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Alterations in the Gut Microbiota Composition in Obesity with and without Type 2 Diabetes: A Pilot Study

Xiaojing Wang, Qinli Guo, Zhaoxiang Liu, Yanlei Wang, Chenxiang Cao, Lixia Jin, Caihong Li, Jianzhong Xiao, Wenhui Zhao

Department of Endocrinology, Beijing Tsinghua Changgung Hospital, Tsinghua University, Beijing, People's Republic of China

Correspondence: Wenhui Zhao, Department of Endocrinology, Beijing Tsinghua Changgung Hospital, LiTang Lu No. 168, Chang Ping District, Beijing, 102218, People's Republic of China, Tel +86-13810719795, Email zwha01637@btch.edu.cn

Purpose: Obesity has become a major public health concern worldwide, increasing the risk of T2DM. Growing evidence indicates gut microbiota dysbiosis is related to metabolic disorders. We aimed to firstly investigate the compositional and functional features of the gut microbiome between obesity with and without T2DM in the Chinese population.

Methods: A total of 32 obese individuals accompanied with T2DM and 18 age and gender-matched obesity with normal glucose tolerance (NGT) were enrolled. Fecal samples were collected, and the gut microbiota profile was determined using the Illumina MiSeq platform based on V3-V4 bacterial 16S rRNA gene.

Results: Compared with obesity- NGT, obesity-T2DM showed a significantly higher alpha diversity. Principal coordinates analysis based on both Bray-Curtis distance and weighted Unifrac revealed that the global microbial composition was significantly different between the two groups (P = 0.007 and P = 0.005, respectively). At the phylum level, Obesity-T2DM patients exhibited a significant decrease in *Bacteroidetes*, and a pronounced increase in *Firmicutes*. Regarding the genus level, *Bacteroides* and *Escherichia-Shigella* were found to increase considerably, while *Prevotella_9* and *Sutterella* had an evident decrease in Obesity-T2DM. Furthermore, Spearman correlation analysis revealed that *Prevotella_9* and *Sutterella* were negatively associated with HbA1c and fasting blood glucose.

Conclusion: We found clear differences in the gut microbiota composition in obesity-T2DM compared with obesity-NGT. Obesity accompanied with T2DM may aggravate the obesity-associated gut microbiota, and gut microbiota is expected to be a promising novel intervention target for obese management. However, larger sample size and more in-depth taxonomic identification studies are warranted.

Keywords: gut microbiota, obesity, type 2 diabetes

Introduction

The prevalence of obesity is increasing dramatically worldwide in adults and children, and obesity has become a major public health concern.[1] With the rapid socioeconomic development and lifestyle changes in China, the number of obesity has more than quadrupled during the past four decades.[2] The most recent national investigation reported that 16.4% of Chinese adults were obese.[2] There is no question that obesity, which predominantly increases the risk of type 2 diabetes mellitus (T2DM), cardiovascular disease, stroke, and cancers, poses a significant burden for the health-care system.[3]

Obesity is caused by an imbalance between energy intake and expenditure. Notably, excessive energy and fat accumulation induce a low-grade chronic inflammation, an important driver of T2DM resulting from progressive loss of β-Cell insulin secretion and insulin resistance.[4,5] However, not all obese individuals develop T2DM.

Growing evidence has indicated that gut microbiota dysbiosis is related to a wide variety of metabolic disorders, including obesity and T2DM.[6] Intestinal microbiota regulate host glucose metabolism by producing metabolically active products such as short-chain fatty acids (SCFAs) and bile acids.[7] Additionally, lipopolysaccharide (LPS), a component of the cell walls of gram-negative bacteria, is intimately involved in the inflammatory process by activating the TLR-4/NF-

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κB signal pathway.[8] Microbiota abnormalities elevate intestinal permeability, allowing LPS translocation to induce systemic inflammation and glucose metabolic dysfunction.[7,9] A meta-analysis also showed significant differences in gut microbiome composition in obese versus non-obesity adults.[10] Furthermore, fecal microbiota transplantation from obese increases the risk of hyperglycemia.[11] Together, these observations suggest that the gut microbiome may play a crucial role in the development of obesity-associated T2DM. In contrast, a study from Germany population found that the gut microbiome was not significantly different between obesity with and without T2DM.[12] These inconsistent results highlighted the importance of exploring gut microbiome in populations with different ethnicities and dietary habits.[13]

As such, evidence regarding the gut microbiota disturbance in obesity accompanied with T2DM is limited. In the present study, we aimed to investigate the compositional and functional features of the intestinal microbiome between obese individuals with and without T2DM in the Chinese population for the first time. Our research findings may be applicable to other populations with dietary habits of high carbohydrate intake similar to Chinese.

