Mini-Review

Intersection of Polycystic Ovary Syndrome and the Gut Microbiome

Maryan G. Rizk,[1] and Varykina G. Thackray[1]

[1]Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Diego, California 92093, USA

ORCiD numbers: 0000-0002-4962-8136 (M. G. Rizk); 0000-0002-6672-6117 (V. G. Thackray).

Abstract

The etiology of polycystic ovary syndrome (PCOS) remains unclear, although studies indicate that both genetic and environmental factors contribute to the syndrome. In 2012, Tremellen and Pearce proposed the idea that dysbiosis of the intestinal (gut) microbiome is a causative factor of metabolic and reproductive manifestations of PCOS. In the past 5 years, studies in both humans and rodent models have demonstrated that changes in the taxonomic composition of gut bacteria are associated with PCOS. Studies have also clearly shown that these changes in gut microbiota are associated with PCOS as opposed to obesity, since these changes are observed in women with PCOS that are both of a normal weight or obese, as well as in adolescent girls with PCOS and obesity compared with body mass index- and age-matched females without the disorder. Additionally, studies in both women with PCOS and rodent models of PCOS demonstrated that hyperandrogenism is associated with gut microbial dysbiosis, indicating that androgens may modulate the gut microbial community in females. One study reported that the fecal microbiome transplantation of stool from women with PCOS or exposure to certain bacteria resulted in a PCOS-like phenotype in mice, while other studies showed that exposure to a healthy gut microbiome, pre/probiotics, or specific gut metabolites resulted in protection from developing PCOS-like traits in mice. Altogether, these results suggest that dysbiosis of the gut microbiome may be sufficient to develop PCOS-like symptoms and that modulation of the gut microbiome may be a potential therapeutic target for PCOS.

Key Words: polycystic ovary syndrome, hyperandrogenism, insulin resistance, gut microbiome, bile acids

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders, affecting 5% to 10% of women of reproductive age worldwide [1, 2]. Diagnosis of PCOS includes 2/3 clinical presentations: (1) clinical or biochemical hyperandrogenism (HA), (2) oligomenorrhea or anovulation, and (3) polycystic ovaries [3]. Women with PCOS are at a higher risk for infertility and pregnancy complications [4–7]. In PCOS, metabolic dysregulation is correlated with HA, occurs independently of body mass index (BMI), and includes obesity, insulin resistance (IR), and dyslipidemia (Fig. 1) [8–10], which leads to an increased risk of developing type 2 diabetes (T2D), hypertension, and nonalcoholic fatty liver disease [6, 11–14]. Due to its prevalence and association with multiple chronic diseases, studying the mechanisms driving the etiology and pathogenesis of PCOS is of utmost importance in facilitating the development of novel therapies for PCOS.

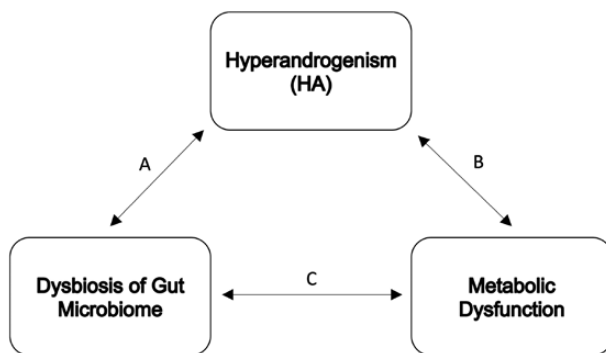The intestinal (gut) microbiome is comprised of a community of diverse bacteria, archaea, fungi, protozoa, and viruses, along with their metabolites. This community plays an important role in host physiology, including immunity, the health of the gut epithelial barrier, production of vitamin B12, and production of short-chain fatty acids (SCFAs) via fermentation of fiber, metabolism, and neurological functions [15]. Alterations in gut microbiota have been associated with metabolic diseases (Fig. 1), autoimmune diseases, neurological disorders, and cardiovascular disease [16]. In 2012, Tremellen and Pearce proposed that a connection might exist between dysbiosis of the gut microbiome and the metabolic and reproductive manifestations of PCOS [17]. Two studies in 2016 and 2017 first reported evidence that changes in the gut microbiome were associated with PCOS in a mouse model of PCOS and in women with the disorder [18, 19]. Since then, multiple subsequent studies in humans [20–28] and rodents [29–32] provided further evidence that dysbiosis of the gut microbiome is associated with PCOS.

