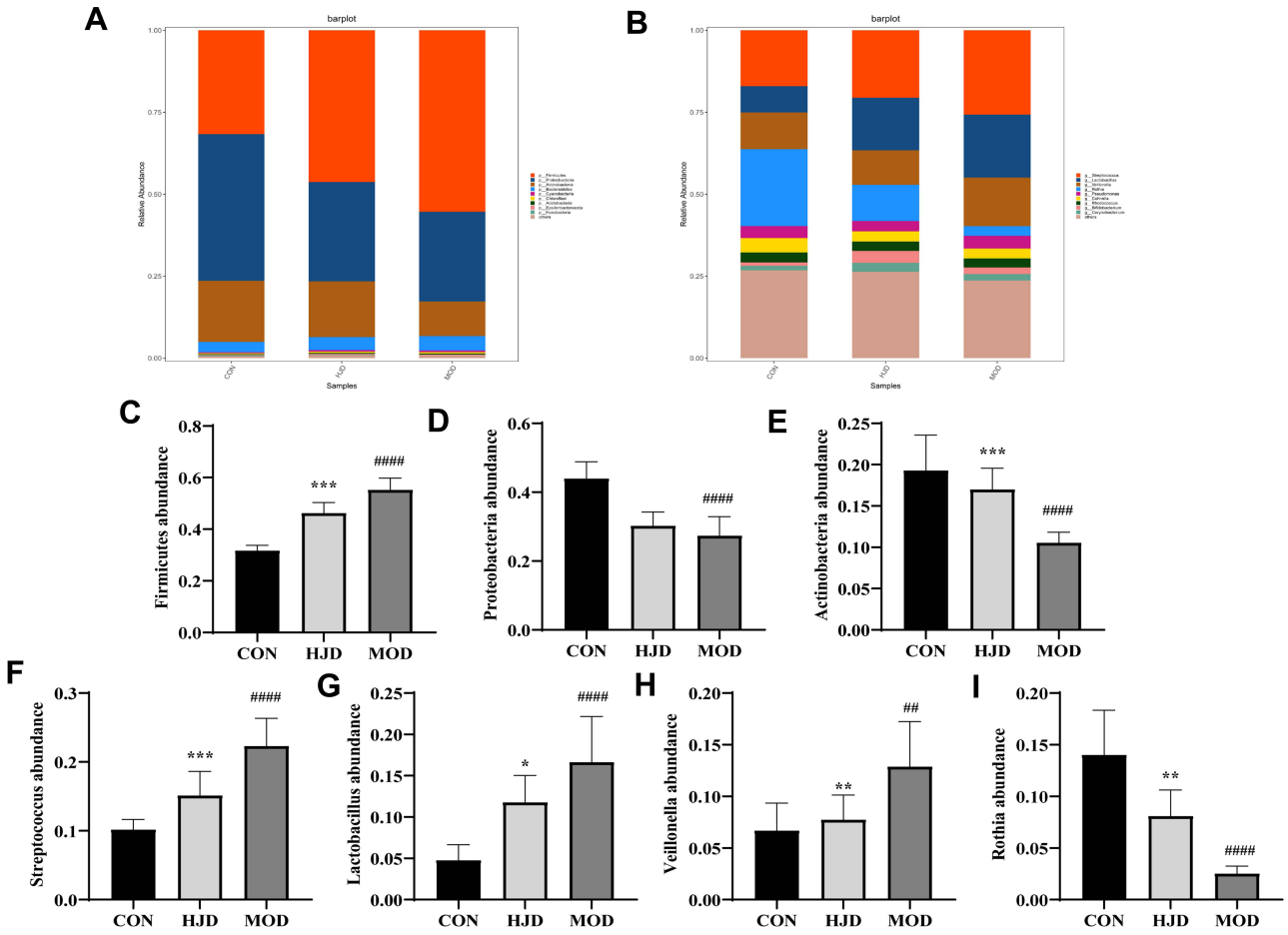

Figure 5 HJD modulates the structure of oral bacteria in T2DM rats at the phylum and genus level. (**A**) Accumulation map of the oral microorganism abundance at the phylum and (**B**) genus level; The abundance of (**C**) Firmicutes, (**D**) Proteobacteria, (**E**) Actinobacteria, (**F**) Streptococcus, (**G**) Lactobacillus, (**H**) Veillonella, and (**I**) Rothia. $^{\#\#\#\#}$P<0.0001, $^{\#\#}$P<0.01, vs CON; ***P<0.001, **P<0.01, *P<0.05, vs MOD.

