

Exploration of the Relationship Between Gut Microbiota and Polycystic Ovary Syndrome (PCOS): a Review

Erforschung der Beziehung zwischen intestinaler Mikrobiota und polyzystischem Ovarsyndrom (PCOS): eine Überblicksdarstellung




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ABSTRACT

Polycystic ovary syndrome (PCOS) is an endocrine and metabolic syndrome (MS) with a complex etiology, and its pathogenesis is not yet clear. In recent years, the correlation between gut microbiota (GM) and metabolic disease has become a hot topic in research, leading to a number of new

ideas about the etiology and pathological mechanisms of PCOS. The literature shows that GM can cause insulin resistance, hyperandrogenism, chronic inflammation and metabolic syndrome (obesity, diabetes) and may contribute to the development of PCOS by influencing energy absorption, the pathways of short chain fatty acids (SCFA), lipopolysaccharides, choline and bile acids, intestinal permeability and the brain–gut axis. As part of the treatment of PCOS, fecal microbiota transplantation, supplementation with prebiotics and traditional Chinese medicine can be used to regulate GM and treat disorders. This article reviews possible mechanisms and treatment options for PCOS, based on methods which target the GM, and offers new ideas for the treatment of PCOS.

ZUSAMMENFASSUNG

Das polyzystische Ovarsyndrom (PCOS) ist ein endokrines und metabolisches Syndrom (MS) mit einer komplexen Ätiologie. Die Pathogenese von PCOS ist noch immer ungeklärt. In den letzten Jahren hat sich die Forschung verstärkt auf die Interaktion zwischen intestinaler Mikrobiota und Stoffwechselerkrankungen konzentriert, was zu einigen neuen Ideen über die Ätiologie und die pathologischen Mechanismen von PCOS führte. In der Literatur wird berichtet, dass die intestinale Mikroflora Insulinresistenz, Hyperandrogenämie, chronische Entzündungen und metabolisches Syndrom (Adipositas, Diabetes) verursachen kann und durch ihre Auswirkungen auf Energieaufnahme, kurzkettige Fettsäuren, Lipopolysaccharide, Cholin und Gallensäuren, die Permeabilität der Darmschleimhaut sowie die Darm-Hirn-Achse möglicherweise zur Entwicklung von PCOS beitragen kann. Für die Behandlung von PCOS können fäkale Mikrobiomtransfers, eine Supplementierung mit Präbiotika und traditionelle chinesische Medizin zur Regulierung der Darmflora und Behandlung von Störungen eingesetzt werden. Dieser Artikel gibt eine Überblicksdarstellung möglicher Mechanismen und Behandlungsoptionen für PCOS, basierend auf Methoden, die auf eine Änderung der intestinalen Mikrobiota abzielen, und bietet neue Ideen für die Behandlung von PCOS.

