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ORIGINAL RESEARCH

Characteristic Gut Microbiota in PCOS-IR Patients and Its Association with Endocrine Features

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Purpose: The aim of this study is to explore the changes of gut microbiota, the metabolic characteristics and sex hormones in polycystic ovary syndrome with insulin resistance (PCOS-IR), and to clarify the role of gut microbiota in the occurrence of this condition.

Methods: We established a rat model of PCOS-IR using dehydroepiandrosterone (DHEA) combined with a high-fat diet, and recruited patients who met the clinical diagnostic criteria for PCOS-IR. We measured metabolic parameters and sex hormone profiles, and analyzed gut microbiota characteristics via high-throughput 16S rRNA sequencing. We also conducted microbial transplantation experiments to verify the causal relationship between gut microbiota and PCOS-IR.

Results: In PCOS-IR rats, we observed significant endocrine-metabolic disturbances and alterations in gut microbiota β-diversity, characterized by an enrichment of *Fusobacterium*. Transplantation of this dysbiotic microbiota to healthy rats reproduced key PCOS-IR features, confirming a causal role. In people with PCOS-IR, we found a distinct gut microbial profile compared to both healthy individuals and those with PCOS without IR, with *Fusobacterium* consistently identified as a key genus across species.

Conclusion: Our findings show that gut microbiota disturbance leads to endocrine and metabolic features resembling PCOS-IR. The gut microbiota, particularly *Fusobacterium*, could serve as a clinical marker and potential therapeutic target for people with PCOS-IR. This study provides mechanistic insights into how gut microbiota contributes to PCOS-IR pathogenesis.

Keywords: polycystic ovary syndrome, insulin resistance, gut microbiota, metabolism

Introduction

Polycystic ovary syndrome (PCOS) is a common gynecological endocrine disorder with a prevalence of 6–10% among women. As its diagnostic criteria are centered on reproductive abnormalities, the prevalence is notably higher, reaching and 9–18% among women of reproductive age.[1,2] It is characterized by hyperandrogenemia (HA), persistent anovulation and polycystic changes of the ovaries, with hirsuteness, acne, and infertility affecting the patients' physical and mental health.[3] Insulin resistance is one of the pathological features of PCOS patients, which is present in about 60% of normal-weight PCOS patients and in 94% of overweight PCOS patients.[4] IR can cause metabolic abnormalities and increase the risk of type 2 diabetes, metabolic syndrome, and endometrial cancer.[5–7] In addition, IR also exacerbate the symptoms of HA, thus promoting the development of PCOS.[8] Therefore, it is of great clinical significance to study PCOS with insulin resistance (PCOS-IR).

Gut microbiota, as a community of microorganisms living in the gastrointestinal tract, plays an important role in host metabolism and immunity.[9,10] Studies on the pathophysiological mechanism of PCOS have shown that gut microbiota

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disorders can ultimately interfere with normal follicular development.[11] Notably, gut microbiota disorders are also early risk factors for IR.[12–14] The potential mechanisms linking gut dysbiosis to these central features of PCOS may involve a cascade of events. Dysbiosis can impair the production of gut-derived metabolites, such as short-chain fatty acids, which are crucial for maintaining intestinal barrier integrity. A compromised barrier allows bacterial lipopolysaccharide to translocate into circulation, triggering chronic low-grade inflammation and contributing to systemic insulin resistance.[11–13] This resulting hyperinsulinemia, in turn, stimulates ovarian androgen production and disrupts the normal follicular development process, leading to the characteristic polycystic ovaries and anovulation.[11] Additionally, alterations in bile acid metabolism by gut microbiota may further influence metabolic homeostasis.[14] Recent research has found that curcumin, inulin, and probiotics regulate gut microbiota and thus treat PCOS-IR.[15–17] It could be seen that gut microbiota is closely related to PCOS-IR. However, key questions remain unanswered. The causal relationship between gut microbiota dysbiosis and PCOS-IR is still unclear—whether these microbial changes are a driver or a consequence of the condition. Besides there is a pressing need to identify specific microbial biomarkers that could aid in the early detection and personalized treatment strategies for PCOS-IR. Dehydroepiandrosterone (DHEA) combined with high fat diet (HFD) mimic the manifestations of clinical PCOS, including elevated serum testosterone (T) and luteinizing hormone, dyslipidemia, and abnormalities in the ovarian microenvironment with polycystic changes in the ovaries.[18] In this study, we analyzed the 16S rRNA sequencing data of PCOS-IR patients and PCOS-IR rats constructed by DHEA combined with HFD, to clarify the gut microbiota characteristics and biomarker in a state of PCOS-IR, with the aim of providing insights into the current microbiological research on PCOS-IR and its treatment.

Materials and Methods

Animals

The study protocol was approved by the Animal Ethics Committee of Shanghai Changhai Hospital (Ethical Approval No.: CHEC [A.E] 2022–011). High fat diet (XTHF60) and normal standard diet (XTCON50J) was obtained from Jiangsu Xietong Pharmaceutical Bio-Engineering Co,Ltd. (Nanjing, Jiangsu, China). The dietary formula was shown in the Supplementary Tables 1 and 2. Female Wistar rats (3 weeks old) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All animals were housed in a specific pathogen-free conditions, maintaining controlled temperature and humidity conditions (22 ± 2°C, 55 ± 5% humidity), with a 12:12 h light: darkness cycle. After a 1-week acclimatization period, the rats were randomly assigned to four groups (n = 6 per group): blank group, PCOS group, IR group, and PCOS-IR group. The blank group was fed with standard maintenance chow and subcutaneously injected with 0.2 mL of sesame oil per 100 g body weight daily. The PCOS group received the same chow with daily subcutaneous injections of 0.2 mL of DHEA suspension. Rats in the IR group were fed a high-fat diet (60% fat-derived calories) and injected with sesame oil. The PCOS-IR group received both the high-fat diet and DHEA suspension. All interventions were performed for 35 consecutive days.[19]

Estrus Cycle

Vaginal exfoliative cytology was performed continuously in all four experimental rat groups, beginning on day 15 post-modeling and continuing until the end of the experiment. The morphology of the exfoliated cells was observed using an inverted microscope, and the estrous cycle of the rats was determined and plotted as an estrous cycle chart.

Serum Hormone Assay

After the last intervention, rats in the interoestrus period were selected and fasted overnight for 12 hours. They were sacrificed, and blood samples were collected via abdominal aortic puncture into centrifuge tubes. The serum was obtained after centrifuging at 4°C and 3500 rpm for 15 minutes. The levels of T, fasting insulin (FINS), and fasting blood glucose (FBG) in the serum were detected using ELISA kits, and the index of insulin resistance (HOMA-IR) was calculated using the method of FINS×FBG/22.5, and if the HOMA-IR was more than 2.6, then it would be considered as IR.

Fecal Microbiota Transplantation

Normal rats drank water containing 1 g/L ampicillin, 1g/L neomycin, 1g/L metronidazole and 0.5g/L vancomycin for 2 weeks, so as to enable the formation of a pseudo sterile environment in their intestinal tracts. To prepare fecal microbiota, 200 mg of feces were collected daily from normal rats and PCOA-IR rats, and then suspended and homogenized in 1 mL PBS. Centrifuge at 4000 rpm for 3 minutes to collect the bacteria-rich supernatant. Subsequently, the bacterial precipitate was obtained by centrifugation at 12000 rpm for 10 minutes and resuspended in 700 μL of saline containing 20% (v/v) glycerol. Pseudo-germ-free rats fed with a normal diet were divided into two groups and orally administered with fecal microbial suspensions from normal rats and PCOA-IR rats, respectively.