In this review, we highlight recent findings on the association between the gut microbiome and PCOS, the relationship between HA and the gut microbiome in PCOS, the relationship between substrates and metabolites of the gut microbiota and PCOS, and potential gut microbiota-altering treatments as therapies of PCOS. To do so, we used the following search terms in NCBI PubMed: "microbiome," "microbiota," and "polycystic ovary syndrome." We confined our search criteria to primary research articles of human and rodent studies on PCOS and the gut microbiome between 2016 and 2020.

Figure 1. Correlations between hyperandrogenism (HA), dysbiosis of the gut microbiome, and metabolic dysfunction. **A:** Link between HA and dysbiosis of the gut microbiome. Recent studies in women and rodent models demonstrated that HA is correlated with dysbiosis of the gut microbiome, including changes in the overall biodiversity of gut bacteria as well as the relative abundance of certain bacteria. In addition, 1 study reported that introducing stool from women with PCOS or a bacterial species (*B. vulgatus)* in antibiotic-treated mice resulted in HA, suggesting that gut microbial dysbiosis or the overabundance of specific bacteria may be sufficient to induce PCOS-like symptoms. **B:** Link between HA and metabolic dysfunction. Metabolic dysfunction, including weight gain, insulin resistance (IR), and dyslipidemia, occurs predominantly in women with PCOS diagnosed with HA and ovulatory dysfunction, independent of body mass index. **C:** Link between dysbiosis of the gut microbiome and metabolic dysfunction. Gut dysbiosis has been associated with obesity, IR, and impaired lipid metabolism in metabolic diseases, including metabolic syndrome, type 2 diabetes, nonalcoholic fatty liver disease, and PCOS. Despite the tripartite set of correlations between HA, gut microbial dysbiosis, and metabolic dysfunction, the mechanisms of how each player affects the other 2 are still largely unknown. Future studies will be required to decipher how the gut microbiome communicates with the host and vice versa in order to alter or respond to varying levels of steroid and metabolic hormones.

Dysbiosis of the Gut Microbiome is Associated with polycystic ovary syndrome (PCOS)

Alpha diversity of the gut microbiota in humans

The overall composition of gut microbiota can be represented by metrics of alpha diversity, which estimate the species richness and/or evenness of a community, sometimes taking phylogenetic relationships into account. Recent studies have shown that alpha diversity of gut microbiota is altered in women with PCOS compared with healthy women. By sampling the fecal microbial content and sequencing 16S ribosomal RNA (rRNA) genes amplified with universal bacterial primers, multiple studies demonstrated that alpha diversity of gut bacteria decreased in premenopausal women with PCOS as compared with age-matched, healthy women [19, 22, 26–28, 33]. In contrast, 3 studies did not observe significant changes in alpha diversity between women with PCOS and healthy women, potentially due to small sample sizes [21, 24, 34]. Interestingly,

studies that used shotgun metagenomic sequencing of the gut microbiome also did not report changes in alpha diversity [20, 23, 25]. All of the aforementioned studies included women diagnosed with PCOS using the Rotterdam criteria [35, 36] from limited geographical locations in Asia and Europe, including China, Turkey, Austria, Poland, and Spain. A recent study on gut microbial changes (using 16S rRNA sequencing) in adolescent girls (14–16 years old) with PCOS and obesity and weight- and age-matched healthy controls from an ethnically diverse population in the United States reported that PCOS was also associated with a decrease in alpha diversity [37]. This study indicates that decreased biodiversity of gut microbes, like other features of PCOS [38], manifests by adolescence. High alpha diversity was proposed as an indication of productivity and stability in an ecosystem, implying overall health of the community [39]. Decreases in alpha diversity have been observed in immune diseases such as Chron's disease, ulcerative colitis, type 1 diabetes mellitus, celiac disease, and allergies [40–42]; in cardiometabolic diseases such as obesity, T2D, and vascular stiffness [42–44]; colorectal cancer [42]; and autism [42]. Thus, loss of microbial diversity in the gut may serve as a biomarker of disease or as an indication of a functional problem, especially when correlated with changes in metabolites of the gut microbiome, as in T2D [44].