Introduction

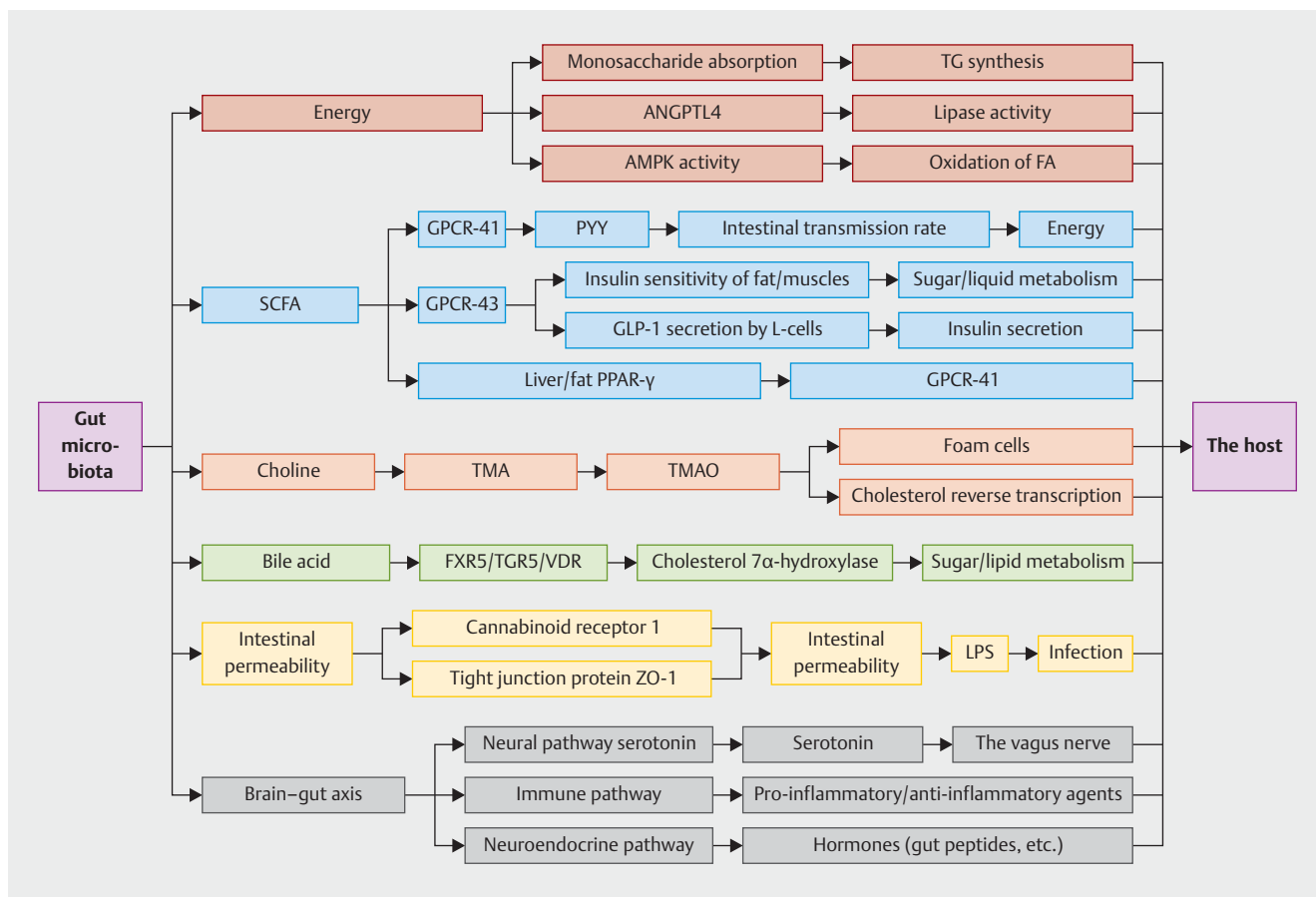
Polycystic ovary syndrome (PCOS) is a common gynecological endocrine disease, with a prevalence of between 8 and 13% depending on the population studied and the definitions used [1]. The main manifestations are irregular menstrual cycles, infrequent ovulation or anovulation, hyperandrogenemia and polycystic ovarian changes. Several metabolic disorders have been linked to PCOS, including insulin resistance (IR), diabetes and obesity [2, 3]. About 50% of PCOS patients present with varying degrees of IR [4], which in turn serves as a link to other endocrine disorders [5]. In recent years, a new consensus on the diagnosis and treatment of PCOS has emerged, and the idea that metabolic abnormalities and other complications of PCOS should be included in the diagnosis and treatment of PCOS has been emphasized by several reproductive endocrine institutions, including the National Institutes of Health (NIH), the American Endocrine Society, the European Endocrine Society and others [6, 7]. The diagnosis, treatment and research scope of PCOS has gone beyond reproduction-related fields and now also involves metabolic disease, cardiovascular disease, tumors and other aspects. It is very important to systematically analyze changes to metabolic states when exploring the pathogenesis of PCOS. Modern research shows that GM disorders are closely related to the occurrence and development of metabolic diseases. In the past two decades, some studies have reported a relationship between GM and metabolic syndrome (MS). Larsen et al. [8] studied the changes in the GM composition of patients with type 2 diabetes and found that the quantity of Firmicutes and Clostridium was significantly decreased compared with the control group. Tumbaugh et al. [9] transplanted the GM of obese mice into sterile mice and found that body fat and IR increased significantly after the transplant, while probiotics, such as Bifidobacteria and Lactobacillus, which help to reduce obesity, cholesterol, and weight, declined [10]. These experiments set up a premise which prompted us to investigate the connection between GM and PCOS.

The human microbiome refers to all microorganisms parasitic on the skin and the digestive, respiratory and urogenital tracts, with the gut flora possessing the largest number of microorganisms and most extensive functions [11]. Although GM are only present in the digestive tract, their role goes far beyond regulating the digestive system. A normal GM provides nutrition to the host through energy transport and material exchanges; a delicate balance is established between GM and the host as part of the long-term coexistence process which enables the host to own acquired immunity and establish immune barriers [12]. GM are also widely involved in the storage of fat, the generation of blood vessels, regulation of the nervous system and bone density, and participate in drug metabolism [13, 14]. Researchers have gradually begun to establish a relationship between GM disorders and the occurrence of diseases. Numerous studies have investigated the relationship between GM disorders and PCOS [15]. Sun et al. [16] discovered that the level of serum dimethylamine was significantly increased in PCOS patients, suggesting that GM activity was higher in the group of women with PCOS. Zhang et al. [17] confirmed that GM were involved in the development of IR and menstrual disorders in PCOS patients by affecting intestinal wall permeability. All the above studies have confirmed the close correlation between GM

and PCOS, and this article aims to systematically discuss and summarize the connection between the two and talk about the treatment which targets at the GM, as shown in ► Fig. 1, in hope of offering new ideas for the treatment of PCOS. Exploring the specific mechanisms is of great clinical significance.

