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PATHOLOGICAL ROLE OF GUT MICROBIOTA AND SERUM METABOLITES IN DEVELOPMENT AND PROGRESSION OF POLYCYSTIC OVARY SYNDROME

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ABSTRACT

Polycystic Ovary Syndrome (PCOS) is a common female endocrinopathy with unknown origins that is characterized by hyperandrogenism, oligo-/anovulation, and ovarian cysts. Obesity, insulin resistance, and systemic low grade inflammation are all common in women with PCOS. In 2012, tremellen and pearce proposed the idea that dysbiosis of the intestinal (gut) microbiota is a contributing factor to PCOS metabolic and reproductive manifestations. The gut microbiota has been shown to play a role in the onset and progression of many diseases, including type 2 diabetes, obesity, coronary heart disease, and so on. In the past five years, studies in both human and animal models have determined that alterations in the taxonomic composition of gut bacteria are associated with PCOS. It sheds light on the pathogenesis of polycystic ovary syndrome (PCOS). This study provided the link between gut microbial composition and serum metabolites contributing to the occurrence and development of PCOS. Altogether, these results suggest that dysbiosis of the gut microbiome may be sufficient to develop PCOS-like symptoms, and the modulation of gut microbiota may be a potential therapeutic target for PCOS.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common heterogeneous endocrine and chronic metabolic disease affecting 3–26% of reproductive-aged women, related to hirsutism, hyperandrogenism, menstrual disorders, and infertility. Approximately 50–70% of anovulatory infertility case in patients, notably those with poor ovulation induction rates, low pregnancy rates, and high abortion rates, are related to PCOS. It is also associated with several metabolic disorders, including insulin resistance (IR), obesity, cardiovascular disease, diabetes, and other long-term metabolic syndromes. Contrary to obesity, around half of PCOS affected women have insulin resistance and compensatory hyperinsulinemia. Increased insulin levels can directly boost testosterone production in the ovaries and increased androgen can cause irregular menstrual, growth of ovarian cysts, hirsutism, and other PCOS- related conditions.

Human gut microbiota performs wide range of functions such as digestion of food, development of host immune system, synthesis of short chain fatty acids (SCFA), vitamins, amino acids, angiogenesis, defense against pathogens, regulation of bone mineral density, fat metabolism and drug metabolism.

Tremellen *et al.,* proposed a hypothesis called DOGMA (dysbiosis of gut microbiota), which explains a potential series of events in pathogenesis of PCOS as follows:

1. A high-fat, high-sugar diet and low dietary-fiber diet leads to an imbalance in the intestinal flora, which damages the connections between intestinal epithelial cells and increases the permeability of the gut mucosa
2. A leaky gut may result in the release of lipopolysaccharide (LPS) into the bloodstream, which may activate the immune system interfere with functioning of insulin receptor, causing IR
3. Insulin Resistance (IR)/Hyperinsulinemia might promote synthesis of testosterone, hence meddling with follicular development

OBJECTIVES:

The main purpose of this study is to identify the role of gut microbiota in the development of PCOS. This study also highlights the latest findings as follows

a) Potential mechanisms underlying the association between IR and gut microbiota in patients with PCOS
b) The relationship between Hyperandrogenism (HA) and the gut microbiota in PCOS and
c) The relationship between the substrates and metabolites of the gut microbiota in PCOS

POTENTIAL MECHANISMS UNDERLYING ASSOCIATION BETWEEN IR AND GUT MICROBIOTA IN PATIENTS WITH PCOS:

Numerous studies in animals and people have demonstrated the strong relationship between IR and the composition of the gut microbiota. As early as 2004, Gordon *et al.,* transplanted a healthy gut microbiome of mice into germ-free mice. Insulin resistance and an increase in body fat were observed in the germ-free mice after feeding. This was the first discovery between insulin resistance with gut flora. Usually, the gut microbiota encourages the synthesis of triacylglycerol and inhibits the oxidation of fatty acids, which may disrupt the body's energy balance and result in IR. Dysbiosis of gut microbiota can enhance intestinal mucosal permeability. Intestinal microbiota can produce a wide range of inflammatory mediators, including lipopolysaccharide (LPS) and branch-chain amino acids (BCAA). The BCAA stimulates the body's immunological response, whereas inflammatory mediators activate the toll-like receptor 4 (TLR4), which reduces insulin sensitivity.

