European Journal of Medical Research

RESEARCH

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Effects of inulin on intestinal flora and metabolism-related indicators in obese polycystic ovary syndrome patients

Xiaorong Li[1,2,5†], Bo Jiang[2,3,5†], Ting Gao[6], Yan Nian[1], Xing Bai[3,5], Jiawen Zhong[2,3,5], Ling Qin[5], Zhengzheng Gao[4], Hao Wang[4] and Xiaohong Ma[1*]

Abstract

Context Polycystic ovary syndrome (PCOS), a common endocrine disorder in women of reproductive age, is closely associated with chronic low-grade inflammation and metabolic disturbances. In PCOS mice, dietary inulin has been demonstrated to regulate intestinal flora and inflammation. However, the efficacy of dietary inulin in clinical PCOS remains unclear.

Objective The intestinal flora and related metabolic indexes of obese patients with polycystic ovary syndrome (PCOS) after 3 months of inulin treatment were analyzed.

Setting and design To analyze the intestinal flora and related metabolic indexes in healthy controls and obese patients with polycystic ovary syndrome after 3 months of inulin treatment.

Results The results showed that dietary inulin improved sex hormone disorders, reduced BMI and WHR levels in obese women with PCOS. In addition, the inulin intervention reduced plasma TNF-α, IL-1β, IL-6, and MCP-1levels. Inulin intervention increased the abundance of *Actinobacteria*, *Fusobacteria, Lachnospira,* and *Bifidobacterium*, as well as decreased the ratio of *F/B* and the abundance of *proteobacteria*, *Sutterella,* and *Enterobacter*. Correlation analyses showed a strong relationship among plasma inflammatory factors, sex steroid hormones, and the intestinal flora of patients.

Conclusions Dietary inulin may improve obese PCOS women disease through the gut flora–inflammation–steroid hormone pathway.

The clinical trial registration number: ChiCTR-IOR-17012281.

Keywords Inulin, Polycystic ovary syndrome (PCOS), Obesity, Gut microbiota, Inflammatory

†Xiaorong Li and Bo Jiang contributed equally to this work and are listed as the first authors.

*Correspondence:
Xiaohong Ma
2947484391@qq.com
Full list of author information is available at the end of the article

Introduction

Polycystic ovary syndrome (PCOS), a common endocrine disorder, is one of the most important causes of infertility in women of childbearing age [1], with a prevalence of approximately 18% (17.8 ± 2.8%) [2], seriously affecting female reproductive, metabolic, and psychological health. The exact pathogenesis of polycystic ovary syndrome is poorly understood, with the main pathological basis as an imbalance in hormone levels with elevated androgen and/or insulin levels, and a chronic low-grade inflammatory response.

Studies have continuously reported that intestinal flora plays a key role in the development of PCOS. Significant changes in intestinal flora diversity and flora fractions have been reported in mice with PCOS or rodent models [3]. Mice developed insulin resistance and ovarian polycystic changes after gavage of feces from PCOS patients [4]. *Bifidobacterium lactis V9* can reduce androgen level in patients with PCOS by modulating the gut–brain axis [5]. Lipopolysaccharide (LPS) released by certain bacteria in the gut translocates into the circulation, leading to insulin resistance and the apoptosis of ovarian granulosa cells [6]. Intestinal flora can cause menstrual disorders and insulin resistance by altering intestinal permeability [7]. Overall, insulin resistance and hyperandrogenemia in PCOS are critically influenced by the gut microbiota.

As a chronic inflammatory disease, the occurrence and development of chronic inflammation of PCOS is closely related to intestinal dysbiosis [8, 9]. It is reported that Bacteroides vulgatus was markedly elevated in the gut microbiota of individuals with PCOS, modifying the gut microbiota may be of value for the treatment of PCOS [4]. It has been reported that gut microbiota-mediated priming/activation of neutrophils has been shown to increase the number of activated/aged neutrophils in the circulation, which secrete pro-inflammatory cytokines and granule proteases that damage tissues and exacerbate disease [10]. It has been widely demonstrated by many researchers that microbiota composition changes and dysbiosis occurs in PCOS animal models and women with PCOS [11].Therefore, how to improve the dysbiosis of intestinal flora in PCOS patients has become the key to treating PCOS.

Probiotics have been strongly demonstrated to show pleiotropic benefits consisting of regulating intestinal flora and suppressing the inflammation, improving glycolipid metabolism [12], enhancing immunity [13], enhancing cognitive function [14], enhancing anti-cancer efficacy, and reducing side effects of chemotherapy drugs [15], and antioxidant damage [16]. As a kind of dietary fiber, inulin has been widely used in food supplementation with properties such as regulating intestinal microbiota, influencing lipid metabolism, and anti-inflammatory and antioxidant properties [17, 18]. Our previous studies had also shown that inulin can improve inflammation and intestinal flora diversity in mice with letrozole-induced PCOS [19]. However, whether this phenomenon still remains valid for patients with PCOS has not been illustrated.

This study aims to investigate the potential value of inulin in the treatment of PCOS by altering the gut microbiota, which may be a new therapy for the control of clinical PCOS.

Materials and methods

Ethics statement

This observational prospective study was carried out at the Reproductive Center of the General Hospital of Ningxia Medical University from August 2017 to August 2020. It was approved by the medical ethics committee of General Hospital of Ningxia Medical University (ethics number: KYLL-2016-017) and conducted according to the principles expressed in the Declaration of Helsinki. The written informed consent was obtained from all enrolled cases and all data for research analyses were anonymized.

Inclusion criteria

(1) Patients who meet the diagnostic criteria for PCOS in the 2003 Rotterdam Consensus Statement [20]: ① Ovulation is sparse or non-ovulation; ② Clinical or biochemical evidence of Hyperandrogenemia; ③ Polycystic changes of the ovary. Two of the above three items can be diagnosed.

(2) Someone who can understand the purpose of the study, and willing to cooperate with the experimenter.

Exclusion criteria

(1) Patients with a combination of endometriosis, premature ovarian failure, ovarian resistance, hyperprolactinemia, ovarian tumors, or other reproductive disorders that are not diagnostic criteria for PCOS;

(2) Patients with uterine malformations or severe organic endometrial lesions and a previous history of pelvic tuberculosis;

(3) Patients with severe combined cardiovascular, cerebrovascular, hepatic, renal and hematopoietic diseases;

(4) Suffers from hypertension, abnormal glycolipid regulation, and other endocrine diseases;

(5) Hyperandrogenemia caused by other possible causes;

(6) People who smoke, drink alcohol, and are allergic to dietary fiber inulin;

(7) Furthermore, patients with a recent history of impact on gut flora were excluded, including those undergoing a weight loss lifestyle, those who had

undergone intensive exercise training within the previous four weeks, and those who had used antibiotics, micro-ecological modulators, hormones, and insulin sensitizers within three months.

Human subjects

Fifty-Five overweight women were enrolled trough public announcement in the Reproductive Center of the General Hospital of Ningxia Medical University from August 2017 to August 2020. The selection criteria are described above. Subjects ($n=55$) were divided into 3 groups: obese PCOS patients (FDB group, $n=19$), obese control group (NFD group, $n=16$), and non-obese control group (NSD group, $n=20$). After the intervention, the 13 patients in the FDB group who strictly adhered to the intervention criteria were renamed to the FDA group. According to the regulations of World Health Organization [21], obesity is defined as BMI ≥ 25 kg/m^2, and non-obesity is defined as BMI < 25 kg/m^2. This study was approved by the ethical committee of General Hospital of Ningxia Medical University (2016-017) and signed the informed consent form with the subjects after ensuring their rights, interests, and safety. The clinical trial was registered with the Chinese Clinical Trials Registry, registration account: chiCTR-TRC-17012281.

Research methods

Intervention

A uniformly trained reproductive endocrinology professional promoted and disseminated health information to all subjects, took fasting blood, and fresh stool samples from all subjects for the first time, explained to the intervention subjects (obese PCOS group) the purpose of the experiment, the duration of the intervention, and precautions to be taken during the intervention, and started the inulin intervention for 3 months. The control group was given about 150 ml of warm water every morning on an empty stomach. The subjects were instructed to take one bar (15 g, inulin produced by Fengning Ping a Hi-Tech Industry Co., Ltd.) every morning and pour it into about 150 ml warm water and drink it on an empty stomach.

After 3 months of routine administration, fasting blood and stool specimens were retained from 13 patients who had taken inulin strictly in accordance with the requirements of this study.

Collection of basic indicators

The height (cm) and weight (kg) were measured after fasting defecation and urination in the morning. A circle around the upper border of the pubic bone to the midpoint of the lower rib cage on both sides is defined as waist circumference (cm) and a circle around the most prominent point of the hip is defined as hip circumference (cm). Body Mass Index (BMI = weight/height^2 (kg/m^2)) and Waist-to-Hip Ratio (WHR = waist/hip circumference) was calculated.