Overview of GM

The microecosystem of the human GM is diverse and dynamic. It consists of about 1000–1500 species of bacteria, around 100 trillion bacteria in all, which colonize the human intestinal tract. This figure equates to nearly 10 times the number of human somatic cells, and the number of genes in their genome is 150 times that of their host [18]. Humans inherit roughly 26600 coding genes from their parents, far fewer than other species. This is in sharp contrast to the complexity of humans in terms of their biology, emotions and behavior, also known as the genome-complexity conundrum. Studies have shown that in the human transcriptome, approximately 4×10^6 mRNAs come from the GM [19], which has also been called “the second genome” [20]. During their long-term symbiosis, the gene complex of human and GM forms and communications at the gene level functionally manifest through extensive participation in various physiological activities, making human physiological activities far more complex than those of other species [21]. Colonization by GM occurs at birth, when babies acquire complex bacteria as they pass through the birth canal. Therefore, the infant GM composition shares obvious maternal characteristics for a time after birth, gradually changing to become more individualized after about one year [22]. When healthy, each person has at least 160 kinds of dominant GM [23]. Although there are individual differences in terms of GM species, the dominant species are basically the same. More than 90% are Firmicutes and Bacteroides, but typically all humans harbor Bacteroides, Prevotella, Porphyromonas, Clostridium, and Eubacterium. Other less abundantly present bacteria include actinomycetes, proteobacteria and methanogenic archaea [24]. GM are divided into three categories according to their interaction with the host: beneficial bacteria, conditioned pathogens and harmful bacteria. Beneficial bacteria include Lactobacilli and Bifidobacterium, which can inhibit harmful microorganisms, enhance immunity, promote absorption, synthesize vitamins, inhibit tumors, reduce infection, and relieve allergic reactions. Harmful bacteria include Staphylococcus, Salmonella and Campylobacter, which can produce toxins, increase the incidence of cancer and induce infections. Opportunistic pathogens include Enterobacter, Escherichia, and Bacteroides [25]. Under normal circumstances, the bacteria in the GM maintain a delicate dynamic balance to prevent the development of various diseases. A range of diverse factors can lead to changes in GM, including age, diet, and the use of antibiotics. With increasing age, the beneficial bacteria in the intestinal tract decrease, while the toxins produced by harmful bacteria increase, leading to accelerated aging [26]. Diet is one of the most influential factors for GM. Zhang et al. [27] reported on the destruction of GM homeostasis caused by a high-fat diet. It has also been found that abnormal gut motility or immune dysfunction in the intestinal tract and the taking of antibiotics, immunosuppressants or anti-tumor drugs has an adverse effect on GM and may ultimately lead to disease.



► **Fig. 1** Regulation of human health by gut microbiota. TG: triglycerides; ANGPTL4: angiotensin-like 4; AMPK: 5' adenosine monophosphate-activated protein kinase; FA: fatty acids; ZO-1: zonula occludens-1; LPS: lipopolysaccharides; GPCR-41/43: G-protein-coupled receptor 41/43; PYY: peptide tyrosine tyrosine; GLP-1: glucagon-like peptide-1; PPAR-γ: peroxisome proliferator-activated receptor γ; TMA: trimethylamine; TMAO: trimethylamine oxide; FXR5: farnesoid X receptor 5; VDR: vitamin D receptor; TGR5: G-protein-coupled receptor for bile acids.

Correlation between GM and PCOS

PCOS is a complex endocrine and metabolic disease. Several PCOS-related genes have been found to be associated with carbohydrate metabolism and the steroid synthesis pathway [28], suggesting an important correlation between metabolic factors and the pathological mechanism of PCOS. GM are involved in various metabolic activities, and accordingly GM are closely associated with the pathogenesis and clinical manifestations of PCOS. Torres et al. [29] studied 73 PCOS patients and found that compared to normal women, patients with PCOS had fewer types of GM, and that this trend was related to an increase in androgens. To study the effect of GM on the host's PCOS phenotype, feces from healthy women and from patients with PCOS were respectively transplanted into two groups of mice by oral lavage. Compared with control mice, the mice transplanted with fecal samples from patients with PCOS developed IR, had an increased number of ovarian cyst-like follicles, a decreased corpus luteum, and higher levels of testosterone and luteinizing hormone. Fertility tests were carried out, in which the number of pups in the first litters after mating were counted. Mice given fecal transplants from women with PCOS produced fewer pups than healthy control mice [30].

Lindheim et al. [31] found that compared with the control group, the relative abundance of *Tenericutes* ML615J-28 and *Bacteroidetes* S24-7 was lower in PCOS patients. Another study found that during the construction of a mice model with letrozole-induced PCOS, the total genus of GM decreased but the number of *Firmicutes* (which are closely related to obesity and MS) increased [32]. From the above experiments, we can infer that GM may be involved in the occurrence and development of PCOS. This could offer a new therapeutic strategy for the clinical diagnosis and treatment of PCOS. Of course, the exact mechanism of the GM in PCOS needs to be supported by further evidence.

