Not all dietary fibers alleviate type 2 diabetes

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Research

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Abstract

Background High intake of dietary fiber was found to be inversely associated with type 2 diabetes (T2D), whereas the difference among different dietary fibers on T2D is still not clear.

Results Here we investigated the effects of 9 types of dietary fiber on type 2 diabetic rats. we found supplementation with β-glucan, arabinogalactan, guar gum, apple pectin, glucomannan, arabinoxylan (especially β-glucan and arabinogalactan treatment) significantly reduced the fasting blood glucose, whereas carrageenan, xylan and xanthan gum had no effect on glycemic control in rats with T2D. Fibers with hypoglycemic effects associated with the increased butyric acid level and abundance of beneficial bacteria (*Anaeroplasma*, *Parabacteroides* and some butyric acid-producing bacteria), as well as improved host metabolism by decreasing 12α-hydroxylated bile acids, acylcarnitines and amino acids (leucine, phenylalanine, citrulline, and etc.). It was also found that only β-glucan can attenuate insulin resistance in T2D via down-regulation of *Prevotella copri* mediated branched chain amino acids biosynthesis.

Conclusions not all dietary fibers were able to improve T2D, which mainly relied on the improvement of composition and functions of gut microbiota.

1. Background

Type 2 diabetes (T2D) is a group of metabolic diseases characterized by hyperglycemia, insulin resistance, and relative insulin deficiency. T2D accounts for 90–95% of all cases of diabetes, which has obtained a major concern worldwide and is a leading cause of death in most countries (1). The prevalence of T2D affects more than 400 million people worldwide, causing profound psychological and physical distress to patients and is related to several severe complications (2, 3).

Dietary fiber is considered to be non-digestible carbohydrates presented inherently in plants, and it also includes isolated or synthetic fibers with physiological benefits for human (4). Dietary fiber is rich in plant-based foods, including whole grains, fruits, vegetables and some legumes. Prospective cohort studies and clinical studies have shown that high intake of dietary fiber is inversely associated with the risk of T2D (5–7). The American Diabetes Association (ADA) recommends that fiber intake in people with diabetes should match the recommendations for the general population, to increase intake to 25 g/day for adult women and 38 g/day for adult men, as the recommendations are associated with reduced risk (20–30%) of developing T2D after correction for confounders (8, 9). Effects of dietary fiber on T2D have been reported to be associated with gastric empty delay, body weight and postprandial glucose responses control, lipid metabolism regulation, enzyme action efficacy (α-amylase and α-glucosidase) inhibition, as well as gut microbiota modification (7, 9–11).

Dietary fibers with different chemical structure and physicochemical properties (such as gel-forming capabilities, viscosity, solution conformation or fermentation rate) have been reported to be associated with different physiological activities. For example, there is a plethora of clinical evidence on using soluble viscous dietary fiber supplements in the regulation of hyperglycemia and reduction of risk of T2D,

whereas non-viscous fiber seems to have limited effects on the regulation of postprandial glucose responses (12, 13). A variety of dietary fiber were administrated in our daily life, and fiber supplemented to patients with T2D in clinical trial are generally mixture of various dietary fiber (7, 14, 15). Furthermore, a 2014 position statement from the ADA deemphasized the impact of fiber in diets, as marginal improvement on glucoregulation (HbA1c, -0.2 to -0.3%) was observed with intakes of > 50 g of fiber/day (8). Also,the role of different dietary fibers on T2D, as well as whether all of dietary fibers have beneficial effects on glycemic control of T2D is still not clear. Consequently, it is urgent to investigate the effects of different dietary fibers on the management of T2D, as well as the underlying mechanism of fiber with different structure/type. In the presented study, A comparative analysis from perspective of physiology, metabolomics and genomics were conducted to evaluate the effects of different types of dietary fiber on type 2 diabetic rats, with the goal of investigating the mechanism for the different fibers on the management of T2D.

2. Methods

2.1 Dietary fiber preparation

Guar gum, carrageenan, apple pectin, xylan, arabinogalactan and xanthan gum were purchased from Sigma-Aldrich (St. Louis, MO, USA). Glucomannan, β-glucan and arabinoxylan were prepared from barley, konjac and seed of *Plantago asiatica* L by our group using previous method, respectively (16–18).

2.2 Animals and experimental design

Male Wistar rats (180–200 g) were purchased from Vital River Laboratories (VRL, Beijing, China). All rats were housed under controlled conditions at 22 ± 2 °C, 55 ± 10% relative humidity, and 12/12 h light/dark cycle with *ad libitum* access to both normal rat chow and water. Rats were given one week to acclimate before experimentation began. All procedures for animal use were approved by the Animal Care and Use Committee, Nanchang University.

After acclimation, 15 rats were fed a standard normal chow diet and the other 150 rats were fed a high fat diet (HFD) (19). Rats were fasted for 12 h after 4 weeks of dietary manipulation, and rats fed on HFD were induced to T2D by the injection of streptozotocin (STZ, 35 mg/kg body weight) from tail vein (19). Rats were considered to be diabetic when the fasting blood glucose (FBG) level exceeded 16.7 mmol/L. The experimental design was shown in Fig. 1A. Briefly, rats were divided into a control group (Con)/diabetes mellitus group (DM): normal/diabetic rats treated with saline; Guar gum, carrageenan, apple pectin, xylan, arabinogalactan, xanthan gum, glucomannan, β-glucan and arabinoxylan group: diabetic rats treated with the corresponding dietary fiber (270 mg/kg).

2.3 Measurement of FBG, serum GSP, C-peptide, insulin and its related parameters

During the experimental period, FBG levels were measured weekly. The FBG level in the rats were determined by Accu-Chek Performa (Roche Diagnostics, Mannheim, Germany) from the tail vein after overnight fasting. After 4-week treatment with different dietary fibers, rats were orally administered 2.0 g/kg glucose after 12 h fasting period. Then oral glucose tolerance test (OGTT) was performed and the area under the blood glucose curve (AUC) was calculated for the evaluation of impaired glucose tolerance (IGT) (16). At the end of the 4-week experimental period, rats were humanly anesthetized, and blood samples were collected and centrifuged at 1,000 *g* for 10 min to collect the serum. The concentrations of insulin, glycated serum protein (GSP) and C-peptide in serum were determined by commercial kits (Nanjing Jianchen Bioengineering Institute, Nanjing SenBeiJia Biological Technology Co., Ltd. Jiangsu, China). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from FBG and fasting insulin levels according to previous method (20).

2.4 Measurements of serum lipid

Levels of serum lipids including total cholesterol (TC), triacylglycerols (TG), high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c), and kidney function parameters (uric acid, creatinine and urea) were determined using an Automatic Biochemical Analyzer (Mindray BS-380, Shengzheng, China). Non-esterified fatty acid (NEFA) content in the serum was determined by an assay kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) according to the manufacturer's instructions.

2.5 Histopathological analysis of pancreas samples

The pancreas samples were fixed in 10% formalin for more than 48 h at room temperature, then the specimens were dehydrated in graded alcohol and embedded with paraffin wax in molds. Paraffin-embedded tissues were sectioned into 4 μm thick using a rotary microtome, and stained with hematoxylin and eosin (H&E) for histological evaluation. The pathological changes in the pancreatic tissues were evaluated using a light microscope (16).

2.6 Measurement of serum hormones and LBP

Levels of serum glucagon-like peptide-1 (GLP-1), peptide YY (PYY), leptin, adiponectin and lipopolysaccharide binding protein (LBP) were measured using commercial ELISA kits (Nanjing Jianchen Bioengineering Institute, Nanjing, China).

2.7 Serum metabolomics analysis

2.7.1 Sample preparation and data acquisition

Metabolomics analysis was employed to investigate the variation of host metabolism after dietary fiber administration to type 2 diabetic rats. Serum samples were slowly thawed at room temperature before metabolomics analysis, then the acetonitrile/water (4:1, v/v) was added to serum samples (4:1, v/v) for proteins precipitation and metabolites extraction. The mixture of serum samples was vortexed for 2 min, and subsequently placed in the ice bath for 10 min and centrifuged at 10000 rpm for 20 min at 4 ℃. The

supernatant was filtered through a membrane filter for ultra-performance liquid chromatography coupled triple time-of-flight mass spectrometry (UPLC-Triple-TOF-MS) analysis. In order to avoid problems from instrument drift and obtain reliable and high-quality data in metabolomics, pooled quality-control samples (QC) were injected periodically for use in downstream data processing and correction. Preparation and application of QC samples were performed according to our previous method (21, 22).

