Inulin and metformin ameliorate polycystic ovary syndrome via anti-inflammation and modulating gut microbiota in mice

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**Abstract.** Polycystic ovary syndrome (PCOS) represents an endocrine disorder, which is closely related with gut microbiota. Inulin, a kind of probiotics, has been proven to alleviate gut microbiota dysbiosis. Metformin, a biguanide agent, shows beneficial effects on chronic metabolic diseases. Our objective was to assess the effects and associated mechanisms of inulin and metformin on attenuation of PCOS in mice. Mice were divided into 4 groups: control group (CON), model group (MOD), inulin group (INU), metformin group (MET). The last three groups were fed 6 mg of dehydroepiandrosterone (DHEA) per 100 g body weight and 60% high-fat diet to generate mice model. After 21 days of intervention, mice were euthanized and associated indications were investigated. Body weight (BW) and testosterone (T) levels were significantly decreased, but estradiol (E2) levels were increased in INU or MET group, respectively. Ovary HE staining demonstrated that inulin or metformin ameliorated PCOS morphology. Inflammatory indicators from plasma and ovary including TNF-α, IL-6, and IL-17A were decreased in INU or MET group. Moreover, IL-10 in ovary of INU or MET group was increased. Sequencing and analysis of gut microbiota showed that compared to MOD group, *Bifidobacterium* was increased, but *Proteobacteria*, *Helicobacter* and *Parasutterella* were decreased in INU group. *Helicobacter* was decreased in MET group. Correlation analysis showed that gut microbiota was correlated with inflammatory factors. Our results revealed that inulin and metformin alleviated PCOS via anti-inflammation and modulating gut microbiota, which may contribute to potential clinical therapy for the disease.

**Key words:** Inulin, Metformin, Polycystic ovary syndrome (PCOS), Anti-inflammation, Gut microbiota
microbiota plays an important role in the progression and pathogenesis of PCOS [4-7]. In a rat model of letrozole-induced PCOS, the composition of gut microbiota was significantly altered inclusive of Lactobacillus, Ruminococcus, Clostridium and Prevotella. Further intervention with both Lactobacillus and fecal microbiota transplantation (FMT) of healthy rats showed beneficial for the treatment of PCOS [5]. The severity of insulin resistance and menstrual disorders as well as high levels of androgens in patients with PCOS are closely linked to alteration of gut microbiota [6]. A high fat diet and hyper-androgenic state induce LPS production from abnormal gut microbiota, causing systemic and ovarian inflammation in PCOS [7, 8].

Chronic inflammation also plays a crucial role in the pathogenesis of PCOS [7, 9, 10]. Multiple inflammatory indicators involving tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP), have remarkably increased in PCOS [9]. Moreover, occurrence and development of chronic inflammation in PCOS are closely linked with altered gut microbiota [10]. Ecological imbalance of gut microbiota leads to increased intestinal permeability. Intestinal-derived LPS enters into the circulation through the “intestine leaking” wall to induce systemic low-grade inflammation, leading to increased testosterone production of ovaries in the formation of PCOS [7, 11, 12].

Probiotic inulin, a plant-stored polysaccharide consisting of β-(2-1)-linked fructose residues, has been proven to alleviate diverse metabolic diseases via modulating gut microbiota. Inulin can alleviate endotoxemia and inflammation in obesity by elevation of the relative abundance of intestinal Aasteraceae, Bicenple cephalosporins and Bifidobacteria [13-15]. Metformin, a biguanide agent, reduces body weight (BW) and endotoxemia of patients by regulating the gut microbiota [16, 17]. However, the treatments of inulin in the inflammation and gut microbiota of PCOS remain poorly understood.

In the present study, we evaluated the effects and associated mechanisms of inulin and metformin on PCOS in mice. Our results may contribute to the understanding of complicated relationship among PCOS, gut microbiota, inflammation, and potential novel strategies for clinical applications.