Patients

Eighteen patients with PCOS-IR and eleven patients with PCOS-NIR who attended the outpatient clinic of Traditional Chinese Medicine and Gynecology Department of Shanghai Changhai Hospital from June 2016 to November 2017 were recruited, and the diagnostic criteria for PCOS were referred to the diagnostic criteria for PCOS as revised by Rotterdam Conference of the Netherlands in 2003; the age of PCOS ranged between 16–35 years old; and the HOMA-IR > 2.6. Excluded were (i) oral contraceptives, anti-androgen drugs, insulin sensitizers in the last 3 months; (ii) pregnancy; (iii) other known causes of hyperandrogenemia and ovulation disorders: 21-hydroxylase deficiency, congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumors, thyroid disorders, and hyperprolactinemia; (iv) any psychiatric or organic disorders; (v) use of corticosteroids or sex steroids; (vi) use of corticosteroids or sex steroids in the last 2 years. Steroids; and (vii) use of antibiotics or probiotics or prebiotics in the past 3 months.[20] Thirty PCOS-free women with age and BMI matching the patients' age and BMI who had normal menstrual cycles were selected as the control group for the same period of outpatient physical examination at Changhai Hospital, excluding those with clinical and/or biochemical hyperandrogenemia; and those with any congenital or psychiatric diseases were excluded. The study was approved by the Ethical Review Committee of Chinese Registered Clinical Trials (Ethical Review No. CHiECRT-20160050), and each subject voluntarily signed an informed consent form before the start of the trial.

Clinical Sample Collection and Testing

Fasting peripheral blood samples were collected from all participants on the third day of their menstrual cycle. For amenorrheic patients, blood collection was performed on a random day after pregnancy was excluded. Serum levels of sex hormones, including luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), T, DHEA, and prolactin (PRL), were measured in the Clinical Laboratory of Changhai Hospital, Shanghai, China. Fecal samples were collected 3 to 5 days after the end of menstruation. All participants were instructed to follow a carbohydrate-rich diet (300 g/day) for 3 days prior to sampling. Approximately 10 g of fresh stool was collected from each subject using a disposable sterile plastic spoon and stored at −80 °C. The gut microbiota was analyzed using 16S rRNA sequencing.

Analysis of Gut Microbiota

DNA Extraction and PCR Amplification

Total microbial genomic DNA was extracted from fecal samples using the E.Z.N.A. soil DNA kit according to manufacturer's instructions. DNA concentration and purity were determined using NanoDrop2000. The hypervariable region V3-V4 of the bacterial 16S rRNA gene were amplified with primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCR reaction mixture including 4 μL 5 × Fast Pfu buffer, 2 μL 2.5 mM dNTPs, 0.8 μL each primer (5 μM), 0.4 μL Fast Pfu polymerase, 10 ng of template DNA, and ddH_2O to a final volume of 20 μL. PCR amplification cycling conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 27 cycles of denaturing at 95 °C for 30s, annealing at 55 °C for 30s and extension at 72 °Cfor 45s, and single extension at 72 °C for 10 min, and end at 4 °C. The PCR product was extracted from 2% agarose gel and purified using the PCR Clean-Up Kit according to manufacturer's instructions and quantified using Qubit 4.0.

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Illumina Sequencing

The PCR products were recovered using a 2% agarose gel and then purified using the AxyPrep DNA Gel Extraction Kit. The recovered products were quantified using a Quantus Fluorometer. Sequencing was performed using Illumina's MiSeq PE300 platform.

Data Processing and Bioinformatics Analysis

Raw FASTQ files were de-multiplexed using an in-house perl script, and then quality-filtered by fastp version 0.19.6 and merged by FLASH version 1.2.7 with the following criteria. Then the optimized sequences were clustered into operational taxonomic units (OTUs) using UPARSE 7.1 with 97% sequence similarity level. Based on the OTUs information, rarefaction curves and alpha diversity were calculated with Mothur v1.30.1. The similarity among the microbial communities in different samples was determined by principal coordinate analysis (PCoA) based on Bray-curtis dissimilarity using Vegan v2.5–3 package. The linear discriminant analysis (LDA) effect size (LEfSe) (http://huttenhower.sph.harvard.edu/LEfSe) was performed to identify the significantly abundant taxa (phylum to genera) of bacteria among the different groups.

Statistical Analysis

Quantitative demographic and clinical variables with a normal distribution were expressed as mean ± standard deviation and compared between groups using the independent-samples *t*-test. For non-normally distributed sequencing data, the Wilcoxon rank-sum test was applied. *p*-values were adjusted for multiple comparisons using the false discovery rate (FDR) method. All analyses were conducted using SPSS software (version 21.0; IBM, Armonk, NY, USA). All statistical tests were two-tailed, and differences were considered statistically significant at $P < 0.05$.

Results

Validation of the PCOS-IR Rat Model

Regarding the estrous cycle, varying degrees of disruption were observed in the PCOS, IR, and PCOS-IR groups, while the blank group exhibited normal cycles. The rates of estrous cycle irregularity were 2/3 in the PCOS group, 1/6 in the IR group, and 5/6 in the PCOS-IR group (Figure 1A). In terms of sex hormone levels, the serum T levels in both the PCOS and PCOS-IR groups were significantly elevated compared to the blank group ($P < 0.01$), whereas no statistically significant difference was observed between the IR group and the blank group. With respect to glucose metabolism, the IR and PCOS-IR groups showed significantly increased levels of FBG, FINS, and HOMA-IR compared with the blank group ($P < 0.05$). No significant differences were found between the PCOS and blank groups. Notably, compared with the PCOS group, the PCOS-IR group exhibited significantly higher T levels ($P < 0.05$); and compared with the IR group, the PCOS-IR group showed markedly increased FINS and HOMA-IR levels ($P < 0.01$) (Figure 1B–E). These findings indicate that the combined PCOS-IR condition results in more pronounced disturbances in hormone levels and glucose metabolism than either PCOS or IR alone.

Gut Microbiota Diversity in PCOS-IR Rats

Compared with the blank group, there was no statistically significant difference between the α-diversity indices of chao1, observed species, shannon and simpson in the PCOS group, the IR group and the PCOS-IR ($P > 0.05$), see Figure 2A. From the perspective of β-diversity, there was a significant analysis of bacterial phyla and genera of the PCOS group and the PCOS-IR compared with the blank group, while there was a partial overlap of the bacterial phyla in the IR group and the blank group, see Figure 2B. In addition, we analyzed the percentage of phyla and genera in each sample, and it was seen that the phyla with higher abundance in each group included *Firmicutes, Bacteroidota, Proteobacteria, Actinobacteria, Actinobacteriota* and *Desulfobacterota*, and the genera with higher abundance in each group included *Muribaculaceae, lachnospiraceae_NK4A136_group*, Bacteroides, *Ruminococcus* and *Clostridia_UCG-014*, as shown in Figures 2C and D.

