Inulin-type Fructans Protect Against High Fat-sucrose Diet-induced Gestational Diabetes Mice in Association with Gut Microbiota Regulation

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Abstract

Background: Inulin-type fructans have been used as prebiotics to alleviate glucose and lipid metabolism disorders. However, few studies evaluate the microbial mechanism of inulin in improving maternal metabolic status during pregnancy.

Methods: We use high fat-sucrose diet-induced gestational diabetes mice as a model to study the effect of inulin-type fructans on the cecal microbiota by 16S rRNA gene amplicon sequencing.

Results: The inulin-type fructans-supplemented diet improved glucose and lipid metabolism disorder parameters in high-fat/sucrose diet (HFD) induced gestational diabetes mice, alleviating fat accumulation and glucose intolerance. The α diversity of gut microbial community of HFD mice was reduced after inulin treatment, while the β diversity tended to return to the level of normal chow diet (NCD) mice. Interestingly, *Verrucomicrobia*, *Bifidobacterium* and *Akkermansia* were obviously enriched, while *Dubosiella* was obviously lessened after inulin treatment. Further analysis indicated that *Dubosiella* was positively correlated with markers of glycolipid metabolism disorders, whereas the inulin-type fructans-supplemented diet partially reversed the changes.

Conclusions: Our results suggest that the inulin-type fructans-supplemented diet alleviates glucose and lipid metabolism disorders mediated by gut microbiota.

Background

Gestational diabetes mellitus (GDM), carbohydrate intolerance and insulin resistance during pregnancy are serious problems that are increasing worldwide [1], which carry significant short-term and long-term adverse health outcomes in both mother and offspring [2–4]. In GDM women, the physiological changes in insulin resistance and lipid profiles are exaggerated and may indicate an underlying metabolic dysfunction that transiently manifests during pregnancy [5, 6] .

Gut dysbiosis plays a vital role in abnormal host metabolism, as recently demonstrated in studies of type 2 diabetes (T2D) and obesity[7]. Prevotella and Bacteroides have been identified as the main species contributing to insulin resistance and glucose intolerance[8]. While the impact of gut microbiota on host metabolism and metabolic diseases is well-documented [9], only recently, studies have focused on microbiota changes to influence metabolic mechanisms during pregnancy[10]. Parabacteroides are significantly more abundant in GDM women than in healthy pregnant women [11]. Novel relationship between gut microbiome composition and the metabolic hormonal environment in overweight and obese pregnant women at the first trimester has also been described [12]. These studies suggest that major shifts in the gut microbiome during pregnancy may play a crucial part in the development of GDM.

Dietary intervention to modulate the gut microbiota has become a potentially effective strategy to improve host health [13]. Dietary fibers, like inulin-type fructans (ITF), are present in many vegetables, and can also be extracted and isolated from chicory roots, to be used as food ingredients [14]. Isolated ITF

have been considered to be typical prebiotics [15]. Prebiotics are defined as non-digestible compounds that through fermentation by the microorganisms in the gut, modulate the composition and/or activity of the gut microbiota, thereby conferring a beneficial physiological effect on the host [16, 17]. In vitro studies and randomized controlled trials have shown that ITF can stimulate the growth of Bifidobacterium populations [18, 19] and certain butyrate-producing species [20, 21] as well as reduce the abundance of Firmicutes [22–24]. In addition, numerous randomized controlled trials have demonstrated direct health benefits from ITF, including the inhibition of pathogens, protective effects against cardiovascular disease, and the improvement of mineral bioavailability [25–27]. However, the relationships among dietary ITF, GDM and gut microbiota are still not clear.

Given that there are few studies aiming to evaluate the microbial mechanism of soluble dietary fiber in improving maternal metabolic status during pregnancy, the current research was undertaken to investigate effects of adding ITF to high-fat/sucrose diet (HFD), on the composition and metabolites of fecal microbiota from 4 weeks before conception and throughout gestation as well as maternal and neonatal health parameters in GDM mice model. It is supposed to provide some microbial mechanistic insights into the application of ITF to a typical gestational diet characterized by high fat/sucrose and energy intake for improving maternal and neonatal health.

Methods

Materials

Six-week-old C57BL/6 J mice were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). ITF were procured from Fengning Ping'an hi tech Industry Co., Ltd (Vilof TM Soluble Dietary Fiber powder) which contains 91% ITF and 9% mixture of sucrose, fructose, and glucose.

Animal treatment and experiment design

Mice were housed in a temperature-and humidity-controlled laboratory. Approval of this animal experiment was approved by the Animal Protection Ethics Committee of Women's Hospital of Nanjing Medical University (No.2018-49). All animal experiments were performed in accordance with Chinese national regulations on the administration of animal experimentation as well as international guidelines on animal experimentation. After one week of acclimatization, mice were randomly divided into three groups (n=5): control (normal chow diet (NCD)+vehicle, n=5), HFD (high-fat/sucrose diet (HFD)+vehicle, n=5) and inulin-type fructans treatment (HFD+ITF, N=5). In order to compare the changes of intestinal flora before and after pregnancy, the three groups were renamed normal chow diet in (NCDG) group, HFDG group and ITFG group after mating. The control mice were fed a low-fat diet (Research Diet AIN-93G, consisting of 20.3%protein, 63.9% carbohydrate, and 15.8% fat) for 4 weeks prior to mating and throughout pregnancy (18 days), while both HFD and treatment groups were fed a high-fat/sucrose diet (Research Diet D12451, consisting of 35.2%protein, 63.9% carbohydrate, and 45% fat). The treatment group received a 3.33g/kg dose of ITF solution each day via oral gavage, whilst the control and HFD

group received the same dose of a vehicle (H_2O_2). All mice were given free access to 100 grams of fresh diet and 250 ml of fresh water daily per cage (Five mice per cage).

Fasting blood glucose (FBG) and Oral glucose tolerance test (OGTT)

Blood samples were collected from the tail vein and blood glucose levels were measured with a glucose meter (Roche Accu-Chek Active, Mannheim, Germany). Fasting blood glucose (FBG) were monitored at different time points, including before dietary intervention, after 4 weeks of HFD, and on GD 0, 10, 14 and 18. Oral glucose tolerance test (OGTT) was performed on GD14. The animals were fasted for 6 h, and then gavaged with 2 g/kg glucose. The blood glucose levels at 0, 30, 60, 90 and 120 min were determined.

Detection of biochemical indexes

Mice were euthanized by CO_2 inhalation on GGD18 (or equivalent) after fasting for 6 h from 8 AM and blood sample was collected. Blood was centrifugated at 3,000 g for 15 min at 4℃ and serum was isolated. The levels of fasting serum insulin (FINS), triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were measured using a commercial detection kit (NJJCBIO Co., Ltd, Nanjing, China) according to the kit instructions.

Based on the measured content of FBG and FINS, the homeostasis model of assessment (HOMA) for IR index (HOMA-IRI) was calculated and compared. HOMA-IR was calculated as (fasting glucose (mmol/L) x fasting insulin (mU/L))/22.5. Meanwhile, area under the curve (AUC) of blood glucose was calculated [28].

Haematoxylin-eosin Staining

Liver and inguinal fat tissues were fixed in 4% paraformaldehyde, decalcified, paraffin-embedded and stored at 4°C. After tissues were sliced into 4 μm sections, haematoxylin-eosin staining was performed. First, sections were stained with haematoxylin for 5-10 minutes, immersed in 70% ethanol for 30 minutes to remove cytoplasm colouring, alkalized with alkaline solution and washed with distilled water for 1 minute. Second, sections were stained with eosin for 30-60 seconds, dehydrated with gradient ethanol, cleared two times with xylene, dried and mounted. Finally, the morphological structures of the liver and inguinal fat tissues were observed under an optical microscope.