Methods

Animal and diet

The experiments were approved by the Ethics Committee of General Hospital of Ningxia Medical University (No.2016-017). 21-day-old female C57BL/6J mice were purchased from Vital River Laboratory Animals Technology Co., Ltd., Beijing, China. All mice were housed in specialized cages in a temperature-controlled (25°C) and 12 h light/dark cycle room. All animal feed was obtained from TROPHIC Animal Feed High-tech Co., Ltd., Nantong, China. Inulin was purchased from Fengning Pingen High-tech Industrial Co., Ltd., Chengde, China. Trans-Dehydroandrosterone (DHEA) was obtained from Macklin Biochemical Co., Ltd., Shanghai, China.

Experimental design

All mice were randomly divided into 4 groups (10 mice/group). (a) Control group (CON), mice were fed the control diet and treated with same amount of normal saline (NS) as negative control; (b) Model group (MOD), mice were fed 6 mg DHEA per 100 g BW and 60% high-fat diet for 20 days with subsequently switched to the control diet, treated with the same amount of NS as the model control of the intervention group; (c) Inulin group (INU), mice were fed the same diet as MOD group and treated with inulin (0.05 g per 100 g BW) for 21 days while switched to a normal diet; (d) Metformin group (MET), mice were fed the same diet as MOD group but treated with metformin (1.9 g per 1,000 g BW) for 21 days while switched to a normal diet. 60% High-fat diet energy was consists of 19.4% protein, 20.6% carbohydrate and 60% fat. The control diet were the pair-fed controls for 60% high-fat diet. After 20 days of modeling and subsequent 21 days of treatments, all mice were euthanized and associated specimens were collected for further analysis.

Vaginal smear

Vaginal smears of all mice were collected every day at AM 9:00 to microscopically evaluate the estrous cycle by Wright-Giemsa staining. The observation period was lasted for 41 consecutive days (from the first day of modeling to the last day of treatment).

Determination of hormone and inflammatory factors

A series of plasma hormones including testosterone (T), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), estradiol (E2), and progesterone (PROG), as well as the levels of TNF-α, IL-6, IL-10, and IL-17A in ovaries, were determined using enzyme linked immunosorbent assay (ELISA). All of indicators from plasma hormone and ovarian inflammatory cytokines were measured according to the manufacturer’s instructions (Shanghai Baoman Biotechnology Co., Ltd, Shanghai, China). The interassay variability of each ELISA kit was <15%, and the intra-assay variability of each ELISA kit was <10%.
**Insulin tolerance tests**

The insulin tolerance tests (ITT) were performed immediately after 21 days of insulin and metformin intervention. After the mice were fasted for 6 hours, blood glucose of the mice was measured. After intraperitoneal injection of insulin (1 IU/kg body weight) immediately, blood glucose was measured at 30, 60 and 90 minutes, respectively. GraphPad Prism software was used to calculated the total area under curve of glucose response (AUC).

**Plasma LPS analysis**

Plasma LPS levels of each group were determined using limulus amebocyte lysate kit (Xiamen Bioendo Technology Co., Ltd. Xiamen, China). Briefly, 50 μL of diluted plasma (1:4 dilution with endotoxin-free water) was dispensed to each well in a 96-well plate. Then, 50 μL/well of the limulus amebocyte lysate reagent was added respectively. The microplate was incubated at 37°C for 30 min. After incubation, 100 μL of chromogenic substrate was warmed to 37°C was added for each well. Additional incubation was extended for 6 min at 37°C. The reaction was stopped by adding 100 μL of a 25% solution of glacial acetic acid. Optical density at 545 nm was used to measure microplate with the reader (Thermo Scientific, USA).

