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Differential effects of inulin and fructooligosaccharides on gut microbiota composition and glycemic metabolism in overweight/obese and healthy individuals: a randomized, double-blind clinical trial

Jie Li^{1†}, Feitong Liu^{1†}, Yuemei Luo^{1†}, Anisha Wijeyesekera², Shan Wang³, Xiaojiao Chen¹, Yamei Lv¹, Jing Jin¹, Huafang Sheng¹, Guopan Wang⁴, Yuanan Wei⁴, Zhuang Li^{1*}, Muxuan Chen^{1*} and Hongwei Zhou^{1,5*}

Abstract

Background Modulating the gut microbiota with prebiotics is a promising strategy for managing metabolic diseases. However, the clinical effects on glycemic metabolism across different populations remain uncertain. In this study, we conducted a randomized, double-blind investigation to examine the impact of inulin and fructooligosaccharides (FOS) on glycemic metabolism in overweight/obese and healthy adults.

Methods A total of 131 adults were included, with 44 receiving inulin, 43 receiving FOS, and 44 receiving placebo over a period of 4 weeks. Blood and fecal samples were collected before and after the intervention, and various metabolic parameters, gut microbiota composition, and metabolites were analyzed.

Results Placebo had no effect on glycemic metabolism or gut microbiota. Inulin significantly reduced glucose levels at 1 h (Cohen's $d=0.71$, $p=0.041$) and 2 h (Cohen's $d=0.73$, $p=0.028$) during oral glucose tolerance test (OGTT), increased fasting insulin (Cohen's $d=0.70$, $p=0.008$), and lowered homocysteine (HCY) levels (Cohen's $d=0.76$, $p=0.014$) in overweight/obese individuals. These effects were not observed in healthy individuals. In contrast, although FOS significantly decreased HCY (Cohen's $d=0.72$, $p=0.023$), it did not improve glycemic metrics in either group. Inulin also reduced the abundance of *Ruminococcus* by 72.0% (from $1.661\% \pm 1.501\%$ to $0.465\% \pm 0.594\%$), positively correlating with improved glycemic outcomes. Propionate levels decreased significantly in both overweight/obese (Cohen's $d=0.89$, $p=0.014$) and healthy participants (Cohen's $d=1.19$, $p=0.020$) following inulin. Functional prediction of gut microbiota revealed upregulation of microbial folate and glutathione metabolism with inulin, and purine metabolism with FOS.

[†]Jie Li, Feitong Liu and Yuemei Luo contributed equally to this work.

*Correspondence:

Zhuang Li

jiandandjx@126.com

Muxuan Chen

muxuanchen@126.com

Hongwei Zhou

hzhou@smu.edu.cn

Full list of author information is available at the end of the article



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Conclusions Practically, inulin may be more suitable for managing glycemic dysregulation in overweight or obese individuals, while FOS may be considered for HCY reduction in individuals with normal glycemic status. Such targeted use of prebiotics could complement existing dietary and pharmacologic strategies in personalized metabolic care.

Trial registration number ChiCTR-IOR-17010574.

Keywords Prebiotics, Inulin (INU), Fructo-oligosaccharides (FOS), Glycemic metabolism, Overweight/obese, Gut microbiota, RCT

Background

The rising global prevalence of metabolic diseases has generated significant interest in exploring innovative strategies [1]. Various approaches are currently utilized to manage glycemic metabolism and ameliorate metabolic disorders, including pharmacological interventions, dietary control, physical activity, and surgical procedures [2–4]. While these interventions have shown some efficacy, they may be linked to adverse effects or lack universal applicability, prompting a quest for alternative approaches.

It is increasingly evident that the gut microbiota plays a pivotal role in metabolic health, leading to the emergence of gut microbiota modulation as a promising avenue [5]. Specifically, prebiotics—nondigestible substrates that selectively promote the growth of beneficial gut bacteria—have attracted attention for their potential influence on metabolic health. Preclinical and clinical studies have demonstrated that prebiotics can enhance glucose homeostasis, improve insulin sensitivity, and alleviate inflammation, presenting a complementary or adjunctive strategy alongside conventional therapies for managing metabolic disorders [6–8]. Furthermore, prebiotics are relatively safe and generally do not lead to serious side effects, making them more readily accepted and adhered to by patients. This suggests that probiotic intervention holds broad applicability in intervening in metabolic health.

Despite the potential advantages of prebiotic interventions, their efficacy remains a subject of significant debate. While numerous studies have highlighted the health-promoting metabolic benefits of prebiotics, others have presented conflicting results. Systematic reviews have emphasized the modest impact of prebiotics on metabolic variables, including glycemic metabolism, casting doubt on their therapeutic potential [7, 9]. Furthermore, recent evidence regarding the effect of inulin-type fructans on cardiovascular risk factors has been inconclusive, highlighting the need for further rigorous clinical trials [7]. Moreover, the majority of prebiotic intervention studies have been conducted in animal models, resulting in a lack of clinical data [10–12]. Consequently, uncertainties persist regarding the clinical impact of prebiotics on glycemic metabolism,

particularly in distinguishing various population groups.

Inulin and FOS are both fructans composed of fructose units, but they differ remarkably in their degree of polymerization (DP) and chain length, which lead to differences in their physicochemical properties and physiological effects [13]. Inulin typically has a longer chain length, with a DP ≥ 10 , resulting in lower solubility and slower fermentation in the distal colon [14]. On the contrary, FOS consists of shorter chains, with a DP 2 to 9, presenting higher solubility and undergoing rapid fermentation in the proximal colon [14]. These differences affect their impact on short-chain fatty acid (SCFA) production, gut microbiota modulation, and subsequently results in different effects on host metabolism [14–16].

Our study aims to address the differential effects of two commonly used prebiotics, inulin and fructooligosaccharides (FOS), on glycemic metabolism. Through a meticulously designed randomized, double-blind clinical investigation, we aim to elucidate the distinct impacts of inulin and FOS on overweight/obese (OW) individuals and healthy (normal weight, NW) controls. By undertaking this endeavor, we seek to contribute to the optimization of prebiotic interventions and their tailored application to specific population subsets in the clinic.

Methods

Study design

This study is a 4-week, CONSORT (Consolidated Standards of Reporting Trials) compliant, randomized, double-blind trial. The study protocol was reviewed and approved by the Chinese Ethics Committee of Registering Clinical Trials (No. ChiECRCT-20170006). All participants signed written informed consent prior to enrollment. The study was registered at www.chictr.org.cn (number ChiCTR-IOR-17010574, registration date February 7, 2017), which included four experimental groups: Fructo-oligosaccharide (FOS), Inulin, Resistant Starch (RS), and Control (placebo). Due to production-related issues encountered early in the trial, the RS group was not included in the actual intervention phase of the trial and therefore not reported in the study.

Sample size calculation

The sample size was determined based on practical considerations, including the estimated effect size from the literature and the feasibility of recruiting sufficient participants. We aimed to enroll a sample size large enough to detect clinically meaningful differences in key outcomes, such as 2-h glucose levels during the oral glucose tolerance test (OGTT) and changes in gut microbiota composition. Based on previous studies investigating the effects of prebiotics on glycemic metabolism and gut microbiota [17–19], we estimated that a minimum of 40 participants per group would be adequate to detect potential differences in these outcomes. This estimation was further informed by prior research [20] demonstrating similar interventions in smaller cohorts with significant effects. Therefore, we estimated that a total sample size of 120 participants would be efficient. Ultimately, 131 participants were recruited and randomized into three groups: inulin ($N=44$), FOS ($N=43$), and control ($N=44$). No interim analyses were conducted, and no formal stopping guidelines were established for this trial.