Detection of plasma lipid and glucose metabolism indicators

All subjects were collected from superficial vein (median cubital vein) blood of the forearm after 10–12 h of overnight fasting and water ban. Before starting the dietary protocol participants came to the Reproductive Center of the General Hospital of Ningxia Medical University for the basal measurements. Samples for lipid metabolism and glucose metabolism tests were submitted to the hospital's blood analysis department for testing. The plasma fasting aspartate transaminase (AST), alanine aminotransferase (ALT), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine (CRE), uric acid (UA), and fasting plasma glucose (FPG) levels were separately measured using a fully automated rapid test biochemistry analyzer (SIEMENS Germany). All test kits are purchased from SIEMENS. The plasma C-reactive protein (CRP) and Fasting insulin (FINS) were measured with immunoassay. Glycosylated Hemoglobin (GHb) was measured using a D-10 high-resolution glycosylated hemoglobin meter (Bio-RAD, USA).

Oral glucose tolerance test (OGTT): After collecting the fasting forearm median cubital vein blood mentioned above, 75 g anhydrous glucose was mixed with 300 ml sugar-free pure water and asked the subjects to take it all within 5 min. The same blood collector 2 h later was asked to draw the patient's forearm median cubital vein blood again and immediately sent to the laboratory for 2 h of glucose detection.

Insulin beta-cell function index (HMOA-β) and insulin resistance index (HOMA-IR) were calculated as HOMA-β = 20 × FINS (mU/L)/(FPG (mmol/L)-3.5) and HOMA-IR = FPG (mmol/L) × FINS (mU/L)/22.5) respectively.

Determination of plasma sex steroid hormones

All participants had 10 mL of blood collected from a superficial forearm vein (median cubital vein) at 8 a.m. on days 3–5 of the menstrual cycle, for further detection of luteotropic hormone (LH), follicle-stimulating hormone (FSH), testosterone (T), progesterone (P), prolactin (PRL), Anti-mullerian hormone (AMH), and Estradiol$_2$ (E_2). The complete set of hormones was detected by chemiluminescence immunoassay (Atellica IM 1600, SIEMENS, Germany) in the laboratory department, test kits were purchased from SIEMENS.

Determination of plasma inflammatory indicators

The preparation work is the same as the above glucose and lipid metabolism. Plasma inflammatory factors including tumor necrosis factor-α, interleukin (IL)-1β, IL-6, and IL-10 were measured by using enzyme linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (Shanghai Jianglai Biotechnology Co., Ltd., China). The monocyte chemoattractant protein-1 (MCP-1) was measured by using ELISA kits (Fankewei Biology, Shanghai, China). The sensitivities of the assays were 1.0, 0.1, 1.0, 0.1, and 0.1 pg/mL for TNF-α, IL-1β, IL-6, IL-10, and MCP-1, respectively. Each sample was tested in triplicate.

Gut microbiota sequencing analysis

The subjects were instructed to discharge the fecal samples into a clean container, and immediately after defecation, fresh and clean feces (about 5–10 g) were collected in an aseptic spoon and put in 4 aseptic airtight containers, and immediately stored at − 80 ℃. All fecal samples were used to measure the sequence of gut microbiota by 16S rRNA in Beijing Novogene Co., Ltd., for detection.

Fecal genomic DNA was extracted by the SDS method, and the purity and concentration of DNA were detected by agarose gel electrophoresis. The sample DNA was diluted to 1 ng/μL, the high-quality DNA purification template was used as polymerase chain reaction template, and barcode with high specificity was selected as a primer. The PCR in the reaction program was amplified by Phusion ®High-FidelityPCR Master Mix with GC Buffer produced by New England Biology Co., Ltd. (NEB), which was the characteristics of high fidelity and high performance and minimizes the error of experimental data. The reaction procedure was carried out in accordance with Bio-rad Bole PCR instrument T100. Then the amplified products were detected using agarose gel electrophoresis. DNA fragments were purified with the GeneJET gel recovery kit of Thermo Scientific company. Libraries were constructed using the Ion Plus Fragment Library Kit 48 rxns (Thermofisher) and sequenced using Ion S5^{TM}XL (Thermofisher) after passing Qubit quantification and library testing.

Statistical analysis

Clinical data were analyzed using SPSS 25.0 (IBM Corp., NY, USA) and inflammation data were analyzed and plotted using GraphPad Prism software 8.0 (GraphPad Software Inc., CA, USA). All experimental data are expressed as mean ± standard deviation of at least three independent experiments. Data were determined by one-way analysis of variance to compare the mean values of variables among the groups. Tukey's post hoc test was used to identify the significance of the pairwise comparison of mean values among the groups. Comparisons between the two groups were made using the rank sum test. Spearman correlation analysis was also performed to understand the relationship between gut microbes and inflammatory and metabolic markers. $P<0.05$ was considered statistically significant.

Result

Analysis of changes in clinical indicators with the inulin treatment

BMI, WHR, TG, UA, FIN, and HOMA-IR levels were significantly elevated in the NFD group compared to the NSD group ($P=0$, 0, 0.034, 0, 0.006,0.003), while sex hormone levels were decreased except FSH and TSTO, and only significant differences in LH/FSH and AMH ($P=0.047$, 0.003). In the FDB group compared with the NFD group, all indicators of glycolipid metabolism increased except for TG, TC, HDL-C, and CRE levels, with a significant difference in the increase in ALT and UA levels and the decrease in HDL-C levels ($P=0.024$,0.002,0.011). In addition, sex hormones LH, LH/FSH, TSTO, PRL, AMH, and E2 levels were increased and FSH levels were decreased, with significant differences in the changes of LH, LH/FSH, TSTO, AMH, E2 and FSH levels ($P=0.02$,0,0.033,0.042). BMI was also significantly higher. After the inulin intervention, AST, TC, LDL-C, HDL-C, CRE, UA, FPG, FIN, HOMA-IR, LH, LH/FSH, TSTO, P, PRL, AMH, BMI, and WHR levels in plasma were all decreased in the FDA group compared to those in the FDB group, with a statistically significant difference in the decrease in BMI ($P=0.046$), while FSH and E2 levels increased. These two indicators showed no significant difference between the FDA and FDB groups (Table 1).

Differences in abundance and diversity of intestinal flora among groups

We found that when the number of sequences increased to 5479, the curve flattened out, indicating that the amount of sequencing data was reasonable (Fig. 1A). The results of the β-diversity analysis suggested that the abundance and diversity of the gut microbiota were reduced in the FDA group compared to the FDB, NFD, and NSD groups ($P=0.0222$, 0.0021, 0.0012) (Fig. 1B). In 68 fecal samples, 407 intestinal microorganisms were found in all groups, while 358, 77, 72, and 11 intestinal microorganisms were found in the NSD, NFD, FDB, and FDA groups alone, respectively. There was significantly more gut microbial species in the NSD group than in the NFD and FDB groups, while the intestinal microbial species decreased further after the inulin intervention compared to the FDB group (Fig. 1C).

Table 1 Basic parameters, metabolic indexes, and hormone levels of patients in each group