High-resolution mass spectrometry system was based on Nexera X2 UPLC system (Shimadzu Corporation, Kyoto, Japan) coupled to Triple TOF 5600 Mass Spectrometer (AB Sciex, Framingham, USA) using electrospray ionization (positive and negative electrospray ionization) for data acquisition. The detailed conditions of UPLC-Triple-TOF/MS were presented as described in our previous report (21).

2.7.2 Acquisition of metabolic profile and metabolite identification

The acquired row mass data were processed using commercial software Prognosis QI (Waters Corporation). Reference run was automatically selected following the manufacturer's instructions. The procedures including deconvolution of total ion chromatogram, data normalization, peak picking, alignment of ion peaks, and formation of extracted ion chromatogram were performed with default parameters. A data matrix containing normalized ion intensities (variables), variable index (paired m/z-retention time), sample names (observations) and isotope distribution was generated by using m/z and retention time as the identifiers for each ion. QC samples were used for signal correction within and between analytical blocks. An univariate approach termed QC-based robust LOWESS (locally weighted scatterplot smoothing) signal correction was applied for sample shift calibration (23). Afterward, the resultant data matrices were further quality assurance by removing peaks with missing values in more than 80% samples and those with isotope ions from each group to obtain consistent variables. Variables with unacceptable reproducibility was set in the QC samples with RSD (relative standard deviation) great than 30% and detected in less than 80% samples, as a standard in the evaluation of repeatability in metabolomics data sets.

Metabolite annotation was made by searching MS and MS/MS information against the HMDB database (version: 4.0), Lipid maps Structure Database (updated: 2017) and METLIN (version: 1.0.6499.51447). The mass error tolerance of ms1 and ms2 was set at 5 ppm. Data matrix was imported into SIMCA-P software (version 14; Umetrics AB, Umeå, Sweden) to acquire clustering information among different groups and select biomarker candidates (OPLS-DA model). Biomarkers candidates were selected on the basis of variable importance in the projection (VIP) threshold of 1. Chemical similarity enrichment analysis (ChemRICH) was used to perform chemical similarity-based statistical enrichment analysis. MetaboAnalyst 4.0 (https://www.metaboanalyst.ca) was used for pathway analysis, and correlation heatmap was carried out by the software package R.

2.7.3 Clustering of co-abundant serum metabolites

Clusters of co-abundant serum metabolites were performed using the R package WGCNA (24). Signed, weighted metabolite co-abundance correlation (biweight midcorrelations after log2 transformation) networks were calculated for all samples. A scale-free topology criterion was used to choose the soft threshold of β = 14 for the correlations as per the WGCNA protocol (25, 26). Clusters were identified with the dynamic hybrid tree-cutting algorithm using a deepSplit of 4. Similar clusters were subsequently merged when the correlation coefficient between the clusters' eigenvectors exceeded 0.85 (26).

2.8 Gut microbiota analysis

2.8.1 DNA extraction and 16S rRNA gene V3-V4 region sequencing

Total DNA was extracted from colonic contents (200 mg of frozen feces) by QIAamp DNA Stool Mini Kit. The V4 region of the bacteria 16S ribosomal DNA gene was amplified by primers 515F 5'-barcode-GTGCCAGCMGCCGCGGTAA)-3' and 806R 5'- GGACTACHVGGGTWTCTAAT -barcode-3', and the barcode is a six-base sequence unique to each sample. Amplicons were extracted from 2% agarose gels and purified using the Common Agarose Gel DNA Recovery Kit (TIANGEN, CO., LTD, China). Purified PCR products were quantified by Qubit 3.0 (Life Invitrogen). Mix the DNA as recommended and the preparation of DNA library included the repair ends and selected the library size, adenylate 3' ends, ligate adapters and enriched DNA fragments. Agilent 2100 Bioanalyzer System (Highly Sensitive DNA Chip) was used for the quality control of the library. The pooled DNA product was paired-end sequenced on the Illumina MiSeq platform according to the standard protocols.

QIIME (Quantitative Insights into Microbial Ecology, v1.9.0, http://qiime.org) software was used for quality control splicing, filtering and other pre-processing of the raw data, and Usearch method was used to remove chimeric sequences. Raw fastq files were demultiplexed, quality-filtered using QIIME with the following criteria: (i) The 250 bp reads were truncated at any site receiving an average quality score < 20 over a 10 bp sliding window, discarding the truncated reads that were shorter than 50 bp. (ii) exact barcode matching, 2 nucleotide mismatches in primer matching, reads containing ambiguous characters were removed. (iii) only sequences that overlap longer than 10 bp were assembled according to their overlap sequence. Reads which could not be assembled were discarded.

2.8.2 Sequencing data analysis

Compared with Greengenes database, OTU table was generated and filtered according to the data volume. Then QIIME software was used for alpha diversity (including Chao1, ACE and Shannon indices) and microbial taxa distribution analysis. LEfSe analysis of relative abundance matrix at the genus level is submitted on the Galaxy online analysis platform (http://huttenhower.sph.harvard.edu/galaxy/). The difference among the microbial genus (significantly changed by different dietary fibers) of different groups was calculated using student t test. The top 300 OTUs ranked by abundance were further clustered by WGCNA. Metagenomic predictions were made using PICRUSt (Phylogenetic Investigation of

Communities by Reconstruction of Unobserved States) and summarized as KEGG (Kyoto Encyclopaedia of Genes and Genomes) pathways.

2.9 Measurement of short chain fatty acids (SCFAs)

SCFAs in rat colonic contents were measured as described in our previous report (19). Briefly, colonic contents (200.0 mg) were diluted with deionized water at a ratio of 1:9. Diluted colonic contents were vortexed and subjected to ultrasound for the extraction of SCFAs. Samples were placed in the ice bath and then centrifuged at 4800 *g* for 20 min. The procedure was repeated and the supernatant were collected for the gas chromatography analysis.

2.10 Statistical analysis

Statistical analysis was performed by SPSS 17.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) for multiple comparisons followed by Tukey's test were used to determine significance of differences between groups. A difference with $p < 0.05$ was considered to be significant.

3. Results

3.1 Effects of different dietary fibers on glycemic control in type 2 diabetic rats

Reduction of body weight is an obvious symptom during onset and progression of T2D. In the present study, normal rats showed steadily increased body weight whereas diabetic rats exhibited opposite trend. The body weight loss can be alleviated by some dietary fiber treatment in diabetic rats, as the body weight of arabinoxylan, apple pectin and arabinogalactan groups were significantly higher than DM group after 4 weeks intervention (**Additional file 1: Table S1**, $p < 0.01$). At the end of the experimental period, the rats in DM group reached the highest FBG. The levels of FBG were significantly decreased after guar gum, glucomannan, β-glucan, arabinoxylan, apple pectin and arabinogalactan treatment, especially treatment with arabinogalactan and β-glucan exhibited the best hypoglycemic effect. Carrageenan, xylan and xanthan gum intervention did not show significant improvement on FBG for type 2 diabetic rats (Fig. 1B). Concentrations of serum GSP were also in agreement with the results of FBG, as the blood glucose-associated parameter was effectively decreased by β-glucan, apple pectin, xylan and arabinogalactan treatment (Fig. 1C). Furthermore, only arabinogalactan treatment significantly improved impaired glucose tolerance in type 2 diabetic rats from the OGTT test, suggested that fiber with different structure/properties had different effects on glycemic control under diabetic condition (Fig. 1D).

The levels of insulin in DM group were significantly higher than Con group, indicating the T2D-induced insulin resistance. In the present study, only guar gum administration significantly decreased the concentration of insulin in diabetic rats, and supplementation with others fiber have no effects on the levels of insulin and C-peptide (proinsulin can lead to the release of equimolar amounts of insulin and C-peptide) (Fig. 1E-F). However, fiber with significantly hypoglycemic effect (guar gum, glucomannan, β-

glucan, arabinoxylan, apple pectin and arabinogalactan) also improved insulin resistance in type 2 diabetic rats (Fig. 1E). Furthermore, we found some dietary fibers (guar gum, β-glucan, arabinoxylan and arabinogalactan) clearly showed favorable pancreas-protective effects, as the destroyed cell populations, intracellular degranulation and inflammation were improved in pancreas (**Additional file 1: Fig. S1**). An upset plot was constructed based on the improvement of these 6 glycemic control-related parameters by different dietary fibers (Fig. 1G). We found 4 dietary fibers improved half of these parameters, among which arabinogalactan showed best hypoglycemic effect on T2D, followed by apple pectin, β-glucan and guar gum.