The Pathway Mechanism of GM Leading to PCOS

Energy absorption

GM degrades carbohydrates into simple sugars and then ferments them into hydrogen, CO₂, CH₄, and short chain fatty acids (SCFAs) to provide energy to the host [33]. Obese individuals take more energy from food, which then aggravates the symptoms of obe-

sity, creating a vicious circle. This phenomenon is associated with a disordered GM. Cani et al. [34] found that a GM disorder in obese patients may increase the host's energy intake by converting substances that cannot be utilized into substances that can be absorbed. SCFAs produced by the fermentation of carbohydrates by the GM can stimulate the release of peptide YY (PYY) in the ileum and colon. PYY inhibits intestinal peristalsis, decreases the secretion of pancreas and promotes the absorption of energy in the intestinal tract [35,36]. Turnbaugh et al. [37] analyzed the GM of lean and obese mice with the same diet and found that obese mice had more Firmicutes and fewer Bacteroides than lean mice, and that this distribution of GM was able to absorb energy better. However, after wild aseptic mice were given a GM transplant from obese mice, their energy intake increased and they presented with symptoms of obesity. This study confirmed the relationship between GM, energy metabolism and obesity. Data showed that more than half of PCOS patients were overweight or obese [38,39]. Moreover, researchers have confirmed that the richness and phylogenetic diversity of GM species in patients with PCOS is significantly reduced compared with that of control groups [40]. Multiple meta-analyses have confirmed that this is closely related to the obesity phenotype [41,42]. To further investigate the relationship between obesity, PCOS and GM, one study compared groups of obese women with PCOS, non-obese women with PCOS, obese women without PCOS and normal controls. The results showed differences in β diversity between obese and non-obese women with PCOS [43], but these changes were not observed in non-obese and obese control women. It was speculated that GM may have a specific effect on obesity and energy metabolism in the context of PCOS. From the above experiments, we can conclude that GM disorders can accelerate the process of PCOS by affecting energy absorption.

Short-chain fatty acid (SCFA) metabolism pathway

Gut microbiota decompose organic materials to produce three major types of SCFAs, including acetate, propionate and butyrate. Den Besten et al. [44] found that SCFAs can activate peroxisome proliferator-activated receptor gamma (PPAR- γ) in liver and muscle, thereby regulating glucose uptake and fatty acid oxidation. GM can also affect insulin sensitivity through inflammatory responses mediated by branched-chain amino acids (BCAAs) [45] and SCFAs [46]. Zheng et al. [47] found in three prospective cohort studies that the more BCAAs people ingested, the more likely they were to develop type 2 diabetes [48]. One possible mechanism could be 3-hydroxyisobutyrate (3-HIB), a catabolite of valine, which stimulates the uptake of fatty acids in muscle tissue, leading to fat accumulation and IR [49]. Pedersen et al. [50] revealed the relationship between GM and BCAA metabolism. It was found that a type of *Prevotella* in the human intestinal tract was involved in the synthesis of BCAAs. Mice fed a high-fat diet had higher levels of BCAAs in their blood after two weeks, and after three weeks they developed various levels of IR. This animal model exactly simulates the metabolic state of PCOS patients who are obese or who consume a high-fat diet over the long term. SCFAs can combine with free fatty acid receptor-2 (FFAR-2) and FFAR-3 expressed in tissues such as the intestines, fat, skeletal muscle, liver, the immune system and the nervous system to in-

hibit the secretion of appetite-stimulating hormone (ASH) by the gastric mucosa [51]. ASH not only inhibits the secretion of GnRH and of sex hormones [52] but also blocks the transformation of sex hormones. Docanto et al. [53] found that ASH can inhibit the expression of aromatase CYP19A1 in adipo-stromal cells and prevent the transformation of androgen into estrogen. The lower the level of ASH, the higher the androgen level [54]. A meta-analysis indicated that ASH levels of PCOS patients were lower than those of women without PCOS [55]. It would appear that an abnormal SCFA metabolism caused by an abnormal GM is associated with IR and the hyperandrogenemia present in PCOS. High androgen levels in turn can aggravate the abnormal GM, creating a vicious cycle. Kelley et al. [56] found that hyperandrogenemia reduced the diversity of the GM in mice with letrozole-induced PCOS. Similarly, Moreno-Indias et al. [57] found that GM diversity was reduced in female rats exposed to a high-level androgen environment within 24 h after birth, and that the risk of metabolic diseases in adulthood was increased. Therefore, we draw the conclusion that GM affects the occurrence and development of PCOS through the SCFA pathway.