Index	Normal Group		PCOS Group		*P* value			
	NSD (*n* = 20)	NFD (*n* = 16)	FDB (*n* = 19)	FDA (*n* = 13)	*a*	*b*	*c*	*d*
Basic parameters								
Age	27.55 ± 3.79	28.81 ± 2.79	27.26 ± 2.64	26.38 ± 3.66	0.274	0.101	0.317	0.07
BMI (kg/m^2)	20.49 ± 1.9	26.75 ± 1.57	28.09 ± 2.82	27.23 ± 3.77	0*	0.088	0.046*	0.792
WHR	0.84 ± 0.04	0.89 ± 0.03	0.88 ± 0.04	0.87 ± 0.05	0*	0.765	0.38	0.707
Lipid metabolism index								
AST (U/L)	18.39 ± 4.85	20.91 ± 14.55	23.72 ± 9.33	22.03 ± 8.95	0.482	0.495	0.463	0.282
ALT (U/L)	15.32 ± 8.2	16.2 ± 6.48	25.81 ± 13.94	26.08 ± 15.2	0.735	0.024*	0.859	0.058
TG (mmol/L)	0.98 ± 0.57	1.42 ± 0.62	1.37 ± 0.48	1.61 ± 0.74	0.034*	0.782	0.249	0.483
TC (mmol/L)	4.03 ± 0.54	4.23 ± 0.45	4.04 ± 0.55	3.95 ± 0.7	0.248	0.296	0.625	0.056
HDL-C (mmol/L)	1.39 ± 0.23	1.29 ± 0.27	1.06 ± 0.22	0.96 ± 0.19	0.22	0.011*	0.889	0.001*
LDL-C (mmol/L)	1.85 ± 0.42	2.02 ± 0.42	2.06 ± 0.36	1.97 ± 0.54	0.241	0.738	0.142	0.826
CRE (umol/L)	51.31 ± 7.02	48.78 ± 8.39	47.96 ± 7.5	42.81 ± 13.34	0.33	0.763	0.552	0.211
UA (umol/L)	251.1 ± 42.59	318.69 ± 51.3	389.79 ± 69.29	373.15 ± 72.12	0*	0.002*	0.311	0.056
Sex hormone index								
LH (mIU/L)	4.13 ± 1.64	3.27 ± 1.99	5.78 ± 2.96	5.67 ± 3.38	0.162	0.02*	0.552	0.054
FSH (mIU/L)	6.34 ± 1.37	7.16 ± 2.1	5.68 ± 2.05	5.73 ± 1.69	0.167	0.042*	0.576	0.028*
LH/FSH	0.68 ± 0.34	0.47 ± 0.25	1.07 ± 0.46	1.04 ± 0.64	0.047*	0*	0.906	0.012*
T (ng/dL)	38.9 ± 11.81	41.3 ± 12.6	52.02 ± 15.56	51.99 ± 13.96	0.56	0.034	0.701	0.048*
P (ng/mL)	0.62 ± 0.23	0.62 ± 0.23	1.24 ± 2.05	0.63 ± 0.19	0.946	0.237	0.208	0.539
PRL (ng/mL)	11.25 ± 5.77	10.19 ± 5.1	11.12 ± 6.3	9.41 ± 5.05	0.581	0.644	0.152	0.565
AMH (ng/mL)	5.68 ± 2.36	3.24 ± 1.99	6.84 ± 3.16	5.17 ± 1.86	0.003*	0*	0.136	0.017*
E2 (pg/L)	39.18 ± 15.63	36.49 ± 13.85	48.79 ± 17.88	53.03 ± 14.19	0.594	0.033*	0.249	0.002*
Glucose metabolism index								
FPG (mmol/L)	4.75 ± 0.43	4.95 ± 0.59	5.01 ± 0.49	5.21 ± 0.52	0.246	0.751	0.1	0.254
FIN (mU/L)	9.51 ± 4.08	13.76 ± 4.62	17.77 ± 8.9	20.5 ± 10.23	0.006*	0.208	0.807	0.096
HOMA-β	141.77 ± 53.7	195.4 ± 79.95	265.68 ± 190.05	256.29 ± 152.63	0.071	0.19	0.422	0.369
HOMA-IR	2.01 ± 0.83	3.05 ± 1.15	3.97 ± 2.08	4.82 ± 2.67	0.003*	0.24	0.507	0.066
OGTT (mmol/L)	5.45 ± 0.89	6.21 ± 1.62	7.37 ± 3.48	6.77 ± 1.14	0.081	0.23	0.507	0.035*
GHb (mg/dL)	5.13 ± 0.23	5.22 ± 0.45	5.34 ± 0.67	5.28 ± 0.85	0.403	0.552	0.572	0.186

Data are expressed as the mean ± SEM. *$P \leq 0.05$ was considered significantly different. (a) NFD *vs.* NSD. (b) FDB *vs.* NFD. (c) FDA *vs.* FDB. (d) FDA *vs.* NFD. (*BMI* body mass index; *WHR* waist-to-hip ratio, *AST* aspartate transaminase, *ALT* alanine aminotransferase, *TG* triglyceride, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *CRE* creatinine, *UA* uric acid; LH; FSH; *T* testosterone, *P* progesterone, *PRL* prolactin, *AMH* anti-mullerian hormone, E_2 estradiol$_2$, *FPG* fasting plasma glucose, *FINS* fasting insulin, *HOMA-β* insulin beta-cell function index, *HOMA-IR* insulin resistance index, *OGTT* oral glucose tolerance test, *GHb* glycosylated hemoglobin)

Diversity of the overall composition of the gut microbiota

PCoA analysis showed that the community composition structure was similar between groups with no significant differences, indicating that inulin did not significantly improve the gut microbial community in obese PCOS patients (Fig. 2).

Analysis of intestinal microflora abundance changes and differential microflora

At the phylum level, *Firmicutes* and *Bacteroidetes* constituted the two dominant phylum in the four populations, followed by *Proteobacteria* and *Actinobacteria* in higher abundance, and the rest accounted for a low abundance (Fig. 3A, C). The predominant *Firmicutes* and *Bacteroidetes* showed no significant change among the diverse groups. The ratio of *Firmicutes* to *Bacteroidetes* (*F/B*) and the relative abundance of *proteobacteria* were significantly higher in the NFD and FDB groups than in the NSD group, and highest in the FDB group (Fig S1A, B). After inulin intervention, the ratio of *F/B* and the relative abundance of *proteobacteria* were significantly lower in the FDA group than in the FDB group. The relative abundances of *Actinobacteria* and *Fusobacteria* were significantly lower in the NFD and FDB groups than in the NSD group. While after inulin intervention, the relative abundance of *Actinobacteria* and *Fusobacteria* in the

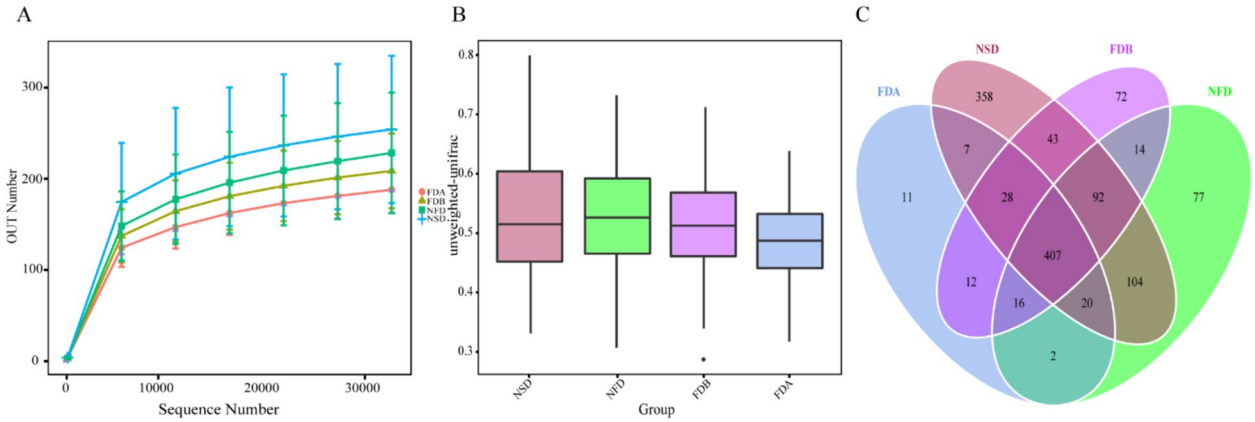

Fig. 1 Sequencing data plausibility analysis and species diversity analysis. **A** Rarefaction curve, reflecting the plausibility of the sequencing data; **B** Box plot of β-diversity, reflecting species differences among groups; **C** Venn diagram, indicating the number of unique and common OTUs in the different groups

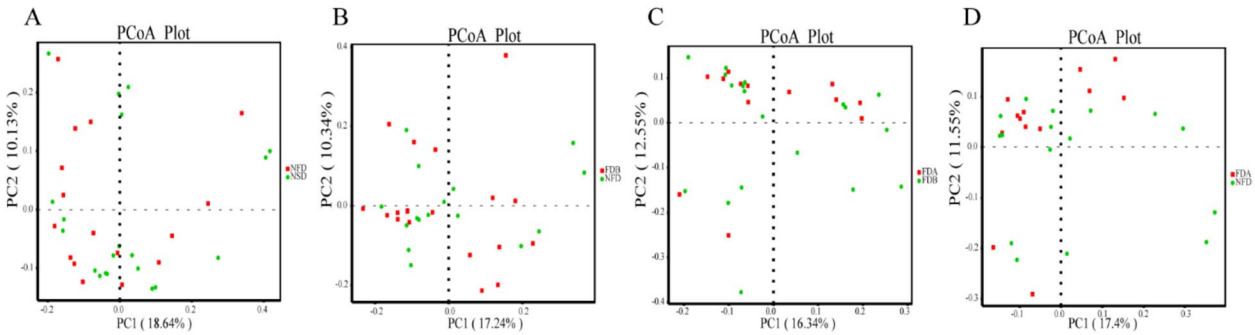

Fig. 2 PcoA analysis showing the difference in terms of species in fecal samples. Beta diversity was on Unweighted-Unifrac. **A**: NFD vs. NSD; **B**: FDB vs. NFD; **C**: FDA vs. FDB; **D**: FDA vs. NFD

FDA group was increased significantly compared to the FDB group (Fig S1C, D). Collectively, inulin had important effects on the ratio of *Firmicutes/Bacteroidetes, as well as* the abundance of *proteobacteria*, *Actinobacteria,* and *Fusobacteria* in obese PCOS patients.