3.2 Effects of different dietary fibers on lipid profile, serum hormone and kidney function

It is well known that patients with T2D often had dyslipidemia, as the disorder of lipid metabolism always led to the accumulation of plasma TC, TG, LDL-c and decreased HDL-c. Majority of dietary fiber significantly decreased the concentrations of TC, TG and LDL-c in type 2 diabetic rats (except guar gum, carrageenan, apple pectin, xanthan gum on TC; guar gum and xanthan gum on TG; guar gum and xanthan gum on LDL-c). For the HDL-c, there was no significant difference between Con group and DM group, but treatment with arabinoxylan, apple pectin and xanthan gum significantly increased the level of HDL-c in diabetic rats ($p < 0.01$). Specifically, only arabinoxylan significantly improved all of the four lipid parameters in diabetic rats, and levels of NEFA were not decreased by any dietary fiber treatment (Fig. 2A-E).

GLP-1 and PYY are gut-derived hormones with positive effects on glucose homeostasis. Despite there was no significant difference on GLP-1 between normal and diabetic rats, whereas treatment with carrageenan, glucomannan, β-glucan and arabinoxylan led to the increase of the gut hormone (Fig. 2F). Furthermore, reduced PYY in diabetic rats can be significantly increased by guar gum and glucomannan supplementation (Fig. 2G). Serum leptin was significantly increased in DM group compared with Con group, and only guar gum and carrageenan could decrease the adipose tissue hormone (Fig. 2H, $p < 0.01$). Another adipose tissue hormone, adiponectin, was not influenced by diabetes or dietary fiber supplementation under diabetic condition (Fig. 2I). Importantly, increased LBP indicated high level LPS leak into blood circulation from intestine, leading to the host inflammation and metabolic endotoxemia (27). In addition to xanthan gum treatment, dietary fiber supplementation could effectively decrease the concentration of serum LBP, which showed the potential anti-inflammation effects of dietary fiber on T2D (Fig. 2J).

3.3 Changes in the serum metabolomic features

3.3.1 Global metabolomic profiling among different groups

Untargeted metabolome profiles were generated to identify the serum metabolome features of T2D and investigate the effects of different dietary fibers on host metabolism. The abundance of sample RSD was less than 11% after LOWESS normalization (**Additional file 1: Fig. S2**). OPLS-DA model was constructed

by normalized features, and the score scatter plots showed clear differentiation among normal rats, non-treated and dietary fiber-treated diabetic rats (Fig. 3A). Permutation test was exploited to validate the OPLS-DA model. All the permuted R^2 and Q^2 values to the left were lower than the original points to the right, and the Q^2 regression line in blue had a negative intercept, indicating that the OPLS-DA model was not overfitting (**Additional file 1: Fig. S3**).

3.3.2 Effects of different dietary fibers on the metabolomic profile

WGCNA was used for the analysis of clusters of co-abundant serum metabolites. The application of the MS-based analytical platforms provided information about 301 metabolites (131 known and 170 unknown) based on the OPLS-DA model. These serum metabolites were binned into 29 co-abundance clusters across all the samples. We found 14 of the 29 metabolite clusters (48%) was significantly associated with FBG, IGT or HOMA-IR (Spearman rank correlation test, $p < 0.05$, Fig. 3B, **Additional file 2: Table S1**). Nine metabolite clusters were found to be significantly associated with at least 2 of the 3 parameters, whereas only 2 metabolite clusters were significantly associated with the 3 parameters simultaneously. Among these 14 clusters, the metabolite clusters were separated into two groups which were either positively or negatively correlated with glycemic control and insulin resistance, here referred as T2D positive-metabotype and T2D negative-metabotype, respectively (Fig. 3C, **Additional file 2: Table S2**). The clusters with T2D positive-metabotype mainly including amino acids (M11: leucine, citrulline, proline), fatty acids and acylcarnitines (M3 and M13: stearic acid, palmitoyl-L-carnitine, acetylcarnitine), whereas smetabolites clusters in T2D negative-metabotype were mainly comprised of tryptophan metabolism (M18), amino acids (M26; lysine and glutamine), and a number of lysophosphatidylcholines (LPCs; M17, M23, M27). Moreover, these clusters with T2D positive/negative-metabotype showed a relatively strong correlation with serum lipids, glycemic and kidney function-related parameters, but weakly correlated with gut and adipose hormones, as well as insulin-related parameters (Fig. 3D).

By abundance comparison, we found 12 of the 14 clusters in T2D positive/negative-metabotype modules were generally highly/lowly abundant in the diabetic rats (except M2 and M17 in T2D negative-metabotype, which were not considered in abundance comparison) (**Additional file 2: Table S3**). Dietary fiber treatment showed different improvement effects on these clusters by abundance comparison. β-glucan treatment improved 6 of the 12 clusters (50%), followed by xanthan gum and arabinogalactan/glucomannan/apple pectin treatment, with 5 and 4 clusters (42% and 33%), respectively (Fig. 3E). Interestingly, majority of dietary fiber supplementation were mainly focused on the improvement of clusters in T2D positive-metabotype, such as the reduction of fatty acids, carnitines and amino acids (e.g. leucine, citrulline, proline). However, β-glucan treatment was found to have effects on the amelioration of T2D negative-metabotype associated metabolite clusters, including the increase of LPCs and various organic compounds (M16, e.g. gluconic acid, glutathione amide disulfide etc. **Additional file 2: Table S3)**.

Results of chemical similarity-based metabolites enrichment were in agreement with the results of T2D positive/negative-metabotype from WGCNA. The classes such as lysophosphatidyl ethanolamine (LPE), cholic acids, amino acids (leucine, proline, kynurenine), carnitine, indoles, saturated fatty acids, and acidic amino acid (glutamate, isovalerylglutamic acid) were increased, whereas the levels of LPCs, basic amino acids (glutamine, lysine, aminocaproic acid), and some unsaturated fatty acids were decreased in T2D (Fig. 4A **and B, Additional file 1: Table S2**)

3.3.3 Effects of different dietary fibers on the improvement of metabolites and metabolic pathway

A total of 131 metabolites were found significantly changed in type 2 diabetic rats in comparison with normal rats (**Additional file 2: Table S4**). Based on these significantly changed metabolites, we performed pathway analysis according to metabolites enrichment and pathway topology analysis. A total of 11 pathways were found with the impact-value threshold above 0.1 (FDR < 0.05), and these T2D-related disturbed metabolic pathways were mainly belonging to amino acid metabolism (10/11, Fig. 4C, **Additional file 1: Table S3**). Among these pathways, linoleic acid metabolism had the highest impact factor of 1, followed by glutamine and glutamate metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, arginine biosynthesis, starch and sucrose metabolism, with impact factors of 0.5, 0.5, 0.42 and 0.42, respectively. In order to find the potential ameliorative effects of different dietary fibers on T2D, we perform comparative analysis based on the improved metabolites and their involved metabolic pathways. From the perspective of improved metabolites, treatment with arabinoxylan, apple pectin, xylan, arabinogalactan, xanthan gum and β-glucan improved more than half of the metabolites, among which arabinogalactan and xanthan gum improved 83 (63%) and 81 (62%) of the 131 significantly changed metabolites, respectively (Fig. 4E). Based on these improved metabolites, we found arabinogalactan and β-glucan supplementation have favorable effects on the improvement of T2D induced metabolic disorder, as the two fibers improved 8 and 7 of the 11 significantly disturbed pathways in type 2 diabetic rats (FDR < 0.01, Fig. 4D). Also, metabolism of cysteine and methionine was improved by all types of dietary fiber treatment, and carrageenan supplementation only improved this metabolic pathway **(**Fig. 4D).