**Hematoxylin-eosin (HE) staining**

After sacrifice of mice, the ovaries were isolated and immediately fixed with formalin. The tissue was then dehydrated and embedded in paraffin. Paraffin-embedded sections were performed for HE staining. To evaluate ovary damage, every 10th section was mounted and air-dried at room temperature. It was particularly important that the DNA sample cannot be too dry. DNA sample was precipitated at –20°C after shaking up and down. Then the mixture was centrifuged according to previous centrifugation conditions. The obtained precipitate was washed twice with 1 mL 75% ethanol. Then the precipitate was blown dry on a clean bench or air-dried at room temperature. It was particularly important that the DNA sample cannot be too dry. DNA samples were dissolved in ddH₂O. If the sample was difficult to dissolve, it needed to be incubated at 55–60°C for 10 min. Finally, 1 μL of RNase A was added to the dissolved DNA sample, which was allowed to placed at 37°C for 15 min to obtain bacterial DNA. The extracted DNA was stored at –20°C until application.

**Sequencing and analysis of gut microbiota**

After six weeks of treatment, 5 mice from each group were randomly selected and placed in sterilized cages. Fresh feces of each mouse was respectively collected and immediately stored at –80°C for subsequent DNA extraction.

Extraction of bacterial DNA by Cetyltrimethylammonium Bromide (CTAB): the appropriate amount of lysozyme and sample were added to 1,000 μL CTAB lysate. The mixture was placed in a 65°C water bath and mixed by inversion several times in order to facilitate complete lysis of the sample. Next, phenol (PH 8.0), chloroform, and isoamyl alcohol were added to the supernatant after centrifugation so that the ratio of the three was 25:24:1. They were mixed by inversion and centrifuged at 12,000 × g for 10 min. In the same way, chloroform and isoamyl alcohol (24:1) were added to the obtained supernatant followed by centrifugation. The collected supernatant was added with isopropanol. The mixture was precipitated at –20°C after shaking up and down. Then the mixture was centrifuged again according to previous centrifugation conditions. The obtained precipitate was washed twice with 1 mL 75% ethanol. Then the precipitate was blown dry on a clean bench or air-dried at room temperature. It was particularly important that the DNA sample cannot be too dry. DNA samples were dissolved in ddH₂O. If the sample was difficult to dissolve, it needed to be incubated at 55–60°C for 10 min. Finally, 1 μL of RNase A was added to the dissolved DNA sample, which was allowed to placed at 37°C for 15 min to obtain bacterial DNA. The extracted DNA was stored at –20°C until application.

The DNA Sequences involving V3 and V4 of 16S rDNA hypervariable regions were amplified by Phusion® High-Fidelity PCR Master Mix with GC Buffer (New England Biolab, America) using the following primers (5’ to 3’): 341F-CCTAYGGGRBGASCAG, 806R-GGACTACNNGGTTACTAAT. The PCR product was analyzed and separated on a 2% agarose gel, which was purified using GeneJE Gel Recovery Kit (Thermo Scientific, America). The library was constructed using the TruSeq® DNA PCR-Free Sample Preparation Kit in order to Qubit quantitation and library detection. After passing the test, the library was sequenced using illu-
Vaginal cytology

Alterations in vaginal smears of different estrous cycles in mice were shown in Fig. 1. Vaginal cytology for the first 20 days showed that the estrous cycle of PCOS mice was disordered and stopped in the diestrus phase. Intriguingly, intervention of inulin or metformin alleviated the estrous cycle disorder of PCOS in mice.

Inulin and metformin attenuated ovarian histopathological injury and reduced plasma LPS levels

According to HE staining of ovarian tissue in each group, granulosa layers of ovarian tissue in MOD group showed reduced cystic degenerating follicles. Whereas inulin and metformin increased the formation of granulosa layers and corpora lutea, demonstrating that inulin or metformin contributed to improving the pathological changes of ovarian tissue (Fig. 2).

Plasma LPS was significantly increased in MOD group, compared with that in CON group \( (p = 0.0004) \). Meanwhile, the abnormal elevated LPS in PCOS was decreased in INU \( (p = 0.0023) \) or MET \( (p = 0.0006) \) group, suggesting that inulin and metformin possessed ability to attenuate LPS-induced endotoximia in PCOS (Fig. 3).