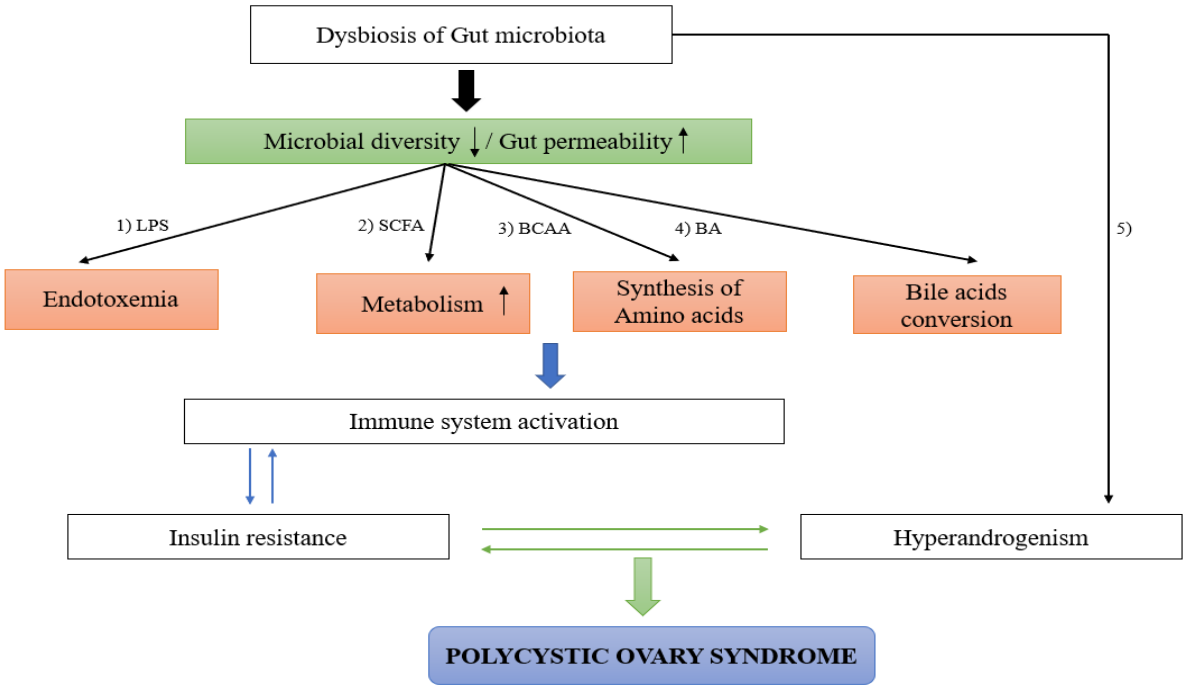

Fig 1: Mechanism & Pathogenesis of PCOS induced by gut microbiota.

1.Gut microbiota affect lipopolysaccharide (LPS) and its receptor CD14 which results in IR; 2. SCFAs protect intestinal barrier integrity and act on beta cells to promote insulin secretion, thus improving metabolism; 3. The metabolic disorder of amino acids might trigger IR by changing glucose metabolism or inducing inflammation; 4. Bile acids (BAs) are signaling molecules that control glucose metabolism and promote insulin sensitivity; 5.PCOS is developed and exacerbated by a vicious cycle between hyperandrogenemia and IR.

LPS: lipopolysaccharide; SCFA: short-chain fatty acid; BCAA: branch-chain amino acid; BA: Bile acids

THE RELATION BETWEEN HYPERANDROGENISM(HA) AND GUT MICROBIOTA IN PCOS:

The intrinsic causes of PCOS development, IR and compensatory hyperinsulinemia are crucial in the production of excess androgen in PCOS. The excess insulin production can trigger insulin receptor of pituitary gland, to release luteinizing hormone (LH), and promote secretion of androgen by ovary and adrenal gland. Additionally, it can raise levels of free testosterone and decrease the production of Sex hormone-binding globulin (SHBG). Excess androgen secretion may lead to hirsutism, acne, alopecia symptoms, and may inhibit the growth and development of ovarian follicles. Insulin can also boost the activity of insulin-like growth factor-1 (IGF-1) receptor in the ovary, which increase its levels of free IGF, increasing the production of androgen.