Analysis of Pearson Correlation Coefficient

As mentioned above, bi-directional relationships have been found between oral microbiota and food preferences, including preferences for fat and sugar. Thus, Pearson correlation coefficient was used in this study to explore the correlation between oral microbiota and food preferences. The results revealed that preferences for fat and sugar are positively correlated with the abundance of Streptococcus. While no significant correlation was discovered between these preferences and other oral microbiota (see Figure 7).

Discussion

As one of the most common metabolic diseases, T2DM features hyperglycemia, hyperlipidemia and IR. HFD/STZ-induced T2DM rats model, the most widely accepted model for the study of pathophysiology of T2DM,[25] were successfully established in this study. In the experiment, the therapeutic effects of HJD on the metabolism of blood glucose and lipid as well as the function of pancreatic β-cell in diabetic rats were confirmed based on the biochemical indicators of serum. Furthermore, significantly stronger preferences for fat and glucose in diabetic rats were observed in contrast to that in non-diabetes, which was consistent with the previous research.[13] The decreased preferences for fat and glucose were found after eight-week intervention of HJD. Hence, it was believed that the therapeutic effect of HJD on diabetes was partially based on its role in lowering preferences for sugar and fat.

Oral and systemic diseases have a marked impact on the structure of oral microbial colony. Growing evidences revealed that oral bacteria are likely to be vital pathological factors of chronic diseases like DM.[26] In this research, high-