Inulin and metformin improved insulin resistance in PCOS

Insulin sensitivity was significantly lower in MOD group than that in CON group \( (p = 0.0011) \), as determined by area under the curve AUC glucose during insulin tolerance tests. Meanwhile, insulin sensitivity was improved in INU group and in MET group compared to MOD group \( (p = 0.0137) \).

**Table 1** Routine parameters of mice with diverse treatments in PCOS

<table>
<thead>
<tr>
<th>Measurements</th>
<th>CON</th>
<th>MOD</th>
<th>INU</th>
<th>MET</th>
<th>Unpaired ( t ) tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight, g</td>
<td>20.48 ± 0.34</td>
<td>22.72 ± 0.34</td>
<td>21.66 ± 0.22</td>
<td>20.77 ± 0.21</td>
<td>0.0002; 0.0181; 0.0001</td>
</tr>
<tr>
<td>T, ng/mL</td>
<td>520.7 ± 45.26</td>
<td>920.35 ± 56.38</td>
<td>741.56 ± 39.61</td>
<td>578.15 ± 50.46</td>
<td>0.0006; 0.0319; 0.0019</td>
</tr>
<tr>
<td>FSH, mIU/mL</td>
<td>53.81 ± 3.45</td>
<td>45.71 ± 2.81</td>
<td>51.94 ± 3.52</td>
<td>52.42 ± 3.26</td>
<td>0.1059; 0.2040; 0.1577</td>
</tr>
<tr>
<td>LH, mIU/mL</td>
<td>6.71 ± 0.70</td>
<td>8.47 ± 0.38</td>
<td>7.13 ± 0.51</td>
<td>6.80 ± 0.65</td>
<td>0.0599; 0.0697; 0.0576</td>
</tr>
<tr>
<td>PRL, ng/mL</td>
<td>37.34 ± 2.31</td>
<td>32.51 ± 0.46</td>
<td>33.89 ± 0.75</td>
<td>37.13 ± 2.22</td>
<td>0.0742; 0.1580; 0.0763</td>
</tr>
<tr>
<td>E2, μmol/L</td>
<td>51.71 ± 1.050</td>
<td>40.64 ± 3.39</td>
<td>50.42 ± 0.66</td>
<td>52.22 ± 0.51</td>
<td>0.0143; 0.0221; 0.0097</td>
</tr>
<tr>
<td>PROG, ng/mL</td>
<td>12.80 ± 0.70</td>
<td>10.04 ± 0.52</td>
<td>11.44 ± 0.68</td>
<td>11.63 ± 0.75</td>
<td>0.0137; 0.1420; 0.1207</td>
</tr>
</tbody>
</table>

a: CON vs. MOD, b: MOD vs. INU, c: MOD vs. MET

minia HiSeq 2500 platform by Beijing Novogene technology co., LTD., China.

**Statistical analysis**

All experimental data were analyzed using Prism (GraphPad Software Inc., CA, USA). The results were represented as mean ± SEM. According to the data conforming to a Gaussian distribution and equality of the variance, the independent \( t \)-tests were adopted for analysis of significant difference between two groups. In contrast, Mann-Whitney \( U \) tests were used to identify the significant difference in gut microbiota between two groups. Spearman’s correlation analysis was used to identify between gut microbiota and inflammatory indicators. Result were considered significant at \( p < 0.05 \).