Fecal DNA extraction

On 1 day prior to mating and GD18, fecal samples were collected in individual sterilized cages and immediately frozen in liquid nitrogen. About 100 mg of stool samples were used to extract total genome DNA according to the DNA extraction kit (DP328, Tiangen Company, Beijing, China). The concentration and purity of the extracted bacterial DNA were detected using Qubit 2.0 Fluorometer (Thermo Scientific, USA). The 16S rRNA gene V4 region-specific primer are 515F (GTGCCAGCMGCCGCGGTAA) and 806R GGACTACHVGGGTWTCTAAT. The PCR products of sterile water were considered as the negative control

for 16S rRNA seq. The PCR products were purified using the Gene JET Gel Extraction Kit (Thermo Scientific). The library was constructed using Ion Plus Fragment Library Kit 48 reactions (TermoFisher, USA). After Qubit quantification and testing, the library was sequenced by TermoFisher's Ion S5^{TM}XL.

Gut microbiota analysis

Raw data were obtained after data were processed using Cutadapt (V1.9.1, http:// cutadapt. readthedocs.io/en/stable/). Ten, chimera sequences were removed to obtain clean reads. OTUs were assigned for sequences with ≥ 97% similarity. OTUs were annotated using the SILVA132 database (http://www.arb-silva.de/). The taxonomic information was obtained, and the community composition was counted at seven taxonomic levels: kingdom, phylum, class, order, family, genus and species. Alpha-diversity was analyzed by Chao 1 (http://scikit-bio.org/docs/latest/generated/ skbio.diversity.alpha.chao1.html#skbio.diversity.alpha.chao1) with QIIME software (version 1.9.1). Beta-diversity metrics were calculated by the NMDS model based on Bray-Curtis distance. One-way ANOSIM analysis with multiple pairwise post-tests on all groups at the same time was performed to test whether the difference between the extra groups was greater than that between the intra groups and to assess the significance of the difference in separation. The Chao1, Bray-Curtiss indexes, NMDS and ANOSIM were calculated at OTU level. Differentially abundant genera were analyzed by meta stats (https://omictools.com/metastats-tool) with a nonparametric test, followed by the Benjamini and Hochberg false discovery rate approach to filter relevant p-values.

Statistical analysis

Data represent mean ± standard error of the mean. For parametric variables, the unpaired two-tailed Student t-test was used to assess the differences in mean values between two groups. For three groups, statistical analysis was performed with ANOVA with Tukey post hoc test. For nonparametric variables, the statistical significance of the differences was evaluated by the Mann–Whitney test or Kruskal–Wallis test. For the repeated measures in case of growth and OGTT results, multivariate ANOVA was performed with a post hoc test using Bonferroni method. $P<0.05$ was considered statistically significant. GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA) was used to do the statistical analyses.

Results

The inulin-supplemented diet can improve metabolic disorder related symptoms in high fat-sucrose diet-induced gestational diabetes mice

To investigate the effect of inulin treatment on glycolipid metabolism disorders in high- fat/sucrose diet induced gestational diabetes mice, we examined the body weight, daily food intake and glycolipid metabolism related parameters. The body weight, FBG, FINS, TG, TC, LDL-C and the area under the curve (AUC) of the oral glucose tolerance test (OGTT) of the HFDG group mice were significantly elevated

compared with those of NCDG group mice (Figure 1A-G, Figure 2A-D), indicating severe glucose intolerance and dyslipidemia.

In contrast, ITFG group mice fed the ITF-supplemented diet showed improved metabolic parameters (Figure 1A-G, Figure 2A-D). After ITF intervention, body weight, serum TG, TC and LDL-C on GD18 reduced significantly by 4.54 g, 0.48 mmol/l, 1.04 mmol/l and 0.494 mmol/l ($p< 0.05$, vs HFDG group) (Figure 1C, Figure 2A, B, D). Additionally, the AUC of OGTT on GD 14, FBG and serum insulin on GD 18 were lowered by 7.95 mmol/L/h, 2.04 mmol/l and 3.46 mIU/L ($p< 0.05$, vs HFDG group), indicating a significant improvement in glucose tolerance (Figure 1G, D, E). According to hepatic and adipose tissues staining (Figure 2E), HFDG group mice exhibited severe hepatic lipid droplets and adipocyte hypertrophy, which were alleviated after ITF treatment. Overall, the above results indicate that ITF have a beneficial effect that ameliorates glycolipid metabolism disorders in HFD mice.

Changes of Fecal Microbial Diversity

We used the 16S rRNA gene amplicon sequencing method (V4 region) and generated 2,131,728 reads for a total of 25 samples, with an average of 85269± 22,171 reads per sample. At each stage, NCD-HFD-ITF and NCDG-HFDG-ITFG pairs shared less common OTUs with each other, respectively. The Venn graph exhibited common OTUs for NCD-HFD-ITF and NCDG-HFDG-ITFG pairs decreased from 579 before mating to 438 on GD18 of gestation, respectively (Figures 3A).

To assess fecal microbial community structure, richness (Chao 1 index) and diversity (Simpson index) were calculated (Figure 3B, C). For the Chao 1 index, the data of ITF group exhibited significantly higher than NCD and HFDG groups ($p<0.05$, $p<0.01$). A remarkable increment in Simpson index with ITF supplementation was found compared with HFD and HFDG groups in the present study ($p<0.05$). All the results above provide the view that ITF addition could effectively improve the decline of Chao 1 index and Simpson index induced by high fat addition.

We then used Principal Co-ordinate Analysis to categorize the Operational Taxonomic Units (OTU) data into two main factors that explained 64.42% of the variance (Figure 3E), which showed that the microbiome in NCD (NCD and NCDG), HFD (HFD and HFDG) and ITF (ITF and ITFG) treatment groups significantly differed from one another while the two groups of the same treatment shared some overlapping regions before and after conception, which indicated that the overall gut microbial community had been significantly modified. The four groups exhibited significant, tight clustering according to NCD or ITF diet. Independent biological replicates were generally consistent, but more variable among mice fed by HFD (Figure 3D).

Changes of the Relative Abundance at Phylum Level

The phylum Bacteroidetes was dominant among the 9 phyla (>1% in at least one sample) present in the gut microbiota from the six groups of mice, and the ratio of *Firmicutes/ Bacteroidetes* was increased in HFD and HFDG mice over NCD and NCDG groups, but lower in the ITF and ITFG groups compared with HFD and HFDG mice (Figure 4). The gut microbiota in obese individuals has usually shown an increased *Firmicutes/ Bacteroidetes* ratio [21]. Therefore, the decreased *Firmicutes/ Bacteroidetes* ratios of ITF and ITFG mean that this feature in obesity could be reversed by the ITF-supplemented diet. High fat treatment decreased the relative abundance of *Proteobacteria* before mating (p<0.01). ITF supplementation increased the relative abundance of *Verrucomicrobia* compared with HFD before mating and on GD18 of gestation (p<0.01). Relative abundances of *Deferribacteres* group of HFD and *Cyanobacteria* group of NCD were not detected in fecal samples on GD18 of gestation. Moreover, relative abundances of *Actinobacteria* decreased in HFD before mating, while increased substantially when reaching the perinatal period. The majority of genera were affected by gestation stage indicating that their relative abundances changed greatly over the pregnancy progress.

Changes of the Relative Abundance at Genus Level

The relative abundances at genus level (>1% in at least one sample) were present in Figure 5. Fat addition (HFD and HFDG) increased the relative abundances of *Dubosiella* and *Lactobacillus* and decreased that of *Romboutsia* and *Alloprevotella* compared to the NCD (NCD and NCDG). The abundance of *Bifidobacterium* increased, whereas that of *Dubosiella* decreased with the intervention of ITF before and after conception. Our results also indicated that the abundance of *Akkermansia* was significantly higher in the ITF-treated (ITF and ITFG) groups than any other group. The heat map analysis of microbial community composition at the family level confirmed that the abundance of *Dubosiella* that cause obesity and metabolic syndrome-related inflammation were reduced (Figure 6).