Materials and Methods

Study Population

The case-control study enrolled 50 obese individuals, including 32 with T2DM and 18 with normal glucose tolerance (NGT), who were scheduled to undergo bariatric surgery at the Beijing Tsinghua Changgung Hospital between June 2019 and December 2021. The participants were included if they met the following criteria: age between 18 and 60 years; BMI ≥ 28kg/m^2 (Obesity was diagnosed based on Chinese criteria); drug-naïve T2DM was diagnosed according to the WHO 1999 diagnostic criteria for diabetes. The exclusion criteria were as follows: obesity due to any endocrine disease such as Cushing syndrome, hypothyroidism, etc; antibiotics, probiotics, prebiotics, and symbiotic use during the past 3 months; any gastrointestinal disease or gastrointestinal surgery history. The study was conducted following the Declaration of Helsinki and approved by the local Ethics Committee of Beijing Tsinghua Changgung Hospital, and written informed consent was obtained from all participants.

Clinical Assessment and Measurements

A trained nurse measured height, weight and waist circumference (WC). Venous blood samples were collected after 12 h of overnight fasting. An oral glucose tolerance test (OGTT) was performed on each participant. Glucose and lipids were analyzed using the automatic biochemical analyzer (Roche C702, Germany). Serum insulin and C-peptide levels were determined using direct chemiluminescence immunoassay (Roche E801, Germany). Additionally, glycosylated Hemoglobin (HbA1c) was measured using high-performance liquid chromatography (BIO-Rad VARIANT II). Homeostatic model assessments of islet B cell function (HOMA-B) and basal insulin resistance (HOMA-IR) were calculated as follows: HOMAB = fasting serum insulin (mU/L) × 20/(fasting plasma glucose in mmol/L-3.5), HOMA-IR = fasting serum insulin (mU/L) × fasting plasma glucose (mmol/L)/22.5.

Gut Microbiota Sequencing and Analysis

Stool samples were collected in sterile containers according to the standard protocol and stored immediately at −80°C before processing. Microbial DNA was extracted using the CTAB/SDS method. DNA concentration and purity were assessed using spectrophotometry (Nanodrop ND1000). Variable regions V3-V4 of bacterial 16s rRNA were amplified by polymerase chain reaction (PCR) using the forward primer 341F (CCTAYGGGRBGCASCAG) and the reverse primer 806R (GGACTACNNGGGTATCTAAT). PCR products were purified with Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Prep Kit (Illumina, USA) following manufacturer's recommendations. Finally, the libraries were sequenced on an Illumina NovaSeq 6000 platform, generating 250 bp paired-end reads.

Sequencing raw reads were filtered using QIIME software. The filtered high-quality reads were assigned to operational taxonomic units (OTUs) based on a 97% similarity threshold, and then the RDP classifier version 2.2 algorithm annotated the representative sequences. Alpha diversity reflecting intra-individual bacterial diversity was evaluated with Shannon, Simpson, Chao1and ACE indices. Beta diversity was analyzed by principal coordinates analysis (PCoA) based on Bray-Curtis distance and weighted Unifrac. Linear discriminant analyses Effect Size (LEfSe) and metastats analysis

were applied to assess differences in microbiota composition. Moreover, the correlations between the relative abundance of bacterial taxa and clinical parameters were performed using Spearman correlation. Two-tailed $p < 0.05$ were considered statistically significant. A false discovery rate (FDR) was applied to correct the significant p-values.

Statistical Analysis

Normally distributed continuous variables were expressed as mean ± standard deviation, and independent sample t-tests were used to evaluate differences in clinical parameters between obesity with T2DM and NGT individuals. Skewed data are presented with the median and interquartile range and compared with Mann–Whitney *U*-test. A Chi-square test was conducted for categorical variables. The statistical analyses were performed using SPSS version 20.0.

Results

Anthropometric and Biochemical Parameters

A total of 50 obese patients were included in this study. The age, sex distribution, BMI and WC were similar between the obesity-T2DM and obesity-NGT groups. The HbA1c, fasting blood glucose, total cholesterol and triglyceride levels of obese-T2DM patients were significantly higher than obesity-NGT participants, while high-density lipoprotein cholesterol (HDL-c) levels were significantly lower. The detailed participant characteristics are shown in Table 1.