Lipopolysaccharide metabolic pathway

Lipopolysaccharides (LPS), also known as bacterial endotoxins, are a unique component in the cell wall of gram-negative bacteria. Bacteroides and Escherichia in the human intestine are gram-negative bacteria [58]. After being absorbed into the blood, LPS can bind to Toll-like receptor 4 (TLR4) on the surface of immune cells through the transmission of LPS-binding protein (LBP), CD14 and bone marrow differentiation factor (MD-2). This can lead to the activation of downstream signaling pathways which promote the expression of TNF- α , IL-6, etc. [59], affecting insulin sensitivity and leading to IR. In one study, two groups of mice were fed either a normal or a high-fat diet. After 4 weeks, mice fed a high-fat diet became obese and showed signs of IR; compared with the control group, the concentration of blood LPS in the high-fat diet group was 2–3 times higher, and the GM had changed synchronously. LPS was then subcutaneously injected into wild mice fed a normal diet in this study. After 4 weeks, the control mice had become obese and developed IR [60]. We find that an abnormal GM affects the metabolism, promoting IR and obesity through the LPS pathway. IR is an important clinical characteristic of PCOS [61] and occurs in 50–70% of patients with PCOS [62]. From the above experiments, we can conclude that the GM mediates IR through LPS. Some researchers believe that IR is the central link in the abnormal metabolism of PCOS and that it promotes a chronic inflammatory state in patients with PCOS. The long-term low release of pro-inflammatory factors can promote hyperandrogenism and obesity in PCOS patients [63,64]. Conversely, high androgen levels in PCOS patients also increase inflammation. Su et al. [65] confirmed that androgen activates the ERK1/2/NF- κ B signaling pathway and enhances the response of 3 T3-L1 adipocytes to LPS, resulting in the increased secretion of IL-6. Zhu et al. [66] compared the levels of serum LBP in PCOS patients with that of healthy women and found that LBP levels in PCOS patients were significantly increased, irrespective of body mass, providing strong evidence for the involvement of LPS in the pathogenesis of PCOS.

Choline pathway

PCOS patients not only suffer from diabetes and obesity, but also have an increased risk of heart disease [67]. A meta-analysis of 104 392 subjects showed a significant correlation between PCOS and coronary heart disease (CHD) (OR = 1.44; 95% CI: 1.13–1.84; $p = 0.004$) [68]. Studies have shown that GM may contribute to the occurrence and development of cardiovascular disease through the choline pathway. Choline is a necessary nutrient for the human body and is mainly obtained from red meat and eggs. It is a component of cell membranes and predominantly involved in lipid metabolism. Choline in food is first fermented to trimethylamine (TMA) by the GM, and then metabolized to trimethylamine oxide (TMAO) by flavin-containing monooxygenase (FMO) in the liver. TMAO is an osmotic substance which plays an important role in maintaining the balance of water and electrolytes both inside and outside cells. TMAO is also involved in regulating cholesterol metabolism, IR, platelet aggregation and inflammatory reactions [10]. Increased TMA-producing bacteria in the intestine can lead to higher TMAO levels, which can result in reverse transport of foam cells and cholesterol, resulting in atherosclerotic lesions and cardiovascular disease [69]. PCOS patients often present with hyperlipidemia and IR [70], which can damage vascular endothelial function and induce thrombosis. These metabolic abnormalities increase the risk of CHD. However, the specific molecular mechanism leading to this pathological state has not yet been explored. Now studies have revealed the mechanism of how GM affects cardiovascular health through the choline pathway. Whether this mechanism plays a role in the occurrence of cardiovascular complications in PCOS patients remains to be verified.

Bile acid pathway

Bile acids emulsify fats and promote digestion and the absorption of fat-soluble substances in the gastrointestinal tract, while bacteria are essential for the transformation of bile acids. GM regulates bile acid metabolism through farnesoid X receptor (FXR), vitamin D receptor (VDR) and G-protein-coupled receptor for bile acid (TGR5), and participates in the synthesis, metabolism and reabsorption of bile acids. The GM regulates fat synthesis and the level of TG by inhibiting the activity of cholesterol 7 α -hydroxylase (CYP7A1) [71, 72]. Abnormal lipid metabolism is often present in women with PCOS, with a reported incidence of as much as 50% [73], and it mainly manifests in the form of increased levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL) and hormone-sensitive lipase (HSL). Bile acid and decreased concentrations of HDL and Apo A-I [74]. According to the gene and genome analysis of Kyoto Encyclopedia, bile acid metabolism is one of the main metabolic pathways affected by GM in patients with PCOS. Qi et al. [30] conducted an analysis of bile acids in patients with PCOS and normal controls and found that, compared to the control group, glycodeoxycholic acid (GDCA) and tauroursodeoxycholic acid (TUDCA) were significantly decreased in the PCOS group. Correlation analysis showed that *Bacteroides forsythus* were negatively correlated with GDCA and TUDCA, and interestingly, *Bacteroides forsythus* were able to uncouple the binding bile acids synthesized in the liver [75]. Compared to the control group, there was a significant increase in the number of bile salt hydrolase genes (encoding bile salt hydrolase enzymes)

in PCOS patients. These data suggest that *Bacteroides forsythus* are significantly expressed in the GM of patients with PCOS, affecting GDCA and TUDCA metabolism in their hosts. In addition, deoxycholic acid induces IL-22 secretion by intestinal lymphocytes via GATA binding protein 3, which in turn improves the PCOS phenotype. This finding is consistent with lower levels of IL-22 in patients with PCOS. In addition, some studies have shown that GM can affect insulin sensitivity through an inflammatory response mediated by bile acids [76]. Based on the above studies, we find that GM affects glucose and lipid metabolism and chronic inflammatory states in PCOS patients by influencing bile acids, leading to other endocrine disorders.