**Results**

**Routine parameters of mice in each group**

There was no significant difference in body weights among diverse groups before treatment. However, after 21 days of intervention, BW in INU or MET group were lower than that in MOD group (INU vs. MOD, \( p = 0.0181 \); MET vs. MOD, \( p = 0.0001 \)), demonstrating that inulin can reduce BWs in PCOS, but not as effective as metformin. T levels in MOD group were significantly higher than that in CON group \( (p = 0.0006) \). T levels in INU and MET groups were significantly reduced, compared to MOD group (INU vs. MOD, \( p = 0.0319 \); MET vs. MOD, \( p = 0.0019 \)). Compared to CON group, E2 levels in MOD group were significantly lower \( (p = 0.0143) \). INU or MET treatment promoted the increase of E2 levels compared with that in MOD group (INU vs. MOD, \( p = 0.0221 \); MET vs. MOD, \( p = 0.0097 \)). PROG levels in MOD group were significantly decreased, compared with CON group \( (p = 0.0137) \). In addition, there were no significant difference among treated and control groups in FSH, LH, PRL, and PROG levels (Table 1).
Inulin and metformin ameliorate PCOS

We found a significant increase of plasma and ovary TNF-α (plasma: $p = 0.0002$, ovary: $p = 0.0087$), IL-6 (plasma: $p = 0.0088$, ovary: $p = 0.0068$), IL-17A (plasma: $p < 0.0001$, ovary: $p = 0.0150$), as well as a decrease of plasma and ovary IL-10 (plasma: $p = 0.0365$, ovary: $p = 0.0055$) in MOD group than that in CON group. In contrast, the IL-6 levels in INU (plasma: $p = 0.0362$, ovary: $p = 0.0235$) or MET group (plasma: $p = 0.0029$, ovary: $p = 0.0171$) were significantly reduced, compared to MOD group. Similarly, TNF-α concentra-

**Fig. 1** Changes in estrous cycle of mice of each group. a: Vaginal smears of proestrus stage. b: Vaginal smears of estrus stage. c: Vaginal smears of metestrus stage. d: Vaginal smears of diestrus stage. e: Representative estrous cycle of each group, 1: diestrus stage, 2: proestrus stage, 3: estrus stage, 4: metestrus stage. Original magnification, $\times 100$.

**Fig. 2** Effects of inulin or metformin on ovarian tissues in PCOS with Hematoxylin-eosin (H&E) staining. a: CON, b: MOD, c: INU, d: MET. The boxed areas in a, b, c, d ($40\times$) was shown changes in cystic follicles, with higher magnification ($200\times$) in e, f, g, h. Changes in the corpus luteum (i and j). TCL: theca cell layer, GCL: granular cell layer, L: luteum. Original magnification ($40\times$).

Inulin and metformin decreased the levels of inflammatory cytokines of plasma and ovary in PCOS
Inulin and metformin modulated gut microbiota in PCOS

Accumulating evidences have demonstrated that the gut microbiota plays a crucial role in the occurrence and development of PCOS [4-7]. In order to investigate whether amelioration of inulin in PCOS was closely related to the alterations in the composition of gut microbiota, we conducted a metagenomic analysis of mice feces. Rarefaction curves were adopted to evaluate the rationality of the sequencing data (Supplementary Fig. 4).

Fig. 3 Effects of inulin or metformin on plasma lipopolysaccharide (LPS) levels. Data are displayed as mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001.

Fig. 4 Insulin tolerance test. a. Serum glucose levels, b. AUC of glucose. *p < 0.05, **p < 0.01.

Fig. 5 Detection of plasma or ovary inflammatory cytokine levels in diverse groups of mice. a–d: Plasma in mice of each group was collected respectively for detection of TNF-α (a), IL-6 (b), IL-10 (c), and IL-17A (d). e–h: Ovary in mice of each group was collected respectively for detection of TNF-α (e), IL-6 (f), IL-10 (g), and IL-17A (h). Data were expressed as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, NS, not significant.
S1), which tended to be flat when the sequences number increases to 8,429, showing that the amount of sequencing data were reasonable.

The whole composition bacterial community was analyzed using Unweighted-Unifrac of Pcoa (Fig. 6) and Weighted-Unifrac of NMDS (Supplementary Fig. S2). PCoA results showed a significant difference of species in fecal samples among the CON, MET and INU groups indicating that inulin altered gut microbiota of PCOS. In addition, NMDS analysis presented similar results.