Microbial Diversity in the Obesity with T2DM and NGT Participants

A total of 3745 OTUs were observed in the two groups. The obesity-T2DM group exhibited larger amount of OTUs, and shared 1911 common OTUs with the obesity-NGT group (Figure 1). In the alpha diversity analysis, despite a significantly higher ACE index in the obesity-T2DM group in comparison with obesity-NGT group (Figure 1), no significant differences in Shannon, Simpson and Chao1 index were found between the two groups (Figure S1). Overall bacterial community composition (that is, beta diversity) was analyzed by using weighted UniFrac and Bray-Curtis distance PCoA followed by permutational multivariate analysis of variance (PERMANOVA) test. We found that the bacterial beta diversity of obesity-T2DM significantly differs from that of obesity-NGT (P = 0.007 and P = 0.005, respectively) (Figure 1), indicating that gut microbiota profile of obesity-T2DM was substantial diverse from that of obesity-NGT.

Table 1 General Characteristics of Obesity with and without Type 2 Diabetes

	Obesity-DM	Obesity-NGT	P Value
N (Female)	32 (16)	18(12)	0.37
Age (years)	40±11.91	35.78±9.37	0.202
BMI (Kg/m^2)	36.37±7.88	40.28±5.64	0.071
WC (cm)	111.25(103.00,126.50)	111.00(102.25,132.00)	0.986
HbA1c (%)	7.90±1.71	5.59±0.39	**<0.001**
FBG (mmol/L)	8.64±3.83	5.24±0.57	**0.001**
PBG (mmol/L)	14.14±4.49	6.09±1.25	**<0.001**
FINS (mIU/L)	22.00(14.40,28.60)	17.95(11.55,32.02)	0.713
PINS (mIU/L)	95.25(33.81,173.00)	80.93(48.76,145.61)	0.842
FCP (ng/mL)	2.68±0.93	2.35±1.13	0.32
PCP (ng/mL)	7.56±4.42	7.30±3.16	0.837
TC (mmol/L)	5.12±1.18	4.48±0.72	**0.043**
TG (mmol/L)	2.82±2.2	1.34±0.73	**0.008**
LDL-c (mmol/L)	3.35±1.20	3.01±0.71	0.275
HDL-c (mmol/L)	0.97±0.14	1.13±0.30	**0.012**

Notes: P< 0.05 is highlighted in bold.

Abbreviations: WC, Waist circumference; HbA1c, glycated hemoglobin; FBG, fasting blood glucose; PBG, 2-hour OGTT plasma glucose; FINS, fasting plasma insulin; PINS, 2-hour OGTT plasma insulin; TC, total cholesterol; TG, triacylglycerol; LDL-c, low-density lipoproteins; HDL-c, high-density lipoproteins.