3.4 Effects of different dietary fibers on gut microbiota

As shown in the results above, we found metabolites such as amino acids, bile acids and indoles were significantly changed, and the metabolites in T2D-associated phenotypes were involved in the disturbed metabolism of amino acids, which is the co-metabolites of gut microbiota and host. Thus, we then investigated the changes of the gut microbiota in the normal rat, non-treated and fiber-treated diabetic rat by sequencing the 16S rRNA V3-V4 region of colonic contents. A clear separation was observed among Con, DM, and fiber-treated groups in PCA score plot, suggested the disturbed gut microbiota composition occurred in rats, depending on the pathological condition and treatment intervention (**Additional file 1: Fig. S4A)**. For the bacterial communities at the phylum level, T2D-induced significantly increased abundance of *Actinobateria* and *Lentisphaerae*, and decreased the abundance of *Bacteroidetes*,

Cyanobacteria, *Tenericutes* and TM7 (**Additional file 1: Fig. S4B and Fig. S5A**). Though there was no significant difference for the F/B (*Firmicutes*/*Bacteroidetes*) ratio in all groups, the ratio was increased in DM group (0.83) compared to Con group (0.56). However, the ratio was down-regulated in all groups (from 0.51–0.72) after the intake of different dietary fibers (**Additional file 1: Fig. S5B)**. There was also difference for the bacterial communities at the genus level (**Additional file 1: Fig. S4C**). In addition, it could be found that the apple pectin and guar gum supplementation showed the highest alpha-diversity indexes, whereas the xanthan gum treatment showed the lowest (**Additional file 1: Fig. S4D, E, F**).

The top 300 OTUs ranked by abundance were further clustered by WGCNA, and 213 of which after dynamic hybrid tree-cutting were used for the construction of clustered network (Fig. 5A). These OTUs were clustered into 10 clusters based on the spearman correlation coefficients. Of these, we found the abundance of cluster brown, green and magenta were decreased significantly in diabetic rats in comparison with normal controls (Wilcoxon rank sum test, $p < 0.05$, Fig. 5B). Of the OTUs in the three clusters, 81.2% belonged to S24-7 and *Prevotellaceae* (**Additional file 2: Table S5**), members of which may exert health benefits by the degradation of complex carbohydrates and production of SCFAs (28, 29). Notably, the abundance of cluster purple, blue, pink, yellow were significantly higher in the diabetic rats. This clusters are comprised *Helicobacteraceae*, *Desulfovibrionaceae*, *Lactobacillaceae*, *Clostridiales*, and *Bifidobacterium*, and some of which have been reported as pathogens or opportunistic pathogens (28). Treatment with different dietary fibers were mainly associated with improvement of abundance in cluster yellow, brown and green (decreased abundance in cluster yellow, and increased abundance in cluster brown and green), with glucomannan improved all of the three clusters simultaneously (Fig. 5C).

For better understanding the specific difference among the different groups, we compared the gut microbiota composition at the genus level. Fourteen genera in diabetic rats were found significantly changed compared with normal rats (**Additional file 1: Table S4**, $p < 0.01$, $q < 0.05$). Based on the bacteria with abundance great than 1% between Con and DM group, 6 of that 14 genera were found significantly changed (Fig. 5D, **Additional file 1: Table S4, Fig. S6)**. It could be obviously found that DM group exhibited an acute intestinal dysbiosis characterized by an obvious increase in *Bifidobacterium*, *Blautia* and *Allobaculum* (*Bifidobacterium* and *Allobaculum* are not detected in normal rats). In addition, the levels for the genus *Prevotella* (family Paraprevotellaceae), *Oscillospira* and *Ruminococcus* were significantly decreased in DM group (Fig. 5D, $p < 0.01$, $q < 0.05$).

Different dietary fibers showed varied effects on the modification of gut microbiota composition in type 2 diabetic rats. The relative abundance of the genus *Bifidobacterium* and *Allobaculum* (represented genera in DM group) were significantly increased in colon microbiota of the diabetic rats, whereas the intake of guar gum, carrageenan, arabinoxylan, and apple pectin effectively decreased its abundance (Fig. 5E). Genus *Oscillospira* (represented genus in Con group) was found significantly decreased in diabetic rats, whereas only the guar gum and carrageenan intake up-regulated the diabetes-induced decrease on this genus. Although there was no significant difference between the relative abundance of genus *Prevotella* (family Prevotellaceae) in colon microbiota of Con (28%) and DM (23%) group, its abundance was dramatically increased from 22.9–45.1% after the supplementation of different dietary fibers (Fig. 5E**)**.

Furthermore, the abundance of some bacteria that associated with health benefits were also increased based on the variation of the structure/type of dietary fiber. For example, the abundance of *Anaeroplasma* (a potential anti-inflammatory probiotic) was increased by β-glucan, apple pectin, and arabinogalactan treatment (30). The two butyrate-producing bacteria, *Butyricimonas* and *Lachnobacterium* (31), were also increased with supplementation of β-glucan and glucomannan. These improvements may associate with the alleviative effects of dietary fiber on T2D, as the β-glucan, arabinogalactan, and glucomannan treatment showed favorable anti-diabetic effects in our study. Also, other dietary fibers were also specifically related to the change/improvement of gut microbiota composition, which represented in the increased abundance of *Phascolarctobacterium*, *Akkermansia*, and *Faecalibacterium* by carrageenan, xylan, and apple pectin treatment, respectively (Fig. 5E**)**. In addition, abundance of some SCFA-producing bacteria were not influenced by T2D, whereas administration of dietary fiber actually increased their abundance (e.g., increased *Roseburia* by guar gum and apple pectin; *Clostridium* by guar gum and β-glucan; *Parabacteroides* by β-glucan and apple pectin; *Bacteroides* by β-glucan, carrageenan and apple pectin, *Anaerostipes* by carrageenan and xylan) based on the different type.

SCFAs of colonic contents were also detected as these small molecules can improve the host physiological status by targeting multiple pathways. We found T2D-induced significant increase in acetic acid whereas reduction in butyric and valeric acid (Fig. 5F). Intake of different dietary fiber showed varied response on the production of SCFAs by gut microbiota. Carrageenan, glucomannan and β-glucan intake notably decreased acetic acid, whereas arabinoxylan and arabinogalactan administration significantly increased its concentrations, in comparison with DM group. Also, β-glucan, guar gum, arabinogalactan and xanthan gum significantly increased propionic, butyric and valeric acid in diabetic rats simultaneously (Fig. 5F). Furthermore, the correlations between the gut microbiota (genus and OUT clusters) and SCFAs were presented in Spearman's correlation heatmap. At the genus level, *Allobaculum*, *Bifidobacterium*, and *Enterobacter* exhibited significant positive correlations with acetic acid production ($p < 0.05$ or $p < 0.01$). Similarly, cluster yellow was also found positive correlation with acetic acid. The results were in agreement with correlations in genus, as cluster yellow contained 78% of *Allobaculum* and *Bifidobacterium*. The butyric acid showed positive correlations with *Butyricimonas* whereas negative correlations with *Phascolarctobacterium. Enterobacter* also positively correlated with propionic acid and valeric acid (Fig. 5F).

3.5 Multi-omics network analysis revealed the relationship between gut microbiota and serum metabolites in T2D

The correlation between gut microbiota and serum metabolites was investigated to further explore the characteristics of gut microbiota on T2D. Given a p value of 0.05, we found 7 of the 10 gut microbiota clusters were significantly correlated with 10 metabolic modules, and these metabolic modules also significantly correlated with FBG, HOMA-IR and IGT through Spearman correlation coefficients (Fig. 6A, **Additional file 2: Table S6**). Cluster brown, green and magenta (significantly enriched OUT clusters in Con group) were positively correlated with T2D negative-metabotype modules (such as amino acid and LPCs),

but negatively correlated with T2D positive-metabotype modules (such as fatty acids and carnitines). Cluster brown, pink and yellow was found closely related to the majority of serum modules, implied gut microbiota in these groups might influence T2D by interacting with different metabolites. Importantly, we found only cluster blue showed strong positive correlation with amino acids (M11, such as leucine and citrulline, Fig. 6A, r = 0.721, $p = 0.002$), which ultimately positively correlated with insulin resistance and FBG. Cluster blue mainly composed of species *Prevotella copri* (significantly enriched in DM group), and the species only significantly and positively correlated with leucine (Fig. 6B, r = 0.451, $p = 0.027$). Importantly, only β-glucan supplementation significantly decreased the abundance of *Prevotella copri* and the concentration of leucine simultaneously (Fig. 6B). Metabolomics analysis showed diabetics rats had disturbed BCAAs (valine, leucine and isoleucine) metabolism, including significantly increase leucine (Fig. 4B **and C**). Results of microbial functions from PICRUSt analysis also showed gut microbiota in DM group possessed more functions involved in biosynthesis and less function involved in degradation of BCAAs, suggested gut microbiota in type 2 diabetic rats contributes to the increased BCAAs into the circulation (Fig. 6C). Only β-glucan intervention improved microbiota-mediated BCAAs metabolism (decreased biosynthesis and increased degradation) simultaneously (Fig. 6C).