At the genus level, *Bacteroidetes* and *Faecalibacterium* were the most widely distributed genera in the intestinal tract of patients in each group. The overall relative abundance of intestinal genera in the NFD group was significantly lower than that in the NSD group, with a statistically significant decrease in the abundance of *unidentified_Ruminococcaceae* ($P=0.02$). In addition, the relative abundance of *Roseburia*, *Dialister*, *Blautia*, *Agathobacter*, *unidentified_Lachnospiraceae*, *Parabacteroides*, *Lactobacillus*, *Streptococcus, Intestinibacter*, *Romboutsia*, *Fusicatenibacter*, *Dorea,* and some other conditionally pathogenic bacteria had higher relative abundance in the NFD group than in the NSD group, with *Megamonas, Allisonella,* and *Howardella* having significantly higher relative abundance ($P=0.041$, 0.002, 0.046) (Fig. 3D, E). The relative abundances of *Bacteroidetes*, *Fusobacterium*, *unidentified_Ruminococcaceae*, and *Lachnospira* were lower in the FDB group than in the NSD and NFD groups, while the relative abundances of these genera were increased in the FDA group compared to the FDB group after inulin intervention, with the relative abundance of *Lachnospira* being significantly higher in the FDA group than in the FDB group ($P=0.04$) (Fig. 3G). Besides, the relative abundance of *Sutterella*, *Lactobacillus, Lactococcus,* and *Enterobacter* was significantly lower in the FDA group than in the FDB group ($P=0.047$, 0.021, 0.002, 0.022) (Fig. 3E). Interestingly, the opposite trend was observed for the genera *Megamonas*, *Enterococcus*, *Blautia*, *unidentified_Lachnospiraceae*, *Fusicatenibacter*, and *unidentified_Erysipelotrichaceae*, of which the relative abundances were higher in the FDB group than in the NSD and NFD groups, and decreased in the FDA group after inulin intervention, but without statistically significantly difference (Fig. 3D). In addition, we found that the relative abundance of *Lactococcus* was significantly higher in the FDB group than in the NFD group ($P=0.022$), while the relative abundance

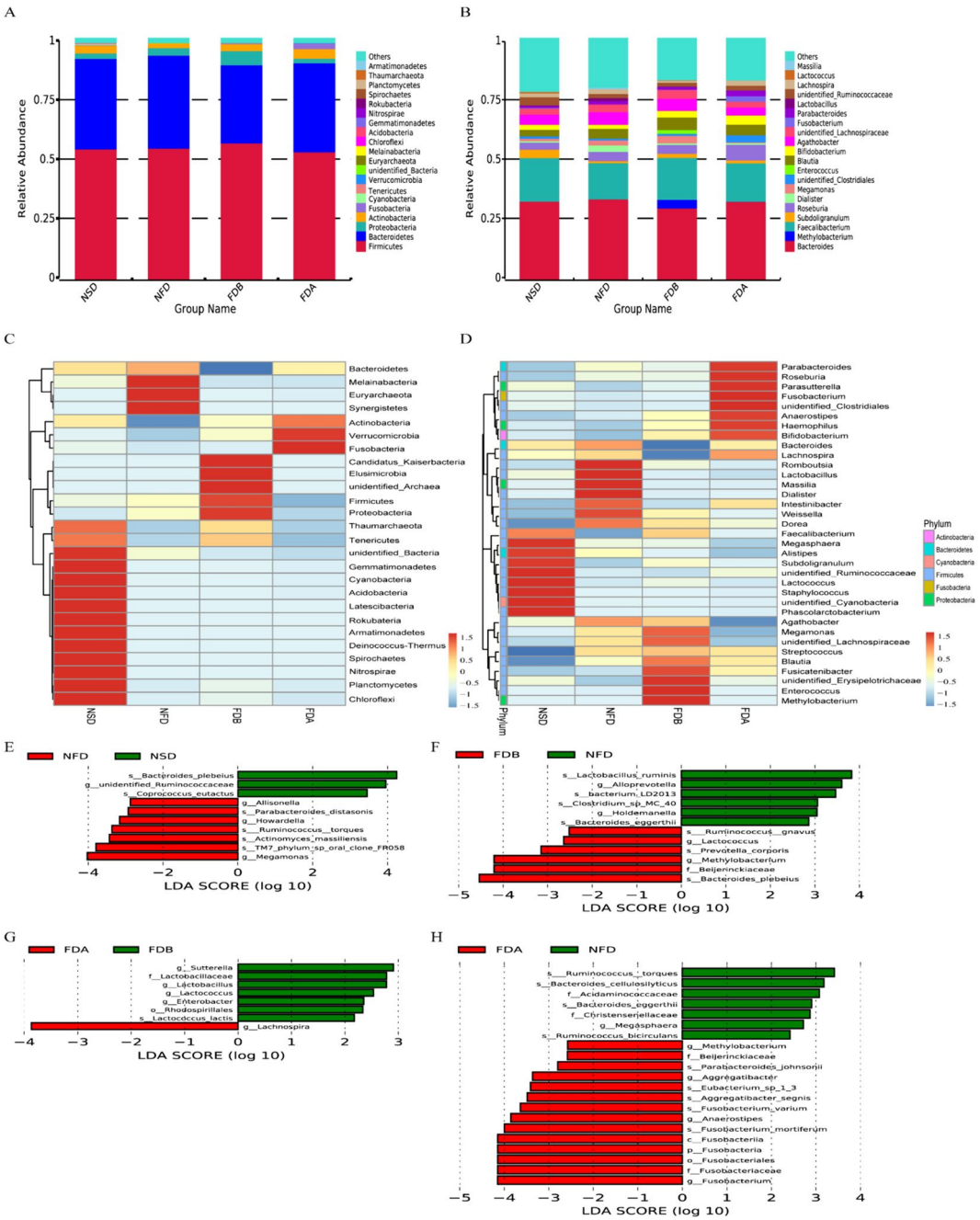

Fig. 3 Relative abundance of microbial species at the phylum and genus level in intestinal feces of different people and the biomarker of significant differences between groups. **A**, **B** Analysis of the relative abundance of intestinal microorganisms at the phylum and genus level; **C**, **D** Heat map of the relative abundance of gut microorganisms at the level of the top 35 phylum and genus at $P \leq 0.05$. **E–H** Analysis of Biomarkers with significant differences between groups based on LDA Effect Size. (**E**: NFD vs. NSD; **F**: FDB vs. NFD; **G**: FDA vs. FDB; **H**: FDA vs. NFD.)

of *Alloprevotella* and *Holdemanella* was significantly lower in the FDB group than in the NFD group ($P=0.023$, 0.043) (Fig. 3F). Surprisingly, the relative abundance of *Methylobacterium* was significantly higher in the FDB group than in the other four groups and was significantly different when compared to the NFD group ($P=0.036$) (Fig. 3B, F). Overall, dietary inulin dramatically changed the abnormal proportions of genus components in obesity PCOS by increasing the abundance of *Lachnospira*, and *Fusobacterium* as well as decreasing *Sutterella*, *Lactobacillus*, *Lactococcus*, and *Enterobacter*.

Changes in plasma inflammatory levels

Plasma levels of the pro-inflammatory factors TNF-α, IL-1β, IL-6, MCP-1, and the anti-inflammatory factor IL-10 were significantly higher in the NFD group compared to the NSD group ($P=0.0005$, 0.0008, 0.0045, 0.0361, <0.0001). Compared to the NFD group, plasma levels of the inflammatory factors TNF-α, IL-1β, IL-6 and MCP-1 in the FDB group were further increased ($P=0.0131$, 0.0222, 0.0182, 0.0348), while the level of IL-10 decreased. Excitingly, plasma TNF-α, IL-1β, IL-6, and MCP-1 levels were significantly lower in the FDA group after the inulin intervention than in the FDB group ($P=0.0034$, 0.0215, 0.0024, 0.0266), but there was no significant change in IL-10 level. The above further confirms that both obesity and PCOS disease were accompanied by an inflammatory state, which could be improved by dietary inulin (Fig. 4).

Correlation analysis

Due to the low abundance of some differential genus, only the top 40 genus in terms of abundance were selected for analysis among all differential genus. We found that *unidentified-Ruminococcaceae* abundance was negatively correlated with TG, UA, BMI, WHR, IL-1β, IL-6, and IL-10 levels, respectively ($P=0.043$, 0.025, 0.014, 0.001, 0.001, 0.006, 0.002), and positively correlated with HDL-C ($P=0.026$). *Megamonas* abundance was positively correlated with HDL-C levels and negatively correlated with HOMA-β and OGTT levels ($P=0.027$, 0.024, 0.039). *Lactococcus* abundance was positively correlated with TNF-α and IL-6 levels ($P=0.01$, 0.031). *Methylobacterium* abundance was positively correlated with TNF-α, IL-1β, IL-6, AST, ALT, UA, LH/FSH, and HOMA-β, respectively ($P=0.041$, 0.013, 0.028, 0.025, 0.009, 0, 0.037, 0.023) and negatively correlated with TC, HDL levels ($P=0.022$, 0.016). *Lactobacillus* abundance was negatively correlated with IL-10, TG, FPG ($P=0.033$, 0.024, 0.04). *Anaerostipes* abundance was significantly positively correlated with UA, E2, FIN, HOMA-IR, BMI, WHR levels, respectively ($P=0.031$, 0.005, 0.011, 0.013, 0.001, 0.005) and significantly negatively correlated with HDL-C ($P=0$). *Fusobaterium* abundance was significantly positively correlated with IL-β, CRP, AST, and WHR levels, respectively ($P=0.025$, 0.003, 0.014, 0.02).