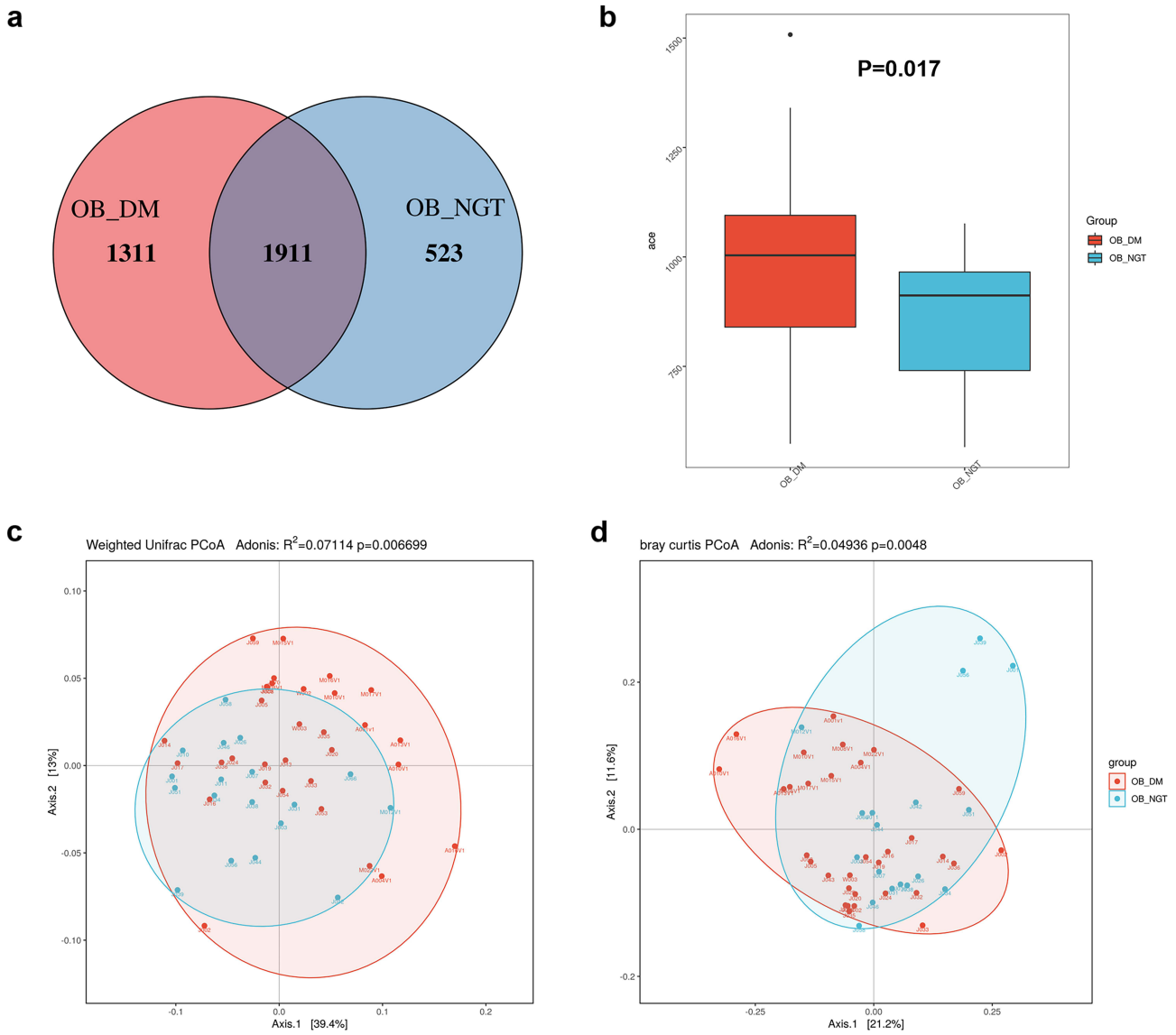

Figure 1 Diversity and richness of the gut microbiota in obesity-DM group and obesity-NGT group. (**a**) Venn diagrams showing OTU distribution; (**b**) alpha diversity estimated by the ACE index; (**c** and **d**) Principal-coordinate analysis based on weighted UniFrac and Bray-Curtis distance.

The Difference in Microbiota Composition in the Obesity-T2DM and NGT Group

The dominant phyla of the two groups were *Bacteroidetes* and *Firmicutes*, followed by *Proteobacteria, Fusobacteria* and *Actinobacteria*. Obesity-T2DM patients exhibited a significant decrease in the abundance of *Bacteroidetes*, and a significant increase in the abundance of *Firmicutes* and *Actinobacteria* compared to obesity-NGT. Regarding the genus level, *Bacteroides, Escherichia-Shigella* and *Roseburia* increased considerably in the Obesity-T2DM group. Nevertheless, *Prevotella_9* and *Sutterella* had an evident decrease in Obesity-T2DM compared to obesity-NGT (Figure 2). The other significantly different bacteria from the phylum level down to the genus level based on Metastasis analysis are given in Table S1.

Lefse analysis (LDA score > 3.0 as the cutoff) was conducted to further determine the different taxa between the Obesity-T2DM and Obesity-NGT groups. The results showed that 16 genus, 3 family, and 1order including *Bacteroides* and *Bacteroidaceae* were enriched in the Obesity-T2DM group, while *Bacteroidetes, Bacteroidia, Bacteroidales, Prevotella_9* and *Sutterella* were enriched in the Obesity-NGT group (Figure 3).