At phylum level (Fig. 7a), we analyzed top six of phyla and found that **Firmicutes** and **Bacteroidetes** were the most common phylum in diverse groups. The proportion of **Bacteroidetes** was increased in CON (p = 0.0317), INU (p = 0.5317) or MET (p = 0.0952) group, compared to MOD group. There’s no alteration of **Firmicutes** in CON (p = 0.1508), INU (p = 0.9444) or MET (p = 0.8016) group, compared to MOD group. In addition, we found that **Proteobacteria** was significantly reduced in CON (p = 0.0079), INU group (p = 0.0317) or MET (p = 0.0556) group, compared with MOD group (Fig. 7c).

At the genus level (Fig. 7b), we analyzed top forty bacteria and found that **Bifidobacterium** showed a negative correlation with TNF-α (p = 0.0006, Fig. 8a), IL-6 (p = 0.0040, Fig. 8b) or IL-17A (p = 0.0120, Fig. 8c), respectively. **Helicobacter** abundance was positively correlated with plasma inflammatory cytokines (TNF-α, p = 0.0184; IL-6, p = 0.0080; IL-17A, p = 0.0156, Fig. 8d–f). **Bifidobacterium** showed a negative correlation with TNF-α (p = 0.0004, Fig. 9a), IL-6 (p = 0.0150, Fig. 9c) and IL-17A (p = 0.0053, Fig. 9b). **Parasutterella** abundance was positively correlated with ovary pro-inflammatory cytokines including TNF-α, IL-6, IL-17A (Fig. 9b: TNF-α, p = 0.0278; Fig. 9d: IL-6, p = 0.0020; Fig. 9i: IL-17A, p = 0.0132), but negatively correlated with ovary anti-inflammatory cytokine IL-10 (p = 0.0041, Fig. 9f). Meanwhile, **Helicobacter** abundance was positively correlated with IL-6 (p = 0.0022, Fig. 9e) and negatively correlated with IL-10 (p = 0.0092, Fig. 9g).

**Discussion**

In the present study, we investigated the efficacy of dietary inulin treatment in PCOS. By in vivo 21-day treatment of PCOS in mice, our study indicated that inulin and metformin showed more effective in the decrease of ovarian damage, suggesting that the intervention displayed preventive and therapeutic potential. Our further study revealed that amelioration of PCOS phenotype including hormonal disturbance may closely associated
with the reduction of inflammation and the alteration of
gut microbiota.

In this paper, dietary inulin and metformin were dem‐
onstrated to possess capacity to alleviate PCOS through‐
out a series of routine indicators including pathological
examination, reduced BWs, as well as rectification of
abnormal hormones (T and E2), which were consistent
with previous studies [19-24].

Abnormal level of LPS derived from gut microbiota
dysbiosis dramatically affects systemic metabolism
through changing the intestinal epithelial barrier, leading
to bacterial endotoxin into the blood, thereby upregulat‐
ing pro-inflammatory signals. LPS induces inflammatory
immune response to ultimately lead to chronic ovarian
inflammation [3, 7]. In our study, plasma LPS was sig‐
nificantly decreased in INU and MET groups, suggesting
that inulin and metformin may reduce intestinal per‐
meability and decrease translocation of LPS from the
intestine to the liver and blood circulation, eventually
contribute to the reduction of ovarian inflammation.