THE RELATION BETWEEN SUBSTRATES AND METABOLITES OF GUT MICROBIOTA IN PCOS:

Gut microbes metabolize substrates that enter the gut, from the diet and produce metabolites that may act directly on the intestines or enter systemic circulation and influence various host tissues such as ovary, skeletal muscle, liver, and adipose tissue whose function is altered in PCOS. Some of the gut bacterial metabolites like secondary bile acids, SCFAs, and TMA are changed in PCOS. For instance, bile acids promote intracellular signaling in numerous organs via binding to receptors, such as FXR (farnesoid X receptor). Conjugated primary and secondary bile acids, lactate, glucose, TMAO and other metabolites are produced by metabolic tissues such as skeletal muscle, the liver, and adipocytes that enter the gut and may modify the composition of the bacteria in the gut by acting as substrates.

DISCUSSION

STUDY – 01Zheng Yu *et al.*, (2022): Conducted a cross-sectional study to identify gut microbiome in PCOS association with serum metabolomics. They recruited 40 participants including 20 PCOS patients and 20 healthy controls aged 18-40 yrs. In this study, the abundance of the firmicutes, bacteroidetes, actinobacteria, tenericutes, prevotella and gemmatimonadetes in the PCOS group were decreased compared to healthy controls and the proportion of proteobacteria, verrucomicrobia, fusobacteria, acidobacteria and cyanobacteria were increased. In this study, we found that among the 15 different serum metabolites in PCOS samples, 13 are related to glycerophospholipid metabolism pathway and 2 to energy metabolism in contrast to healthy controls. Characteristic metabolites found in the PCOS group were linoleoylglycerophosphocholine, phosphoniodidous acid, PC, bilirubin, ganglioside GA2, -nicotinic acid nucleotide, and citric acid. This study provides the close correlation between gut microbiota and serum metabolites which is used to develop effective treatment for PCOS.

STUDY – 02Gailing Li *et al.*, *(2022):* Conducted a study to examine the alterations of gut microbiome and fecal fatty acids in PCOS patients. They have prospectively collected fecal and serum samples of 58 participants including 31 PCOS patients and 27 healthy controls. The results of the study showed that the abundance of 13 bacterial groups including klebsiella, peptostreptococcacese and gammaproteobacteria was significantly higher in PCOS patients ($P < 0.05$) than in healthy controls, this indicates that PCOS had gut flora dysregulation and unique gut flora structural changes which play an important role in pathogenesis or disease progression of PCOS. In this study, the serum levels of D-LA and DAO in PCOS patients were increased compared with healthy controls, and levels of TNF-α and LBP were also significantly increased. Based on these findings, it is anticipated that gut dysbacteriosis in PCOS affects gut permeability changes, which subsequently result in a systemic inflammatory response, which in turn leads to the pathogenesis and development of PCOS. The results also showed that presence of SCFA (short chain fatty acids) metabolites like acetic acid, propionic acid in stool sample of PCOS patients are significantly higher than those in healthy controls, which indicate the alterations of gut flora in PCOS patients may cause the metabolic alterations of SCFAs. Finally, this study elucidated the correlation between gut flora alterations and inflammatory indicators, and gut permeability indicators, and also the correlation between gut flora and SCFA metabolism in PCOS patients.

STUDY – 03Fangfang He *et al.*, *(2021):* Conducted a study to identify the association between gut microbial composition and PCOS by taking 26 women with PCOS and 10 normal women aged 18-35 yrs. At the family level, significant variations were observed in abundance of lactobacillus, enterococcaceae, peptostreptococcaceae, and micrococcaeae among the three groups. In the PCOS-IR group, the abundance of peptostreptococcaceae, enterococcaceae, and micrococcaeae was higher than that of the other two groups. PCOS-NIR group had the abundance of lactobacillaceae. In contrast to the other two groups, PCOS-IR patients had considerably greater relative abundances of rothia, ruminococcus, lachnospira, and enterococcus, while Prevotella was significantly less common (P 0.05).Prevotella was the predominant bacterial species in the HC group (LDA score > 2.0) and (P 0.05). Prevotella is a bacterium which produces short-chain fatty acids (SCFA), regulates the uptake of nutrients and hormone levels in the gut. Additionally, it takes role in energy metabolism, and its decreased abundance is strongly correlated with rising testosterone levels and pro-inflammatory cytokines. In this study, the HC group had the highest abundance of Prevotella, maintaining the balance of intestinal flora, while the NIR-PCOS and IR-PCOS groups had a reduced abundance of Prevotella. Further investigation showed that Enterococcus was the most abundant genera in the PCOS-IR group, and its abundance was positively correlated with insulin resistance index.