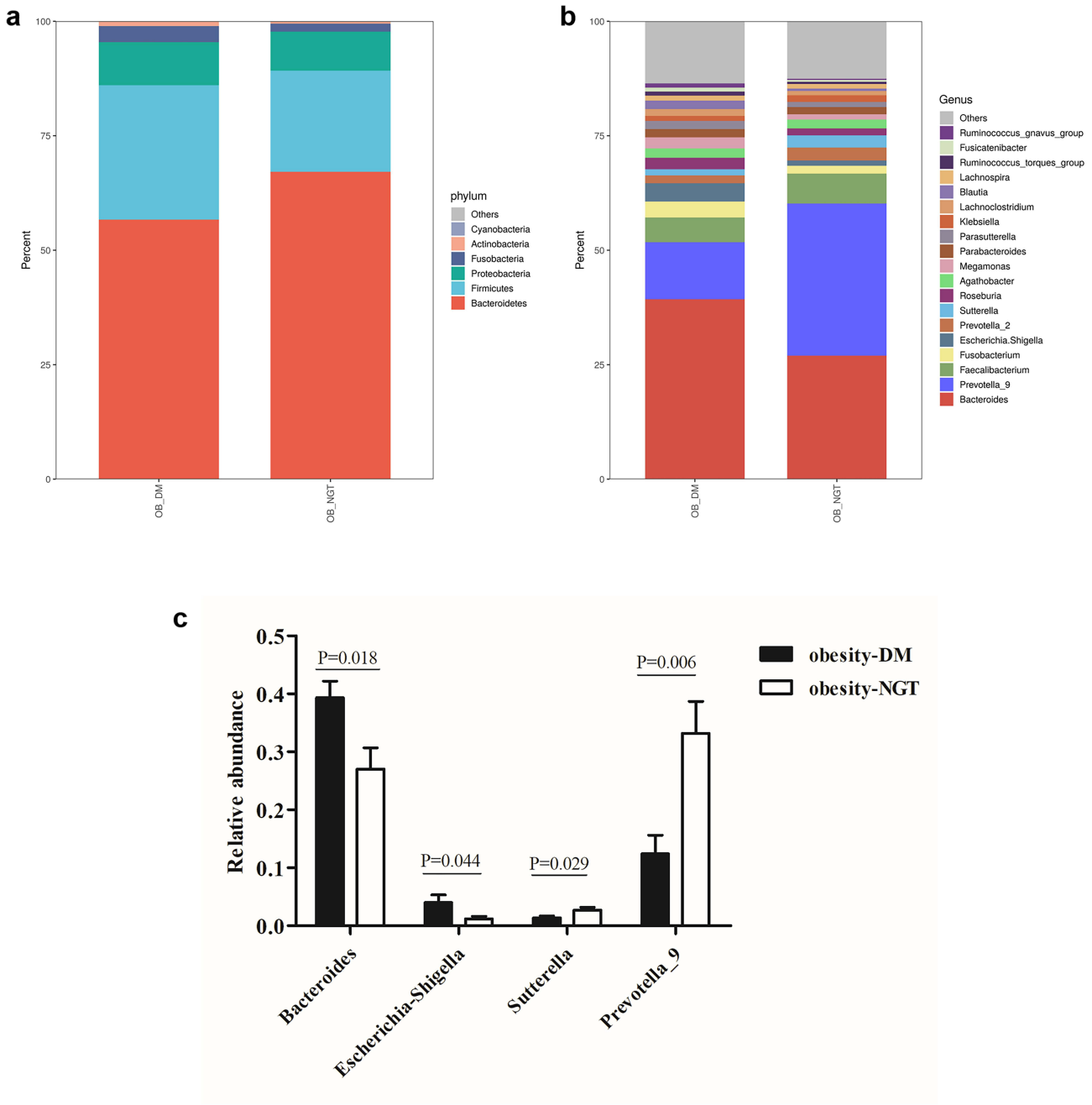

Figure 2 Composition of gut microbiota in obesity-DM group and obesity-NGT group. (**a** and **b**) The relative abundance of bacteria at the phylum level and genus level; (**c**) Comparison of differentially abundant significant genera.

Correlation Between the Gut Microbiota and Glucose and Lipids Parameters

Spearman correlation was conducted to evaluate the association between the relative abundance of differently expressed taxa and glucose and lipid metabolism indices. *Bacteroidetes, Bacteroidia, Bacteroidales, Prevotella_9* and *Sutterella* showed significantly negative correlations with HbA1c, fasting blood glucose and triglyceride levels. Besides, *Bacteroidetes, Bacteroidia, Bacteroidales* and *Prevotella_9* exhibited positive correlations with HDL-c. Additionally, *Lachnospiraceae, Firmicutes, Blautia* and *Roseburia* were positively associated with HbA1c and OGTT-2h glucose. Moreover, positive correlations were found between *Bacteroides, Bacteroidaceae* and HOMA-IR (Figure 4).