Next, to identify the changes in specific bacterial taxa after the inulin-supplemented diet intervention before and after conception, we utilized the linear discriminant analysis (LDA) effect size (LEfSe) to compare the cecal microbiota composition between the NCD, HFD and ITF groups, LDA score was selected to discriminate specific taxa in different groups. Compared with the HFD group, the ITF mice had a higher abundance of *f-Ruminococcaceae, f-prevotellaceae, o-Verrucomicrobiales*, *g-Akkermansia*, *c-Verrucomicrobiae*, *p-Verrucomicrobia* and *f-Akkermansiaceae* but lower abundance of *g-Unidentified clostridiales*, *f-Unidengtified Clostridiales, g-Dubosiella*, *c-Erysipelotrichia*, *o-Erysipelotrichales* and *f-Erysipelotrichaceae* (Figure 7A-E). Correspondingly, *g-Bacteroides*, *f-Ruminococcaceae* and *f-Bacteroidaceae* were enriched in ITFG group on GD18 of gestation (Figure 8A-C).

Correlations between glycolipid metabolism indicator and bacterial abundance

At phylum level, we analyzed the correlations between significant glycolipid metabolism indicator and gut microbiota on GD18 of gestation. *Bacteroidetes* abundance was negatively correlated with FBG, FINS, TG and TC, whereas *Firmicutes* abundance was positively correlated with FBG, FINS and TG (Figure 9A). Moreover, *Actinobacteria* abundance was positively correlated with FINS and TC (Figure 9A).

At genus level, the relative abundance of *Dubosiella* showed positively correlated with FBG, FINS and TC (Figure 9B). *Romboutsia* abundance was positively correlated with FBG (Figure 9B).

Discussion

Disorder of the gut microbiota has been considered one of the reasons for metabolic disorders. The composition of the microbiome also changes during pregnancy. It has recently been proposed that intestinal microflora and their metabolic activities (intestinal dysbiosis) may play a critical role in body weight control, energy homeostasis, fermentation, and absorption of non-digestible carbohydrate, and also in the development of IR; therefore, may also participate in the pathogenesis of several metabolic disorders, such as obesity, diabetes mellitus, and GDM [29–32]. Prebiotics can exert positive effects on the maintenance of host metabolic homeostasis, which are mainly mediated by gut microbiota [33, 34]. ITF, one of the crucial prebiotics, has been demonstrated the effectiveness in treatment of T2DM [35, 36], while data on the effects of synbiotic supplementation on markers of insulin metabolism and lipid concentrations in GDM are scarce. The aim of this study was to determine whether ITF taken before and during pregnancy, would impact the development of HFD-induced glucose intolerance during pregnancy.

To induce features of gestational diabetes, mice were fed a high-fat/sucrose diet (HFD) for 4 weeks before and during pregnancy. This model has previously been used to induce features of GDM in mice, such as insulin resistance and dyslipidemia [37–39]. A period of only 4 weeks of HFD exposure before pregnancy is not sufficient to cause a diabetic phenotype; however, continued feeding throughout pregnancy leads to progressive glucose intolerance and insulin resistance, mimicking human disease. This mouse model allowed a factorial design to determine the interaction of treatments, as well as more thorough examination of potential mechanisms and whole-tissue analysis, which would not be possible in human trials.

Our results demonstrated that ITF possessed the therapeutic efforts on HFD-induced GDM mice. The decrease of BWs after treatment of ITF indicated that dietary inulin may potentially improve the nutrition absorption and energy metabolism in the gastrointestinal tract. The metabolic routine indicators including FBG, insulin, OGTT, TG, TC and LDL-C were totally alleviated in the treatment of ITF, revealing that ITF can regulate the metabolic disorders. In consistent with our findings, inulin-fed mice increased the production of short chain fat acids (SCFAs) to benefit the balance of gut microbiota in the alleviation of diabetic mice [40]. Inulin dose-dependently decreased caloric intake, improved glucose tolerance, increased the abundance of Bacteroidetes and Bifidobacterium spp. with metabolic effects being largely independent of caloric restriction [41]. A randomized-controlled clinical trial revealed that inulin

administration significant decreased in the levels of FBG, GHb, IL-6, TNF-α and plasma LPS in T2DM patients [42, 43].

Accumulating studies have been performed to reveal the underlying mechanisms of efficient treatment of ITF in GDM. The majority of mechanisms are attributed to gut microbiota alteration, immune inflammation, abnormal lipid metabolism and oxidative stress. During these mechanisms, growing evidences have demonstrated the gut microbiota play a critical role in the development of GDM [44–47]. In the present study, the Alpha diversity index reduced by HFD could be effectively improved by inulin addition. Stanislavski et al reported gestational weight gain has been associated with lower alpha diversity [48]. The Beta diversity analysis of unweighted UniFrac illustrated the distinct clustering of the relative abundances of operational taxonomic units (OTUs) after ITF treatment. Moreover, similar results from PCoA analysis were obtained.

At phylum level, a higher ratio of Firmicutes to Bacteroidetes (F/B) was observed in the HFD group, which was supported by a study showing that the F/B ratio in overweight human adults was lower than that in lean controls [49]. An imbalance in the Firmicutes/Bacteroidetes ratio has already been related to dysbiosis conditions [50, 51]. The decreased Firmicutes/Bacteroidetes ratios of ITF and ITFG mean that this feature in obesity could be reversed by the ITF-supplemented diet. Our analyses showed that after ITF treatment, an enhancement of relative abundance of *Verrucomicrobia* in HFD group before mating and on GD18 of gestation, as well as an obviously lessened *Actinobacteria* on GD18 of gestation. The *Verrucomicrobia* is a member of the PVC (*Planctomycetes-Verrucomicrobia-Chlamydiae*) superphylum which includes phylogenetically related bacteria with unusual characteristics such as the existence of a complex and dynamic endomembrane system that, in some aspects, makes them closer to eukaryotic cells. A recent study showed that the healthy chilean subjects reveals a high abundance of the phylum *Verrucomicrobia* [52]. Positive correlations of *Actinobacteria* with FINS caused aggravation of insulin resistance in the disease was reversed by inulin intervention.

At genus level, ITF supplementation showed significant effect on increasing the abundance of *Bacteroides* which have been demonstrated to ameliorate inflammation in recent studies [53–55]. SCFAs derived from gut microbiome, including acetates, propionates and butyrate, are pivotal for rectifying host metabolism and immunity [56]. Significant elevation of SCFAs-generating *Bacteroides* revealed that our ITF treatment may restore gut dysbiosis by promotion of *Bacteroides*. Another genus that we found in abundance in fecal samples of the controls was *Akkermansia*. Recent studies described this as an important eubiotic genera, with systemic beneficial effects to the host [57, 58], including the control of metabolic syndromes [59, 60]. In rodents, probiotics supplementation with *Akkermansia* improved glucose tolerance and insulin sensitivity [61]. Our results suggest that *Akkermansia* might have another impact on host physiology during pregnancy than otherwise described or that we find another subspecies of *Akkermansia*. The applied 16S rRNA gene amplicon sequencing methods does, however, not make it possible to investigate this finding at a deeper taxonomic resolution. We observed that HDF mice have increased *Dubosiella* which has been previously described in dysbiotic conditions such as GDM and

obesity [62–66]. Positive correlations of *Dubosiella* with FBG, FINS and TC demonstrated that these bacteria may promote the glycolipid metabolism disorders, but could be reversed by ITF treatment.

Conclusions

GDM is accompanied by alterations in gut microbiota composition and increasing inflammatory cascade. Dietary inulin ameliorates GDM via suppressing inflammation and modulating gut microbiota, especially in pre-diabetic and early diabetic stages, thus potentially serves as an inexpensive intervention for the prevention and treatment of T2DM patients.