In addition, we found that plasma TNF-α expression level was significantly and positively correlated with UA, T, E2, FIN, and HOMA-IR levels ($P=0$, 0, 0.01, 0.04,0.032) and negatively correlated with HDL

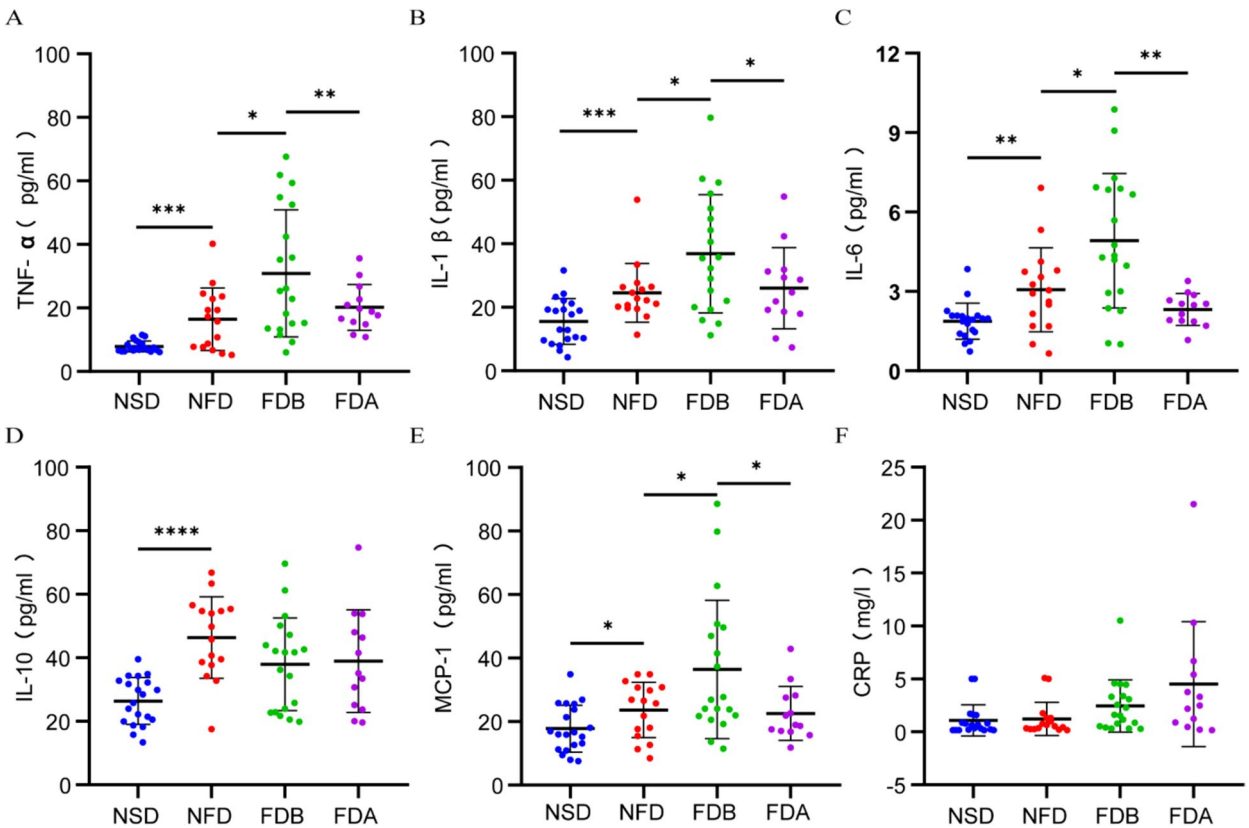

Fig. 4 Detection of plasma inflammatory factors levels in diverse groups. Data are expressed as mean ± SEM. *$P<0.05$, **$P<0.01$, ***$P<0.001$

($P=0.006$). Plasma IL-1β expression levels were significantly and positively correlated with plasma AST, ALT, FIN, and HOMA- IR. WHR levels were significantly positively correlated ($P=0.001$, 0.007, 0.002, 0.001, 0.001) and negatively correlated with HDL-C levels ($P=0.032$). Plasma IL-6, UA, T, and WHR levels were significantly positively correlated ($P=0.001$, 0.038, 0.037), negatively correlated with HDL ($P=0.04$). Plasma IL-10 expression level was significantly positively correlated with TG, TC, LDL-C, and BMI levels ($P=0.023$, 0.013, 0.047, 0.014). Plasma MCP-1 expression level was significantly positively correlated with ALT, UA, OGTT, GHb, and BMI levels ($P=0.012$, 0, 0.018, 0.011, 0.001) and negatively correlated with FSH ($P=0.018$). Plasma CRP expression levels were significantly positively correlated with AST, ALT, WBC, LH/FSH, P, FPG, FIN, HOMA-IR, OGTT, and BMI levels ($P=0.009$, 0.032, 0.001, 0.033, 0.032, 0.012, 0.001, 0, 0, 0.001). UA and BMI were positively associated with all inflammatory factors, while HDL levels were significantly negatively associated with all inflammatory factors(Fig. 5). Taken together, there were close correlations among gut bacteria, inflammation, sex steroid hormones, and clinical metabolic indicators.

Discussion

In the present study, we observed and analyzed the abnormal changes and correlation of clinical metabolic indexes, intestinal flora, and inflammatory factor levels in obese women with PCOS before and after inulin intervention to investigate the therapeutic effects and possible mechanisms of inulin in obese women with PCOS. We demonstrated that dietary inulin modulated steroid hormone homeostasis, and gut microbiota components and suppressed inflammation in obese women with PCOS. This provided a theoretical basis for the use of inulin as an inexpensive intervention for obese PCOS.

The development of PCOS as a chronic endocrine metabolic disorder is mainly characterized by the disruption of sex steroid hormones [22]. The main pathological feature of PCOS is hyperandrogenemia due to elevated serum testosterone (T) and luteinizing hormone (LH) levels [23]. Elevated LH levels drive the synthesis of sex steroid hormones (androgens and estrogens) by ovarian

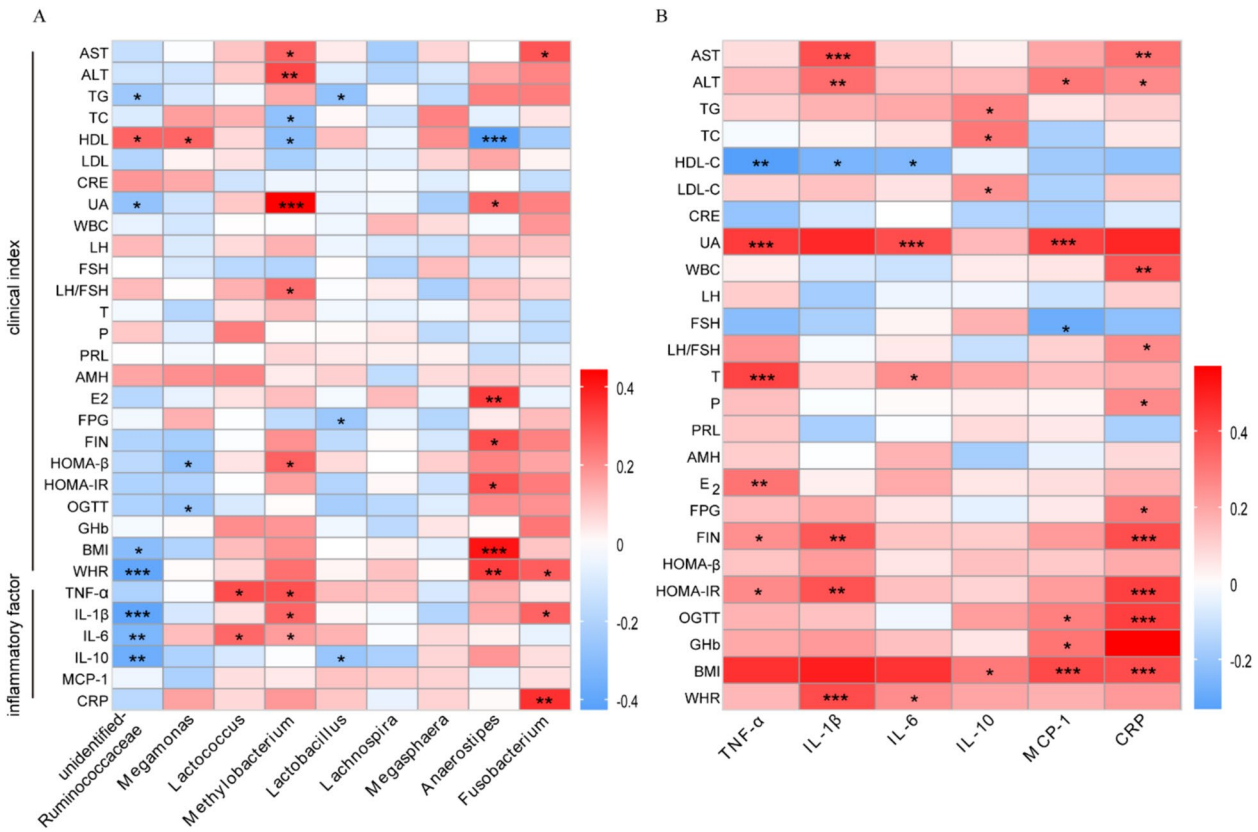

Fig. 5 Correlation analysis between the relative abundance of gut microbiota with plasma inflammatory factors and clinical indicators. **A** Heat map of correlation analysis of clinical indicators and inflammatory factors with differential gut microbial abundance; **B** Heat map of correlation analysis between inflammatory factors and clinical indicators. (*$P<0.05$, **$P<0.01$, ***$P<0.001$)