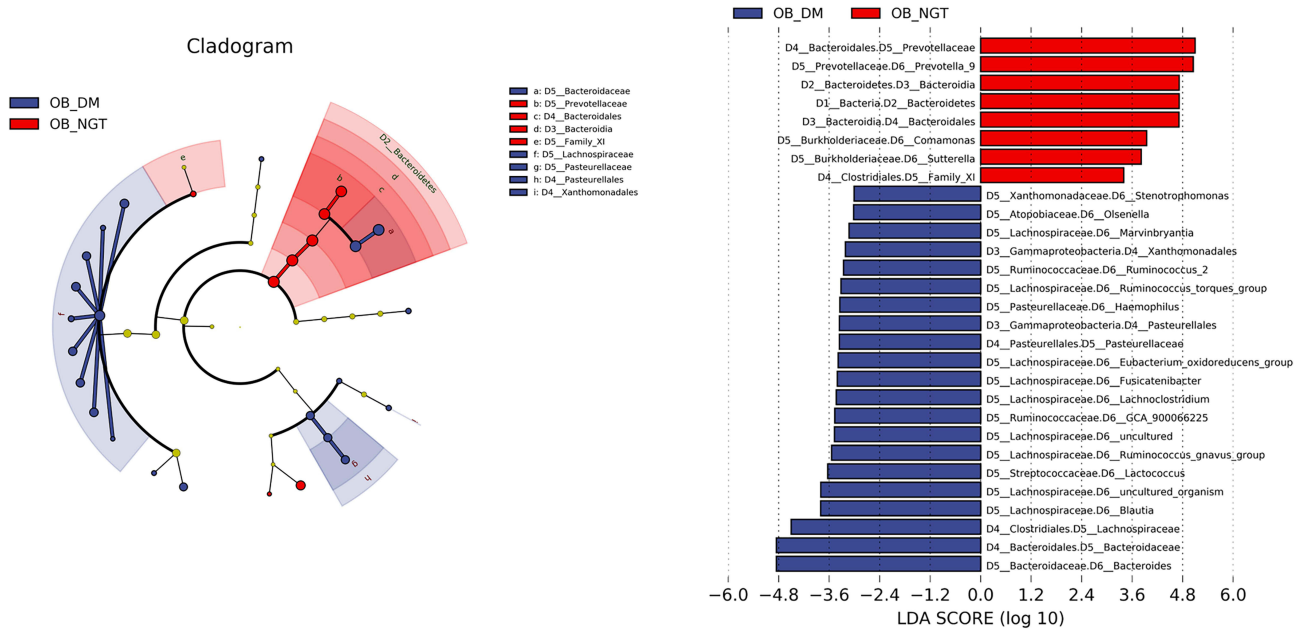

Figure 3 Cladogram and Linear discriminant analysis effect size analysis in obesity-DM group and obesity-NGT group.

Discussion

Although a large number of studies have shown gut microbiome alterations in obesity or T2DM, there is limited evidence to compare intestinal bacterial differences between obesity with T2DM and NGT. The present study demonstrated, for the first time, that the gut microbiota profile of obesity-T2DM individuals was substantially diverse from that of obesity-NGT individuals in Chinese populations. Importantly, we found that gut bacteria conducive to glucose metabolism, such as *Sutterella* and *Prevotella_9*, were significantly decreased in obesity with T2DM, while *Bacteroides* exerting pro-inflammatory effects and inducing insulin resistance was enriched.

Alpha diversity reflects intra-individual bacterial diversity. Lower alpha diversity had been identified in T2DM and obesity in previous studies,[10,14] but there is still much debate on the alpha diversity of T2DM or obese gut microbiome.[15] Interestingly, the present study firstly observed that the ACE index reflected bacterial community richness was higher in obesity-T2DM than in obesity-NGT. In agreement with our findings, metformin and berberine significantly reduced alpha diversity and gut microbiota richness in obese rats.[16] Greater bacterial diversity in obese Japanese subjects compared with non-obese subject was also observed.[17] In contrast, Thingholm LB et al reported that alpha diversity was not significantly different between obesity with and without T2DM.[12] The inconsistent results may be due to differences in diabetic medications (None of the subjects in our study took antidiabetic drugs), dietary habits and genetic factors, and other factors. In short, the relationship between higher alpha diversity and disorders is complex. Perhaps what matters is not just the bacterial richness but also the specific composition of the gut microbiota. Overabundance of potential pathogenic bacteria may contribute to the high alpha diversity in obesity-T2DM. Further investigations are required to explore the relationships between decreased alpha diversity and metabolism disease.

Our findings indicate that clear differences in the gut microbial composition of obesity-T2DM compared to obesity-NGT. Notably, obesity with T2DM showed a significant decrease in the relative abundance of *Sutterella* and *Prevotella_9*. The genus *Sutterella* is a gram-negative, anaerobic, non-spore-forming bacterium. Similar to our findings, decreased abundance of *Sutterella* had also been demonstrated in T2DM and type 1 diabetes (T1DM) compared to healthy people.[18] Furthermore, a recent study reported that the gut microbiota of normoglycemic pregnant women was associated with an increased abundance of the genus *Sutterella*.[19] Importantly, an increased abundance of *Sutterella* was observed in the caecum of T2DM rats after Roux-en-Y gastric bypass surgery, which may contribute to glucose metabolism improvement after surgery.[20] Another animal study demonstrated that the application of metformin could selectively increase the abundance of *Sutterella*.[21] Thus, these studies suggest that the genus *Sutterella* has beneficial