Beta diversity of the gut microbiota in humans

In addition to alpha diversity, beta diversity (how similar or different the composition of 1 gut microbial community is compared to another community) can also be estimated using distance metrics that take or do not take phylogenetic relationships or abundance into account. Using 16S rRNA gene sequencing, multiple studies reported that beta diversity was altered in fecal samples obtained from women with PCOS compared with healthy women [19, 22, 28]. However, in other studies, no significant difference in beta diversity was detected in women with PCOS compared with healthy women [21, 26, 33, 34], potentially due to small sample sizes. Comparing adolescent girls with PCOS and obesity to BMI-matched controls, changes in beta diversity were also observed between the 2 groups [37]. Unlike alpha diversity, changes in beta diversity were observed in 2 studies where shotgun metagenomics were used to sequence gut microbiota in women with PCOS [23, 25]. In contrast, 1 study using metagenomic sequencing did not observe differences in gut microbial beta diversity between women with and without PCOS [20]. Overall, these results indicate that differences in beta diversity also appear to be associated with PCOS.

Relative abundance of bacterial taxa in humans

In addition to looking at gut microbial diversity at the community level, various studies assessed differences in the relative abundance (RA) of specific bacterial taxa. In the healthy gut, the phyla *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Verrucomicrobia* are the most dominant, while *Proteobacteria* and *Tenericutes* exist at low abundance [45]. Table 1 summarizes cohort characteristics of women included in studies of PCOS and the gut microbiome, while Table 2 summarizes the different taxa that were significantly altered in women with PCOS from the studies reviewed herein and is organized by bacterial phyla. Of the genera within phylum *Bacteroidetes*, *Bacteroides* were positively associated with PCOS in 5/7 studies and *Parabacteroides* were positively associated with PCOS in 2 studies [20, 23–25, 28, 33], while the family S24-7 was negatively associated with PCOS in 2 studies [19, 33]. Of the genera within phylum *Firmicutes*, family *Clostridiaceae* was positively associated with PCOS in 2 studies [22, 25] and family *Veillonellaceae* in 2 other studies [27, 33]. Of the genera within phylum *Proteobacteria*, *Escherichia*, and *Shigella* were positively associated with PCOS in 2 studies [20, 22].

Alpha and beta diversity in rodent models of PCOS

In addition to human studies, changes in overall gut microbial diversity were also observed in PCOS-like rodent models compared with placebo controls using 16S rRNA gene sequencing. As recently reviewed [46], hyperandrogenic rodent models of PCOS have been created using treatment with dihydrotestosterone (DHT) or the nonsteroidal aromatase inhibitor, letrozole. In a letrozole-induced pubertal PCOS mouse model, alpha diversity of the gut microbiome was lower in letrozole-treated mice than placebo controls, while beta diversity was also changed between the 2 groups [18]. In contrast, letrozole-induced PCOS in adult mice and rats showed no change in alpha diversity [47, 48]. Although adult mice treated with letrozole showed a shift in beta diversity, changes in specific bacterial taxa were distinct between the pubertal and the adult PCOS mouse models [47], and there were no changes in beta diversity observed in adult rats treated with letrozole [48]. In a cohort of 6-week old rats treated with letrozole, changes in beta diversity were observed when compared with placebo-treated rats, while alpha diversity was not different between the 2 groups [49]. These results suggest that the age at which PCOS is induced in rodent models may be critical in order to recapitulate the metabolic dysregulation and gut microbial changes that resemble those found in women with PCOS.