Subject recruitment

Participants were recruited from local communities in Guangzhou, China, through advertising flyers, medical record reviews, or dietitians' recommendations at Healthy Management Center. Potential participants were interviewed by a trained researcher with a structured screening questionnaire. The enrollment and intervention were conducted at the local Zhujiang Hospital, Southern Medical University, from October 2016 to March 2017. The study enrolled participants involved in normal weight ($18.5 \text{ kg/m}^2 \leq \text{body mass index (BMI)} \leq 23.9 \text{ kg/m}^2$) Chinese group (NW), overweight ($24.0 \text{ kg/m}^2 \leq \text{body mass index (BMI)} \leq 27.9 \text{ kg/m}^2$), or obese ($\text{body mass index (BMI)} \geq 28.0 \text{ kg/m}^2$) Chinese group (OW). Exclusion criteria were as follows: patients with any endocrine disorders; patients with severe respiratory infection; patients with any intestinal disease and cancer disease; history or evidence of any cardiovascular and renal diseases; recent intake of antibiotics or drugs known to influence gut microbiota within 1 month; pregnant or lactated women; vegetarians and vegans.

Dietary intervention

After confirming that participants were following the inclusive criteria and obtaining written consent, the participants were enrolled in a run-in period for 4 weeks. After that, the participants were randomized into the FOS group, the Inulin group and the Control group using a stratified randomization method by R software, based on gender and age. Each group was conducted with a

daily supplement of 15 g FOS (Quantum Hi-Tech Biological Co., Ltd, China), Inulin (Oreye, Belgium), and maltodextrin (Qinhuangdao Lihua starch Co., Ltd, China) as placebo and lasted for 4 weeks. The dosage was determined based on our previous clinical trials in the healthy young population, which reported using 16 g/day has no risk of adverse effects [20]. Subjects were still recommended to take a half dose in the first 2 days to promote adaption to the prebiotics and minimize gastrointestinal symptoms. All FOS, Inulin, and maltodextrin products were provided in identical opaque packages (named A, B, or C). The products were suggested to add to drinks such as coffee, tea, or milk. During the whole study, the participants were asked to maintain their lifestyle and eating habits, avoiding the consumption of yogurt, which contains prebiotics. The randomized allocation and the labeling work were performed by staff who were not involved in the recruitment and analysis of the study. Participants, investigators, and laboratory technicians were blinded to the treatment assignments until the conclusion of the trial.

Assessment of harms

Adverse events were defined as any unfavorable or unintended signs, symptoms, or diseases temporally associated with the use of the prebiotic interventions. Participants were asked to report any adverse events throughout the 4-week study period. The Wechat follow-up (Shenzhen Tencent Computer System Co., Ltd) was performed daily to report gastrointestinal symptoms or other discomforts. All reported events were recorded and evaluated by the study team for severity and potential relationship to the intervention.

Trial protocol

Participants and the public were not involved in the design, conduct, or reporting of this research. At the beginning (week 0) and the end of the treatment (week 4), body weight, systolic blood pressure (SBP), diastolic blood pressure (DBP), and waist and hip circumferences were measured and blood and feces were both sampled. Blood was collected and sent to the hospital laboratory to detect the following clinical parameters: fasting glucose, fasting insulin, total cholesterol (TC), high-density and low-density lipoprotein cholesterol (HDL and LDL, respectively), triglycerides (TG), high-sensitive C-reactive protein (hs-CRP), homocysteine (HCY), and uric acid (UA). For oral glucose tolerance test (OGTT), participants were fasted overnight for 10 h: 75 g glucose in drinking water and measurement of glycemia and insulin at 1 h and 2 h after ingestion. Blood glucose was measured by glucose oxidase method. Blood TC, TG, and UA were measured by standardized enzymatic colorimetric

methods. Blood HDLC and LDLC were measured by enzymatic clearance assay. Blood hs-CRP was measured by immune-turbidimetric method (QuikRead CRP, Orion Diagnostica Oy, Espoo, Finland). Blood HCY was measured by enzyme cycling methods (Roche Diagnostics, Indianapolis, IN, USA). Blood insulin was determined by chemiluminescent micro-particle immunoassay (Roche Diagnostics, Indianapolis, IN, USA), with sensitivity of 0.2 $\mu\text{U/mL}$. Each volunteer provided stool specimen in feces collector (Fun-Poo Biotech, Shenzhen) and transported immediately with ice bags to the laboratory in the hospital within 2 h excretion. The fecal samples were stored in biological specimen banks at -80°C until further processing. The Wechat follow-up (Shenzhen Tencent Computer System Co., Ltd) was performed daily to verify the compliance and record possible side effects.

Metabolites extraction and ^1H -NMR metabolomics analysis

0.2 g of fecal sample was thawed and added 1 mL of sterile HPLC water. The sample was vortexed at the highest speed for 3 min until completely homogenized. We concentrated at 13,000 rpm at 4°C for 10 min to settle the solid particles. We mixed 400 μL of the fecal extracts with 200 μL of phosphate buffer [0.2 M (pH 7.4) in D_2O plus 0.001%TSP (3-(trimethylsilyl)-[2,2,3,3,-2H4]-propionic acid, δ 0.00)], and transferred 550 μL of the mixtures into 5-mm NMR tubes. We process samples from different groups in random order under the same condition in order to avoid any lab- and instrumental-related bias. All ^1H -NMR spectroscopy data were acquired on a Bruker Avance DRX 500 MHz NMR spectrometer (Bruker Biopsin, Germany) operating at 500 MHz. Fecal water spectra were acquired using a standard 1D pulse sequence [recycle delay (RD)–90°–t1–90°–Tm–90°–acquire free induction decay (FID)] with water suppression applied during RD of 2 s. For each spectrum, a total of 128 scans were carried out with a spectral width of 14.0019 ppm. The FIDs were multiplied by an exponential function corresponding to 0.3 Hz line broadening.

All spectra were manually phased, baseline corrected, and calibrated to the chemical shift of TSP using TopSpin (Bruker Biopsin, Germany). NMR spectroscopy data were digitalized by Matlab R2017b, and the area normalized by using Matlab R2017b normalization scripts. The identified ^1H -NMR metabolite signals were integrated for peak area (which is proportional to metabolite concentration) and normalized by dividing each integral by the internal standard TSP spectrum integral to provide a normalized integral (N.I.).

Gut microbiota compositional and functional analysis

Each volunteer provided stool specimen in feces collector (Fun-Poo Biotech, Shenzhen) and transported

immediately with ice bags to the laboratory in the hospital within 2 h excretion. Then the fecal samples were stored in biological specimen banks at -80°C until further processing. Microbial total DNA was extracted using the Fecal DNA nucleic acid extraction kit (Shenzhen Bioeasy Biotechnologies, Inc., China) as per the manufacturer's instructions. The V4 variable regions of bacterial 16S rDNA gene were amplified by polymerase chain reaction (PCR) using the following barcoded: forward primer 515 F (GTGYCAGCMGCCGCGGTAA) and barcoded reverse primer 806R (GGACTACNVGGG TWTCTAAT). The PCR cycle conditions were described previously [21]. The PCR products were sequenced using the Illumina HiSeq 2500 platform. Paired-end reads were assembled using SeqPrep and quality filtered with the threshold of 19 in QIIME 1.9.1 `split_libraries_fastq.py` [22]. The sequences removed barcodes and primers were assigned and clustered with Deblur [23] in negative filtering mode, using all default parameters except trim length, which was set to 250 nt. After negative filtering (removing reads which contain PhiX and adapter sequences), RDP was used in QIIME to add taxonomy to the obtained biome table and rarefied the table to 1190 sequences per sample. The remaining sOTUs were aligned using PyNAST, and the phylogenetic tree was constructed using FastTree from the result of alignment. The 16S rRNA gene sequences of the samples were mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database for annotation, and the abundance of metabolic pathways was predicted based on the Phylogenetic Investigation of Communities Reconstructed from Unobserved States (PICRUST2) [24].

Statistical analyses

Statistical analyses were performed with SPSS 20.0 (IBM, USA) and GraphPad Prism 8.0 (GraphPad Software Inc., USA). We performed the Shapiro–Wilk and Kolmogorov–Smirnov (distance) normality test, and the homogeneity of variances across groups was compared using Bartlett's and Brown-Forsythe tests. For parametric data, two-tailed paired Student's t test, two independent t test, or one-way ANOVA with Bonferroni post hoc test was used to compare differences between groups where appropriate. For nonparametric data, a Wilcoxon signed-rank test, Mann–Whitney U test, or Kruskal–Wallis test following Dunn's post hoc test was applied. We applied Bonferroni or Dunn's adjustment to correct for multiple comparisons in statistical analyses. For 16S rDNA amplicon sequencing data, false discovery rates (FDRs) were controlled using the Benjamini–Hochberg method. To assess global differences in overall gut microbiota composition between groups and timepoints, we used Permutational Multivariate Analysis of Variance

(PERMANOVA) based on Weighted UniFrac distances. The differential abundances of genera comparing pre- and post-intervention were determined by Wilcoxon signed-rank test. Correlation analyses were performed using a Pearson correlation coefficient. Multiple comparison was corrected by the Benjamini–Hochberg method. A value of $P < 0.05$ (two-tailed) was considered as statistically significant.