Prebiotic effect of ITF might be involving in the host transcriptome changing mediated by altering the gut microbiota. Based on the results of 16S rRNA gene amplicon analysis and transcriptomic analysis, we explored the complex interplay between the cecal microbiota and host glycolipid metabolism. Giving ITF (3.33 g/kg/day) to high fat-sucrose diet-induced gestational diabetes mice can alleviate glucose and lipid metabolism disorders, increase the abundance of *Verrucomicrobia*, *Bifidobacterium* and *Akkermansia*, and reduce the abundance of *Dubosiella*. However, further studies are required to reveal the precise mechanism(s) behind these effects. A previous study suggested that the AMPK signaling pathway not only plays a pivotal role in regulating metabolism in adipose tissue, liver, and skeletal muscle but also plays a vital role in the metabolic regulation mediated by gut microbiota [67]. The alteration of this similar pathway in the cecum may also play an indispensable role in the interaction between the cecal microbiota and the host metabolism system. Overall, signaling pathways related to energy metabolism in the gut are not negligible.

Abbreviations

GDM: Gestational diabetes mellitus; ITF: Inulin-type fructans; NCD: Normal chow diet; HFD: High-fat/sucrose diet; FBG: Fasting blood glucose; IR: Insulin resistance; OGTT: Oral glucose tolerance test; AUC: Area under the curve; TG: Triglycerides; TC: Total cholesterol; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; OTUs: Operational Taxonomic Units. AMPK: Adenosine 5‘-monophosphate (AMP)-activated protein kinase.

Declarations

Declaration of interests

The authors declare that they have no competing interests.

Authors’ contributions

Miao Miao and Xin Zeng conceived and designed the experiments. Xinyan Wang, Chong Fan and Ting Luan performed the experiments. Qing Wang, Yue Zhang and Can Rui performed the bioinformatics analysis. Lina Yan and Wenwen Hou performed the statistical analysis and interpreted the data. Miao Miao wrote the initial manuscript. Yongmei Dai and Ping Li reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

This study was approved by the Animal Protection Ethics Committee of Women’s Hospital of Nanjing Medical University (No.2018-49). All animal experiments were performed in accordance with Chinese national regulations on the administration of animal experimentation as well as international guidelines on animal experimentation. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

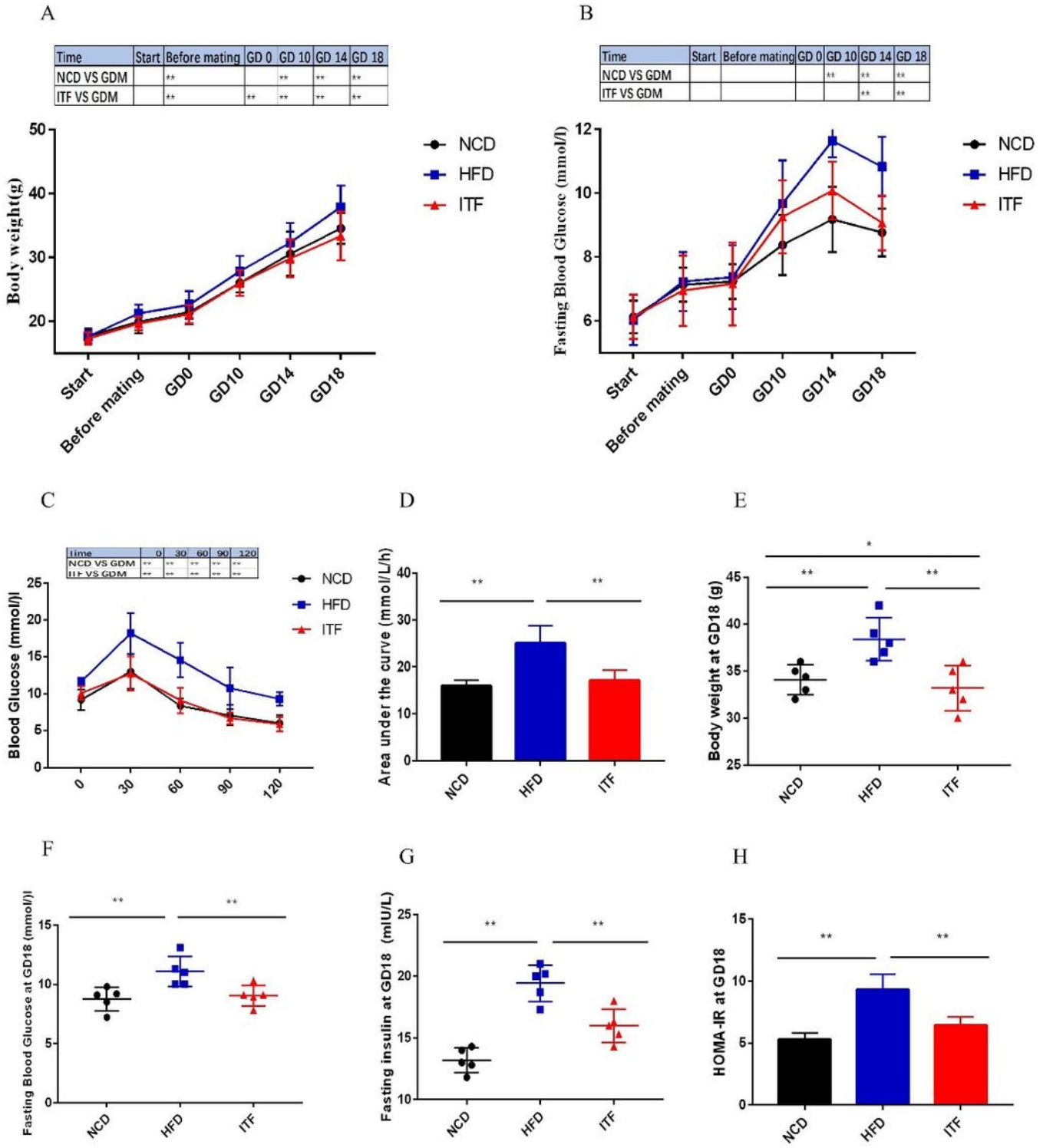

Figure 1

Improvement in metabolic parameters in high fat-sucrose diet-induced gestational diabetes mice by ITF Body weight. B. Fasting blood glucose. C. Plasma glucose profile D. Mean AUC measured during the OGTT. E. Body weight at GD18. F. Fasting blood glucose at GD 18. G. Fasting insulin at GD 18. H. HOMA-IR at GD 18. AUC, area under the curve; OGTT, oral glucose tolerance test; Data are presented as mean ±

SEM. Data were analyzed using two-way ANOVA followed by the Bonferroni post hoc test for A, B and F and with one-way ANOVA followed by the Tukey post hoc test for C, D, E and G. *P <0.05, **P<0.01.