Table 1. Cohort characteristics of human studies on PCOS and the gut microbiome

Country	Cohort Groups	N	Diagnosis	Age (years)	T (nmol/L)	BMI (kg/m2)	HOMA-IR	Method	Ref
Austria	Controls	19	Rotterdam	32.0	1.1	22.3	0.8	16S rRNA (V1–V2)	[19]
	PCOS	24	Criteria	27.0	1.3	24.9	1.7		
China	Nonoverweight controls	7	Rotterdam	30.3	1.1	20.6	n/a	Metagenomics	[20]
	Overweight controls	7	Criteria	28.6	0.7	27.1	n/a		
	Nonoverweight PCOS	7		27.1	1.8	21.0	n/a		
	Overweight PCOS	7		29.1	1.4	27.9	n/a		
Spain	Nonobese controls	8	Rotterdam	27.3	1.6	23.4	1.6	16S rRNA (V4)	[21]
	Obese controls	8	Criteria	27.3	2.0	35.9	3.3		
	Nonobese PCOS	7		23.0	2.5	24.4	1.5		
	Obese PCOS	8		29.9	2.4	37.0	2.6		
China	Nonobese controls	12	Rotterdam	32.2	0.8	21.9	1.7	16S rRNA (V3–V4)	[22]
	Obese controls	6	Criteria	33.0	1.0	27.5	3.5		
	Nonobese PCOS	12		25.5	4.5	21.6	1.1		
	Obese PCOS	21		29.3	5.4	30.0	3.3		
China	Controls	43	Rotterdam	29.6	1.56[a]	23.7	1.6	Metagenomics	[23]
	PCOS	50	Criteria	29.9	2.11[a]	24.7	3.1		
China	Controls	8	Rotterdam	26.4	0.7	20.8	1.4	16S rRNA (V3–V4)	[24]
	NIR-PCOS	8	Criteria	26.1	1.9	22.6	1.9		
	IR-PCOS	9		25.1	2.1	22.6	4.1		
China	Controls	26	B-ultrasound	26.7	0.8	n/a	n/a	16S rRNA (V3–V4)	[25]
	PCOS	38	Oligomenorrhea Hormones	27.6	6.0	n/a	n/a	Metagenomics	
China	Nonobese controls	30	Rotterdam	22.1	1.66[a]	n/a	n/a	16S rRNA (V3–V4)	[26]
	Obese controls	11	Criteria	25.3	1.8[a]	n/a	n/a		
	Nonobese PCOS	30		25.1	2.67[a]	n/a	2.4		
	Obese PCOS	30		26.9	2.63[a]	n/a	6.4		
China	Controls	9	Rotterdam	27.9	1.3	20.9	1.7	16S rRNA (V3–V4)	[27]
	Nonobese PCOS	10	Criteria	25.7	1.9	20.7	1.4		
	Obese PCOS	8		27.1	2.2	29.5	3.8		
Poland	Controls	48	Rotterdam	29.4	1.04[a]	23.7	1.8	16S rRNA (V4)	[28]
	PCOM	42	Criteria	29.8	1.04[a]	22.6	1.7		
	PCOS	73		27.4	0.56[a]	25.6	2.3		
Austria	Controls	20	Rotterdam	32.0	1.1	22.3	0.8	16S rRNA (V1–V2)	[33]
	PCOS	24	Criteria	27.0	1.3	24.9	1.7		
Turkey	Controls	15	Rotterdam	22.0	0.97[a]	31.5	2.1	16S rRNA (V3–V4)	[34]
	PCOS	17	Criteria	20.0	2.22[a]	19.6	2.0		
USA	Obese controls	21	NIH Criteria	14.5	0.69[a]	35.0	4.1	16S rRNA (V3–V4)	[37]
	Obese PCOS	37		16.1	1.49[a]	36.0	4.5		

V1, V2, V3, and V4 are variable regions of the bacterial 16S ribosomal ribonucleic acid (rRNA) gene. Primers are designed to target these regions for 16S rRNA gene sequencing to identify specific bacterial genera.