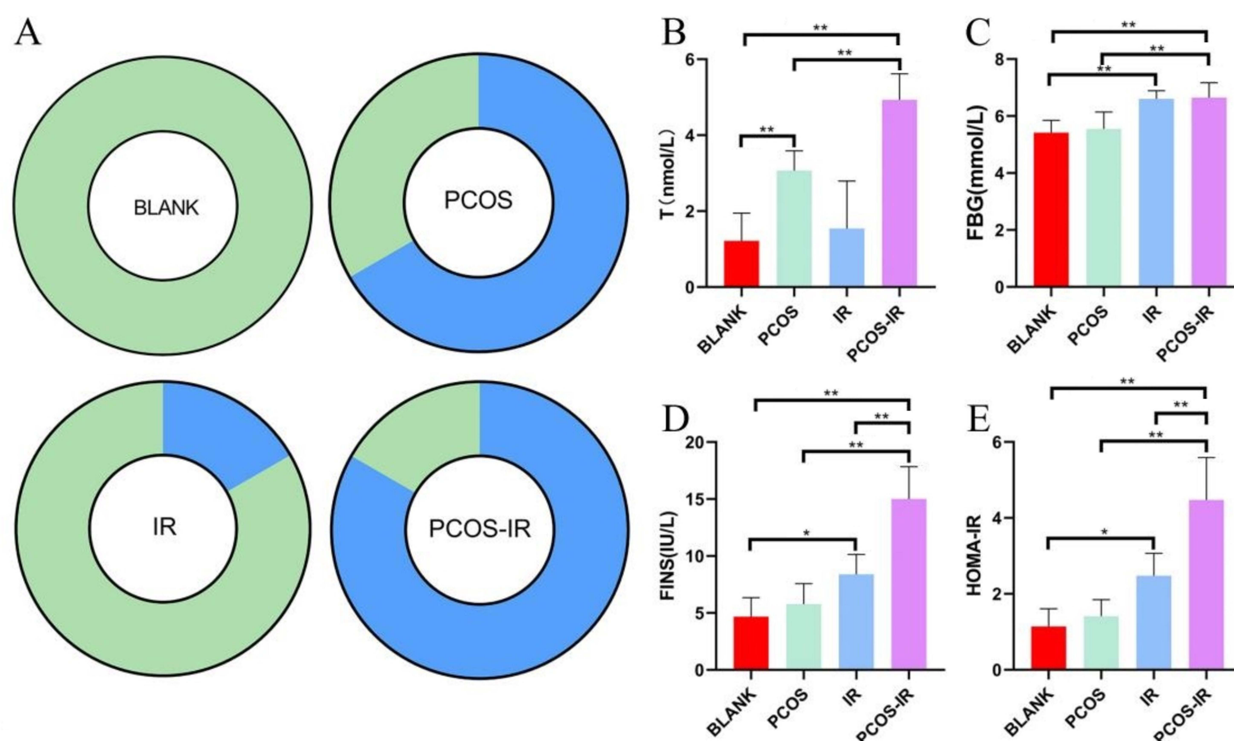

Figure 1 Metabolic and endocrine disturbances in PCOS and IR models. (**A**) Estrous cycle in different groups. (**B**) Serum testosterone (T) levels. (**C**) Serum fasting blood glucose (FBG) levels. (**D**) Serum fasting insulin (FINS) levels. (**E**) HOMA-IR index. Data are presented as the mean ± SD; *P < 0.05, **P < 0.01.

Characteristic Gut Microbiota in PCOS-IR Rats

LEfSe multi-level species discrimination and LDA were used to identify characteristic gut microbiota. It was found that 17 characteristic genera were enriched in the PCOS-IR group compared with the blank group. These included *Christensenellaceae_R_7_group, Negativibacillus, NK4A214_group, Rikenellaceae_RC9_gut_group, Sutterella, Fusicatenibacter, Bilophila, Collinsella, Bacteroides, Faecalitalea, Dubosiella, Rikenella, Prevotellaceae_NK3B31_group, Lachnospiraceae_UCG_010, Coriobacteriaceae_UCG_002, UCG_007*, and *Fusobacterium* (Table 1). Differential abundance analysis revealed that, compared with the blank group, the PCOS group exhibited one differential phylum and 23 differential genera, while the PCOS-IR group showed three differential phyla and 25 differential genera (Supplementary Tables 3–4). No significant differences in bacterial phyla or genera were observed in the IR group (Supplementary Table 5). Notably, differences were also observed between the PCOS-IR and PCOS groups. Specifically, compared with the PCOS group, the PCOS-IR group exhibited one differential phylum and 35 differential genera (Supplementary Table 6).() In summary, the gut microbial profile of the PCOS-IR group was distinct from that of the PCOS group, suggesting that PCOS-IR may represent a distinct subtype of PCOS and should be investigated as an independent pathological entity.

Hormonal and Metabolic Parameters in PCOS-IR Patients

Compared to the control group, the PCOS-IR and PCOS-NIR groups exhibited significantly elevated levels of LH, LH/FSH ratio, and testosterone (T) (P < 0.05), while no significant differences were observed in FSH, estradiol (E2), and prolactin (PRL) levels (P > 0.05). Furthermore, compared with the PCOS-NIR group, the PCOS-IR group showed significantly higher levels of metabolic indicators including HOMA-IR, fasting insulin (FINS), and the incremental area under the curve for insulin (IAUC) (P < 0.01), with no significant difference in fasting blood glucose (FBG) (P > 0.05), as illustrated in Figure 3.

Gut Microbiota Diversity in PCOS-IR Patients

The rarefaction curves tended to plateau, indicating that the sequencing depth was sufficient to capture the majority of species present in the samples (Figure 4A). The rank-abundance curves demonstrated a marked reduction in gut