Results

Anthropometric and physiological data for participants at the beginning and the end of intervention

A total of 143 individuals were assessed for eligibility for the study, with 12 individuals excluded (9 individuals did not meet the inclusion criteria and 3 individuals declined to participate). One hundred thirty one individuals were recruited in the study (Fig. 1), 43 participants were allocated to FOS, 44 participants were allocated to inulin, and 44 participants were allocated to control. Among 43 participants allocated to FOS, 31 were NW adults (1 participant discontinued intervention) and 12 were OW adults. Among 44 participants allocated to inulin, 30 were NW adults (6 participants discontinued intervention), and 14 (1 participant discontinued intervention) were OW adults. Among 44 participants allocated to control, 26 were NW adults and 18 were OW adults. Baseline characteristics of the volunteers at the start or the end of prebiotics intervention are shown in Table 1.

Subjects went through a run-in period for 4 weeks, followed by a 4-week prebiotics intervention of inulin or FOS. The feces and blood samples were collected before and after the prebiotics interventions (experimental setup, Figs. 2A and 3A). No serious adverse events were reported during the study period. After a 4-week intervention, there were no significant effects of these prebiotics on weight, BMI, UA, lipid profile (TC, TG, HDLC, LDLC), and hs-CRP, except that the systolic blood pressure was increased while waist and hip circumference were reduced after FOS treatment in NW group, and hip circumference was reduced after inulin treatment in NW and OW group (Table 1).

A 4-week inulin intervention effectively improves glycemic metabolism in overweight/obese individuals

A 4-week placebo had no effects on glycemic metabolism (Additional file 1 Fig. S1). Strikingly, although inulin did not have significant effects on the levels of insulin at 1 h (Cohen's d , 0.19; 95% CI, -18.020 to 31.970 ; $p = 0.548$) or 2 h (Cohen's d , 0.34; 95% CI, -23.680 to 7.326 ; $p = 0.270$) after OGTT, the area under the curve (AUC) of insulin (Cohen's d , 0.12; 95% CI, -24.23 to 34.29 ; $p = 0.710$), or the fasting blood glucose (FBG) (Cohen's d , 0.05; 95% CI, -0.404 to 0.343 ; $p = 0.861$) in OW group, it significantly decreased the levels of glucose at 1 h (Cohen's d , 0.71; 95% CI, -1.773 to -0.045 ; $p = 0.041$) and 2 h (Cohen's d , 0.73; 95% CI, -1.404 to -0.096 ; $p = 0.028$) after OGTT,

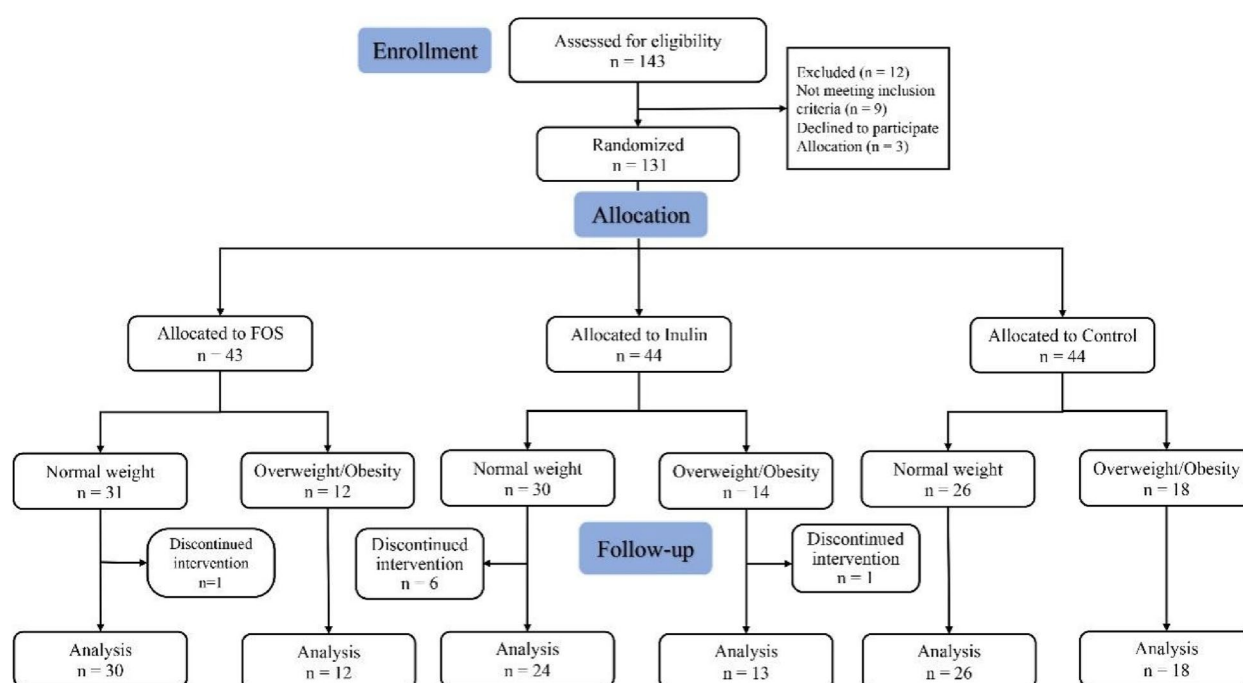


Fig. 1 Design of clinical intervention trial. Flow chart of participants' enrollment process and number of participants analyzed in this study

Table 1 Anthropometric and physiological data for participants at the beginning and the end of intervention

Characteristics	FOS group			Inulin group			Control group		
	NW	W4	OW	NW	W4	OW	NW	W4	OW
Gender	18 F 12 M		6 F 6 M	13 F 11 M		7 F 6 M	16 F 10 M		7 F 11 M
Age,y	33.7 ± 7.7		35.6 ± 8.6	31.5 ± 6.3		39.2 ± 10.1	34 ± 9.0		35.6 ± 8.6
Weight,kg	57.9 ± 8.1	57.3 ± 8.5	70.3 ± 10.2	57.9 ± 7.3	69.8 ± 10.9	75.5 ± 21.3	56.5 ± 6.7	56.3 ± 6.6	71.7 ± 7.4
BMI,kg/m2	21.6 ± 1.4	21.3 ± 1.6	26.3 ± 2.2	21.7 ± 1.4	26.1 ± 2.3	28.3 ± 7.3	21.3 ± 1.5	21.2 ± 1.6	26.5 ± 2.3
Waist,cm	75.3 ± 6.9	73.4 ± 6.0*	86.5 ± 11.0	74.6 ± 4.5	83.2 ± 9.1	93 ± 18.9	72.2 ± 5.1	72.3 ± 5.8	88.4 ± 7.6
Hipline,cm	92.7 ± 3.9	91.4 ± 4.0*	101.3 ± 5.1	93.5 ± 3.9	99.5 ± 6.2	105.2 ± 13.9	92.3 ± 3.2	91.6 ± 3.4	100.8 ± 4.8
SBP,mmHg	115.7 ± 12.5	119.0 ± 13.9*	120.3 ± 13.5	116.0 ± 12.0	121.6 ± 9.1	136.2 ± 20.9	117.8 ± 4.4	119.8 ± 12.0	124.5 ± 9.2
DBP,mmHg	74.0 ± 8.6	76.3 ± 10.6	75.8 ± 6.8	71 ± 8.1	80.2 ± 9.0	86.5 ± 11.7	70.7 ± 7.8	72.9 ± 7.7	77.3 ± 7.8
UA,μmol/L	312.2 ± 64.0	327.6 ± 88.4	412.1 ± 117.4	301.7 ± 82.2	389.2 ± 112.0	344.1 ± 93.3	315.0 ± 73.9	300.2 ± 78.5	371.2 ± 111.5
TC,mmol/L	4.3 ± 0.7	4.8 ± 1.2	4.9 ± 1.1	4.3 ± 0.8	5.1 ± 1.2	4.5 ± 0.7	4.6 ± 1.1	4.7 ± 1.0	5.1 ± 0.8
TG,mmol/L	0.9 ± 0.5	1.4 ± 2.7	1.2 ± 0.6	0.8 ± 0.4	1.1 ± 0.5	1.3 ± 0.7	0.9 ± 0.4	1.0 ± 0.5	1.4 ± 0.6
HDLc,mmol/L	1.5 ± 0.4	1.6 ± 0.3	1.4 ± 0.5	1.7 ± 0.3	1.4 ± 0.4	1.3 ± 0.4	1.7 ± 0.4	1.6 ± 0.4	1.3 ± 0.3
LDLc,mmol/L	2.6 ± 0.6	2.8 ± 0.8	3.3 ± 1.0	2.5 ± 0.6	3.2 ± 1.2	2.9 ± 0.8	2.8 ± 1.0	2.7 ± 0.8	3.3 ± 0.7
hs-CRP,mg/mL	0.8 ± 1.9	0.7 ± 0.7	2.7 ± 2.5	1.0 ± 2.8	2.0 ± 2.0	2.8 ± 4.4	0.9 ± 1.9	1.0 ± 2.2	1.1 ± 1.0