STUDY - 04Yue-Lian *Yang et al., (2021):* Conducted a study to identify intestinal flora's key role in insulin resistance and its contribution to the development of PCOS. In this study, they recruited 56 individuals with PCOS and 31 healthy controls. Treatment-naive PCOS patients and healthy controls have significantly different levels of bacterial alpha and beta diversity. The abundance of *Bacteroides* was significantly higher in treatment-naive PCOS patients than in healthy controls. As well treatment-naive PCOS patients showed a characteristic dysregulation of LH and testosterone secretion, with higher LH and testosterone levels than controls ($P = 0.000$ and 0.033). According to the study, the intestinal flora of people with PCOS may be distinguished by a high relative abundance of bacteroidetes. The intestinal flora is a key factor in the development of insulin resistance in PCOS and it promises a potential target for PCOS treatment.

STUDY – 05 Kreete Lull *et al., (2021):* Conducted a study to examine the gut microbiota in polycystic ovary syndrome and its association with metabolic traits. They collected serum and fecal samples from 304 women including 102 women with PCOS and 202 non-PCOS control women. Participants clinical and biochemical characteristics were evaluated at ages 31 and 46. The results of the study showed that the balance of 4 genera was used to distinguish between PCOS from non-PCOS. The abundance of 2 genera from ruminococcaceae UCG-002, clostridiales, and clostridiales Family XIII AD3011 group, were correlated with numerous PCOS-related markers. In contrast to women with normal glucose tolerance, prediabetic PCOS women showed significantly reduced alpha diversity (Shannon diversity P =.018) and a higher abundance of genus dorea. This study shows that the abundance of Dorea was substantially correlated with metabolic characteristics such as BMI, glucose, and insulin levels, indicating the species involvement in metabolic disorders. However, further investigations clarified the link between gut microbiota, metabolites, and the development of PCOS.

STUDY – 06 Ling Zhou *et al., (2020):* Conducted a study to investigate characteristic gut microbiota and predicted functions in women with PCOS by recruiting 30 obese and 30 non-obese women with PCOS, 30 healthy and 11 healthy obese as control. Serum and fecal samples of all participants were collected and examined. The Hirsutism score, LH/FSH, and serum T levels increased in obese and non-obese PCOS women compared to their controls ($P < 0.05$). According to 16S rRNA gene sequencing, patients with PCOS have altered abundance and diversity of gut microbiota. The linear discriminant analysis (LDA) revealed that Lactococcus significant gut microbiota in non-obese PCOS and Coprococcus_2 in obese PCOS patients. Correlation heatmap analysis indicated that insulin and sex hormones levels in serum were highly correlated to the changes in gut microbiota of obese and non-obese PCOS patients. Predictive microbial function analysis was utilized to reveal the altered metabolic processes of gut microbiota. Interestingly, both obese and non-obese PCOS patients had enriched citrate cycle pathways when compared to their controls. Together, the result of this study showed that the gut microbiota may contribute to the occurrence and development of PCOS in obese and non-obese women.

STUDY - 07*Bo Zeng et al., (2019)*: Conducted a study to identify the structural and functional profile of gut microbial communities in PCOS patients by taking 29 premenopausal women in which across all stool samples collected,12 bacterial phyla were identified and the most abundant phyla were 6 bacteroidetes (63.86%), firmicutes (31.87%) and proteobacteria (3.69%). At the family level, the relative abundance of bacteroidaceae was significantly higher in both PCOS-IR and PCOS-NIR patients when compared to the HC group ($P < 0.05$; $P < 0.001$), while the family of the prevotellaceae was significantly decreased in the PCOS-NIR ($P 13 < 0.05$) and the PCOS-IR groups ($P < 0.001$). The bacteroidaceae displayed a positive correlation with HOMA-IR, testosterone, inflammatory cytokines, LH, and lipids levels, while it had a negative correlation with FSH and estradiol ($P < 0.05$). In contrast, the prevotellaceae showed a negative correlation with the levels of HOMA-IR, testosterone, inflammatory cytokines, LH, and lipids, while it had a positive correlation with estradiol ($P < 0.05$). In the results, we also found the increase of bacteroidaceae was associated with the level of inflammation and insulin resistance in PCOS patients, suggesting that the imbalance of bacteroidaceae could be a promising target for drug design and therapy in PCOS patients, especially PCOS-IR. In further investigations, compared to the PCOS-NIR group, the biosynthesis pathways of lipopolysaccharides are increased in PCOS-IR patients, which in turn increases the risk of inflammation in patients. Indeed, it was observed in our results that the inflammatory cytokines, including C-reactive protein, IL-6, and TNF-α, are significantly elevated in PCOS-IR patients. Together, the findings of the study showed that there was a considerable dysbiosis of the gut microbial community in PCOS-IR patients, which is evidenced by a decline in alpha diversity, an increase in pro-inflammatory bacteroides, and a decrease in prevotellaceae.