Abbreviations: BMI, body mass index; B-ultrasound, brightness-mode ultrasound; HOMA-IR, homeostatic model assessment for insulin resistance; IR, insulin-resistant; n/a, not applicable; NIH, National Institutes of Health; NIR, non-insulin-resistant; PCOM, polycystic ovarian morphology; PCOS, polycystic ovary syndrome; Ref, reference number; rRNA, ribosomal ribonucleic acid.

[a]Converted from ng/mL, ug/L, or ng/dL.

Table 2. Changes in bacterial taxa associated with PCOS in women

Phylum	Family	Genus	Change	Ref
Actinobacteria	*Bifidobacteriaceae*	*Bifidobacterium*	↓	[25]
Actinobacteria	*Coriobacteriaceae*	*Collinsella*	↑	[25]
Actinobacteria			↑	[37]
Bacteroidetes	*Bacteroidaceae*	*Bacteroides*	↑	[20]
Bacteroidetes	*Bacteroidaceae*	*Bacteroides*	↑	[23]
Bacteroidetes	*Bacteroidaceae*	*Bacteroides*	↑	[24]
Bacteroidetes	*Bacteroidaceae*	*Bacteroides*	↑	[28]
Bacteroidetes	*Bacteroidaceae*	*Bacteroides*	↑	[33]
Bacteroidetes	*Bacteroidaceae*	*Bacteroides*	↓	[22]
Bacteroidetes	*Bacteroidaceae*	*Bacteroides*	↓	[37]
Bacteroidetes	*Porphyromonadaceae*	*Odoribacter*	↓	[28]
Bacteroidetes	*Porphyromonadaceae*	*Parabacteroides*	↑	[20]
Bacteroidetes	*Porphyromonadaceae*	*Parabacteroides*	↑	[25]
Bacteroidetes	*Porphyromonadaceae*	*Porphyromonas*	↑	[28]
Bacteroidetes	*Porphyromonadaceae*		↓	[37]
Bacteroidetes	*Prevotellaceae*	*Alloprevotella*	↓	[26]
Bacteroidetes	*Prevotellaceae*	*Prevotella*	↑	[25]
Bacteroidetes	*Prevotellaceae*	*Prevotella*	↑	[37]
Bacteroidetes	*Prevotellaceae*	*Prevotella*	↓	[24]
Bacteroidetes	*Prevotellaceae*	*Prevotella*	↓	[33]
Bacteroidetes	*S24-7*		↓	[19]
Bacteroidetes	*S24-7*		↓	[33]
Bacteroidetes			↑	[27]
Firmicutes	*Clostridiaceae*	*Clostridium*	↑	[25]
Firmicutes	*Clostridiaceae*	*ClostridiumIV*	↑	[22]
Firmicutes	*Erysipelotrichidae*	*Catenibacterium*	↑	[21]
Firmicutes	*Erysipelotrichidae*	*Kandleria*	↑	[21]
Firmicutes	*Lachnospiraceae*	*Blautia*	↑	[28]
Firmicutes	*Lachnospiraceae*	*Blautia*	↓	[20]
Firmicutes	*Lachnospiraceae*	*Blautia*	↓	[25]
Firmicutes	*Lachnospiraceae*	*Coprococcus*	↑	[26]
Firmicutes	*Lachnospiraceae*	*Oribacterium*	↑	[21]
Firmicutes	*Lachnospiraceae*	*Roseburia*	↓	[28]
Firmicutes	*Lachnospiraceae*		↓	[27]
Firmicutes	*Lactobacillaceae*	*Lactobacillus*	↓	[22]
Firmicutes	*Oscillospiraceae*	*Oscillibacter*	↓	[22]
Firmicutes	*Ruminococcaceae*	*Faecalibacterium*	↑	[28]
Firmicutes	*Ruminococcaceae*	*Faecalibacterium*	↓	[20]
Firmicutes	*Ruminococcaceae*	*Faecalibacterium*	↓	[25]
Firmicutes	*Ruminococcaceae*	*Ruminococcus*	↓	[28]
Firmicutes	*Ruminococcaceae*	*Subdoligranulum*	↑	[27]
Firmicutes	*Ruminococcaceae*		↑	[34]
Firmicutes	*Ruminococcaceae*		↓	[22]
Firmicutes	*Streptococcaceae*	*Lactococcus*	↓	[26]
Firmicutes	*Streptococcaceae*	*Streptococcus*	↓	[22]
Firmicutes	*Streptococcaceae*		↑	[37]
Firmicutes	*Veillonellaceae*	*Megamonas*	↑	[27]
Firmicutes	*Veillonellaceae*	*Megasphaera*	↑	[33]
Firmicutes		*Anaerococcus*	↓	[28]
Proteobacteria	*Comamonadaceae*	*Comamonas*	↑	[20]
Proteobacteria	*Enterobacteriaceae*	*Escherichia*	↑	[20]
Proteobacteria	*Enterobacteriaceae*	*Escherichia*	↑	[22]
Proteobacteria	*Enterobacteriaceae*	*Shigella*	↑	[20]