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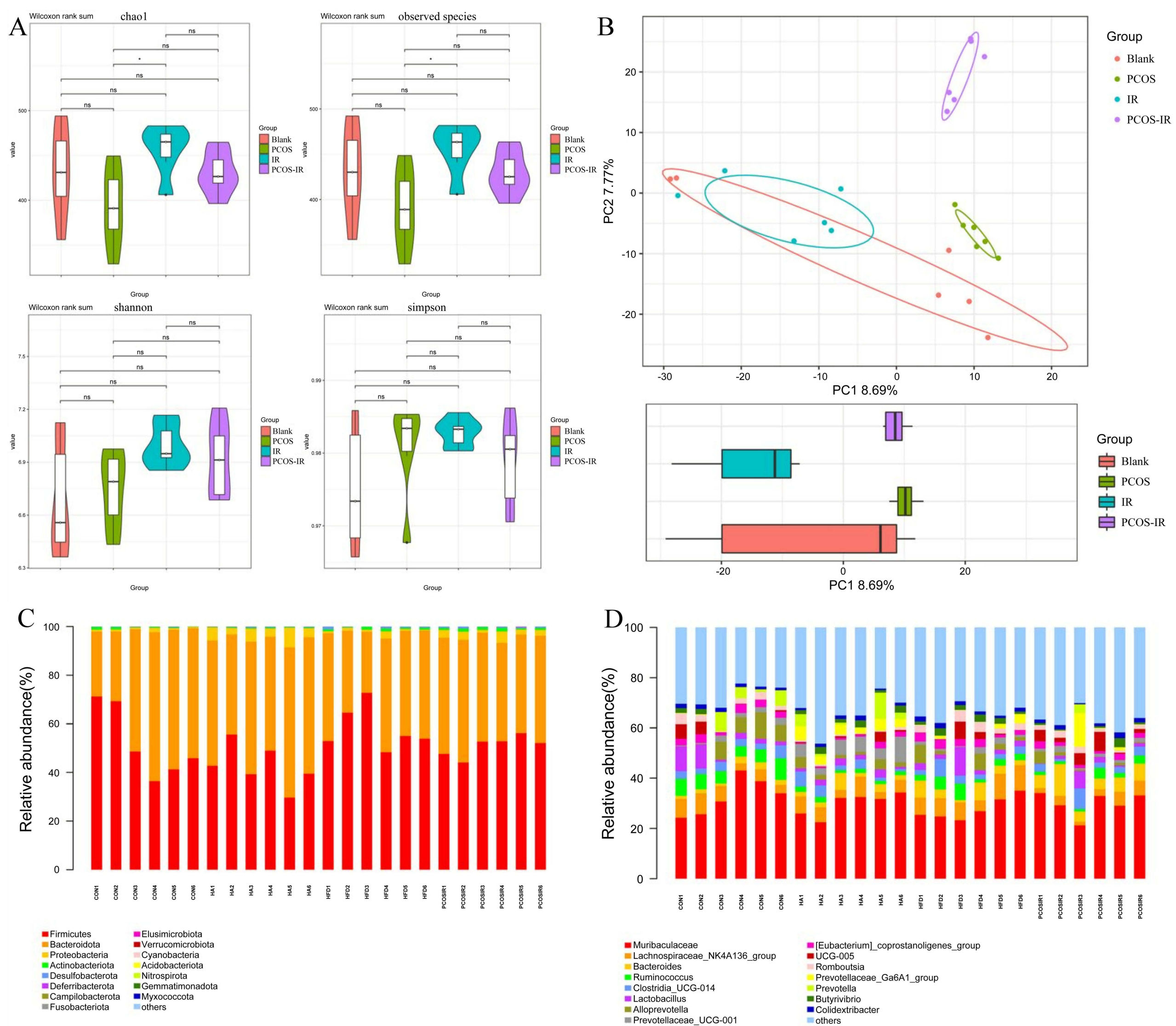

Figure 2 Gut microbiota diversity analysis in four groups. (**A**) α-diversity indices (Chao1, Observed species, Shannon, Simpson). (**B**) β-diversity PCoA analysis. (**C**) Relative abundance of species at phylum level. (**D**) Relative abundance of species at genus level. Data are presented as the mean ± SD; *P < 0.05.
Abbreviation: ns, no significance.

microbiota diversity in both the PCOS-IR and PCOS-NIR groups compared with healthy controls (Figure 4B). β-diversity analysis revealed distinct microbial community structures among the three groups (Figure 4C). In terms of α-diversity, the observed species (Sobs) index was significantly lower in the PCOS-IR group compared to healthy controls ($P < 0.05$). Although the ACE, Chao1, and Bootstrap indices also appeared reduced in the PCOS-IR group, the differences were not statistically significant ($P > 0.05$). No significant differences in Sobs, ACE, Chao1, or Bootstrap indices were observed between the PCOS-NIR group and healthy controls ($P > 0.05$; Figure 4D–G).

Differential Gut Microbiota in PCOS-IR Patients

At the phylum level, the relative abundance of gut microbiota differed among the three groups (Figure 5A). At the genus level, the top five genera in the PCOS-IR group were *Bacteroides* (26.02%), *Faecalibacterium* (13.15%), *Prevotella_9* (9.55%), *Phascolarctobacterium* (4.08%), and *Lachnoclostridium* (2.94%) (Figure 5B). In the PCOS-NIR group, the dominant genera were *Bacteroides* (22.53%), *Faecalibacterium* (14.52%), *Prevotella_9* (7.72%), *Blautia* (4.26%), and *Phascolarctobacterium* (3.67%) (Figure 5C). The top five genera in the healthy control group were *Bacteroides* (20.83%), *Faecalibacterium*

Table 1 LDA Discriminant Histogram of Gut Microbiota Between the Blank Rats and PCOS-IR Rats

Bacteria	Groups	LDA value	*p*-value
Turicibacter	Blank	3.257380	0.003948
Defluviitaleaceae_UCG_011	Blank	3.041646	0.004763
UCG_010	Blank	3.656236	0.024975
Ruminococcus	Blank	4.288577	0.006485
DNF00809	Blank	3.497390	0.022230
Lachnospiraceae_UCG_006	Blank	3.065736	0.002093
UCG_003	Blank	3.194448	0.037373
Christensenellaceae_R_7_group	PCOS-IR	3.720737	0.016309
Negativibacillus	PCOS-IR	3.343761	0.037041
NK4A214_group	PCOS-IR	3.950437	0.003948
Rikenellaceae_RC9_gut_group	PCOS-IR	3.127590	0.049510
Sutterella	PCOS-IR	3.464759	0.022487
Fusicatenibacter	PCOS-IR	3.394733	0.002093
Bilophila	PCOS-IR	3.178074	0.003948
Collinsella	PCOS-IR	3.155460	0.002093
Bacteroides	PCOS-IR	4.339386	0.003948
Faecalitalea	PCOS-IR	3.075207	0.002093
Dubosiella	PCOS-IR	3.345446	0.010406
Rikenella	PCOS-IR	3.318530	0.041596
Prevotellaceae_NK3B31_group	PCOS-IR	3.368958	0.003948
Lachnospiraceae_UCG_010	PCOS-IR	3.333123	0.003346
Coriobacteriaceae_UCG_002	PCOS-IR	3.246760	0.003346
UCG_007	PCOS-IR	3.323758	0.032630
Fusobacterium	PCOS-IR	3.374035	0.003885

(15.12%), *Prevotella_9* (11.98%), *Agathobacter* (3.29%), and *[Eubacterium]_eligens_group* (2.68%) (Figure 5D). Compared with healthy controls, the PCOS-NIR group showed significantly reduced relative abundance in five genera and increased abundance in eight genera ($P < 0.05$, Figure 5E). In the PCOS-IR group, 17 genera exhibited significantly decreased abundance and two genera showed increased abundance ($P < 0.05$, Figure 5F). Furthermore, compared with the PCOS-NIR group, the PCOS-IR group had significantly reduced relative abundance of two species ($P < 0.05$, Figure 5G). These findings indicate that the gut microbiota composition of the PCOS-IR group differs significantly from that of the PCOS-NIR group. This supports the notion that PCOS-IR represents a distinct subtype that warrants independent investigation.

Characteristic Gut Microbiota in PCOS-IR Patients

Characteristic bacterial taxa were identified using LEfSe and LDA-based multi-level species discrimination. Compared with healthy females, *Fusobacterium* and *Faecalibaculum* were identified as characteristic genera in the PCOS-IR group (Figure 6A and B). In the PCOS-NIR group, *Tyzzerella, norank_o__Gastranaerophilales*, and *Morganella* were identified as characteristic genera when compared with healthy controls (Figure 6C and D). Notably, *Fusobacterium* was identified as a characteristic genus in both PCOS-IR patients and PCOS-IR rat models. This overlap suggests that *Fusobacterium* may serve as a potential pathogenic genus associated with PCOS-IR.