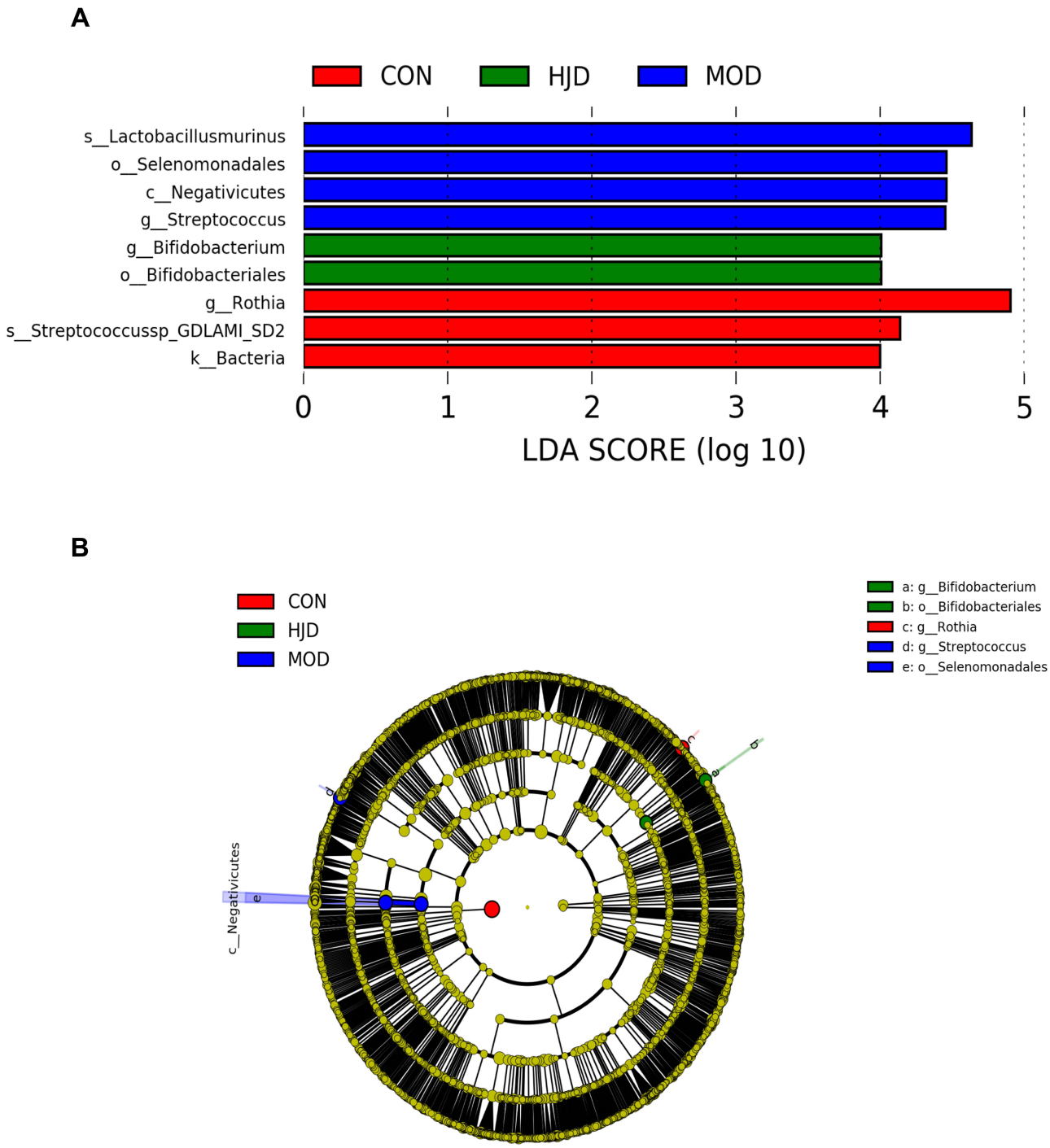

Figure 6 HJD changes the oral microbiota in T2DM rats based on the LDA Effect Size Analysis. (**A**) LDA scoring was calculated for taxa with differential richness of oral microbiota in each group (LDA >4); (**B**) Cladogram from LEfSe analysis of oral microorganism between the three groups.

throughput sequencing was applied to observe the differences in oral bacteria between diabetic rats and non-diabetic ones. In addition, whether and how HJD influenced the abundance and structure of oral microbial colony were also studied in the research. The results confirmed the impacts of DM on the dysbiosis of oral microbiota in diabetic rats and showed a significantly higher diversity of oral microbiota in samples of diabetic rats in contrast to that of rats in the CON group, in agreement with what several clinical studies had reported previously.[12,27] However, those results were contrary to the conclusion reported by Shaalan et al,[28] who found decreasing abundance and diversity of oral microbiota in