Data are expressed as mean ± SD
M male, F female. *p<0.05 as W0 compared with W4 in NW or OW group

(See figure on next page.)

Fig. 2 A 4-week inulin intervention effectively improves glycemic metabolism in overweight/obese individuals. **A** Outline of the design and sampling of intervention, **B** blood glucose at fasting and every 1 h after the OGTT, **C** blood insulin at fasting and every 1 h after the OGTT, **D** blood glucose concentration at 1 h after the OGTT, **E** blood glucose concentration at 2 h after the OGTT, **F** the glucose AUC, **G** blood insulin concentration at 1 h after the OGTT, **H** blood insulin concentration at 2 h after the OGTT, **I** the insulin AUC, **J** FBG, **K** fasting insulin concentration, **L** HCY. Data are shown as box plot with whiskers at min/max in **D–L**, NW, normal weight; OW, overweight/obese; FBG, fasting blood glucose concentration; HCY, homocysteine. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. ⁵ $P < 0.05$ as OW_W0 compared with OW_W4; ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$ as NW_W0 compared with OW_W0

the glucose AUC_{0–2 h} (Cohen's d , 0.79; 95% CI, -2.352 to -0.184 ; $p = 0.026$) and HCY (Cohen's d , 0.76; 95% CI, -4.874 to -0.541 ; $p = 0.014$), and it significantly increased level of fasting insulin (Cohen's d , 0.70; 95% CI, 0.577 to 8.111; $p = 0.008$) in OW group (Fig. 2B–L).

A 4-week FOS intervention does not ameliorate glycemic metabolism

After a 4-week intervention (Fig. 3A), FOS intervention did not have significant effects on diabetic parameters in NW or OW group, including the levels of glucose and insulin at 1 and 2 h after OGTT, the glucose AUC_{0–2 h} and insulin AUC_{0–2 h}, FBG (Fig. 3B–J). Nevertheless, FOS intervention significantly increased the level of fasting insulin (Cohen's d , 0.79; 95% CI, 0.537 to 4.863; $p = 0.019$) in OW group and decreased the level of HCY in both NW (Cohen's d , 0.48; 95% CI, -1.915 to -0.206 ; $p = 0.017$) and OW (Cohen's d , 0.72; 95% CI, -3.855 to -0.345 ; $p = 0.023$) groups (Fig. 3K, L). These results indicate the differential effects of inulin and FOS on diabetic parameters of OW adults. Inulin exerts more beneficial effects on glucose metabolism than FOS does.

Inulin alters gut microbiota composition, which is associated with improved parameters of glycemic metabolism

A 4-week placebo had no effect on gut microbiota composition in NW or OW groups (Additional file 1 Fig. S2). The observed species and Shannon index (α diversity, Fig. 4A) were significantly lower after inulin intervention than that before intervention in OW group, whereas FOS treatment had no significant modification on α diversity using the Wilcoxon signed rank test within OW group (Additional file 1 Fig. S3A). Based on the Weighted Unifrac distance analysis, the gut microbiota composition in OW group changed significantly after inulin intervention (Fig. 4B). There were significant separations in PCoA between NW and OW groups before and after FOS intervention (Additional file 1 Fig. S3B). The 5 most abundant gut microbiota in FOS intervention groups were *Bacteroides*, *Faecalibacterium*, *Prevotella*, *Clostridiales_Lachnospiraceae*, and *Dialister* (Additional file 1 Fig. S3C). Next, to identify the gut microbiota that were significantly changed after

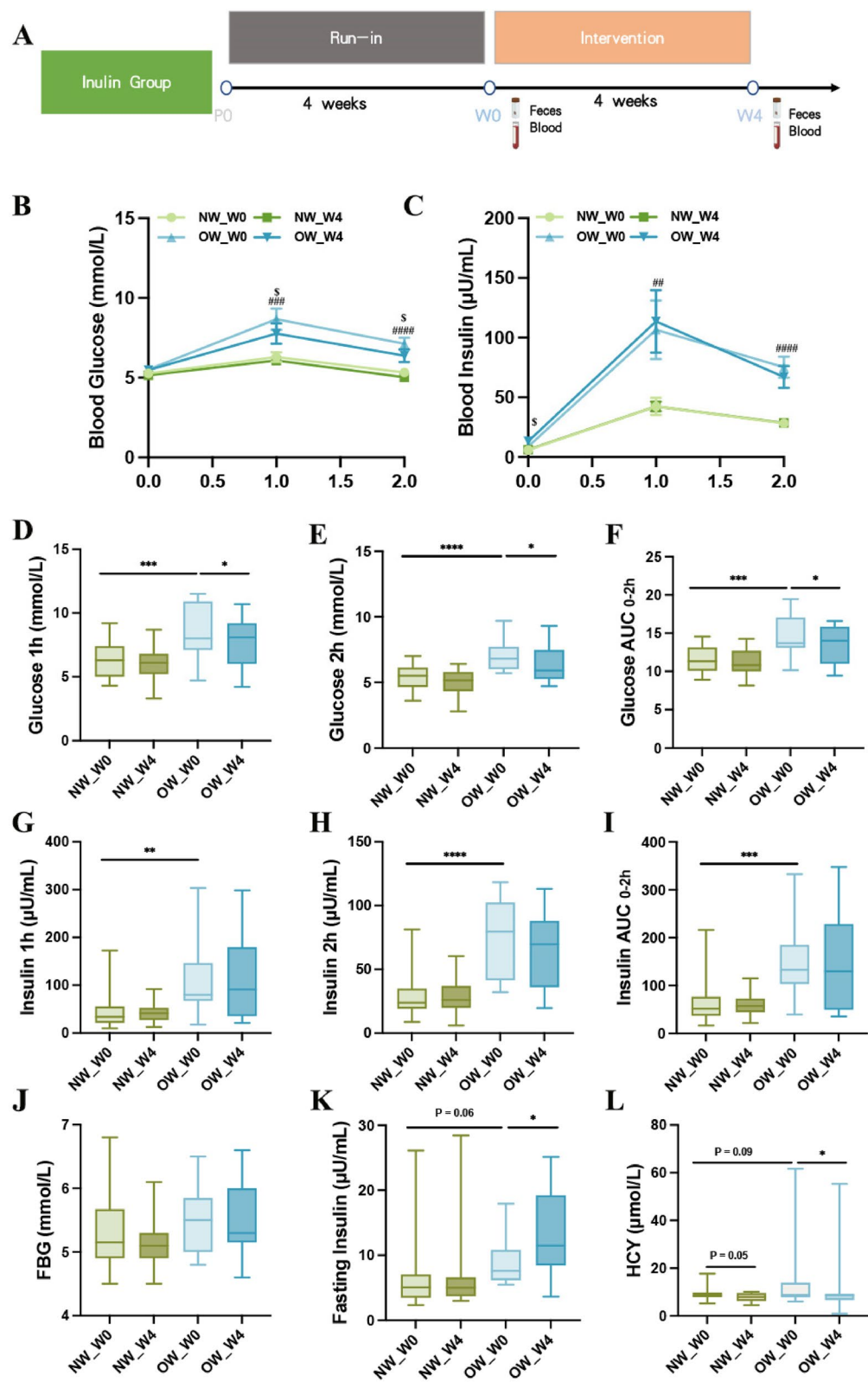
prebiotics intervention, we performed LefSe analysis. We found that the abundances of *Bacteroidales*, *Bacteroidia*, and *Lactobacillus* were significantly increased while the abundances of *Firmicutes*, *Clostridia*, and *Ruminococcus* were significantly decreased after inulin intervention in OW group (Fig. 4C). To find the gut microbiota that is possibly responsible for the beneficial effects on blood glucose metabolism after inulin intervention, we performed a correlation analysis between the changes in the abundance of the genus that altered significantly after inulin intervention and the changes in the levels of glucose at 1 and 2 h after OGTT, the glucose AUC_{0–2 h} and level of HCY. We found that the change in the abundance of *Ruminococcus* was significantly associated with changes in the level of glucose at 1 h after OGTT and the glucose AUC_{0–2 h} (Fig. 4D), suggesting a detrimental role of *Ruminococcus* in the regulation of blood glucose. Furthermore, redundancy analysis (RDA) supported a positive correlation between *Ruminococcus* and diabetic parameters including glucose at 1 and 2 h, the AUC of OGTT glucose and HCY (Fig. 4E). Collectively, these data provide evidence for the involvement of *Ruminococcus* in the glucose metabolism.