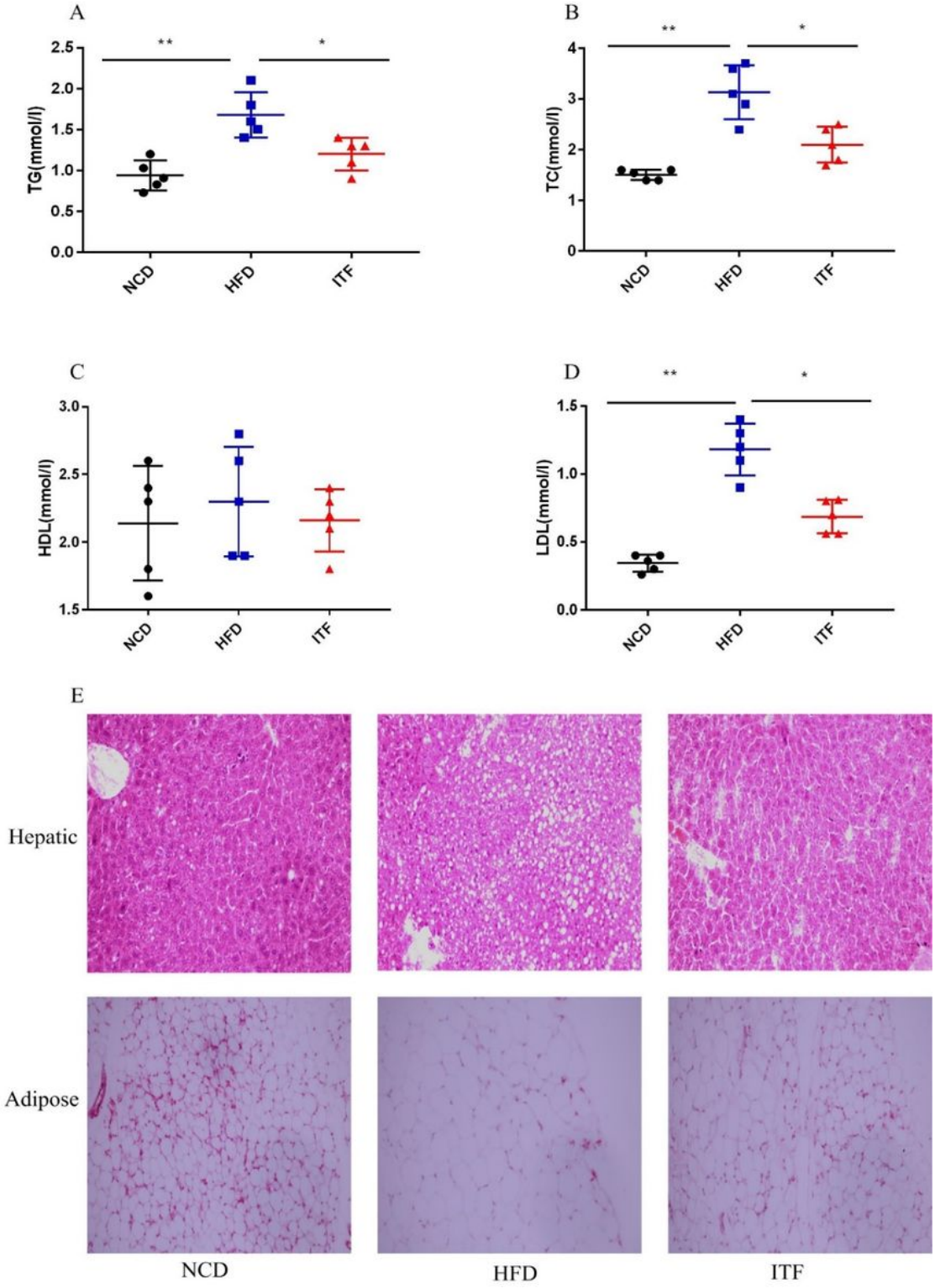

Figure 2

Improvement in metabolic parameters in high fat-sucrose diet-induced gestational diabetes mice by Inulin-type fructans A. Serum TG. B. Serum TC. C. Serum HDL-C. D. Serum TC LDL-C. E. Representative H&E-stained images of the hepatic and adipose tissues (× 200). TG, triacylglycerol; TC, total cholesterol;

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. Data were analyzed using one-way ANOVA followed by the Tukey post hoc test for A-D. *P <0.05, **P<0.01.

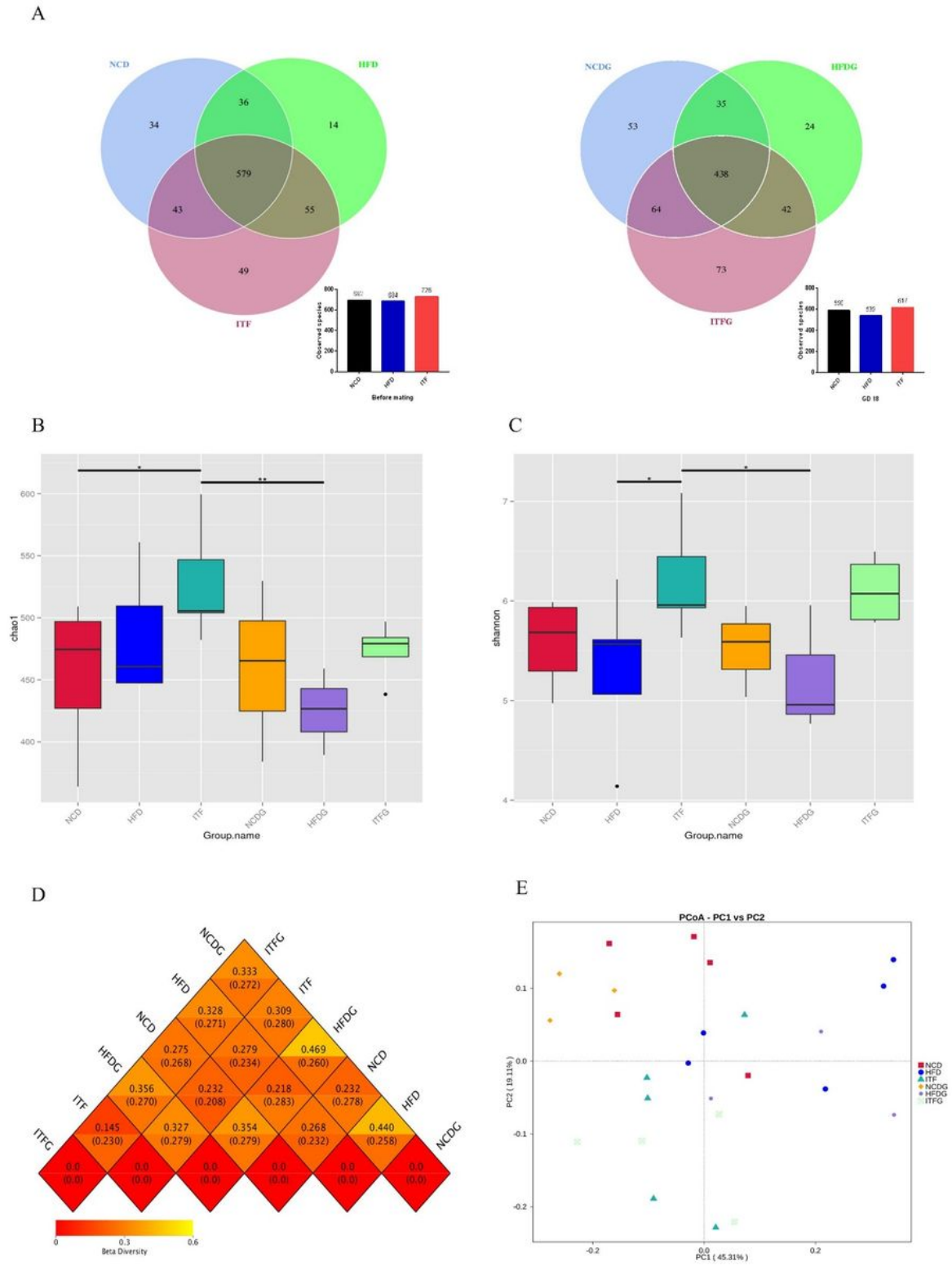

Figure 3

Inulin-type fructans modifies the composition of the cecal microbiota in ob/ob mice A. OTU number before mating and on GD18. B. Chao1 index of microbiota C. Shannon index of microbiota. D. Heat map of beta diversity index E. The beta diversity of gut microbiota analyzed by Principal Co-ordinate Analysis.

Data were analyzed using one-way ANOVA followed by the Tukey post hoc test for B and C. *P <0.05, **P<0.01.