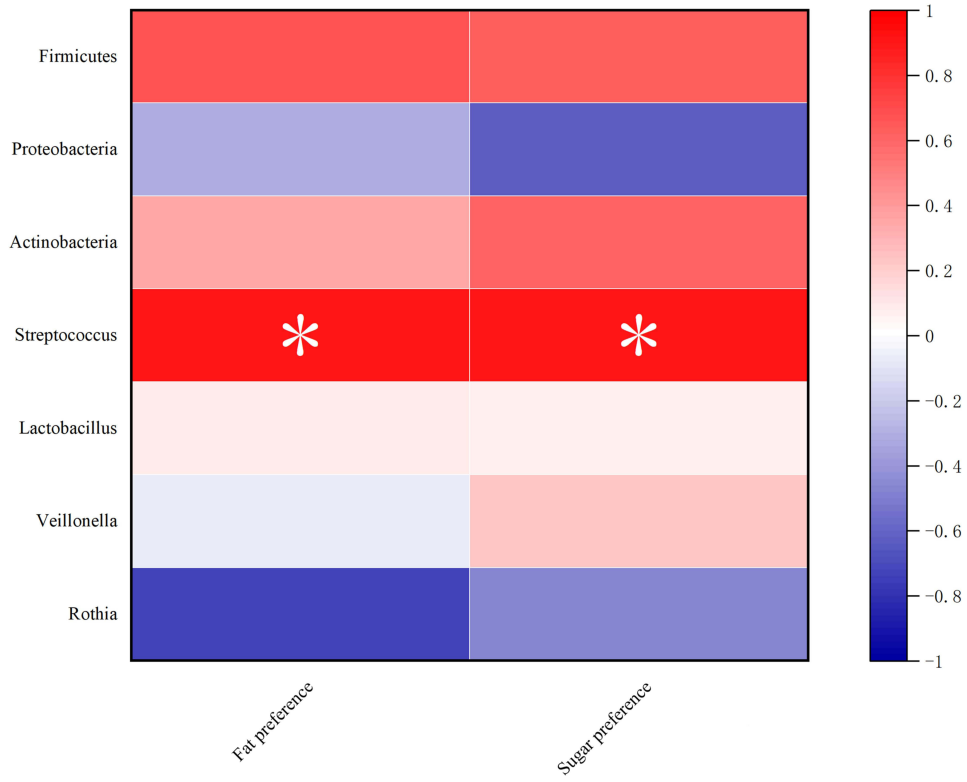

Figure 7 The bi-directional relationships between the oral microbiota and food preferences was reflected by Pearson correlation analysis. *P<0.01.

subjects with T2DM. At the phylum level, it was found that the main species of oral bacterial colony of both diabetic rats and healthy ones were constituted by Firmicutes, Proteobacteria and Actinobacteria, which was similar to the findings of previous study.[29] Besides, an increasing abundance of Firmicutes, as well as a decreasing abundance of Proteobacteria and Actinobacteria were found in saliva from diabetic rats in the present research. And HJD intervention could significantly decrease the abundance of Firmicutes and increase that of Actinobacteria.

Being the most abundant phylum in microbial colonies of mouth and intestine, Firmicutes are usually related to the transport of nutrients, and promote the absorption and fermentation of short chain fatty acid (SCFA) in non-digestible carbohydrates.[30] It has been demonstrated that SCFA can increase the sensitivity of insulin for its effects reducing of the levels of free fatty acids by inhibiting the phosphorylation of insulin receptor substrate.[31] Actinobacteria, as gram-positive bacteria, can be generated by many bioactive metabolites. It is reported that Actinobacteria exist in two-thirds of the antibiotics worldwide.[32] It is also described that Actinobacteria can produce enzyme inhibitors. For example, amylase inhibitors generated by Actinobacteria play an important role in treating carbohydrate-dependent diseases like DM.[33] Furthermore, Long et al suggested that the abundance of Actinobacteria was negatively correlated with the risk of DM.[34] It is reported in previous studies that diabetic patients had a higher abundance of Firmicutes, including genera of Streptococcus, Lactobacillus and Veillonella.[35,36] Similarly, an increasing abundance of Streptococcus, Lactobacillus and Veillonella and a lower abundance of Rothia in samples of saliva from diabetic rats were detected compared with that of the non-diabetes in this research, and HJD could reverse these trends effectively. Streptococci are known as the primary component of supragingival dental plaque, whose growth relies on the fermentation of glucose to organic acids like lactic acid.[37] As what Wilson et al had reported in the previous study, Lactobacilli, a kind of aerotolerant anaerobes, possess the ability to produce lactic acid through the fermentation of sugars.[38] Additionally, Veillonella, one of the commonly recognized beneficial bacteria, has been reported to have a function of changing lactic acids into weaker ones and generating NO^{2}- from NO^{3}-, which is considered to be beneficial for both oral and physical health.[39] Rothia, a nitrate-reducing oral bacterium, has proved to be vital in maintaining a balanced environment of oral cavity, including generating alkali molecules to avoid acid condition.[40] Lundberg et al previously discovered that Rothia played an important role in nitrogen oxides (NO) synthesis,