Gut Microbiota Transplantation Induces Endocrine Features of PCOS-IR in Rats

Compared with the control group, rats receiving gut microbiota transplantation from the PCOA-IR group exhibited significantly elevated levels of serum testosterone (T) ($P < 0.0001$), luteinizing hormone (LH) ($P < 0.001$), the LH/FSH ratio ($P < 0.001$), fasting insulin (FINS) ($P < 0.001$), and HOMA-IR index ($P < 0.001$). In contrast, no significant differences were observed in serum follicle-stimulating hormone (FSH) and fasting blood glucose (FBG) levels ($P > 0.05$) (Figure 7).

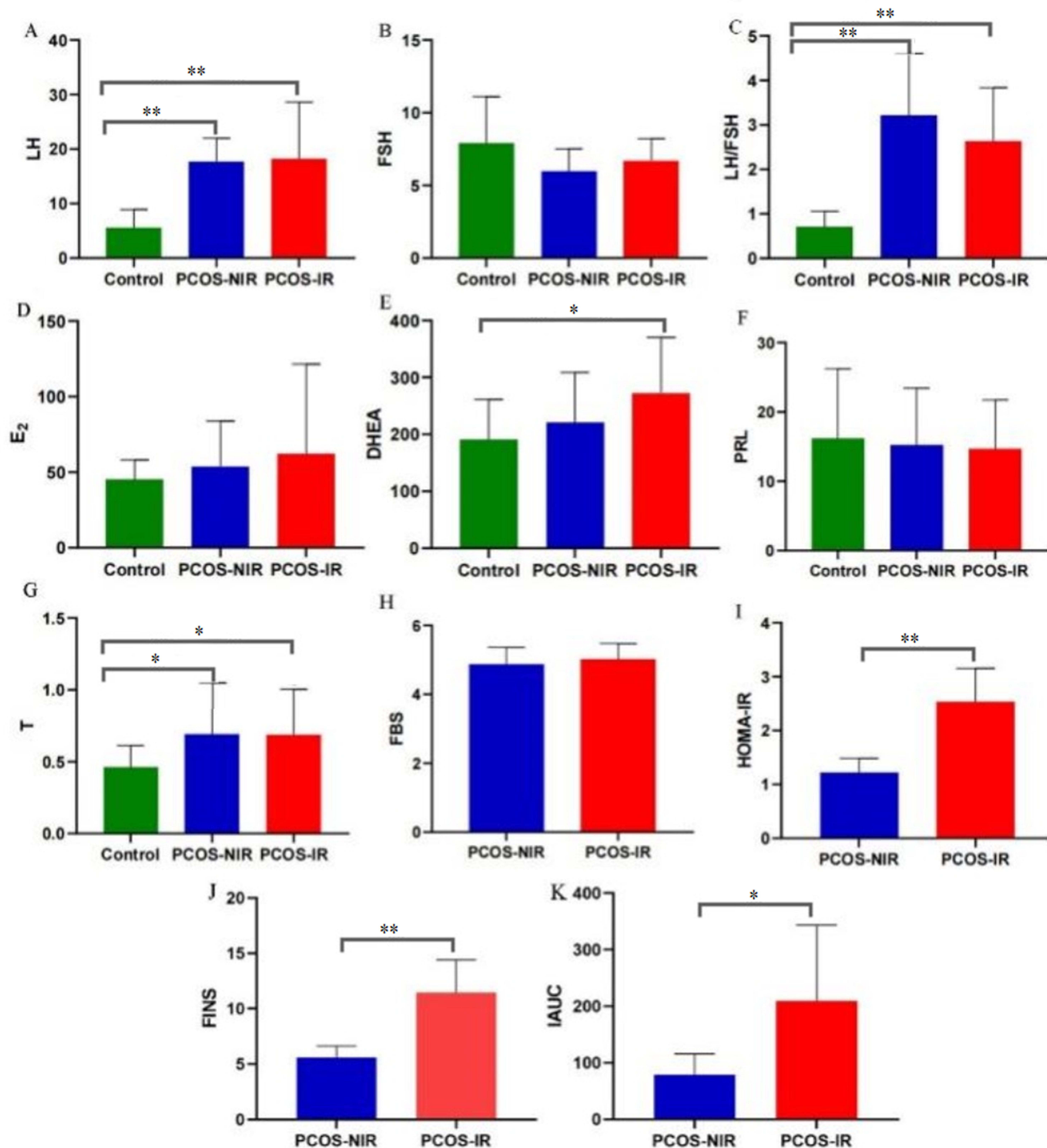

Figure 3 Hormone profiles and metabolic parameters in patients with PCOS and healthy controls. (**A**) LH level. (**B**) FSH level. (**C**) LH/FSH ratio. (**D**) Estradiol (E2) level. (**E**) DHEA level. (**F**) PRL level. (**G**) Testosterone (**T**) level. (**H**) FBG level. (**I**) Homeostatic model assessment of insulin resistance (HOMA-IR). (**J**) Fasting insulin (FINS). (**K**) Incremental area under the insulin curve (IAUC). Data are presented as the mean ± SD; *P < 0.05,**P < 0.01.

These results indicate that alterations in the gut microbiota could induce endocrine abnormalities characteristic of PCOS-IR, suggesting that gut microbiota may be a key contributing factor in the development of PCOS-IR.

Discussion

PCOS is one of the most common endocrine-metabolic disorders in women of reproductive age. Although PCOS is closely associated with long-term complications such as abnormalities of glucose and lipid metabolism and increased