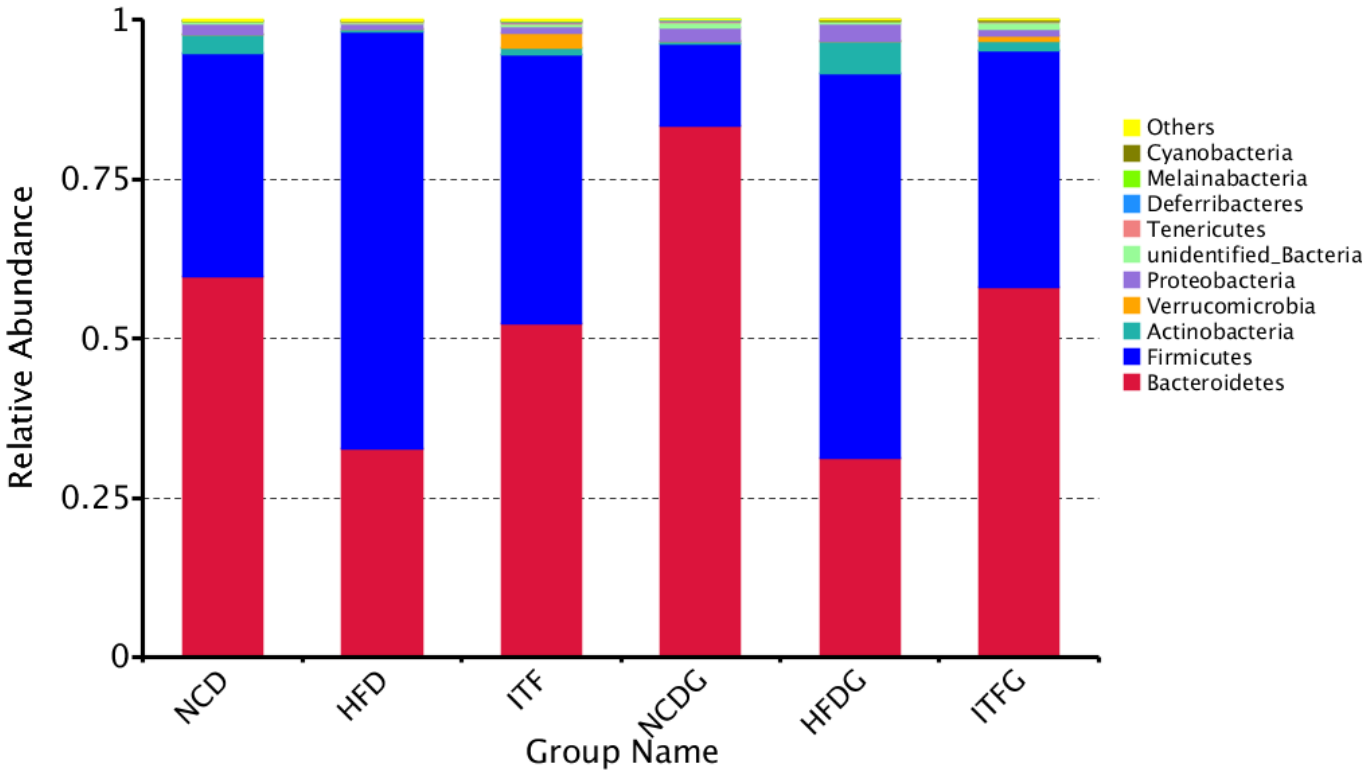

Figure 4

Relative abundance of microbial species of the top 10 phylum in the feces of mice