theca cells, further exacerbating hyperandrogenemia [24]. Elevations in T levels will also lead to impaired progesterone sensitivity in the inferior colliculus, with an increased GnRH pulse frequency, and a decrease in progesterone (P) levels [25]. Secondly, the ovaries of patients with PCOS mainly exhibit impaired follicular development, leading to an excessive accumulation of antral follicles and small sinus follicles, further manifested by decreased levels of folliculopoietin (FSH) expression and increased levels of anti-Müllerian hormone (AMH) [26]. Therefore, the LH/FSH ratio is considered to be a major biomarker for the diagnosis of PCOS disease [27, 28]. AMH levels are more sensitive than ultrasound sinus follicle count (AFC), which reflects antral and small sinus follicles (<2 mm) that are barely visible on ultrasound, and AMH levels may replace the more expensive and less accessible ultrasound in the diagnosis of PCOS [29]. In the present study, we found that plasma T, LH, AMH, E_2 levels, and LH/FSH ratio were significantly higher in obese women with PCOS compared to non-PCOS obese women, while FSH levels were significantly lower, and all indicators were significantly corrected after inulin intervention. Although there was no statistically significant difference, this does not negate the fact that dietary inulin improved steroid hormone homeostasis in obese PCOS patients, and the results may be more significant by increasing our sample size and the duration of the inulin intervention.

In addition, a large body of data suggests that patients with PCOS also often have dyslipidemia and insulin resistance, which may be caused by hyperandrogenemia [30, 31]. Studies have reported that higher levels of endogenous testosterone can raise LDL-C levels and lower HDL-C levels [32]. At the same time, high levels of androgens can cause increased insulin resistance, which leads to a decrease in insulin-mediated intramuscular glucose utilization and reduced insulin sensitivity, further exacerbating insulin resistance levels. These are consistent with our current findings. The alterations in plasma AST, TC, and LDL-C levels before and after the intervention suggested that dietary inulin mitigated lipid metabolism in obese women with PCOS, despite the increases in ALT, TG, and HDL-C levels. Besides, we found that dietary inulin could reduce plasma CRE and UA levels. Studies have demonstrated that UA can form NLRP3 inflammatory vesicles and release various pro-inflammatory factors that further impair insulin signaling, thereby mediating the development of insulin resistance (IR) and hyperandrogenemia and triggering ovarian ovulation disorders [33]. This indicates that dietary inulin is a safe probiotic supplement that does not cause toxic damage to liver and kidney function and has some protective effects. For clinical diagnosis and treatment in patients with polycystic ovary syndrome, we should pay attention to uric acid level change. FPG, FIN, and HOMA-IR levels were significantly higher in the obese population and obese PCOS population, which was consistent with previous studies [34, 35]. Although dietary inulin did not reduce plasma glucose and insulin resistance levels in obese women with PCOS, their fasting blood glucose did not fluctuate beyond normal values after the intervention. Excitingly, dietary inulin effectively reduced plasma OGTT levels in patients, suggesting that inulin improved insulin sensitivity.

Growing evidences have demonstrated that intestinal microbes and their metabolites are closely related to the occurrence and development of PCOS [36–38]. Studies have reported that compared with healthy people, patients with PCOS gut microbes beta diversity decreased, and beta diversity and high negative correlation between androgen hematic disease [39]. If we are to validate the existence of such disparities, we need to further broaden the geographical scope and increase the sample size of the included population. Consistent with previous findings that dietary supplementation with a single fermentable substrate can reduce indicators of fecal bacterial diversity in humans [40] and improve metabolic responses [41]. Our findings suggest that inulin does not increase overall gut microbial species richness in obese women with PCOS, but can significantly alter the composition of the gut microbial community.

The gut microbiota in healthy populations consists of two major phylum, *Firmicutes* and *Bacteroidetes*, while obese humans exhibit a higher *Firmicutes/Bacteroidetes* (*F/B*) ratio [42–44]; elevated *F/B* ratios are associated with a variety of diseases [45–47], and vary with human aging [48]. Our results also showed a consistent trend, but the increased ratio of *F/B* in obese PCOS patient was rectified by dietary inulin administration, including *Bacteroides* and *Megamonas* genus. *Proteobacteria* is a Gram-negative bacterium whose outer membrane is composed mainly of lipopolysaccharides (LPS), and a phylum that contains a variety of pathogenic bacteria including *Enterobacter*, *Salmonella*, *Vibrio cholera*, and *Helicobacter pylori*, with the elevated abundance of *Proteobacteria* in a variety of diseases [49–51]. However, *Actinobacteria* is often used in the research and development of antibiotics and has a crucial role in maintaining intestinal homeostasis. *Bifidobacterium* within the phylum *Actinobacteria* is widely used in the development of various pharmaceuticals and foods, showing beneficial effects in many pathological conditions [52]. Dietary fiber supplementation has been reported to significantly increase its abundance and reduce obesity [53–55]. It has also been reported that a water extract of Ganoderma lucidum mycelium (WEGL) can downregulate

the levels of *proteobacteria* in mice fed a high-fat diet, thereby achieving a reduction in body weight, inflammation, and insulin resistance [56]; an inulin intervention in an obese people was found to increase the abundance of *Actinobacteria* [57]. In the present study, we maintained consistent results that inulin intervention downregulates intestinal *proteobacteria* abundance and upregulated *Actinobacteria* abundance in obese PCOS women. Furthermore, at the genus level, we used the LEfSe method to compare the gut flora composition after the inulin intervention with that before the intervention and we found that inulin restored the gut ecological dysbiosis in PCOS by significantly upregulating the abundance of intestinal *Lachnospira* flora and downregulating the abundance of *Sutterella*, *Lactobacillus*, *Lactococcus*, and *Enterobacter* in the obese PCOS population. Surprisingly, the inulin intervention also significantly downregulated the abundance of *Lactobacillus* and *Lactococcus* in the intestine of obese PCOS patients. When the groups were analyzed together, the abundance of *Lactobacillus* was highest in the FDB group, while *Lactococcus* was the most abundant in the intestine of the healthy population. *Lactobacillus* is usually added to dairy products as a safe beneficial bacterium and its pathogenicity has rarely been reported. In combination with the lifestyle habits of the study subjects, contamination from dietary sources cannot be excluded. In contrast, the abundance of these two genera decreased further after the inulin intervention, perhaps as a result of the effects of prolonged supplementation with a single dietary fiber. Besides, we found that supplementation with dietary inulin upregulated the abundance of *Bifidobacterium*. The results imply that obese women with PCOS have varying degrees of gut flora disorders and that dietary inulin may have anti-obesity and improve PCOS by altering the ratio of *F/B* in the gut of obese women with PCOS and by altering the relative abundance of other specific bacterial species. Excitingly, in this study, we found that *Methylobacterium* was significantly enriched in the gut of obese women with PCOS, with a clear reduction in abundance after the inulin intervention. Regrettably, the post-intervention changes were not statistically significant compared to the pre-intervention, which may be related to the size of our sample. *Methylobacterium* is present in all corners of our living environment as conditionally pathogenic bacteria and are often contracted by immunocompromised individuals [58]. The abundance of this genus has been found to be significantly higher in patients with ulcerative colitis and constipating irritable bowel syndrome, but there is no clear indication that the abundance of this genus interacts with inflammation [59, 60]. It is not known whether upregulated *Methylobacterium* abundance in this study was associated with external infection or by endogenous infection of the intestine. Once it is clear that it is endogenously upregulated, *Methylobacterium* abundance may be a biomarker for the diagnosis of obese PCOS patients. However, we need to involve larger samples for validation and further studies to understand the role of individual components of the gut microbiota in its pathogenesis.