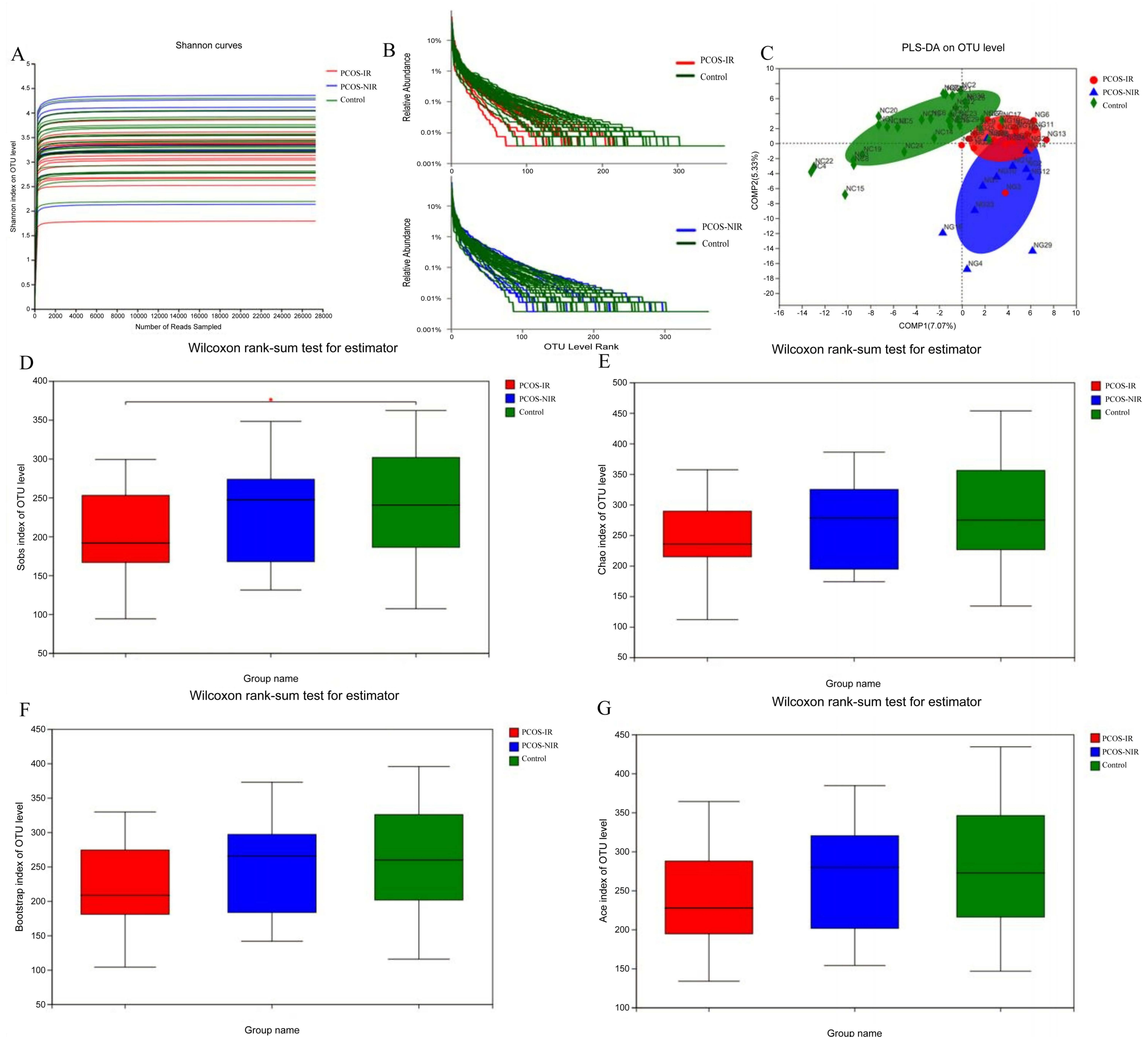

Figure 4 Gut microbiota diversity analysis in patients with PCOS and healthy controls. (**A**) Rarefaction curves. (**B**) Rank-abundance curves. (**C**) β-Diversity PCoA analysis. (**D**) Sobs index. (**E**) Chao index. (**F**) Bootstrap index. (**G**) ACE index.

cardiovascular risk, the multifactorial pathogenesis of PCOS remains incompletely elucidated, posing ongoing challenges in reproductive endocrinology. Notably, IR is a core pathological feature and the mechanistic role of IR in the development of PCOS has become an important research focus.[21] Our investigations of endocrine and metabolic parameters in both PCOS-IR patients and experimental models revealed that their serum androgen levels (T, DHEA) were significantly higher than those of the PCOS-only group, and insulin-related indexes (FINS, FBG, HOMA-IR, IAUC) were significantly higher than those of the IR-only group. These findings demonstrate that the degree of metabolic disturbance in PCOS-IR is more serious than that in either isolated PCOS or IR alone, which may be due to the interaction between the hyperandrogenic state and the hyperpancreatic state, forming a self-perpetuating pathogenic cycle.[22] Therefore, PCOS-IR warrants recognition and investigation as a distinct clinical entity rather than being studied solely through the lens of its individual components.

Emerging evidence underscores the gut microbiota as a critical modulator in PCOS pathogenesis. A prospective cohort study of 24 PCOS patients and 19 healthy controls with 16S rRNA gene sequencing found that endotoxemia triggered by increased intestinal permeability was significantly associated with markers of chronic inflammation, IR,

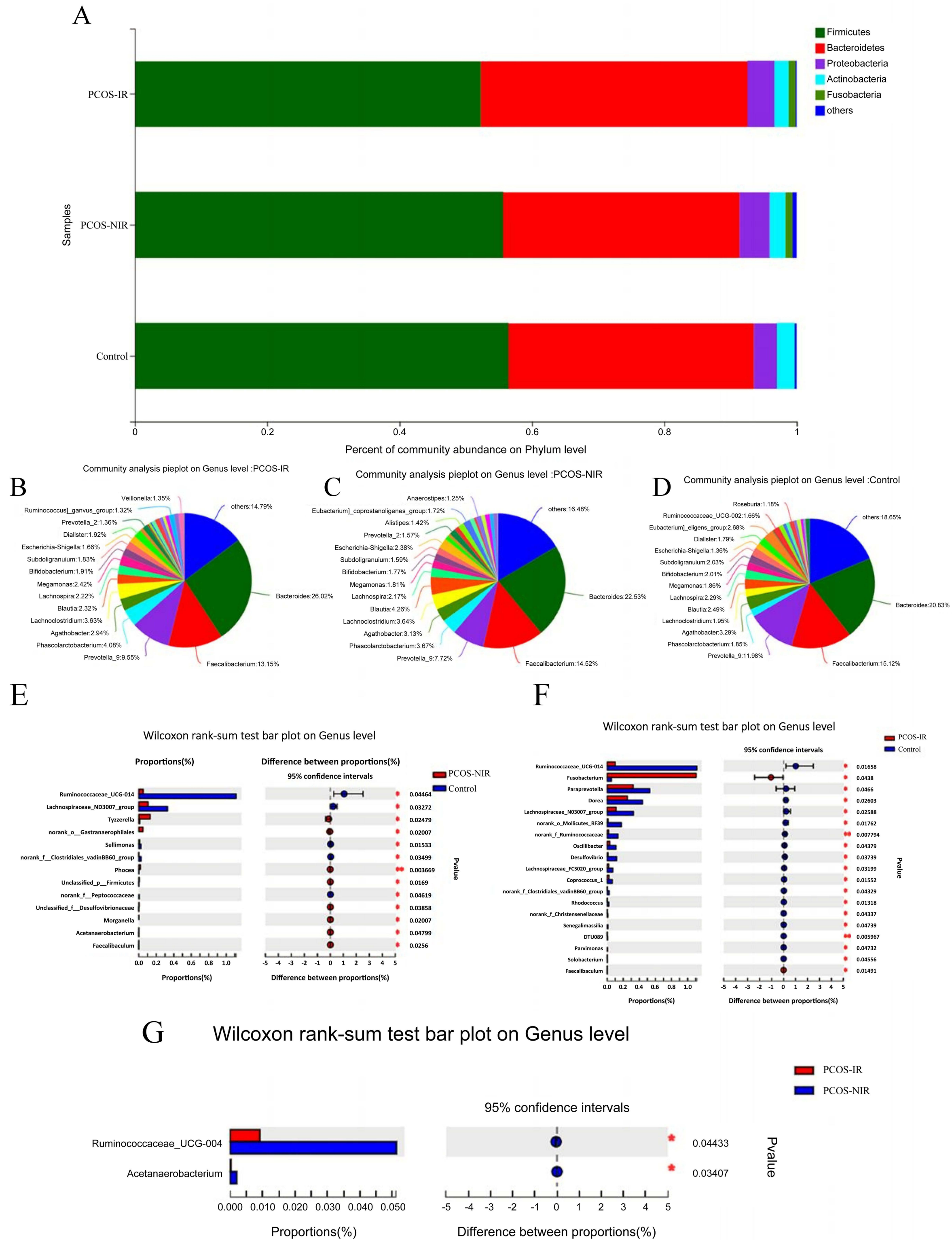

Figure 5 Taxonomic composition of gut microbiota in patients with PCOS and healthy controls. (**A**) Phylum-level composition of species among three groups. (**B-D**) Genus-level composition among three groups. (**E**) Significantly altered genera in PCOS-NIR vs controls. (**F**) Significantly altered genera in between PCOS-IR vs controls. (**G**) Significantly altered genera in PCOS-IR vs PCOS-NIR.