4. Discussions

Growing evidence suggested dietary fiber is a key component in healthful eating patterns, and consumption of foods rich in fiber is recommended for nutritional therapy of patients with T2D. In clinical trials, supplementation with dietary fiber was found inversely associated with risks of diabetes, along with the improvement on glycemic control, lipids profiles and host homeostasis (7, 8). However, mixed fibers with diverse type from dietary sources are generally used for treatment intervention in clinical trials, the effects of individual dietary fiber with different structure on T2D are seldom discussed previously.

The health benefits of dietary fibers were associated with their physicochemical properties, including chemical structure, molecular weight, glycosidic linkages, monomer constituents, water solubility, viscosity, fermentability and etc. Based on these different properties, viscosity was considered as an important characteristic on the management of T2D (12). High viscous fibers are considered have the ability to increase viscosity in the intestine and delay gastric emptying and reduce the rate of nutrient/glucose absorption, thereby attenuate postprandial glucose and insulin responses compared with non-viscous fibers (12, 32). This has been reflected in 2018 American Diabetes Association “Standards of Medical Care in Diabetes”, which recommended an increased viscous fiber (such as in oats, legumes, and citrus) intake in patients with T2D (33). We investigated the association between glycermic control and viscosity among the 9 types of dietary fiber, however, there was no significant correlation between viscosity and glycermic control ($r = 0.37$, $p = 0.06$, **Additional file 1: Fig. S7**). Importantly, the FBG trended to increase with the increased viscosity of dietary fiber, as the fibers with high viscosity (carrageenan and xanthan gum) had no effect on glycemic control. Also, we still had not found significant correlation between viscosity and glycermic control after excluding fiber with high viscosity (guar gum, carrageenan and xanthan gum, > 400 Pa.s), but the FBG tended to decrease when the fiber’s viscosity was less than 200 Pa.s (**Additional file 1: Fig. S7)**.

Aberrant gut microbiota composition was found in the onset and progression of T2D, generally characterized by reduced abundance of butyrate-producing bacteria and the increased concentrations of some *Lactobacillus* species or opportunistic pathogens, such as *Desulfovibrio* sp. and *Clostridium* (4, 34, 35). We proved that the composition and structure of the gut microbiota were altered with the development of T2D in rats in comparison with healthy control. We observed diabetic rats possessed decreased *Bacteroidetes* and increased *Actinobacteria*, along with slight increase of *Firmicutes* at the phyla level (**Additional file 1: Fig. S5**). Increased abundance of *Firmicutes* and *Actinobacteria* and decreased number of *Bacteroidetes* were found to be associated with increased energy harvesting, serum LPS levels, insulin resistance, and other comorbidities (such as diabetes and obesity) of the metabolic syndrome (36, 37). All the 9 types of dietary fibers decreased the abundance of *Actinobacteria* whereas increased the abundance of *Bacteroidetes*, among which guar gum, carrageenan, apple pectin, xylan, arabinogalactan and xanthan intake showed significant difference simultaneously on the two bacteria (**Additional file 1: Fig. S5**). Gut microbiota of diabetic rats was aberrant at multiple levels compared with healthy control. *Butyricimonas*, *Oscillospira*, *Roseburia*, and *Prevotella* (family *Paraprevotellaceae*) at genus level had a lower abundance in the DM group (Fig. 5D **and E**, $p < 0.05$). *Butyricimonas* and *Roseburiais* are known as butyrate-producing bacteria with anti-inflammatory effects, and have been found lower in patients with diabetes and high insulin resistance (38, 39). Improved metabolic syndrome have been shown to be associated with increased abundance of *Roseburia* and level of butyrate after gut microbiota transplantations (40). The abundance of *Roseburia* was increased by guar gum, apple pectin, with *Butyricimonas* was also increased by β-glucan treatment (Fig. 5E). Intake of the 3 dietary fibers decreased FBG in diabetic rats, suggested the butyrate and butyrate-producing bacteria might play an important role on blood glucose regulation.

Fiber supplementation was associated with the increased abundance of fiber-degrading bacteria. We observed members belonging to *Prevotellaceae*, such as genus *Prevotella*, was increased by all of dietary fiber treatment (increased 30–97% compared with DM group), which was in agreement with previous study that increased proportion of *Prevotella* was associated with the diet rich in plant-derived complex carbohydrates and fibers (41). Fiber with different properties selectively increased the abundance of beneficial gut microbes, whereas fiber with favorable hypoglycemic effects also showed some commonness on the modification of gut nicrobiota. β-glucan, apple pectin and arabinogalactan (possessed favorable hypoglycemic effects) commonly increased the abundance of *Anaeroplasma* and *Parabacteroides*. Anaeroplasma is a potent probiotic for the management of chronic inflammation as the bacteria has ability to strength the intestinal barrier by enhancing mucosal IgA (30). *Parabacteroides* also showed metabolic benefits such as improvement of hyperglycemia and hepatic steatosis on high-fat diet-fed mice via production of succinate (by activation of intestinal gluconeogenesis) and secondary bile acids (by activation of the FXR pathway and repair of gut barrier integrity) (42). Importantly, the abundance of *Lachnobacterium* was specifically increased by glucomannan and β-glucan treatment in this study. The bacteria were reported to produce lactic acid and butyric acid from glucose and arabinose, but not from pectin, galactose and xylan (31). Therefore, glucomannan and β-glucan which were both rich in glucose could promote the growth of *Lachnobacterium*, and thereby exert benefits on diabetes.

Production of SCFAs by fermentation of carbohydrates is proposed as a key factor on maintaining a mutualistic relationship between microbial ecosystem and host, and deficiency in SCFAs production has been associated with progression of T2D (7, 43, 44). The production of the butyrate was associated with improved insulin response in patients with T2D, therefore the increased level of butyric acids and abundance of butyric acid-producing bacteria by guar gum, β-glucan and arabinogalactan treatment might be responsible for their hypoglycemic effects (45). Acetic acid and propionic acid were also the end products after fiber fermentation. The decreased propionic acid and increased acetic acid was observed in diabetic rats compared to normal rats. Propionic acid was mainly produced by the fermentation of glucose, xylose and arabinose, which showed beneficial effects on the maintenance of glucose homeostasis through inhibition of β-cell apoptosis and potentiation of glucose-stimulated insulin release (19, 46). Consequently, fibers such as β-glucan, arabinoxylan, xylan and arabinogalactan that rich in glucose, xylose and arabinose could increase propionic acid, thereby exert benefits on glucose homeostasis in T2D. Interestingly, we found cluster yellow that composed of OTUs from *Bifidobacterium* and *Allobaculum*, were enriched significantly in diabetic rats and positively correlated with FBG and insulin resistance (Fig. 6A). High abundance of these acetic acid-producing bacteria contributed to the production of acetic acid in diabetic rats compared to normal rats. Although the abundance of *Bifidobacterium* and *Allobaculum* were observed significantly increased in diabetic rats and patients (47–50), these genera were considered as the important functional phylotype of metabolic dysbiosis in a number of studies (7, 51). Further studies are needed to investigate the roles of these bacteria in the development of T2D.