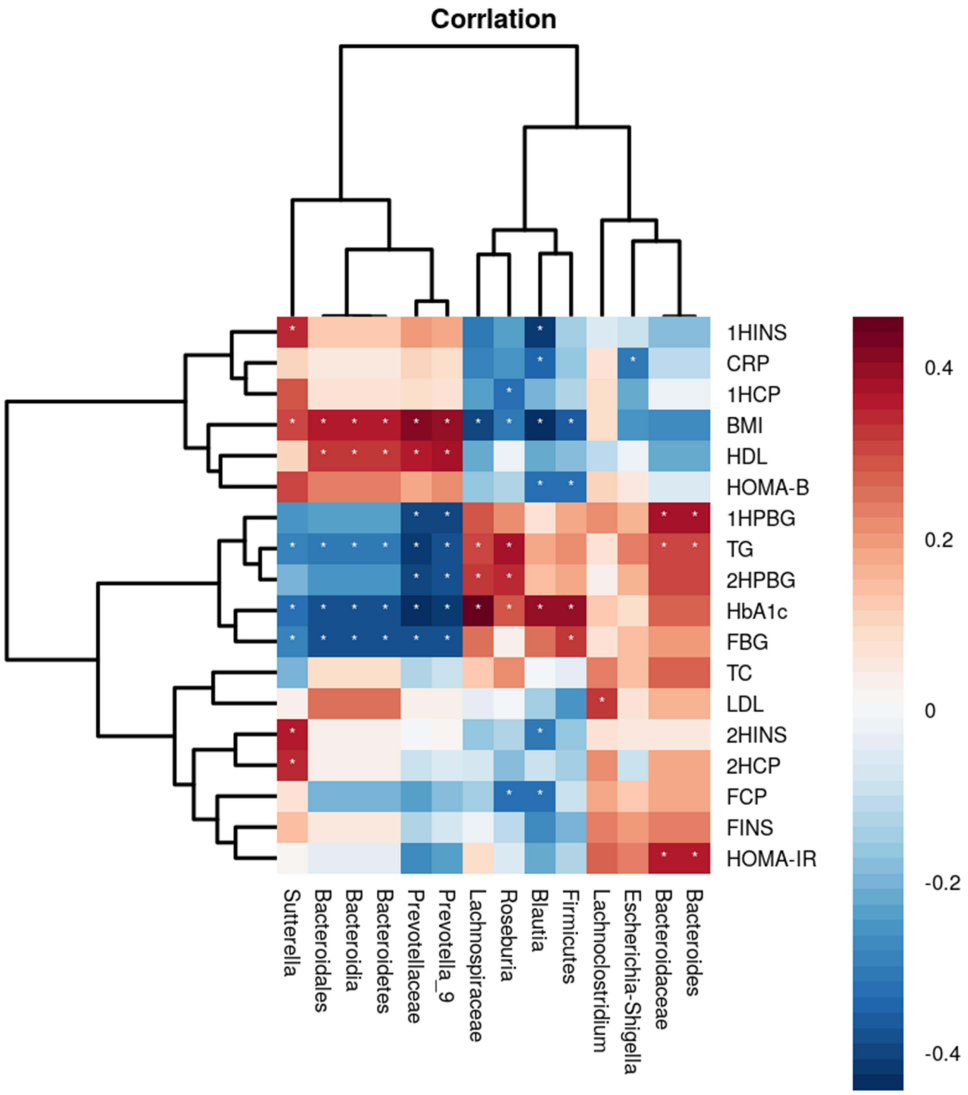

Figure 4 Spearman correlations between different gut microbiota and glucose and lipid metabolic parameters. * P<0.05.

effects on glycometabolism. It also aligns with the fact that *Sutterella* was negatively associated with HbA1c and fasting blood glucose in our study. However, the underlying mechanism by which *Sutterella* affects glucose metabolism remains unclear. Previous studies showed *Sutterella* was positively correlated with glucagon-like peptide (GLP-1) and negatively associated with proinflammatory cytokine and LPS biosynthesis.[21,22] GLP-1 can promote insulin secretion and inhibit glucagon release in a glucose-dependent manner, leading to antihyperglycemic action. Chronic low-grade inflammation is thought to drive insulin resistance and T2DM. Nevertheless, future functional studies on *Sutterella* are needed to better understand its potential role in glucose metabolism.