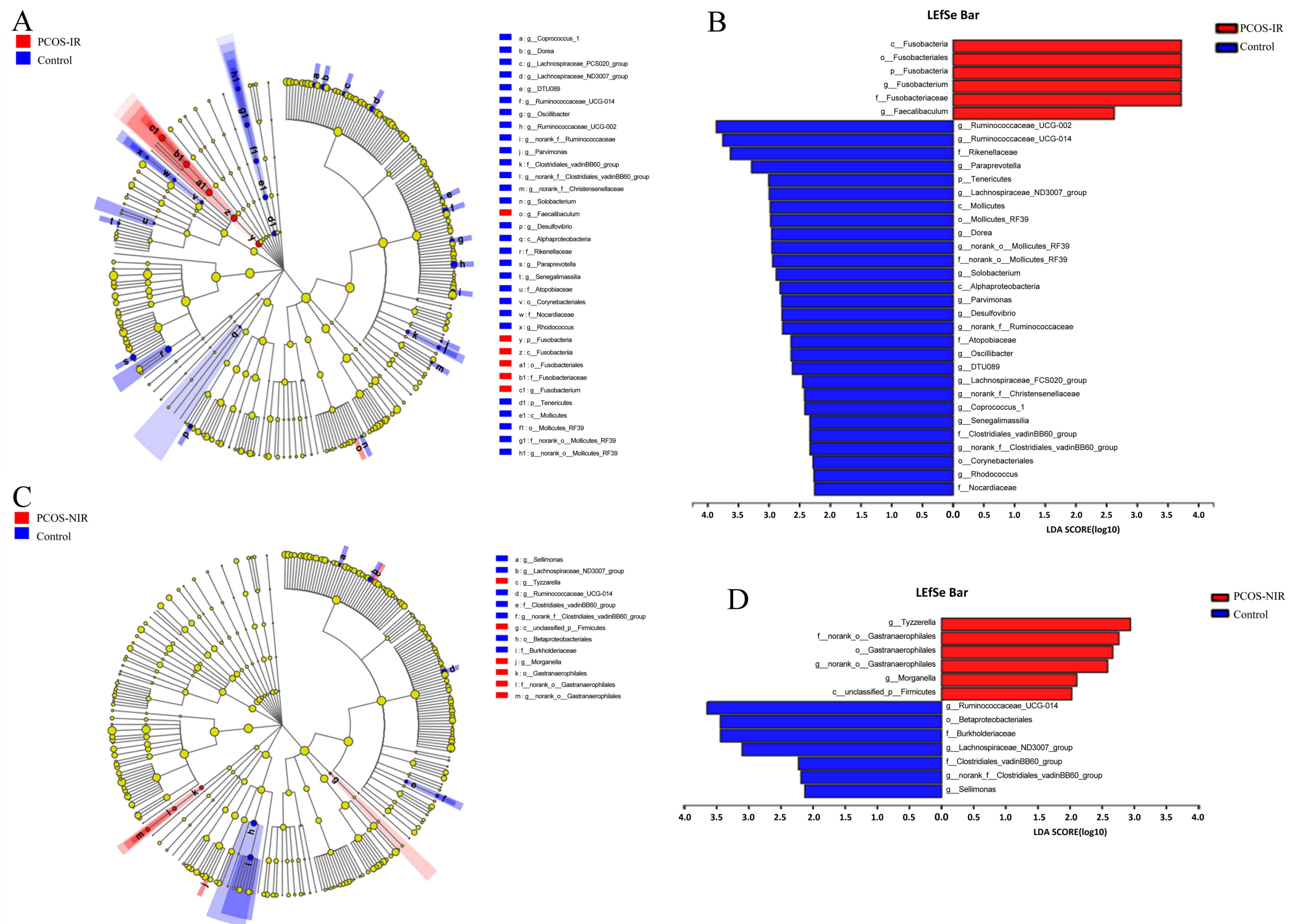

Figure 6 Characteristic gut microbiota in patients with PCOS and healthy controls. (**A**) LEfSe analysis of gut microbiota in PCOS-IR vs Controls. (**B**) LDA analysis of gut microbiota in PCOS-IR vs controls. (**C**) LEfSe analysis of gut microbiota in PCOS-NIR vs Controls. (**D**) LDA analysis of gut microbiota in PCOS-NIR vs controls.

adiposity, and hyperandrogenemia.[23] The abundance of genus *Bacteroides* is pathologically elevated in PCOS patients. This dysbiosis impairs enterohepatic bile acid circulation, reducing serum bile acid levels and consequently suppressing interleukin-22 secretion. This cascade ultimately contributes to IR and infertility.[24] Notably, PCOS subtypes exhibit distinct microbial signatures. Clinical analyses reveal *Coprococcus_2* as characteristic in obese PCOS patients, whereas *Lactococcus* dominates in non-obese subtypes.[25] These compositional divergences suggest subtype-specific microbiota modulate host homeostasis, driving heterogeneous clinical trajectories pf PCOS. In our study, both clinical PCOS-IR patients and experimental models demonstrate significant gut microbiota alterations versus healthy controls, indicating disrupted intestinal homeostasis, which is consistent with the current findings.[26,27] In addition, we observed that a significant reduction in α-diversity of gut microbiota in both PCOS-IR experimental model and clinical patients. The α-diversity serves as an important indicator for assessing the stability, functional redundancy, and community resilience of microbial communities. Higher α-diversity correlates strongly with metabolic homeostasis, immunomodulation and anti-inflammatory capacity in humans.[28] Studies have reported that the gut microbiota in a letrozole-induced PCOS-IR rat model showed a significant reduction in α-diversity and β-diversity restructuring,[29] which was validated in the DHEA/HFD murine models and clinical cohorts. Strikingly, despite different induction methods (letrozole vs DHEA/HFD), both models shared depletion of *Ruminococcaceae* - a major short-chain fatty acid-producing genus. This trans-model conservation implies reduced microbial diversity are a central pathological feature of PCOS-IR.

The causal relationship between gut microbiota dysbiosis and PCOS-IR pathogenesis remains incompletely defined. We performed fecal microbiota transplantation from PCOS-IR donor mice into healthy recipients. It found that recipient mice developed hallmark endocrine-metabolic features of PCOS-IR, such as elevated serum T levels, increased LH/FSH