Table 2. Continued

Phylum	Family	Genus	Change	Ref
Proteobacteria	*Enterobacteriaceae*	*Shigella*	↑	[22]
Synergistetes			↓	[26]
Tenericutes			↓	[19]
Tenericutes			↓	[26]
Verrucomicrobia	*Akkermansiaceae*	*Akkermansia*	↓	[22]

Abbreviations: PCOS, polycystic ovary syndrome; Ref, reference number.

Table 3. Cohort characteristics of rodent studies on PCOS and the gut microbiome

Animal	Treatment	Treatment Format	Start (week)	Duration (week)	N	Method	Ref
C57BL/6N mice	Letrozole	Pellet (50 ug/day)	4	5	10/group	16S rRNA (V4)	[18]
C57BL/6N mice	Letrozole	Pellet (50 ug/day)	4	5	10 placebo; 12 LET	16S rRNA (V4)	[29]
Sprague-Dawley rats	Letrozole	Oral gavage (1 mg/kg/day)	6	3	8/group	16S rRNA (V3)	[30]
C57BL/6N mice	Letrozole	Pellet (50 ug/day)	4	5	8/group	16S rRNA (V4)	[31]
Sprague-Dawley rats	DHT	Injection (83 ug/day)	3	6	5/group	16S rRNA (V3–V4)	[32]
Sprague-Dawley rats	Letrozole	Oral gavage (1 mg/kg/day)	6	11	8/group	16S rRNA (V3–V4)	[49]
Wistar rats	DHT	15 mg silicone tube	3	10	6/group	16S rRNA (V3–V4)	[50]

V1, V2, V3, and V4 are variable regions of the bacterial 16S ribosomal ribonucleic acid (rRNA) gene. Primers are designed to target these regions for 16S rRNA gene sequencing to identify specific bacterial genera.

Abbreviations: DHT, dihydrotestosterone; PCOS, polycystic ovary syndrome; Ref, reference number; rRNA, ribosomal ribonucleic acid.

RA of bacterial taxa in rodents

In addition to looking at changes in the overall gut microbial community in rodents, various studies assessed differences in the RA of specific bacterial taxa in PCOS rat and mouse models. Table 3 summarizes cohort characteristics of rodent models of PCOS where gut microbiota was assessed. Table 4 summarizes the different taxa that were altered in the PCOS models and is organized by bacterial phyla. Of the genera within phylum *Actinobacteria*, *Bifidobacterium* was negatively associated with PCOS in 2/3 studies [18, 49]. Of the genera within phylum *Bacteroidetes*, *Bacteroides* were positively associated with PCOS in 3/4 studies, while *Prevotella* were positively associated with PCOS in 2 studies [30, 32, 49, 50]. Of the genera within the phylum *Firmicutes*, *Blautia* and *Roseburia* were positively associated with PCOS in 2 studies [18, 29], while *Lactobacillus* was negatively associated with PCOS in 2/3 studies [29, 30].