Numerous studies have reported a key role of chronic low-grade inflammation in the development of PCOS disease [35, 61, 62]. In our study, we found that obese people as well as those with PCOS had higher levels of inflammatory factor expression, especially in obese PCOS patients, further confirming the notion that obesity is a chronic inflammatory state [39, 63]. At the same time, we demonstrated that dietary inulin alleviated systemic inflammation by inhibiting pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, MCP-1), suggesting an anti-inflammatory effect of dietary inulin in PCOS. Lipopolysaccharide (LPS), a metabolite of the gut flora, can induce a chronic subclinical inflammatory process and obesity, leading to insulin resistance through activation of TLR4. A reduction in circulating SCFA may also play an important role in reducing insulin sensitivity and promoting the development of inflammation and obesity [36, 64]. Evidence from several studies suggests that probiotic supplementation reduces the level of LPS produced by intestinal pathogenic microorganisms and increases the level of short-chain fatty acids (SCFAs), decreasing intestinal permeability and reducing LPS translocation, further reducing the systemic inflammatory cascade [65, 66]. In addition, some studies have reported that impairment of intestinal tight junction proteins (e.g., ZO-1 and occludins) enhances intestinal permeability and is critical for LPS translocation [67, 68]. Probiotics have been shown to improve intestinal permeability and integrity by upregulating tight junction proteins (ZO-1 and occludins) to inhibit LPS translocation [69]. LPS and SCFA levels, as well as changes in the expression of the TJs or the possible role of dietary inulin as important targets, deserve further research.

Conclusions

This study highlighted that dietary inulin may ameliorated obesity PCOS via the gut microbiota–inflammation–sex steroid hormones axis in human, which may potentially serve as an inexpensive intervention for the control of obesity PCOS patients.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40001-024-02034-9.

Supplementary Material 1. Fig S1. Relative abundance of gut microbial species at the phylum levels in the feces of human.Firmicutes/Bacteroidetes.Proteobacteria.Actinobacteria.Fusobacteria

Acknowledgements
The authors greatly appreciate all the patients for their cooperation.

Author contributions
XL, TG, BJ and HW designed and wrote the paper. YN, XB, JZ, LQ, ZG, and XM performed the research. All authors have read and approved the fifinal manuscript.

Funding
This work was supported by National Natural Science Foundation of China (recipient: XL, grant number: 81660806; 82260947). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability
The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession numbers can be found in NCBI, accession number PRJNA903127.

Declarations

Ethics approval and consent to participate
The clinical study was approved by the Ethics Committee of General Hospital of Ningxia Medical University (No. 2016-017).

Consent for publication
The written informed consents were obtained from all enrolled cases.

Competing interests
The authors declare no competing interests.

Author details
[1]Center for Reproductive Medicine, General Hospital of Ningxia Medical University, 164, Zhiping Road, Yinchuan 750004, Ningxia, China. [2]Key Laboratory of Fertility Maintenance, Ningxia Medical University, 1160, Shengli Street, Yinchuan 750004, Ningxia, China. [3]Key Laboratory of Modernization of Hui Medicine, Ministry of Education, School of Traditional Chinese Medicine, Ningxia Medical University, 1160, Shengli Street, Yinchuan 750004, Ningxia, China. [4]Department of Pathogenic Biology and Medical Immunology, School of Basic Medical Sciences, Ningxia Medical University, 1160, Shengli Street, Yinchuan 750004, Ningxia, China. [5]College of Traditional Chinese Medicine, Ningxia Medical University, 1160, Shengli Street, Yinchuan 750004, Ningxia, China. [6]Chengdu Integrated, TCM&Western Medicine Hospital, 18, Wanxiang North Road, Chengdu 610095, Sichuan, China.

Received: 8 April 2024 Accepted: 21 August 2024
Published online: 31 August 2024

References

1. Wojciechowski P, Lipowska A, Rys P, Ewens KG, Franks S, et al. Impact of FTO genotypes on BMI and weight in polycystic ovary syndrome: a systematic review and meta-analysis. Diabetologiam. 2012;55:2636–45.
2. March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, et al. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod. 2010;25:544–51.
3. Torres PJ, Ho BS, Arroyo P, Sau L, Chen A, et al. Exposure to a healthy gut microbiome protects against reproductive and metabolic dysregulation in a PCOS mouse model. Endocrinology. 2019;160:1193–204.
4. Qi X, Yun C, Sun L, Xia J, Wu Q, et al. Gut microbiota-bile acid-interleukin-22 axis orchestrates polycystic ovary syndrome. Nat Med. 2019;25:1225–33.
5. Zhang J, Sun Z, Jiang S, Bai X, Ma C, et al. Probiotic *Bifidobacterium lactis* V9 regulates the secretion of sex hormones in polycystic ovary syndrome patients through the gut-brain axis. MSystems. 2019;4:e00017–9.
6. Zheng Y, Yu J, Liang C, Li S, Wen X, et al. Characterization on gut microbiome of PCOS rats and its further design by shifts in high-fat diet and dihydrotestosterone induction in PCOS rats. Bioprocess Biosyst Eng. 2021;44:953–64.
7. Zhang D, Zhang L, Yue F, Zheng Y, Russell R. Serum zonulin is elevated in women with polycystic ovary syndrome and correlates with insulin resistance and severity of anovulation. Eur J Endocrinol. 2015;172:29–36.
8. Tilg H, Zmora N, Adolph TE, Elinav E. The intestinal microbiota fuelling metabolic inflammation. Nat Rev Immunol. 2020;20:40–54.
9. He FF, Li YM. Role of gut microbiota in the development of insulin resistance and the mechanism underlying polycystic ovary syndrome: a review. J Ovarian Res. 2020;13:73.
10. Pickard JM, Zeng MY, Caruso R, Núñez G. Gut microbiota: Role in pathogen colonization, immune responses, and inflammatory disease. Immunol Rev. 2017;279:70–89.
11. Thackray VG. Sex, microbes, and polycystic ovary syndrome. Trends Endocrinol Metab. 2019;30(1):54–65. https://doi.org/10.1016/j.tem.2018.11.001.
12. Davidson SJ, Barrett HL, Price SA, Callaway LK, Dekker NM. Probiotics for preventing gestational diabetes. Cochrane Database Syst Rev. 2021;4:CD009951.
13. Maldonado Galdeano C, Cazorla SI, Lemme Dumit JM, Vélez E, Perdigón G. Beneficial effects of probiotic consumption on the immune system. Ann Nutr Metab. 2019;74:115–24.
14. Eastwood J, Walton G, Van Hemert S, Williams C, Lamport D. The effect of probiotics on cognitive function across the human lifespan: a systematic review. Neurosci Biobehav Rev. 2021;128:311–27.
15. Badgeley A, Anwar H, Modi K, Murphy P, Lakshmikuttyamma A. Effect of probiotics and gut microbiota on anti-cancer drugs: mechanistic perspectives. Biochim Biophys Acta Rev Cancer. 2021;1875: 188494.
16. Wang Y, Wu Y, Wang Y, Xu H, Mei X, et al. Antioxidant properties of probiotic bacteria. Nutrients. 2017;9:521.
17. Gupta N, Jangid AK, Pooja D, Kulhari H. Inulin: a novel and stretchy polysaccharide tool for biomedical and nutritional applications. Int J Biol Macromol. 2019;132:852–63.
18. Ahmed W, Rashid S. Functional and therapeutic potential of inulin: a comprehensive review. Crit Rev Food Sci Nutr. 2019;59:1–13.
19. Xue J, Li X, Liu P, Li K, Sha L, et al. Inulin and metformin ameliorate polycystic ovary syndrome via anti-inflammation and modulating gut microbiota in mice. Endocr J. 2019;66:859–70.
20. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19:41–7.
21. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 894:i-253.
22. sCondorelli RA, Calogero AE, Di Mauro M, Mongioi' LM, Cannarella R, et al. Androgen excess and metabolic disorders in women with PCOS: beyond the body mass index. J Endocrinol Invest. 2018;41:383–8.
23. Coutinho EA, Kauffman AS. The role of the brain in the pathogenesis and physiology of polycystic ovary syndrome (PCOS). Med Sci (Basel). 2019;7:84.
24. Blank SK, McCartney CR, Marshall JC. The origins and sequelae of abnormal neuroendocrine function in polycystic ovary syndrome. Hum Reprod Update. 2006;12:351–61.
25. Wang JG, Nakhuda GS, Guarnaccia MM, Sauer MV, Lobo RA. Müllerian inhibiting substance and disrupted folliculogenesis in polycystic ovary syndrome. Am J Obstet Gynecol. 2007;196:77.e1-77.e5.
26. Le MT, Le VNS, Le DD, Nguyen VQH, Chen C, et al. Exploration of the role of anti-Mullerian hormone and LH/FSH ratio in diagnosis of polycystic ovary syndrome. Clin Endocrinol (Oxf). 2019;90:579–85.
27. Yue CY, Lu LK, Li M, Zhang QL, Ying CM. Threshold value of anti-Mullerian hormone for the diagnosis of polycystic ovary syndrome in Chinese women. PLoS ONE. 2018;13: e0203129.
28. Dewailly D, Pigny P, Soudan B, Catteau-Jonard S, Decanter C, et al. Reconciling the definitions of polycystic ovary syndrome: the ovarian follicle