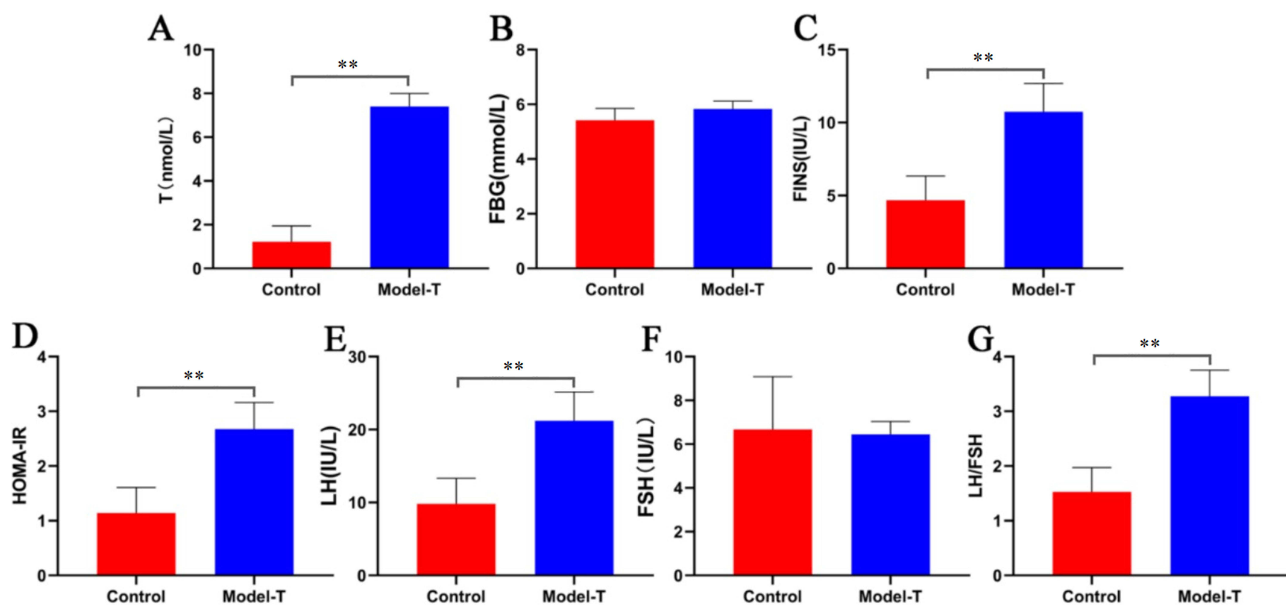

Figure 7 Gut microbiota transplantation from PCOS-IR rats induces endocrine and metabolic alterations in recipient rats. (**A**) Serum testosterone (T) level. (**B**) Luteinizing hormone (LH) level. (**C**) Fasting insulin (FINS) level. (**D**) HOMA-IR index. (**E**) LH level. (**F**) FSH level. (**G**) LH/FSH ratio. Data are presented as the mean ± SD; *P < 0.05,**P < 0.01.

ratio and IR. This demonstrates that gut microbiota dysbiosis is sufficient to drive PCOS-IR pathophysiology. The abundance of genus *Fusobacterium* and *Faecalibaculum* was significantly increased in both clinical PCOS-IR patients and murine models, suggesting that it may act as a potential pathogen of PCOS-IR. Notably, previous studies have shown a strong association between *Faecalibaculum* and metabolic disorders. Its abundance was significantly positively correlated with serum total cholesterol and low-density lipoprotein cholesterol levels in a high-fat diet-induced non-alcoholic fatty liver disease model.[30] Tectorigenin intervention reduced *Faecalibaculum* to improves hepatic lipid metabolism, further supporting its pathogenic role in metabolic diseases.[31] Moreover, the outer membrane of *Fusobacterium* is predominantly composed of lipopolysaccharide (LPS), which acts as a key virulence factor for gram-negative bacteria and drives inflammatory cascade responses through interactions with the host immune system.[32] Disruption of gut microbiota promotes *Fusobacterium* proliferation, elevating lipopolysaccharide (LPS) synthesis and release. This LPS leads to elevated intestinal permeability by directly downregulating tight junction proteins (eg, ZO-1 and occludin) in epithelial cells, which in turn triggers metabolic endotoxemia.[33] Circulating LPS subsequently binds lipopolysaccharide-binding protein, forming a ternary complex with soluble CD14 and myeloid differentiation factor 2. This complex activates the Toll-like receptor 4 signaling pathway, which amplifies systemic inflammation via the release of pro-inflammatory factors, and drives IR via inhibition of the insulin receptor substrate phosphorylation. The process stimulates ovarian theca cells to overproduce androgens, establishing a self-perpetuating vicious cycle and forming the PCOS-IR phenotype.[22,34] However, there is no direct evidence that *Fusobacterium* is associated with the regulation of sex hormone levels. Further investigation is required to determine whether it modulates gonadal axis function via intestinal microenvironment alterations.

Phylum-level analysis revealed significantly reduced abundance of *Tenericutes* in PCOS-IR patients versus healthy controls, aligning with prior reports of its depletion in PCOS cohorts.[23] The abundance of this phylum showed a significant negative correlation with HOMA-IR, suggesting its potential role in modulating insulin sensitivity through metabolic pathways.[35] Notably, no significant difference in the abundance of *Tenericutes*e was observed between PCOS-NIR patients and healthy women, indicating this dysbiotic signature is specific to the PCOS-IR phenotype rather than PCOS alone. This further supports PCOS-IR as a distinct pathological entity requiring targeted investigation. At the genus level, *Fusobacterium* was significantly enriched in the gut microbiota of PCOS-IR patients. *Fusobacterium nucleatum*, a representative species, has been reported to have altered abundance associated with both PCOS and IR.[36,37] This suggests that it could be a potential key

mediator in the development of PCOS-IR. Future studies will expand multicenter cohorts with longitudinal tracking to minimize inter-individual variability while employing: (1) fecal transplantation, (2) integrated metagenomic-metabolomic profiling, and (3) systematic analysis of core genera's metabolic pathways and their ovarian microenvironment modulation networks. This integrated strategy will establish the causal associations between gut microbiota and PCOS-IR, and enable precision microbiome-based interventions targeting core pathological features.

Conclusion

In this study, we delineated the metabolic and gut microbiota profiles of PCOS-IR through integrated animal and clinical investigations. Our results demonstrated that PCOS-IR is characterized by exacerbated hyperandrogenism and insulin resistance compared to PCOS or IR alone, coupled with a distinct gut microbial architecture that differentiates it from both healthy controls and the PCOS-NIR subtype, thereby substantiating its status as a discrete clinical entity. Crucially, *Fusobacterium* was identified as a conserved pathobiont enriched across species, highlighting its role as a key mechanistic driver and a promising non-invasive biomarker. These findings provide novel insights into the metabolic-microbial axis of PCOS-IR and establish a foundational framework for microbiota-targeted strategies. Future research should prioritize the validation of its diagnostic efficacy in large, multi-center cohorts, the definitive establishment of its causal role and underlying mechanisms using germ-free models, and the exploration of targeted interventions such as precision probiotics or prebiotics therapy to restore microbial homeostasis and ameliorate the PCOS-IR phenotype.

Data Sharing Statement

Data and materials supporting the results of this study can be obtained from the corresponding authors.

Ethical Approval and Informed Consent

The study was approved by the Ethical Review Committee of Chinese Registered Clinical Trials (Ethical Review No. CHiECRT-20160050), and each subject voluntarily signed an informed consent form before the start of the trial. This study was carried out in line with the guidelines of the Declaration of Helsinki, so as to secure the ethical treatment of every participant involved.

Besides the animal study protocol was approved by the Animal Ethics Committee of Shanghai Changhai Hospital (Ethical Approval No.: CHEC [A.E] 2022-011), and was conducted in accordance with the principles of ARRIVE guidelines.

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Author Contributions

Wenyi Liang: Writing-original draft, Methodology. Yanning Yao: Supervision, Data curation, Validation. Xinyang Ren: Writing-original draft, Investigation. Anran Xue: Software, Visualization. Mengcheng Cai: Methodology, Validation. Jin Yu: Methodology, Software. Chaoqin Yu: Software, Investigation. Ling Zhou: Conceptualization, Supervision. Dongxia Zhai: Conceptualization, Writing-review and editing, Funding acquisition. All authors took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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