Inulin intervention reduces propionate levels in both overweight/obese and healthy individuals

Finally, we measured the levels of acetate, propionate, butyrate, and lactate in NW and OW groups after inulin or FOS intervention. We found that inulin intervention increased the level of lactate and decreased the level of acetate in NW adults (Fig. 5A, D), and decreased the level of propionate in both NW (Cohen's d , 1.19; 95% CI, -0.685 to -0.087 ; $p = 0.020$) and OW (Cohen's d , 0.89; 95% CI, -0.664 to -0.094 ; $p = 0.014$) adults (Fig. 5B). FOS increased the levels of acetate and lactate in NW adults while had no significant effects on these metabolites of OW adults (Fig. 5A–D).

Functional profiling of gut microbiota

To investigate the potential microbial pathways involved in the differential glycemic responses to inulin and FOS, we performed Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) analysis. This analysis predicted the functional potential



of the gut microbiota following prebiotic interventions. Following inulin supplementation, we observed significant upregulation of several microbial pathways (Fig. 6A). These pathways included Folate biosynthesis, Ubiquinone and other terpenoid-quinone biosynthesis, Glutathione metabolism, Vitamin B6 metabolism, and Inositol phosphate metabolism. On the other hand, FOS supplementation led to the upregulation of the Purine metabolism pathway (Fig. 6B).

Discussion

The rapid advancement of microbiome medicine research has revealed the significant potential of targeting the gut microbiome for treating metabolic diseases, with the established role of gut microbiota in metabolic health. Several microbiome-based treatments, including Fecal Microbiota Transplantation (FMT), probiotics, and prebiotics, have shown promise in improving metabolic health through clinical and preclinical interventions [8, 25–27]. However, inconsistencies in the clinical outcomes of these interventions on metabolic health across various studies suggest that factors like age, sex, and ethnicity may influence the effects of prebiotics on metabolic health in different population subsets [28–31]. To address this issue, we conducted a randomized controlled trial to explore the divergent effects of two commonly used prebiotics, inulin and FOS, on glycemic metabolism in distinct population subsets. Our findings demonstrated differential clinical intervention effects of FOS and inulin on glycemic metabolism, particularly highlighting specific efficacy observed in overweight or obese individuals.

These findings have important implications for future research and clinical applications. Firstly, our results highlight the varying effects of prebiotic interventions on metabolic health depending on the population group. Specifically, we observed significant differences in glucose metabolism between overweight/obese individuals and healthy controls in response to inulin supplementation. This aligns with previous research, suggesting that oligofructose supplementation improves glucose tolerance in overweight and obese adults [32]. The differences in glycemic metabolism between different population subsets may be influenced by factors such as genetic

predispositions, metabolic processes, and gut microbiota composition [30, 31]. Previous study has implied that regional location is strongly associated with microbiota variations [33], which partly explains the differential effects of prebiotics on different cohorts. Therefore, personalized approaches to prebiotic interventions tailored to specific population subsets may be necessary to optimize therapeutic outcomes.

Additionally, our findings contribute to the ongoing debate regarding the efficacy of prebiotics in managing metabolic health. The differential effects of inulin and FOS likely stem from variations in their structural characteristics, fermentation profiles, sourcing, and purification processes, potentially mediated by key gut microbes [34, 35]. Inulin and FOS, both classified as prebiotics, differ in their degree of polymerization, which affects their fermentability and the production of short-chain fatty acids (SCFAs) [36, 37]. These SCFAs can influence the composition and function of the gut microbiota differently, offering a possible explanation for the conflicting views on the therapeutic potential of prebiotics reported in previous literature. For instance, while a study conducted by Slavin et al. highlighted the beneficial role of dietary fiber, including prebiotics, in improving metabolic health [38], other studies have shown mixed results regarding their impact on glycemic control and insulin sensitivity [7]. These discrepancies underscore the importance of understanding how different prebiotics interact with the gut microbiome and subsequently influence metabolic outcomes. Notably, in this study, although FOS did not improve glycemic metabolism, it reduced HCY level, indicating a potential cardiovascular benefit. Elevated HCY is a recognized risk factor for cardiovascular disease, and its reduction with both prebiotics underscores their shared role in supporting cardiovascular health. However, only inulin resulted in improvements in glycemic metabolism, highlighting a divergence in their metabolic effects. Several factors may contribute to this discrepancy: Firstly, in terms of fermentation kinetics, inulin is a longer-chain fructan, which undergoes slower fermentation throughout the distal colon, leading to sustained production of SCFAs such as butyrate, which is closely linked to improved

(See figure on next page.)

Fig. 3 A 4-week FOS intervention does not ameliorate glycemic metabolism. **A** Outline of the design and sampling of intervention. **B** blood glucose at fasting and every 1 h after the OGTT. **C** blood insulin at fasting and every 1 h after the OGTT. **D** blood glucose concentration at 1 h after the OGTT. **E** blood glucose concentration at 2 h after the OGTT. **F** the glucose AUC. **G** blood insulin concentration at 1 h after the OGTT. **H** blood insulin concentration at 2 h after the OGTT. **I** the insulin AUC. **J** FBG. **K** fasting insulin concentration. **L** HCY. Data are shown as box plot with whiskers at min/max in **D–L**, NW, normal weight; OW, overweight/obese; FBG, fasting blood glucose concentration; FOS, fructooligosaccharides; HCY, homocysteine. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. [§] $P < 0.05$ as OW_W0 compared with OW_W4; [#] $P < 0.05$, ^{##} $P < 0.01$, ^{###} $P < 0.0001$ as NW_W0 compared with OW_W0