As critical pro-inflammatory indicators, abnormal high
levels of plasma TNF-α and IL-6 have been demonstrated
to promote the proliferation, differentiation and
maturation of follicles in PCOS patients [25-27]. Altera‐
tion of TNF-α in FF levels is associated with poor-
quality oocytes undergoing IVF which decreased rates of
fertilization, embryonic development, and pregnancy
outcome [25]. IL-6 can reduce intra-follicular estradiol
concentration, fertility and fertilizing capacity [26]. Stud‐
ies have shown that pro-inflammatory cytokine (such as
TNF-α and IL-6) in PCOS are significantly increased
[27]. TNF-α, IL-6 and IL-10 play important role in the
development of inflammation and oxidative stress [27].
Serum IL-17A, TNF-α and IL-6 are closely related to the
number of meiotic I (MI) oocytes in PCOS patients [28].
In this study, we found that the levels of TNF-α, IL-6,
and IL-17A of plasma and ovary in INU and MET
groups were significantly reduced, suggesting that inulin

Fig. 7  Relative abundance of microbial species at phylum and genus levels in mice feces. a: The phylum analysis; b: The genus analysis;
c: The significant analysis in phylum levels. d-f: The significant analysis in genus levels. * p < 0.05, ** p < 0.01, NS, no
significance.
and metformin may alleviate ovarian inflammation via anti-inflammatory cytokines. IL-10, a crucial anti-inflammatory cytokine, showed significant difference in ovary between treated groups and MOD group, suggesting that inulin and metformin may reduced PCOS inflammation partially via regulating IL-10 expression [29, 30].

Dysbiosis of gut microbiota is closely related with the pathogenesis of PCOS [4, 5, 31]. In our study, the proportion of Bacteroidetes and Firmicutes still remained predominant in all groups at phylum level, which was consistent with previous studies [31, 32]. Proteobacteria, a pathogenic bacterium that produces LPS, was significantly decreased in INU group compared to MOD group, indicating that reduction of LPS treated by inulin may due to inhibition of Proteobacteria proliferation [33]. However, the exact role of Proteobacteria in PCOS needs to be further investigated.

Accumulating studies have demonstrated that inulin may increase proportions of Bifidobacterium and Lactobacillus [32, 34]. Probiotic Bifidobacterium possessed capable of suppression of pro-inflammatory cytokine synthesis [35-37]. We also found that inulin elevated this beneficial Bifidobacterium, revealing that inulin may ameliorate PCOS by improving the proportion of Bifidobacterium. In addition, recent studies have showed the possible existence of a correlation between infection with Helicobacter and metabolic syndrome [37]. Furthermore, we found that the decreased number of Helicobacter after treatment with inulin or metformin showed a significantly positive correlation with pro-inflammatory factors (TNF-α, IL-6, and IL-17A). But the physiological role of Helicobacter in metabolic diseases, especially PCOS, is poorly understood. Pathogenic Parasutterella showed a significant decrease after intervention with inulin in our study. Taken together, in the present study, several differential candidates of gut bacteria were identified after treatment with inulin or metformin, of which, the alterations may contribute to improving gut dysbiosis and restoring the microbiota balance. But complicated interactions among above different bacteria and their metabolites with mucosal immune cells (NK cells, regulatory T lymphocytes, γδT cells, marrow derived suppressive cells and dendritic cells) and metabolism still

Fig. 8  Correlation analysis between relative abundance of gut microbiota and plasma inflammatory cytokines. a: Bifidobacterium and TNF-α; b: Bifidobacterium and IL-6; c: Bifidobacterium and IL-17A; d: Helicobacter and TNF-α; e: Helicobacter and IL-6; f: Helicobacter and IL-17A.
remain largely unknown and need to be further investigated.

Conclusions

The present study highlighted that inulin and metformin may ameliorate PCOS via anti-inflammation and modulating gut microbiota in mice, which may potentially serve as an intervention for the prevention and treatment of PCOS.

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Availability of Data and Materials

The datasets used and analyzed in the current study are available from the corresponding author on reasonable request.

Authors’ Contributions

WH, XJ and DXY designed and wrote the paper. LXR, LP, SLP, YXL, ZLL, WZ, DYP, ZL, LH, and ZXX performed research. All authors have read and
approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests. The funding body plays no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Consent for Publication

Not applicable.

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