T2D is a complex metabolic disease characterized by significant pattern differences between normal and diabetic state (Fig. 3A). The metabolites, including acylcarnitines, fatty acids and amino acids (leucine, citrulline, proline), were positively correlated with phenotype of T2D. Whereas LPCs and some amino acids (glutamine and lysine) showed negative correlation with T2D (Fig. 6A). Fiber supplementation showed different improvement effect on host-derived endogenous metabolites (**Additional file 2: Table S4**). β-glucan and arabinogalactan, two fibers showed better hypoglycemic effects, had ability to decrease the concentration of cholic acid and its conjugated forms (taurocholic acid and cholic acid glucuronide). Cholic acid and its conjugated forms, known as 12α-hydroxylated bile acids, was found to be positively associated with insulin resistance in numerous studies (52, 53). However, guar gum supplementation effectively decreased the levels of short-chain (acetylcarnitine and hydroxypropionylcarnitine) and long-chain acylcarnitines (palmitoyl-L-carnitine, tetradecanoylcarnitine and octadecenoylcarnitine), suggesting guar gum have a positive role in the alleviation of incomplete fatty acid β-oxidation in T2D (54–56). β-glucan, apple pectin, arabinogalactan and xanthan gum effectively decreased the concentrations of saturated fatty acids and indole-derived metabolites, whereas guar gum, carrageenan and glucomannan increased levels of some unsaturated fatty acids in diabetic rats. These results suggested fibers with different structures influence host metabolism from different metabolic pathway.

Gut microbiota interacts extensively with the host through metabolic exchange and co-metabolism. Gut microbiota in diabetic rats possessed more biosynthesis-related functions and less degradation-related

functions in BCAAs metabolism, resulting in the increased BCAAs into systemic circulation. Importantly, abundance of *Prevotella copri* and concentration of leucine were significantly increased in colonic content and serum of DM group (Fig. 6B). *Prevotella copri* is identified as a main species driving the association between biosynthesis of BCAAs and insulin resistance, *Prevotella copri* and BCAAs can also induce insulin resistance and aggravate glucose intolerance in T2D (25, 57, 58). Only β-glucan supplementation was found to significantly decrease the abundance of *Prevotella copri* in this study, which also resulted in the lowest level of leucine among all treatments. Also, only the microbiota of β-glucan significantly decreased genes related to BCAAs biosysthesis ($p = 0.03$) and enriched genes related to BCAAs degradation ($p = 0.007$) simultaneously (Fig. 6C). These suggested that β-glucan can attenuate insulin resistance by down-regulation of *Prevotella copri* mediated BCAAs biosynthesis in T2D.

5. Conclusions

Our study suggests that not all dietary fibers alleviate type 2 diabetes. β-glucan, arabinogalactan, guar gum had favorable effects on the alleviation of T2D, and these fibers had more positive effects on the modification of gut microbiota and improvement of host metabolism. Glucomannan, apple pectin and arabinoxylan also showed hypoglycemic effect, whereas xanthan gum, carrageenan and xylan had no effects on glycemic control in type 2 diabetic rats. We also found β-glucan could exert their anti-diabetic effects through down-regulation of *Prevotella copri* mediated BCAAs biosynthesis in type 2 diabetic rats. The increased butyric acid and butyric acid-producing bacteria play a key role in amelioration of diabetes. These results provide the important information to dietary guidelines for the management of T2D.

Abbreviations

T2D: type 2 diabetes; HFD: high fat diet; STZ: streptozotocin; FBG: fasting blood glucose; GSP: glycated serum protein; HOMA-IR: homeostasis model assessment of insulin resistance; TC: total cholesterol; TG: triacylglycerols; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; NEFA: Non-esterified fatty acid; GLP-1: glucagon-like peptide-1; PYY: peptide YY; LBP: lipopolysaccharide binding protein; QC: quality-control; LOWESS: locally weighted scatterplot smoothing; SCFAs: short chain fatty acids; IGT: impaired glucose tolerance; OPLS-DA: orthogonal partial least squares discrimination analysis; BCAAs: branched chain amino acids; LPE: lysophosphatidyl ethanolamine; LPC: lysophosphatidylcholine

Declarations

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Author contributions: Shaoping Nie and Jielun Hu established the study, interpreted the data, and contributed to reviewed/edited the manuscript. Qixing Nie, He Gao, Mingzhi Li, Yonggan Sun, Haihong Chen performed animal study. Sheng Zuo, Qingying Fang, Xiaojun Huang, Junyi Yin helped perform the analysis with constructive discussions. Qixing Nie performed the data analyses and wrote the manuscript. All authors have read and approved the final manuscript.

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Consent for publication: Not applicable.

Availability of data and materials: All data generated or analysed during this study are included in this published article [and its supplementary information

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Figures

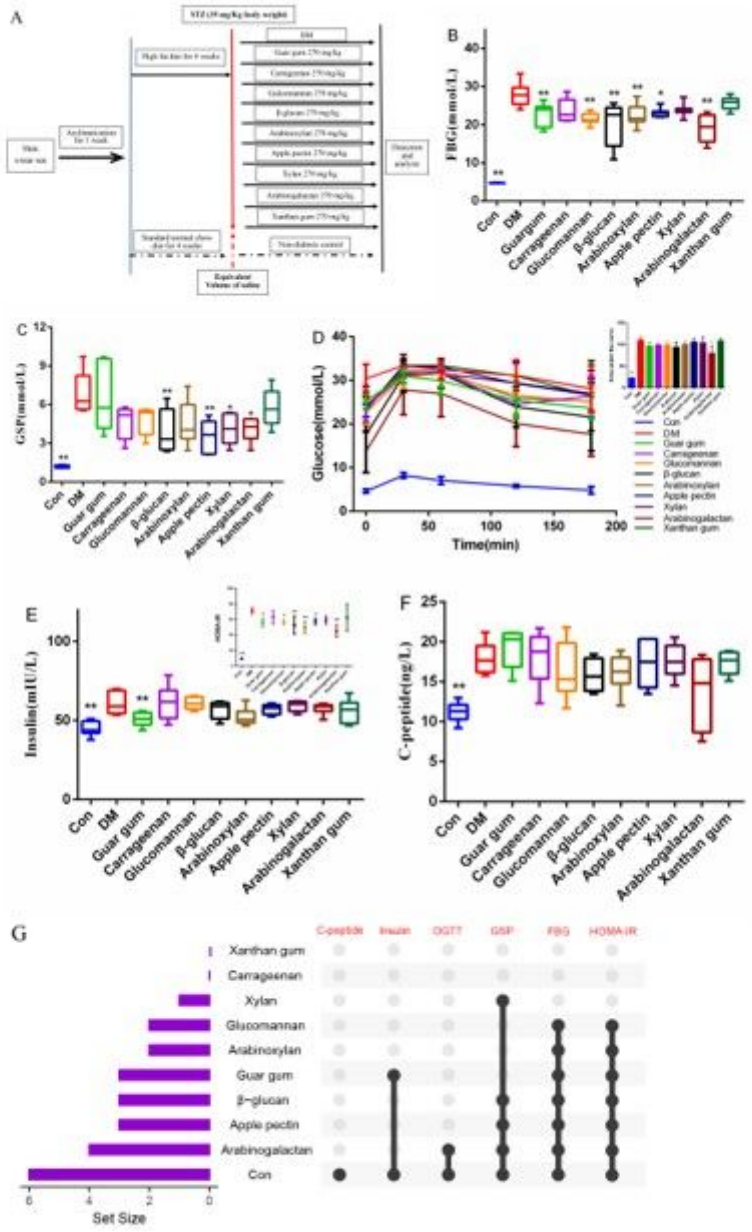

Figure 1

Experimental protocol and design (A); effects of different dietary fiber on glycemic control-related parameters in type 2 diabetic rats: FBG (B), GSP (C), OGTT (D), insulin and HOMA-IR (E), C-peptide (F), and upset plot of these parameters (G). *indicates significant difference compared with DM groups, *p < 0.05, **p < 0.01.