Intestinal permeability

Intestinal mucosal tight junctions are present at the edge and top of mucosal cell membranes and control the openings of intercellular pathways; they are the first line of defense of the cellular bypass pathway [77]. Intercellular tight junctions are physiologically complete, but under pathological conditions such as oxidative stress or inflammatory damage, their structure and function can be destroyed [78]. Studies have shown that inflammatory factors TNF- α and INF- γ can reduce the expression of tight junction proteins zonula occludens-1 (ZO-1) and occludin, leading to a disintegration of tight junctions and increased intestinal permeability [79]. PCOS is considered a chronic inflammatory disease, and the long-term low release of pro-inflammatory factors causes continuous damage to intestinal permeability; this pathological process is closely related to the contribution of the GM. In 2012, Tremellen et al. [80] proposed that one of the pathogenic mechanisms of PCOS could be that obesity and a high-sugar, high-fat diet with low-dietary fiber encourages GM imbalance, thus destroying the junction between intestinal epithelial cells and reducing the expression of ZO-1 and occludin [81]. In addition, the expression of cannabinoid 1 (CD-1) is inhibited, which can also act on tight junction proteins, leading to “leaky gut” [82]. As a result, the process whereby LPS enters the body circulation from gram-negative bacteria accelerates, contributing to an antigen-antibody reaction in vivo which activates the immune system, induces chronic inflammation, hinders the function of insulin receptors, and thereby increases the level of insulin, resulting in high levels of androgens and abnormal follicular development.

Brain–gut axis

The brain–gut axis is the information-communication system between the intestine and the brain, a neuroendocrine-immune network formed by the central nervous system, the intestinal nervous system (ENS), the hypothalamic-pituitary-adrenal axis and the intestinal tract [83]. GM communicate with the host through the gut–brain axis via immune, neuroendocrine and vagal pathways. Under pathological conditions, GM disorders can result in abnormal secretions of peptides, cytokines and inflammatory factors in the intestinal canal, which transmit information to the brain. After analysis and calculation by the brain, instructions are transmitted to various organs as well as to the intestine and the GM [84, 85]. More than 20 of 50 gastrointestinal hormones are known to be involved in the brain–gut interaction [86]. Taking glucagon-like peptide 1 (GLP-1) as an example, studies have shown that GM

and gastrointestinal hormone secretions in PCOS patients are disordered, with decreased levels of GLP-1 [87], which can affect the gastrointestinal system and the CNS through the vagus nerve; GLP-1 plays a vital role in various functions such as delaying gastric emptying time, reducing appetite, enhancing satiety, promoting the proliferation of pancreatic islet β cells and stimulating insulin secretion [88]. Studies have also shown that PCOS patients have common psychological problems such as depression, social phobias, anxiety and aggression [89]. Such psychological conditions are associated with an abnormal brain–gut axis. A particular type of intestinal endocrine cell, enterochromaffin cells, are the major source of the neurotransmitter serotonin (5-HT), with 95% of the body's 5-HT distributed in the intestine [90]. A GM imbalance causes changes in 5-HT secretion, which affects brain development, stress response, and emotional activities such as anxiety and depression by paracrine [91]. Animal experiments have shown that PCOS mice have significantly lower levels of 5-HT, nor-epinephrine (NE) and dopamine compared to normal mice.

We have therefore speculated that the emotional disorders exhibited by some PCOS patients may be related to abnormal transmission of information along the brain–gut–GM axis. The literature shows that the intestinal microbiome can also interfere with host immune regulation through the brain–gut axis. Intestinal lymphoid tissues contain 70–80% of the immune cells of the whole body [92] and provide the anatomical basis for immune regulation. Lang et al. [93] showed that the ratio of T helper cells (Th1/Th2) in the peripheral blood of patients with PCOS-related infertility was higher than that of the control group, and that an imbalance of Th1 and Th2 cells led to poor oocyte quality and ovulation disorders, resulting in lower pregnancy rates for PCOS patients and an increased risk of abortion. Kim et al. [94] found that GM imbalance was significantly correlated with Th1/Th2 ratio, indicating that the balance between GM and helper T cells was both interdependent and mutually restrictive. At present, there is no experimental model which proves that GM regulates immunity through the brain–gut axis, and future research in this area is warranted.

Treatment

At present, the etiology of PCOS has not been clarified, making it difficult to eradicate the disease. Due to the heterogeneity of patient ages and patient demands and the heterogeneity of the clinical manifestations of PCOS, clinical management must adopt an individualized approach to treat symptoms, based on patients' chief complaints, demands and metabolic changes. Treatment approaches include anti-androgen therapy, insulin sensitizer therapy, or ovulation induction therapy to relieve clinical symptoms, improve fertility, maintain health, and improve patients' quality of life. However, current treatment plans still have many shortcomings of one kind or another, and we need to continue to explore the causes of the disease and find a direct way to treat the disease. Currently, there is no treatment plan which aims to regulate GM, although it may be an effective approach to treat PCOS in future. We have therefore summarized potential treatment options for PCOS based on treating the GM to provide new ideas for clinical treatment.

Lifestyle intervention

In recent years, numerous studies and guidelines have recommended lifestyle interventions as a first-line treatment for PCOS. This approach focuses on diet, exercise, behavior, psychology and other channels, all of which can affect the activity of the GM and thus regulate the metabolism of the host.