STUDY-08 Pedro J Torres *et al.*, (2018): Conducted a study to investigate gut microbial diversity in women with polycystic ovary syndrome correlates with hyperandrogenism by taking serum and fecal samples of 163 women including 48 healthy controls, 42 with PCOM (polycystic ovarian morphology) and 73 diagnosed with PCOS. According to study results, PCOS women showed lowerα diversity when compared to controls. Moreover, PERMANOVA demonstrated that hyperandrogenism was substantially linked with changes in gut microbiota. Regression studies revealed that total testosterone, hyperandrogenism, and hirsutism were negatively correlated with α diversity. Finally, these findings imply that hyperandrogenism may significantly impact the gut microbiota in PCOS-affected women.

STUDY – 09Rui Liu *et al., (2017)*: Conducted a study to examine the Dysbiosis of gut microbiota associated with clinical parameters of PCOS by taking 48 premenopausal women including 33 PCOS patients and 15 healthy controls. In this study, we observed that some gram-negative bacteria belonging to the genera bacteroides and escherichia/shigella significantly increased in the gut of PCOS women. In addition, we discovered that akkermensia reduced in PCOS, which can restore host mucus layer. Serotonin and ghrelin, two mediators of the brain-gut axis, have been linked to appetite regulation and psychological well-being in PCOS. This study showed that women with PCOS had decrease in serotonin, ghrelin, and PYY level compared with healthy controls. However, this study demonstrated the association between gut microbiota and PCOS-related clinical parameters.

STUDY- 10 Lisa Lindheim *et al., (2017):* Conducted a study to identify alterations in gut microbiota composition and barrier function associated with reproductive and metabolic defects in women with PCOS by taking 25 PCOS women and 25 healthy women. Stool samples were collected and analyzed. According to 16S rRNA gene amplicon sequencing, PCOS patients showed lower diversity and altered phylogenetic composition compared to controls. The relative abundance of phylum tenericutes, the order ML615J-28 and the family S24-7 (phylum Bacteroidetes) were reduced, which is related to reproductive parameters of PCOS. However, this study provides insights into gut microbiota-gut barrier axis in PCOS and knowledge base for future studies.

CONCLUSION

Polycystic ovary syndrome (PCOS) is one of the most common gynecologic endocrine disorders and is widely considered a top cause of infertility. This review highlights that altered gut microbiota may affect the host by affecting different metabolic functions and hormonal balance and promoting the development of PCOS. This study requires further research to provide basis for drug design and subsequent clinical therapy for PCOS.

ABBREVIATIONS

PCOS : Polycystic Ovary Syndrome;
IR : Insulin Resistance;
SCFA : Short chain fatty acid;
DOGMA: Dysbiosis of gut microbiota;
LPS : Lipopolysaccharide;
HA : Hyperandrogenism;
BCAA : Branched chain amino acid;
TLR-4 : Toll-like receptor-4;
LH : Luteinizing Hormone;
FSH : Follicle stimulating hormone;
IGF-1 : Insulin-like growth factor-1;
SHBG : Sex hormone-binding globulin;
FXR : Farnesoid X receptor;
TMAO : Trimethylamine N-oxide;
PC : Phosphatidylcholine;
TNF-α : Tumor necrosis factor;
LBP : Lipopolysaccharide binding protein;
D-LA : D-Lactate;
DAO : Diamine oxidase;
LDA : Linear discriminant analysis;
HOMA : Homeostatic model assessment;
PYY : Pancreatic peptide YY

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CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

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