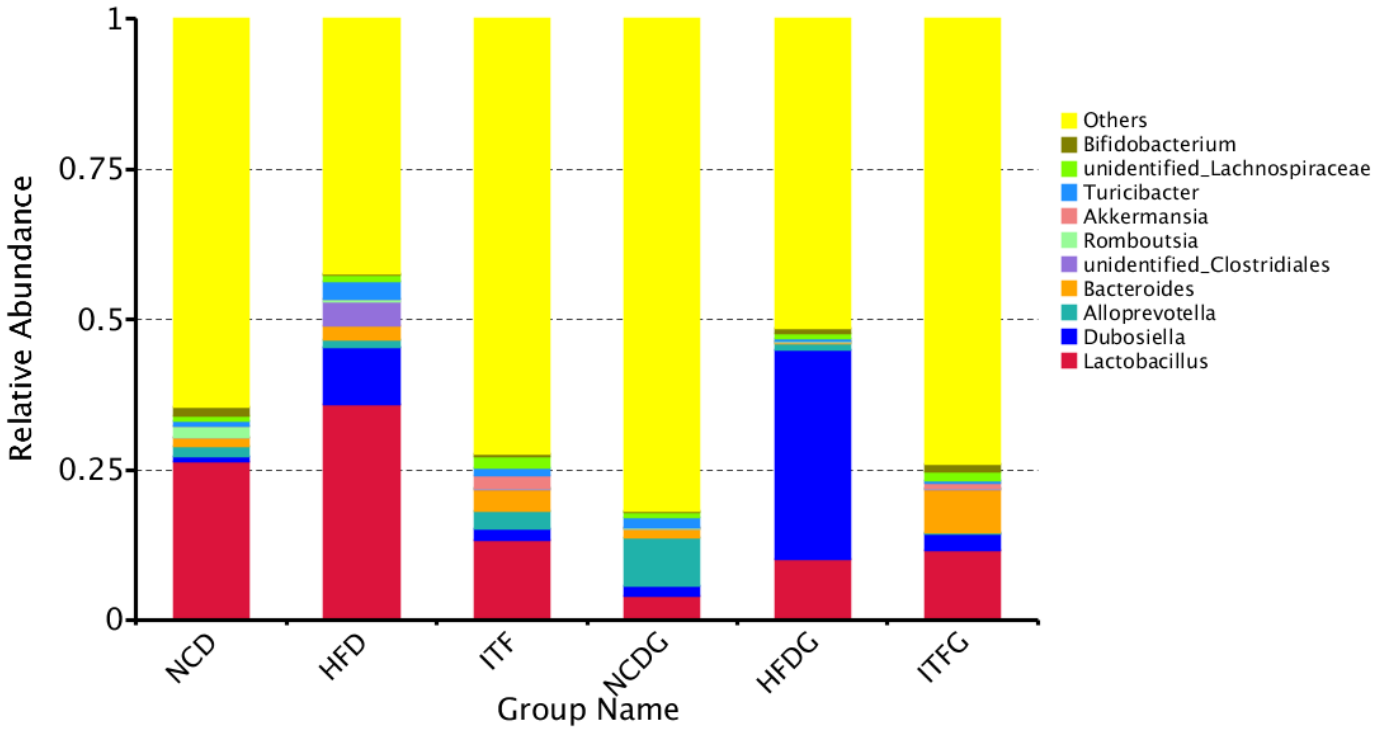

Figure 5

Relative abundance of microbial species of the top 10 Genus in the feces of mice

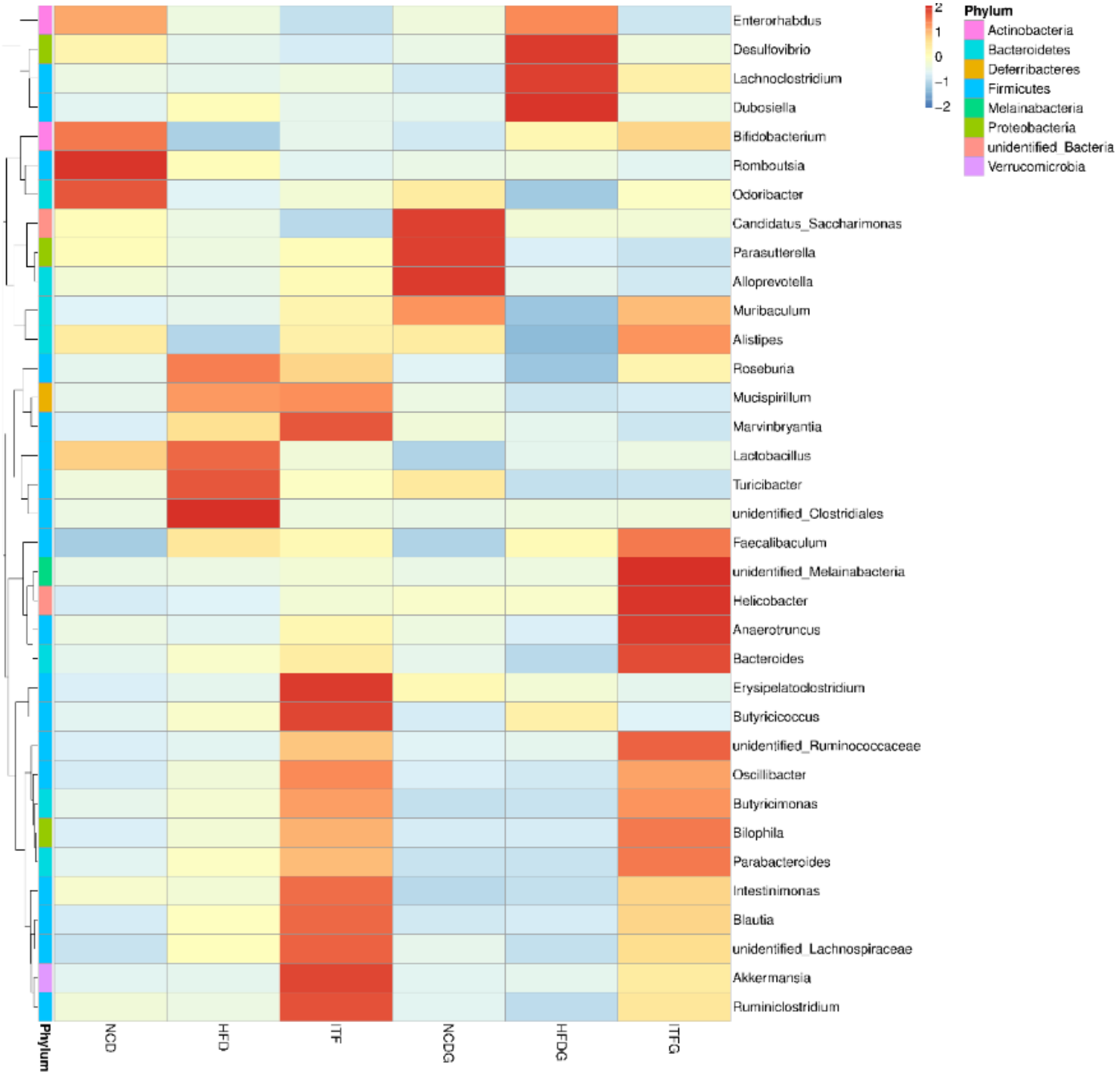

Figure 6

Heat map of microbial species of at Genus level in the feces of mice

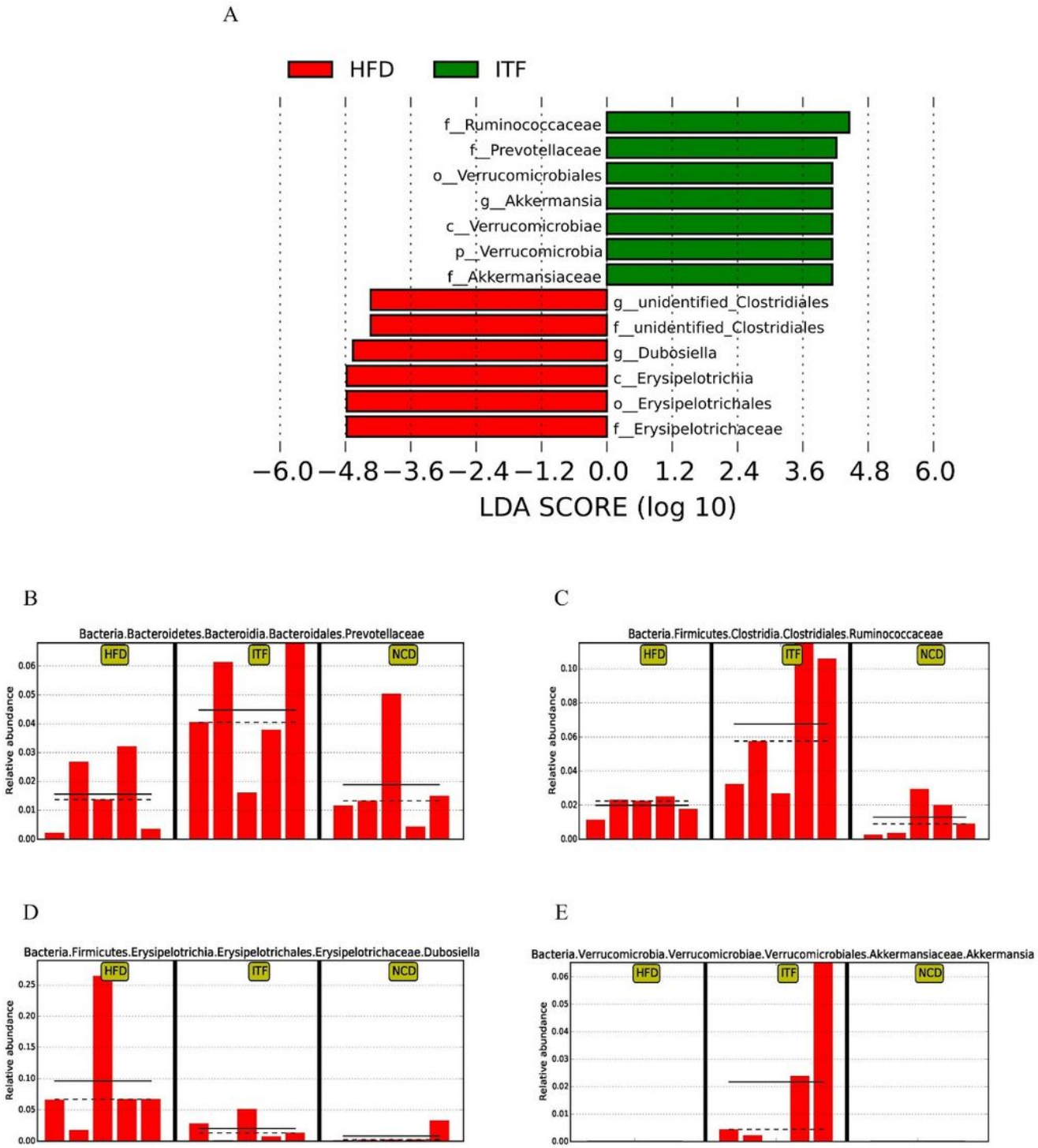

Figure 7

Identification of the most differentially abundance among HFD, ITF and NCD analyzed by the Linear Discriminant Analysis Effect Size (LEfSe) method. A. LDA scores of differentially abundant taxa; B. Relative abundance of Prevotellaceae C. Relative abundance of Ruminococcaceae D. Relative abundance of Dubosiella E. Relative abundance of Akkermansia.