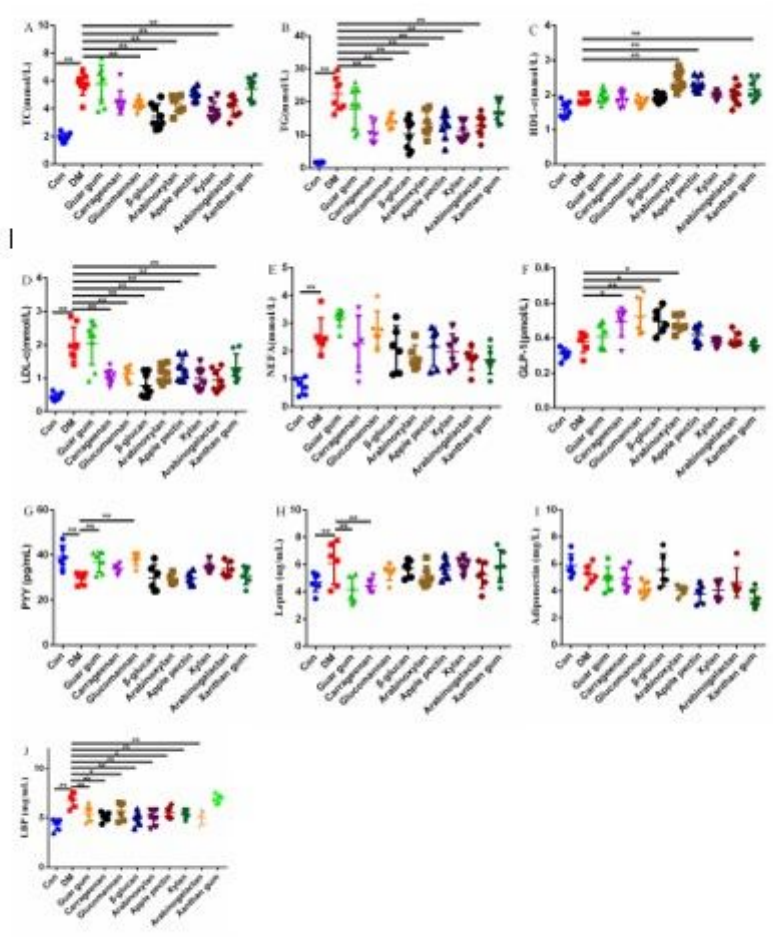

Figure 2

Effects of different dietary fiber on serum lipid profile (A-E), gut and adipose-derived hormones (F-I), and LBP (J). *indicates significant difference compared with DM groups, *p < 0.05, **p < 0.01.

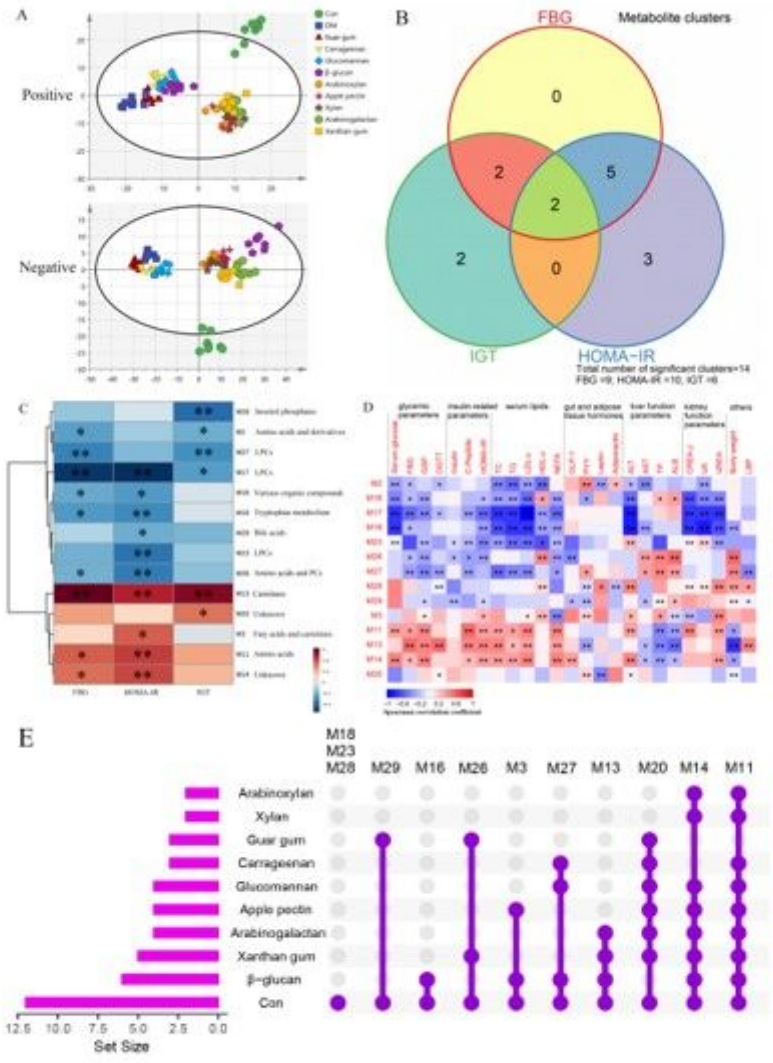

Figure 3

OPLS-DAP score plots (n = 8) of the positive and negative model (A); The number of metabolite clusters significantly associated with FBG, IGT and HOMA-IR (B, $p < 0.05$); Spearman correlations between serum metabolite clusters and major T2D phenotypes (C); Heatmap of association between physiological traits and 14 significantly changed metabolite clusters in T2D (D); Effects of different dietary fiber on the improvement of the significantly changed metabolite clusters (F). *indicates significant difference compared with DM groups, *$p < 0.05$, **$p < 0.01$.

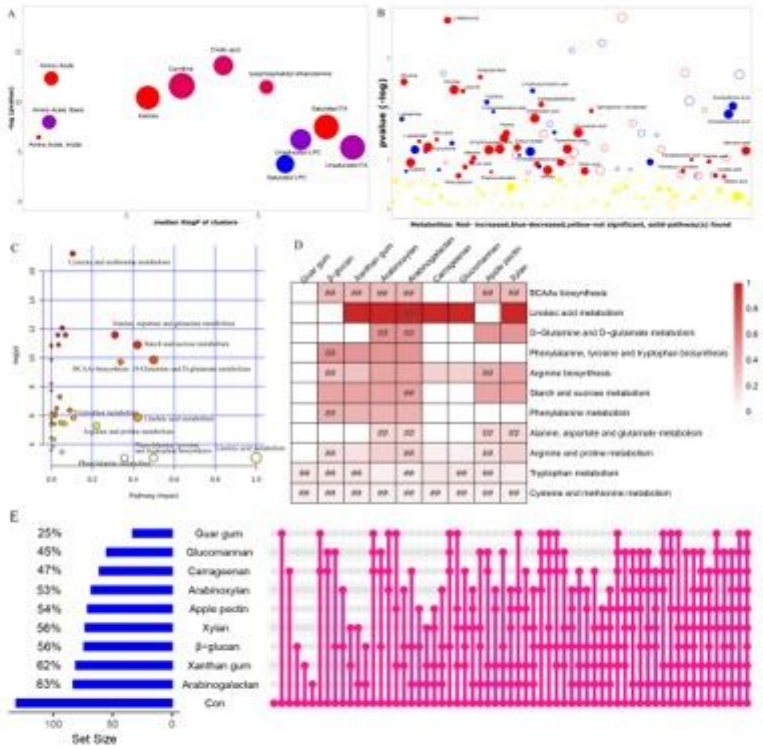

Figure 4

T2D-related significantly changed metabolites classes (A) and metabolites (B) by chemical similarity enrichment analysis; Disturbed metabolic pathways in T2D (C); Effects of different dietary fiber on the improvement of T2D-induced metabolic pathway disturbance and metabolites change (D and E). ##p < 0.01.