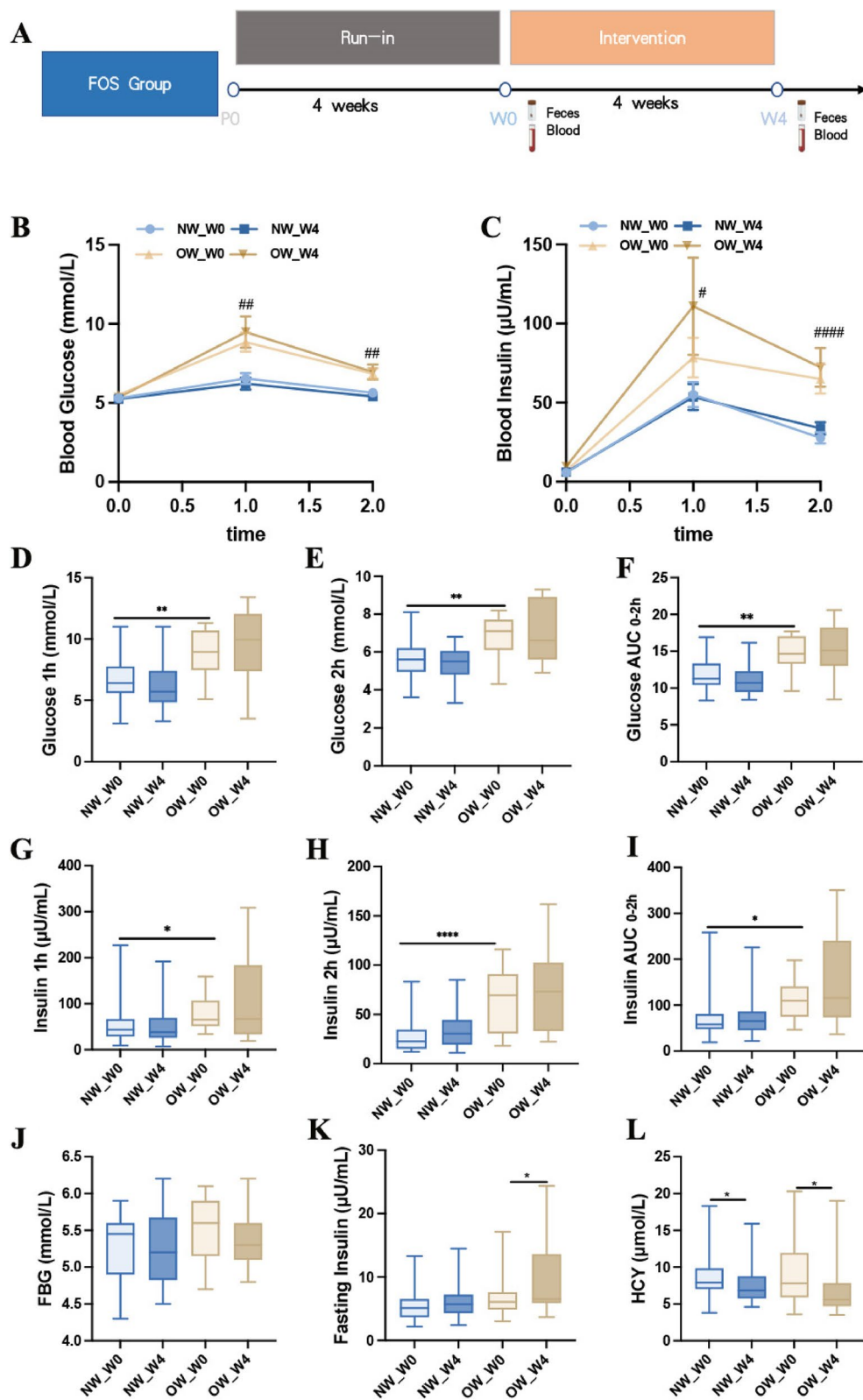


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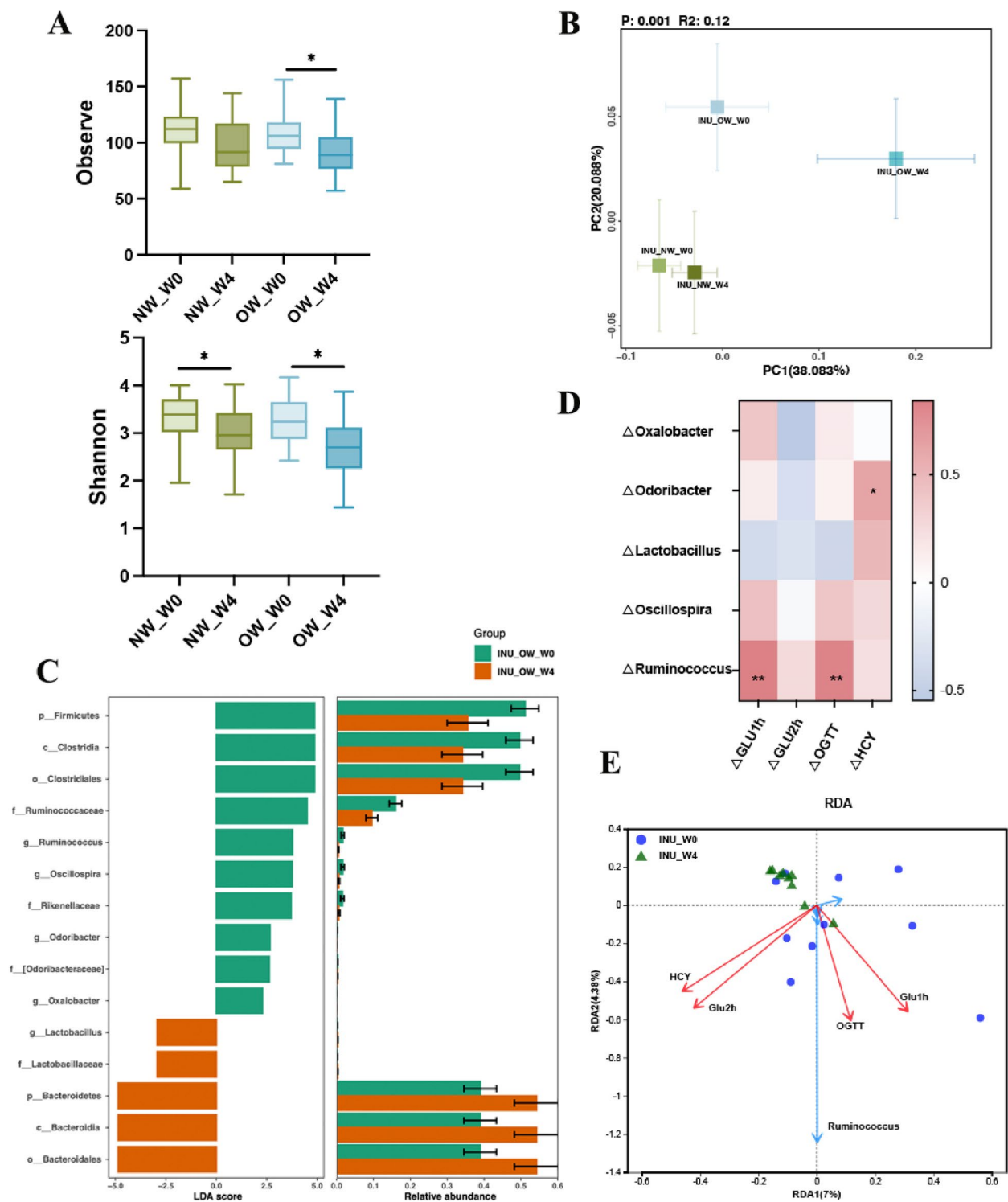


Fig. 4 Inulin alters gut microbiota composition, which is associated with improved parameters of glycemic metabolism. **A** Observed species index and Shannon index. **B** Principal coordinate analysis based on the Weighted UniFrac distance analysis. **C** LEfSe of the gut microbiota before (W0) and after (W4) inulin intervention in OW groups. **D** Correlation analysis between significantly changed genus and glucose concentration at 1 h, 2 h, the area under blood glucose concentration curve and HCY. **E** The redundancy analysis (RDA) that shows the relationships between the significantly changed bacterial community composition at the genus level and the variables for glucose concentration at 1 h, 2 h, AUC OGTT of glucose, and HCY. Data are shown as box plot with whiskers at min/max in **A** and **B**, * $p < 0.05$, ** $p < 0.01$