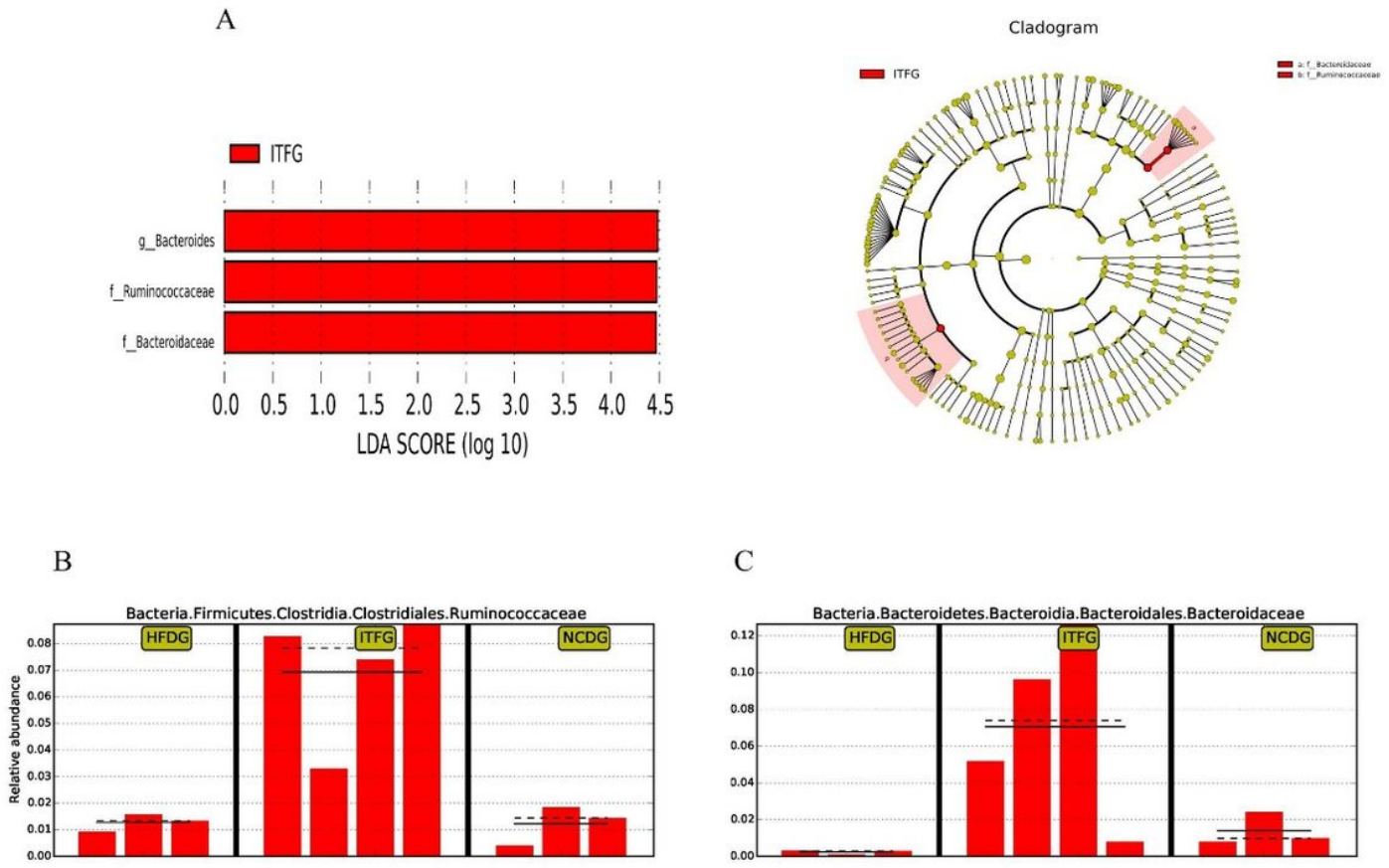

Figure 8

Identification of the most differentially abundance among HFDG, ITFG and NCDG analyzed by the Linear Discriminant Analysis Effect Size (LEfSe) method. A. LDA scores of differentially abundant taxa; B. Relative abundance of Ruminococcaceae C. Relative abundance of Bacteroidaceae.

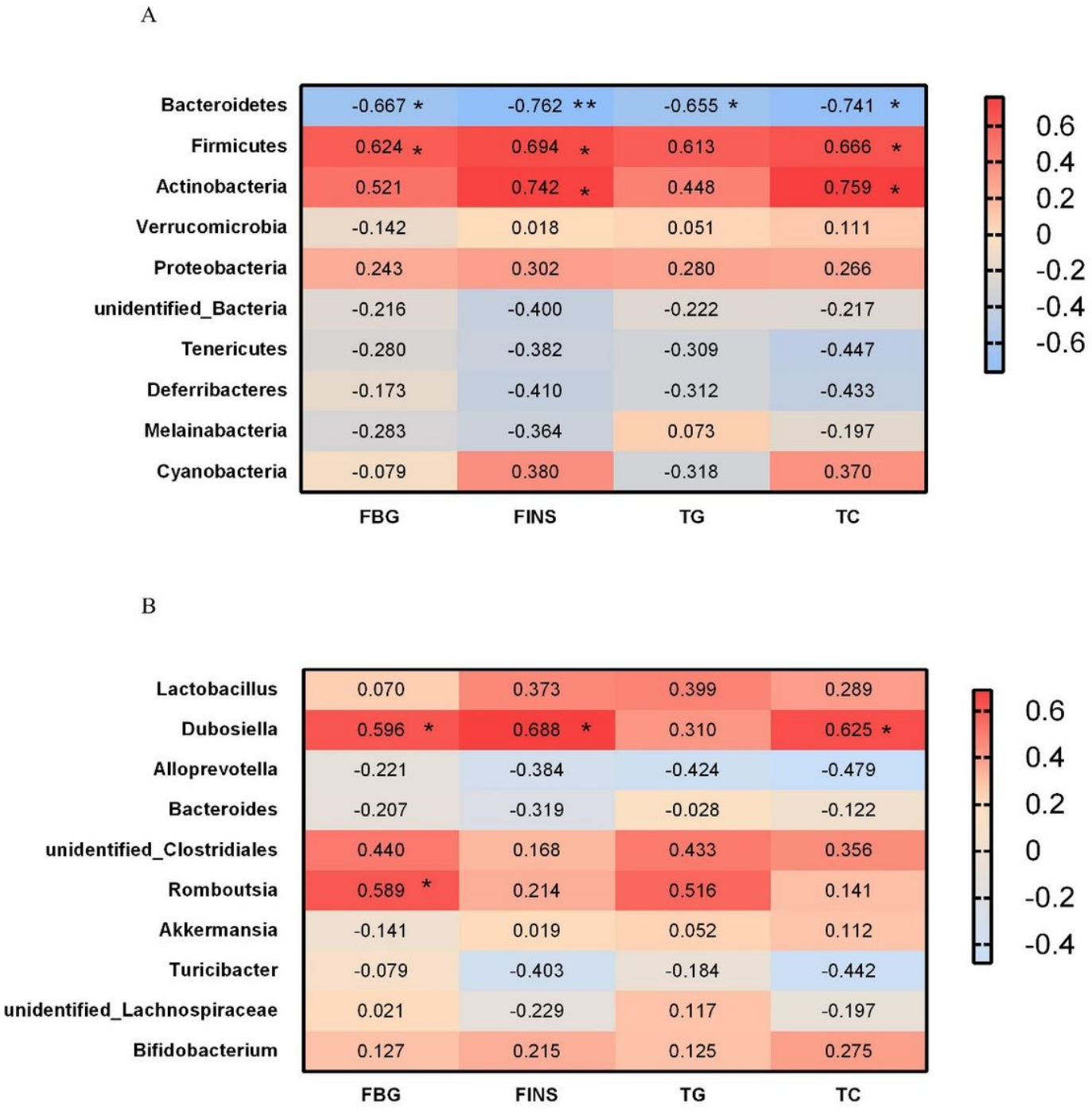

Figure 9

Correlations between glycolipid metabolism indicator and bacterial abundance A. Heatmap of spearman correlations between the levels of metabolites/components and the abundances of gut microbial phyla. B. Heatmap of spearman correlations between the levels of metabolites/components and the abundances of gut microbial genera. FBG, fasting blood glucose; FINS, fasting insulin; TG, triglyceride; TC, total cholesterol. *p < 0.05, **p < 0.01.