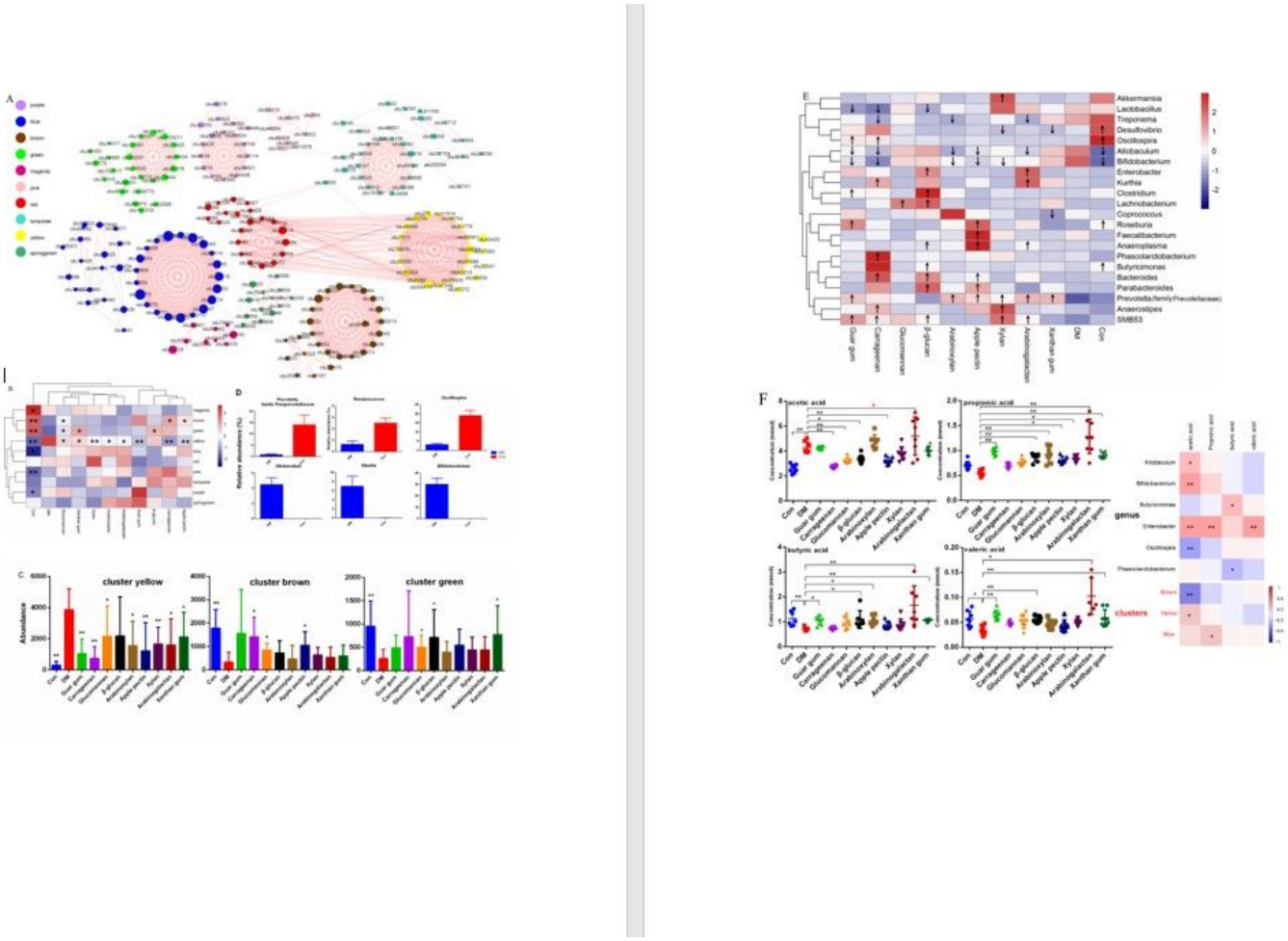

Figure 5

OTU-level network diagram, the node size indicates the mean abundance of each OTU (A); Heatmap and Bar plot illustrating the relative abundances of the 10 clustered co-abundance groups from gut microbiota in different groups (B-C); significantly changed bacteria with abundance great than 1% between normal and diabetic rats (D); Effects of different dietary fiber on the modification of gut microbiota composition in type 2 diabetic rats (E); Concentrations of SCFAs in different groups and spearman correlation between bacterial genus/clusters and SCFAs (F). *indicates significant difference compared with DM groups, *p < 0.05, **p < 0.01; ↓or↑means significantly decreased or increased compared with DM group.

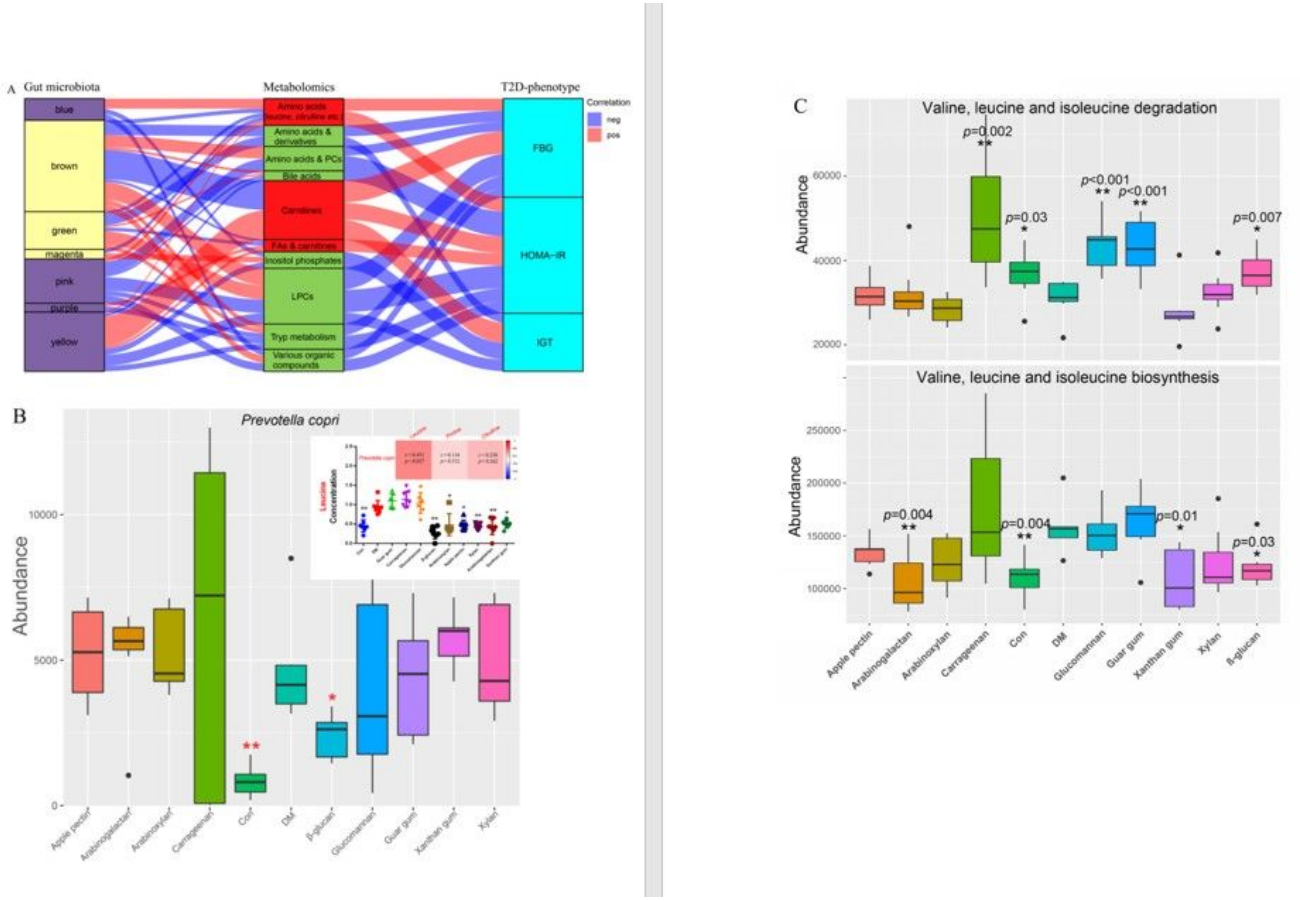

Figure 6

Interrelationship between gut microbiota composition, metabolic profile and main T2D phenotype. Visualization of the correlation network according to Spearman correlation analysis between the gut microbiota of significant bacterial clusters and the parameters represented T2D phenotype was mediated by serum metabolites modules. Red connections indicate a positive correlation, while blue connections show correlations that were negative (Spearman correlation test, $p < 0.05$). In the gut microbiota column, the yellow stratum represents bacterial clusters that were highly enriched in the Con group, and the stratum coloured in purple was increased in DM group. In the metabolomics column, the red stratum represents T2D-positive metabotypes, and the green stratum represents T2D-negative metabotypes (A); Abundance of species Prevotella copri in different groups, level of serum leucine in different groups, and Spearman correlation between Prevotella copri and amino acids (leucine, proline and citrulline) (B); Variation of BCAAs metabolism from PICRUSt gene predicted function (C).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.docx
- Additionalfile2.xls