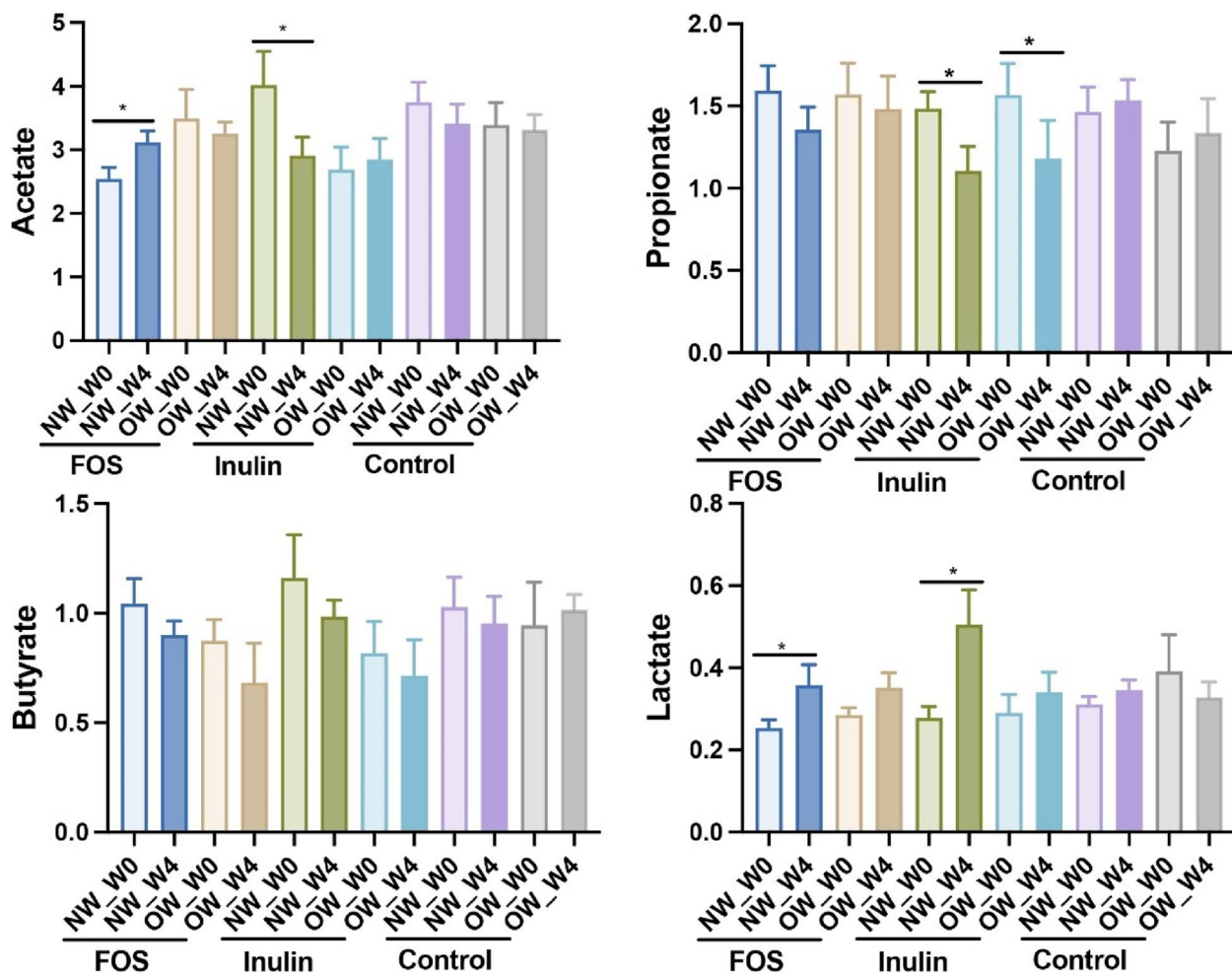


Fig. 5 Inulin intervention reduces propionate levels in both overweight/obese and healthy individuals. The levels of **A** acetate, **B** propionate, **C** butyrate, and **D** lactate. Data are expressed as mean $\pm$ SEM. * $p < 0.05$

insulin sensitivity and glycemic control. In contrast, FOS is rapidly fermented in the proximal colon, potentially resulting in a different SCFA profile less impactful on glycemic regulation [39]. Secondly, although we did not observe increase in butyrate after inulin intervention, possibly because of the short intervention, we did find that inulin robustly promotes bacterial function involved in Folate biosynthesis and Glutathione metabolism that exerts antioxidative stress effects. Thirdly, the observed HCY reduction by both inulin and FOS suggests a common mechanism likely involving enhanced anti-inflammatory effects, while inulin's additional glycemic benefits may be driven by more pronounced butyrate-mediated metabolic effects or modulation of host glucose signaling pathways [16, 40].

In this study, we found inulin intervention reduced propionate levels in both overweight/obese and healthy individuals. Interestingly, propionate has been considered a

health-promoting microbial metabolite [41, 42]. Animal studies have suggested that propionate exerts anti-obesity and anti-diabetes effects by promoting peptide YY (PYY) and glucagon-like peptide 1 (GLP-1) secretion and reducing inflammatory cytokines [43, 44]. On the contrary, other studies have yield contradictory findings. A study conducted by Tirosh et al. demonstrated that propionate triggers sympathetic nervous system and facilitates glycogenolysis and hyperglycemia in rodents by inducing glucagon and fatty acid-binding protein 4 (FABP4) [45]. Furthermore, they conducted a RCT and verified that propionate increases levels of plasma glucagon and FABP4 and induces sympathetic activation, leading to increased blood glucose and insulin resistance [45]. This finding was further substantiated by another RCT, highlighting propionate to be potentially detrimental to metabolic health [46]. More importantly, a recent meta-analysis including 44 human studies investigated the

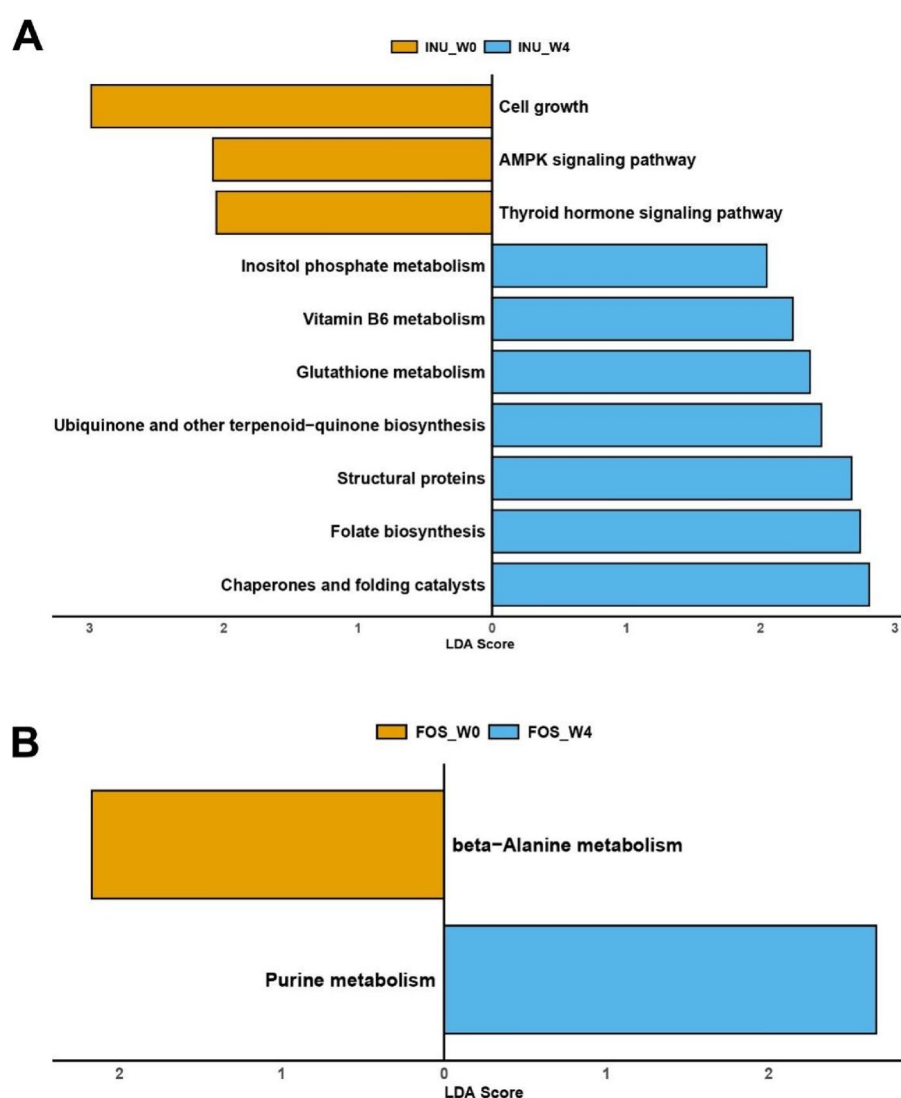


Fig. 6 Functional profiling of gut microbiota. KEGG pathways that were significantly upregulated after **A** inulin intervention and **B** FOS intervention

effects of propionate on glycemic control in humans, it was concluded that propionate has no effects on blood glucose and insulin level in humans [47]. Therefore, further high-quality clinical trials with a large sample size are required to determine the effects of propionate on glycemic metabolism. More importantly, these divergent findings may reflect differences in host metabolic status, gut microbiota composition, and dietary context. Future research is warranted to delineate the conditions under which propionate exerts beneficial versus neutral or adverse metabolic effects.