Dietary changes can rapidly change the relative abundance and species in GM [95], something that has been confirmed in patients with MS [96]. A high-fat, high-sugar diet can result in GM disorders, leading to increased intestinal permeability, endotoxemia, hyperandrogenism and ovulation disorders [97]. Therefore, strict dietary controls are needed. Studies have confirmed that the intake of foods with a low glycemic index can make ovulation and menstrual cycles more regular in patients with PCOS [98] and normalize the metabolic index [98].

A recent meta-analysis [99] revealed that exercise can improve the levels of follicle-stimulating hormone, sex hormone-binding globulin, total testosterone and androgen in PCOS patients, and reduce the modified Ferriman–Gallwey (mFG) score. However, the specific mechanism is not clear. In recent years, the more and more attention has focused on the relationship between exercise and GM. Clarke et al. [100] compared the composition of GM in athletes with that of the general population and found that sports can increase GM diversity. Mika et al. [101] studied the effect of exercise on GM in rats and found that, compared to a normally active group, the GM diversity in rats increased after 6 weeks of wheel running. The phylum abundance of Bacteroidetes in exercising rats increased while the phylum abundance of Firmicutes decreased, both of which are significantly correlated with metabolic syndrome. We hypothesize that exercise changes the GM in PCOS patients and thus results in an improvement of metabolic disorders in hosts. Physicians should therefore encourage PCOS patients to do aerobic exercises at 60–80% of maximum heart rate for more than 30 min at least 4 times per week [102].

Numerous studies have shown that, compared with healthy women, patients with PCOS are more likely to be depressed, anxious and use drugs [103, 104]. Future research should focus on the relationship between PCOS and mental illness [105]. Studies have shown that cognitive behavioral therapy (CBT) can improve the psychological fatigue of patients with PCOS [106]. Given the bidirectional signaling pathway of the brain–gut axis, we speculated that an improvement in emotional brain activity could affect GM and intestinal homeostasis. However, there are very few studies on the impact of psychological interventions in women with PCOS, and research into the effects of psychological interventions on the GM of patients with PCOS are even rarer. This is one area where we intend to carry out further research.

Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) is a method whereby microorganisms from the feces of healthy donors are introduced into the small intestine of patients through a nasogastric tube or a nasointestinal tube to directly and rapidly change the composition of the new host's GM and treat disease [107]. Studies suggest that changes to the GM in the colon can lead to *Clostridium difficile* infections (CDI). FMT has been proposed as a treatment option for CDI, and its effectiveness to treat CDI was reported to be more

than 90%, with few adverse reactions reported [108]. FMT has therefore been recommended in the guidelines as a treatment option for CDI intractable to treatment with antibiotics [109]. FMT can also be used to treat inflammatory bowel disease, chronic constipation and some non-gastrointestinal diseases [110]. Vrieze et al. [111] transplanted the GM of healthy donors into patients with metabolic syndrome, and found that after 6 weeks, the insulin sensitivity of the recipients had increased. At present, there are no clinical reports on the use of FMT to treat gynecological diseases. Guo et al. [112] used FMT in a murine model of PCOS to treat rats. After 36 days, they found significant changes in FMT-treated PCOS rats compared with the untreated control group, with improvements including decreased androgen levels, a significant increase in estradiol and estrone, and normalization of ovarian function. FMT may represent an important new approach to treat PCOS in future, but further study is required.

Probiotic therapy

Probiotics are defined by the Food and Agricultural Organization (FAO) of the United Nations and the WHO as “live micro-organisms, that (when administered in adequate amounts) confer a health benefit on the host” [113]. Probiotics therapy refers to appropriate supplementation with probiotics to treat disease. Traditional probiotics include *Bifidobacterium*, *Lactobacillus* as well as other lactic acid bacteria. Studies have shown that *Lactobacillus reuteri* GMNL-263 can significantly improve IR, increase satiety, promote intestinal gluconeogenesis, reduce fat accumulation [114] and decrease inflammation in rats with alcoholic fatty liver disease [115]. On a macrogenomic level, it has been found that probiotics can return a GM severely affected by a high-fat diet to its normal state. This suggests that probiotics can be utilized to regulate GM and treat metabolic diseases, which also points to a new therapeutic direction for the treatment of the metabolic abnormalities associated with PCOS [116]. In a randomized, double-blind, placebo-controlled trial, 60 PCOS patients were randomized into two groups to receive either probiotic capsules ($n = 30$) or placebo ($n = 30$) for 12 weeks. The probiotic capsules contained three viable freeze-dried strains: *Lactobacillus acidophilus* (2×10^9 CFU/g), *Lactobacillus casei* (2×10^9 CFU/g) and *Bifidobacterium bifidum* (2×10^9 CFU/g). Compared with the placebo group, taking probiotic supplements resulted in a significantly reduced BMI (-0.2 ± 0.2 vs. $+0.03 \pm 0.4$ kg/m², $p = 0.004$), lower serum insulin concentrations (-2.0 ± 5.8 vs. $+1.6 \pm 5.0$ μ U/mL, $p = 0.01$) and lower blood lipid levels (-13.3 ± 51.3 vs. $+13.6 \pm 37.1$ mg/dL, $p = 0.02$) [117]. Another randomized double-blind controlled experiment confirmed that compared with the placebo group, probiotic supplementation with *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium bifidum* (2×10^9 CFU/g each) significantly increased serum levels of sex hormone-binding globulin (SHBG), reduced total serum testosterone levels, reduced modified Ferriman–Gallwey (mFG) scores, and improved chronic inflammatory states as indicated by decreased levels of serum high-sensitivity C-reactive protein (hs-CRP) and malondialdehyde (MDA) concentrations. No adverse effects of probiotic supplementation were observed [118]. A meta-analysis by Zhang [119] showed that probiotics can reduce total cholesterol, triglycerides and low-density lipoprotein cholesterol levels, in-