number and serum anti-Müllerian hormone concentrations aggregate with the markers of hyperandrogenism. J Clin Endocrinol Metab. 2010;95:4399–405.
29. Abruzzese GA, Heber MF, Ferreira SR, Velez LM, Reynoso R, et al. Prenatal hyperandrogenism induces alterations that affect liver lipid metabolism. J Endocrinol. 2016;230:67–79.
30. Ding L, Gao F, Zhang M, Yan W, Tang R, et al. Higher PDCD4 expression is associated with obesity, insulin resistance, lipid metabolism disorders, and granulosa cell apoptosis in polycystic ovary syndrome. Fertil Steril. 2016;105:1330–7.
31. Schooling CM. Testosterone and cardiovascular disease. Curr Opin Endocrinol Diabetes Obes. 2014;21:202–8.
32. Layegh P, Mousavi Z, Farrokh Tehrani D, Parizadeh SM, Khajedaluee M. Insulin resistance and endocrine-metabolic abnormalities in polycystic ovarian syndrome: comparison between obese and non-obese PCOS patients. Int J Reprod Biomed. 2016;14:263–70.
33. Pizzichini M, Di Stefano A, Resconi G, Pompucci G, Marinello E. Influence of testosterone on purine nucleotide turnover in rat kidney. Horm Metab Res. 1990;22:334–8.
34. Barber TM, Golding SJ, Alvey C, Wass JA, Karpe F, et al. Global adiposity rather than abnormal regional fat distribution characterizes women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2008;93:999–1004.
35. Insenser M, Murri M, Del Campo R, Martínez-García MÁ, Fernández-Durán E, et al. Gut microbiota and the polycystic ovary syndrome: influence of sex, sex hormones, and obesity. J Clin Endocrinol Metab. 2018;103:2552–62.
36. González F, Considine RV, Abdelhadi OA, Acton AJ. Saturated fat ingestion promotes lipopolysaccharide-mediated inflammation and insulin resistance in polycystic ovary syndrome. J Clin Endocrinol Metab. 2019;104:934–46.
37. Yurtdaş G, Akdevelioğlu Y. A new approach to polycystic ovary syndrome: the gut microbiota. J Am Coll Nutr. 2020;39:371–82.
38. Li T, Zhang Y, Song J, Chen L, Du M, et al. Yogurt enriched with inulin ameliorated reproductive functions and regulated gut microbiota in dehydroepiandrosterone-induced polycystic ovary syndrome mice. Nutrients. 2022;14:279.
39. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, et al. Richness of human gut microbiome correlates with metabolic markers. Nature. 2013;500:541–6.
40. Vandeputte D, Falony G, Vieira-Silva S, Wang J, Sailer M, et al. probiotic inulin-type fructans induce specific changes in the human gut microbiota. Gut. 2017;66:1968–74.
41. Magne F, Gotteland M, Gauthier L, Zazueta A, Pesoa S, et al. The firmicutes/bacteroidetes ratio: a relevant marker of gut dysbiosis in obese patients? Nutrients. 2020;12:1474.
42. Crovesy L, Masterson D, Rosado EL. Profile of the gut microbiota of adults with obesity: a systematic review. Eur J Clin Nutr. 2020;74:1251–62.
43. Stojanov S, Berlec A, Štrukelj B. The influence of probiotics on the firmicutes/bacteroidetes ratio in the treatment of obesity and inflammatory bowel disease. Microorganisms. 2020;8:1715.
44. Takezawa K, Fujita K, Matsushita M, Motooka D, Hatano K, et al. The Firmicutes/Bacteroidetes ratio of the human gut microbiota is associated with prostate enlargement. Prostate. 2021;81:1287–93.
45. Sawicka-Smiarowska E, Bondarczuk K, Bauer W, Niemira M, Szalkowska A, et al. Gut microbiome in chronic coronary syndrome patients. J Clin Med. 2021;10:5074.
46. Yang T, Santisteban MM, Rodriguez V, Li E, Ahmari N, et al. Gut dysbiosis is linked to hypertension. Hypertension. 2015;65:1331–40.
47. Mariat D, Firmesse O, Levenez F, Guimarães V, Sokol H, et al. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. BMC Microbiol. 2009;9:123.
48. Guan WJ, Yuan JJ, Li HM, Gao YH, Huang Y, et al. *Proteobacteria* community compositions correlate with bronchiectasis severity. Int J Tuberc Lung Dis. 2018;22:1095–105.
49. Shin NR, Whon TW, Bae JW. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 2015;33:496–503.
50. Pammi M, Cope J, Tarr PI, Warner BB, Morrow AL, et al. Intestinal dysbiosis in preterm infants preceding necrotizing enterocolitis: a systematic review and meta-analysis. Microbiome. 2017;5:31.
51. Binda C, Lopetuso LR, Rizzatti G, Gibiino G, Cennamo V, et al. Actinobacteria: A relevant minority for the maintenance of gut homeostasis. Dig Liver Dis. 2018;50:421–8.
52. Parnell JA, Reimer RA. probiotic fiber modulation of the gut microbiota improves risk factors for obesity and the metabolic syndrome. Gut Microbes. 2012;3:29–34.
53. Wang T, Sha L, Li Y, Zhu L, Wang Z, et al. Dietary α-linolenic acid-rich flaxseed oil exerts beneficial effects on polycystic ovary syndrome through sex steroid hormones-microbiota-inflammation axis in rats. Front Endocrinol (Lausanne). 2020;11:284.
54. Bunesova V, Vlkova E, Rada V, Killer J, Musilova S. Bifidobacteria from the gastrointestinal tract of animals: differences and similarities. Benef Microbes. 2014;5:377–88.
55. Chang CJ, Lin CS, Lu CC, Martel J, Ko YF, et al. *Ganoderma lucidum* reduces obesity in mice by modulating the composition of the gut microbiota. Nat Commun. 2015;6:7489.
56. Chambers ES, Byrne CS, Morrison DJ, Murphy KG, Preston T, et al. Dietary supplementation with inulin-propionate ester or inulin improves insulin sensitivity in adults with overweight and obesity with distinct effects on the gut microbiota, plasma metabolome and systemic inflammatory responses: a randomised cross-over trial. Gut. 2019;68:1430–8.
57. Kaneko M, Tominaga Y, Sakamoto K, Shikata H. Hemodialysis vascular access infection caused by *Methylobacterium radiotolerans*: the first confirmed case in Japan. J Infect Chemother. 2020;26:107–9.
58. Matsumoto H, Shiotani A, Katsumata R, Fukushima S, Handa Y, et al. Mucosa-associated microbiota in patients with irritable bowel syndrome: a comparison of subtypes. Digestion. 2021;102:49–56.
59. Tikunov AY, Shvalov AN, Morozov VV, Babkin IV, Seledtsova GV, et al. Taxonomic composition and biodiversity of the gut microbiome from patients with irritable bowel syndrome, ulcerative colitis, and asthma. Vavilovskii Zhurnal Genet Selektsii. 2021;25:864–73.
60. Patel S. Polycystic ovary syndrome (PCOS), an inflammatory, systemic, lifestyle endocrinopathy. J Steroid Biochem Mol Biol. 2018;182:27–36.
61. Rudnicka E, Suchta K, Grymowicz M, Calik-Ksepka A, Smolarczyk K, et al. Chronic low grade inflammation in pathogenesis of PCOS. Int J Mol Sci. 2021;22:3789.
62. Ojeda-Ojeda M, Murri M, Insenser M, Escobar-Morreale HF. Mediators of low-grade chronic inflammation in polycystic ovary syndrome (PCOS). Curr Pharm Des. 2013;19:5775–91.
63. Li K, Zhang L, Xue J, Yang X, Dong X, et al. Dietary inulin alleviates diverse stages of type 2 diabetes mellitus via anti-inflammation and modulating gut microbiota in db/db mice. Food Funct. 2019;10:1915–27.
64. Jayashree B, Bibin YS, Prabhu D, Shanthirani CS, Gokulakrishnan K, et al. Increased circulatory levels of lipopolysaccharide (LPS) and zonulin signify novel biomarkers of proinflammation in patients with type 2 diabetes. Mol Cell Biochem. 2014;388:203–10.
65. Snelson M, de Pasquale C, Ekinci EI, Coughlan MT. Gut microbiome, probiotics, intestinal permeability and diabetes complications. Best Pract Res Clin Endocrinol Metab. 2021;35: 101507.
66. Stan TL, Soylu-Kucharz R, Burleigh S, Prykhodko O, Cao L, et al. Increased intestinal permeability and gut dysbiosis in the R6/2 mouse model of Huntington's disease. Sci Rep. 2020;10:18270.
67. Kim S, Kim GH. Roles of claudin-2, ZO-1 and occludin in leaky HK-2 cells. PLoS ONE. 2017;12: e0189221.
68. Vaghef-Mehrabani E, Harouni R, Behrooz M, Ranjbar F, Asghari-Jafarabadi M, et al. Effects of inulin supplementation on inflammatory biomarkers and clinical symptoms of women with obesity and depression on a calorie-restricted diet: a randomized controlled clinical trial. Br J Nutr. 2022;5:1–28.
69. Liang Z, Di N, Li L, Yang D. Gut microbiota alterations reveal potential gut-brain axis changes in polycystic ovary syndrome. J Endocrinol Invest. 2021;44:1727–37.

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