which was a significant mechanism for regulating cardiovascular states and metabolisms, including the improvement of insulin secretion by β-cell and glucose uptake by muscles.[41] A decreasing abundance of Rothia was observed in prediabetic subjects compared with that of the healthy in their study. Therefore, the abundance changes of these genera indicate the excessive production of lactate in samples of saliva from diabetic rats as a result of altered metabolism of the host. Besides, the results also imply that HJD is likely to change the oral micro-environment through altered acidogenic microorganisms and metabolism of carbohydrate. Bifidobacterium, one of the most popular and commonly used probiotics, has proved to be associated with improved glucose tolerance, glucose-stimulated secretion of insulin and decreasing inflammation.[42] Interestingly, studies on gut microbiota have described that Bifidobacterium, the major genus found in Actinobacteria, is related to decreasing risks of DM and obesity.[43,44] However, as a study reported, Bifidobacterium was observed only in about 33% of the subjects, approximately accounting for an abundance of only 0.1%.[34] Therefore, the reason why no significant difference of Bifidobacterium in any groups was observed in the stacked column could become explainable.

In addition, limited researches exploring the possible etiologic relationships between the oral micro-environment and diabetes have been conducted. It is known that excessive consumption of calories, including fat and sugar, is responsible for higher risks of type 2 diabetes. Stronger preferences for fat and sugar have been considered as a possible reason of diabetes rather than a result.[45] Furthermore, associations between oral microbiota and preferences for fat and sugar have been illustrated in several studies published previously.[15,16] Thus, the correlation between the oral microbiota and preferences for food in diabetic rats was also explored in this study, which is the first report of correlations between oral microbiota and preferences for fat and sugar in STZ-induced diabetic rats.

The sense of taste is crucial for food selection, and helps everyone to differentiate something that he/she likes or not at the first touch with it. Similar to five sorts of tastes, namely the sweet, sour, salty, bitter, umami, taste of fat has been thought to be the basic one. Growing evidences suggest that fat taste can be regulated by fat-rich dietary intervention. The decrease or increase of lipid contained in the diet leads to upregulation or downregulation of taste sensitivity of fat respectively, likely to be related to lipid sensors on the tongue, such as GPR120 and CD36 triggering signaling pathways as what other basic tastes do.[46] In the previous studies, 6-n-propylthiouracil (PROP) has been widely used as a phenotypic marker of genetic variation of taste sensitivities, not only for detection of bitter taste but also the taste of chemosensory component.[47] A previous study has confirmed that decreasing taste sensitivity for fat in obese population is likely to be relevant with the alteration of salivary environment.[19] Shetty et al proposed that decreasing perception of PROP was closely related to higher abundance of Streptococcus in saliva.[48] The results of this study reveal that preferences for fat and sugar are significantly positively correlated with the abundance of Streptococcus in diabetic rats, indicating that diabetes possibly alters preferences for fat and sugar by changing oral microbial colony.

Conclusion

In summary, HJD has a positive effect on the metabolism of glycolipid, function of β-cell and preferences for fat and sugar. Furthermore, HJD is able to regulate the abundance and distribution of oral microbiota, as well as the structure of bacterial colony. In addition, a positive correlation between the abundance of Streptococcus and preferences for fat/sugar was also found in diabetic rats. Thus, it was supposed that the underlying mechanisms of the antidiabetic effect of HJD lie in its ability to improve preferences for fat and sugar through the regulation of oral microbial colony. This is an emerging field, so more profound researches need to proceed in the future to uncover the interaction of preferences for fat and sugar, oral microbiota and diabetes.

Abbreviations

HJD, Huoxue Jiangtang decoction; HFD, high-fat diet; STZ, streptozotocin; FBG, fasting blood glucose; T2DM, Type 2 diabetes mellitus; IR, insulin resistance; TCM, Traditional Chinese medicine; OGTT, Oral Glucose Tolerance Test; AUC, area under the curve; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FFAs, levels and free fatty acids; OTUs, operational taxonomic units; SCFA, short chain fatty acid; ns, no significance.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request for two years after publication.

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Disclosure

The authors report no conflicts of interest in this work.

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