crease high-density lipoprotein cholesterol and consequently reduce the long-term cardiovascular risk of PCOS patients. But they also pointed out the limitations of current studies on probiotics supplementation, including the fact that the probiotics used in each study varied widely and no standard dosages were used, points which will need to be addressed in future studies.

Prebiotics

Prebiotics are non-digestible (oligo)saccharides, defined as “selectively fermented ingredients that allow specific changes, both in the composition and/or activity of the gastrointestinal microflora that confers benefits upon host wellbeing and health” [120]. A number of studies have investigated the effects of prebiotics on different metabolic diseases. Some studies have observed that prebiotics can increase microbial fermentation, reduce appetite, and decrease plasma glucose consumption after meals [121]. Urias-Silvas et al. found that adding prebiotics increased the quantity of *Bifidobacterium* and promoted the secretion of GLP-1 by colon L-cells, thereby improving IR [122]. Inulin, a prebiotic extracted from chicory roots, can improve intestinal microecology and physiological functions and has been shown to increase serum GLP-1 levels, reduce serum IL-6 levels, and normalize the intestinal microecology of diabetic rats [123]. This has led researchers to explore the therapeutic effect of prebiotics on PCOS. Xue et al. [124] divided PCOS model mice into four groups according to an experimental treatment protocol: control group (CON), model group (MOD), inulin group (INU) and metformin group (MET). After 21 days, a significant decrease in body weight and testosterone levels was observed in INU and MET mice; inflammatory markers such as TNF- α , IL-6, and IL-17a had decreased, and the PCO status of these mice had improved significantly. GM sequencing showed that *Bifidobacteria* increased in the INU group compared to the MOD group, while *Proteobacteria* and *Helicobacter* decreased. This suggests that inulin improves the metabolic and inflammatory status of PCOS by regulating the gut microflora, similar to the mechanism of metformin. This finding strongly supports the use of prebiotics in the treatment of PCOS.

Traditional Chinese medicine

Modern research has shown that traditional Chinese medicines contain many ingredients that can improve GM disorders and have a therapeutic effect on PCOS, such as polyphenols, alkaloids, and polysaccharides. Quercetin, a polyphenol, was found to modulate intestinal dysbiosis in rats with high-fat diet-induced obesity by inhibiting the growth of obesity-related bacteria such as *Veillonella*, *Erysipelotrichaceae*, *Bacillus*, and *Eubacterium cylindroides* [125]. Baicalin can reduce metabolic inflammation by lowering the ratio of gram-negative bacteria to positive bacteria in the intestine, limiting the entry of LPS into the blood and reducing the secretion of inflammatory factors [126]. The main component of *Coptis Chinensis* is berberine, an alkaloid. It can decrease the number of SCFA-producing bacteria, including *Allobaculum*, *Bacteroides*, *Blautia*, *Butyrivococcus*, and has a similar therapeutic effect to metformin [127]. Zhang X et al. [128] have proposed that berberine can be used to treat obesity, IR and other metabolic abnormalities by improving GM, and we therefore speculate that one of the mechanisms of berberine in the treatment of PCOS is

its capacity to regulate GM. Polysaccharides, another group of effective ingredients, can regulate intestinal function through their impact on intestinal immunity, the intestinal barrier, and GM. Polysaccharides from *Ophiopogon japonicus* were found to increase the proportion of intestinal probiotics in mice fed a high-fat diet by increasing the number of *Bacteroides* and decreasing the number of *Firmicutes*. MDG-1, a water-soluble β -D-fructan extracted from the roots of *Ophiopogon japonicus*, could decrease the ratio of *Firmicutes*/*Bacteroidetes*, adjust the abnormal gut microbiota to the normal state and alter their metabolic profiles [128, 129]. The weight loss effect of astragalus polysaccharide on obese mice whose obesity was induced by a high-fat diet is related to its regulation of GM. A number of polysaccharide compounds have been shown to promote and regulate the growth of intestinal probiotics in mice, including Chinese yam polysaccharide, dogwood polysaccharide, mulberry leaf polysaccharide, and the polysaccharide *Physalis* [130, 131]. In summary, traditional Chinese medicines, often characterized as multi-component and multi-target compounds, can be used as another therapeutic option to treat PCOS.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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