Accumulating evidence indicates SCFAs (ie, acetate, propionate and butyrate) beneficially contribute to systemic glucose and energy homeostasis by reducing hepatic glucose production and triggering intestinal gluconeogenesis.[22] Additionally, SCFAs regulate mucosal immune function, provide fuel for intestinal epithelial cells and improve epithelial barrier function, thereby preventing LPS translocation from the intestinal barrier.[7,23] Recent research has also shown that obesity microbiota weakened intestinal barriers and instigated gut permeability and inflammation, thus inducing abnormalities in glucose metabolism by ethanolamine/ARID3a/miR-101A/Zo1 axis.[24] It is important to note that *Prevotella_9* belongs to SCFAs-producing bacteria. As such, it beneficially affects intestinal inflammation by inducing anti-inflammation cytokine secretion and favoring Th17 responses.[25] Moreover, this genus-produced succinate improved

glycemic control through activation of intestinal gluconeogenesis.[26] *Prevotella_9* had been reported to be poorly represented in the fecal samples of diabetes and diabetic nephropathy.[18,27,28] Consistent with previous reports, our study found that decreased abundance of *Prevotella_9* in obesity with T2DM was negatively correlated with HbA1c and blood glucose levels. Particularly, *Prevotella* is known to produce propionate, which stimulates the release of GLP-1 and reduces intrahepatocellular lipid content.[29] However, controversial results regarding the effect of *Prevotella* on glycemic control had been reported. *Prevotella* enterotype was associated with a diet rich in carbohydrate and fiber.[13] Significant reductions in total cholesterol and low-density lipoprotein cholesterol were also observed following propionate supplementation.[30] In accordance, our study indicated *Prevotella_9* was negatively associated with triglyceride and positively associated with HDL-c. Certainly, measurement of intestinal SCFAs levels and culture of SCFAs-producing bacteria will help to further understand its role in modulating host metabolism.

On the other hand, we found that *Bacteroides* and *Escherichia-Shigella* dominated in obesity with T2DM patients. The Gram-negative bacteria *Bacteroides* and *Escherichia-Shigella* are considered to be opportunistic pathogens that produce LPS and disrupt the intestinal barrier function of the epithelial cell layer, inducing chronic low-grade inflammation.[31,32] Several studies have also demonstrated a marked increase in the abundance of intestinal *Bacteroides* and *Escherichia-Shigella* in T2DM and T1DM, and *Bacteroides* were positively correlated with glucose.[28,33] These findings are consistent with our study to some extent. Furthermore, in support of our findings, a metagenome-wide association study of gut microbiota in China showed that T2DM patients were characterized by an increase in various opportunistic pathogens including family *Lachnospiraceae*.[34] Our findings indicated that *Lachnospiraceae* enriched in obesity with T2DM and was significantly positively associated with HbA1c and plasma glucose. Collectively, gut microbiota disturbance may play a fundamental role in obesity developing into T2DM. Increasing researches have highlighted the significant and complex role of bacterial metabolites, such as amino acids, bile acids and SCFAs in glucometabolic disturbances.[35] The interplay between microbiota and metabolites potentially influences whole body glucose homeostasis. Modulating the gut microbiota and metabolites is considered a promising and novel approach in the prevention and treatment of obesity and T2DM. Administration of probiotics, prebiotics, and fecal microbiota transplant may be the potential ways to restore microbial disturbances.[35] Oral supplementation of the microbiota-derived amino acid histidine has been shown to exert metabolic benefits.[36] Future studies are required to further determine the optimal dose of these interventions and the most effective strains and metabolites for obesity and glycemic control.

The present study has several limitations that deserve to state. First, the included sample size was relatively small. Differences in low-abundance microbes may not be able to be identified. Thus, a larger cohort is needed to further validate our findings. Second, to minimize the confounding factors, we enrolled age, gender and BMI-matched obesity subjects living in North China. However, minor dietary differences between the obesity-T2DM and obesity-NGT group cannot be excluded. Particularly, the notable effect of dietary iron on the gut microbiome was observed in both human cohort and mice study,[12] but iron intake was not evaluated in our study. Additionally, this is a cross-sectional study. The causal relationship between gut microbiota and the development of T2DM in obese patients can not be established. Finally, only 16s rRNA sequencing was performed. Thus, metagenomics analysis should be applied to investigate the function of gut microbiota.

Conclusions

In conclusion, our research indicates significant differences in the gut microbiota composition in obesity-T2DM compared with obesity-NGT. Enriching potential pathogen-like bacteria and reducing SCFA-producing bacteria may be involved in the progression from obesity to T2DM. Importantly, these findings suggest the gut microbiota is expected to be a promising novel intervention target for obese management. However, larger sample size and more in-depth taxonomic identification studies are warranted in future investigations.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

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Disclosure

The authors declare no conflict of interest.

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