In this study, we have uncovered that the reduction in the abundance of *Ruminococcus* may mediate the beneficial effects of inulin on glycemic metabolism. Due to the technical limitation of 16 s rRNA sequencing whose

resolution is restricted to the genus level, we could not determine *Ruminococcus* at the species or strain level. Based on previous studies reporting the association between *Ruminococcus* and human diseases, we speculate that the *Ruminococcus gnavus* might be the one. Specifically, an elevated abundance of *Ruminococcus gnavus* (*R. gnavus*) has consistently been observed in individuals with metabolic conditions such as obesity [48, 49], type 2 diabetes [50–52], gestational diabetes mellitus [53], coronary artery disease [54], and alcohol-related liver damage [55], as well as gastrointestinal diseases, such as inflammatory bowel disease including Crohn's disease [56–58] and ulcerative colitis [59–62], irritable bowel syndrome [63, 64] and colorectal cancer [65]. These connections imply that *R. gnavus* may have a detrimental impact on

host metabolic health, potentially influencing the positive effects of inulin on glucose metabolism. It has been reported that *R. gnavus* harbors glucorhamnan on the cell surface which can induce production of proinflammatory cytokines [66]. This might lead to chronic systematic inflammation and impaired insulin sensitivity and metabolic disturbance. Moreover, *R. gnavus* is involved in production of secondary bile acids 3-dehydrocolate (3-dehydro-CA) and isocholate (Iso-CA), which are associated with intestinal inflammation [67]. An animal study revealed that supplementation of *R. gnavus* impedes body weight loss and induces liver damage in a high-fat diet-induced obese mouse model that treated with a low-fat diet after the establishment of obesity [68]. The mechanistic role of *R. gnavus* in host's glucose metabolism requires further investigation and targeted interventions aimed at *R. gnavus* might prove more effective. While the reduction of Ruminococcus observed in our study aligns with previous findings linking certain strains to pro-inflammatory states, it is important to note that Ruminococcus is a diverse genus with both pathogenic and commensal strains. Further studies using strain-resolved metagenomics or culture-based methods are needed to clarify the specific roles of individual strains in host metabolism.

Our study provides novel insights into the potential mechanisms underlying the differential glycemic effects of inulin and FOS in overweight and obese individuals. By leveraging PICRUSt analysis, we identified specific microbial pathways that could explain the distinct impacts of these prebiotics on host metabolism. One of the most striking findings was the upregulation of several metabolic pathways following inulin supplementation, including Folate biosynthesis, Glutathione metabolism, and Inositol phosphate metabolism. These pathways have been associated with improvements in insulin sensitivity and reduced oxidative stress, which may contribute to the observed reduction in fasting insulin levels in the inulin group. Folate and vitamin B6 metabolism influence one-carbon metabolism and homocysteine regulation, both of which are critical for optimal insulin function [69, 70]. Moreover, glutathione metabolism helps to counteract oxidative damage, which is known to contribute to insulin resistance in metabolic disorders [71]. Inositol phosphate metabolism has been linked to enhanced insulin signaling and glucose uptake [72]. The upregulation of purine metabolism following FOS supplementation was an interesting contrast. Purine metabolism is linked to the production of uric acid, a molecule that has been implicated in insulin resistance and metabolic dysfunction [73]. This suggests that FOS may exert a less favorable impact on insulin sensitivity compared to inulin, potentially explaining the differential effects on

fasting insulin levels observed between the two interventions. Future studies utilizing more advanced techniques, such as metagenomics and metabolomics, are warranted to further elucidate the precise microbial functions and metabolites responsible for the observed differences in glycemic responses. Additionally, the interplay between microbial metabolites and host signaling pathways should be explored in greater depth to confirm the causal relationships underlying these effects. Given the predictive nature of PICRUSt analysis, future studies incorporating targeted metabolomics and host transcriptomics will be essential to validate the identified pathways, such as folate, glutathione, and inositol phosphate metabolism, and to elucidate the mechanistic interplay between microbial function and host metabolic responses.

Limitation

First, although we observed beneficial effects of inulin on glycemic metabolism after a 4-week intervention, this duration is insufficient to evaluate long-term effects on metabolic parameters and gut microbiota. Future clinical trials with extended intervention periods are needed to assess sustainability and long-term outcomes. Second, a limitation of our study is the use of 16S rRNA sequencing and PICRUSt analysis, which provide only taxonomic and predicted functional data. Metagenomics and metabolomics would offer more detailed and direct insights into the microbial species and metabolic pathways involved in the observed metabolic changes.

Conclusions

These findings highlight the importance of tailored prebiotic interventions, to achieve precise modulation, which involves selecting specific types of prebiotics based on individual metabolic profiles and therapeutic goals. Practically, inulin may be more suitable for managing glycemic dysregulation in overweight or obese individuals, while FOS may be considered for HCY reduction in individuals with normal glycemic status. Such targeted use of prebiotics could complement existing dietary and pharmacologic strategies in personalized metabolic care.

Abbreviations

BMI	Body mass index
DBP	Diastolic blood pressure
F	Female
FBG	Fasting blood glucose concentration
FOS	Fructooligosaccharides
HCY	Homocysteine
HDLC	High-density lipoprotein cholesterol
hs-CRP	High-sensitive C-reactive protein
INU	Inulin
LDLC	Low-density lipoprotein cholesterol
M	Male
NW	Normal weight
OGTT	Oral glucose tolerance test
OW	Overweight/obese

SBP Systolic blood pressure
TC Total cholesterol
TG Triglycerides
UA Uric acid

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12916-025-04189-6>.

Additional file 1. Figure S1-S3. Fig S1-The effects of placebo on glycemic metabolism. Fig S2-The effects of placebo on gut microbiota composition. Fig S3-The effects of FOS on gut microbiota composition.

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Authors' contributions

HZ, MC, JL, FL and YL: designed the research; YL, MC, JL, FL, SW, GW, XC, YL, JJ and HS: conducted the research; YL, JL and MC: collected the data; JL, FL, ZL and AW: analyzed the data and performed the statistical analysis; JL: wrote the manuscript; HZ, ZL and YW revised the manuscript; All authors: read and approved the final version of the manuscript.

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Data availability

Due to ethical and privacy constraints, and in accordance with the limits of participant consent, the de-identified individual participant data from this trial are not publicly available. All data generated in this study are included in this article and supplementary information or are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The trial was approved by the Chinese Ethics Committee of Registering Clinical Trials (No. ChiECRCT-20170006); the registration number: ChiCTR-IOR-17010574. Written informed consents were obtained from the study participants.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Microbiome Medicine Center, Department of Laboratory Medicine, Zhujiang Hospital, Southern Medical University, Guangzhou, Guangdong, China.

²Department of Food and Nutritional Sciences, School of Chemistry, Food and Pharmacy, University of Reading, Whiteknights, Reading RG6 6AP, UK.

³Fun-Poo Biotech, Shenzhen, Guangdong, China. ⁴Quantum Hi-Tech Biological Co., Ltd, Jiangmen, Guangdong, China. ⁵State Key Laboratory of Organ Failure Research, Southern Medical University, Guangzhou, Guangdong, China.

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