Caveats and future directions

The diversity in the clinical presentation of PCOS presents a challenge for studies attempting to decipher consistent patterns in the dysbiosis of the gut microbiome in women with PCOS since not all women diagnosed with PCOS have the same pathological phenotypes. Other challenges of studying dysbiosis of the gut microbiome, in general, are geographical and diet-based differences between study populations, which, in turn, can affect the composition of the gut microbiota. Moreover, the use of different methods for the collection and storage of fecal samples, sequencing, and data analysis probably contribute to inconsistent findings in human gut microbiome studies [51]. For instance, 2 factors that may influence the results obtained from the studies outlined herein is the use of different primers for the sequencing of the hypervariable regions (V1-4) of 16S rRNA gene and the use of different bioinformatics programs for data analysis [51] (Tables 1 and 3). In addition, we note that there is considerable variability in the characteristics of the human cohorts with regards to HA, BMI, and IR, which may also explain some of the inconsistencies with regards to alpha diversity, beta diversity, and the RA of specific bacterial taxa.

Moving forward, consensus methods for studying the gut microbiome in women with PCOS may be required to clearly differentiate differences in the gut microbiome that are due to factors such as geography and diet with those that are related to PCOS. Additionally, since most of the previous studies have relied on 16S rRNA gene sequencing, metagenomic approaches in future studies will be beneficial to investigate whether other gut microbes such as archaea, fungi, and viruses are altered in PCOS and dissect gut microbial gene functions that are associated with PCOS phenotypes. Specifically, metagenomic sequencing of the gut microbiome of adolescent and premenopausal age- and BMI-matched women from diverse ethnic and geographical backgrounds with and without PCOS will be needed to further understand the relationship of PCOS with dysbiosis of the gut microbiome. Mechanistic studies using rodent models of PCOS will also be required to understand how

Table 4. Changes in bacterial taxa associated with rodent models of PCOS

Phylum	Family	Genus	Change	Ref
Actinobacteria	*Bifidobacteriaceae*	*Bifidobacterium*	↑	[29]
Actinobacteria	*Bifidobacteriaceae*	*Bifidobacterium*	↓	[18]
Actinobacteria	*Bifidobacteriaceae*	*Bifidobacterium*	↓	[49]
Actinobacteria	*Coriobacteriaceae*	*Adlercreutzia*	↑	[31]
Actinobacteria			↑	[32]
Actinobacteria			↑	[49]
Bacteroidetes	*Bacteroidaceae*	*Bacteroides*	↑	[32]
Bacteroidetes	*Bacteroidaceae*	*Bacteroides*	↑	[49]
Bacteroidetes	*Bacteroidaceae*	*Bacteroides*	↑	[50]
Bacteroidetes	*Bacteroidaceae*	*Bacteroides*	↓	[18]
Bacteroidetes	*Odoribacteraceae*	*Odoribacter*	↓	[29]
Bacteroidetes	*Porphyromonadaceae*	*Parabacteroides*	↑	[29]
Bacteroidetes	*Porphyromonadaceae*	*Parabacteroides*	↓	[18]
Bacteroidetes	*Prevotellaceae*	*Ga6A1*	↓	[50]
Bacteroidetes	*Prevotellaceae*	*Prevotella*	↑	[30]
Bacteroidetes	*Prevotellaceae*	*Prevotella_9*	↑	[50]
Bacteroidetes	*Prevotellaceae*		↓	[50]
Bacteroidetes	*Rikenellaceae*	*Alistipes*	↑	[32]
Bacteroidetes	*Rikenellaceae*	*Alistipes*	↓	[18]
Bacteroidetes	*S24-7*		↓	[18]
Bacteroidetes			↓	[32]
Cyanobacteria			↑	[32]
Firmicutes	*Christensenellaceae*	*Christensenella*	↓	[31]
Firmicutes	*Clostridiaceae*	*Anaerotruncus*	↑	[32]
Firmicutes	*Clostridiaceae*	*Candidatus Arthromitus*	↓	[31]
Firmicutes	*Clostridiaceae*	*Clostridium*	↑	[32]
Firmicutes	*Clostridiaceae*	*Clostridium*	↓	[30]
Firmicutes	*Dehalobacteriaceae*	*Dehalobacterium*	↓	[18]
Firmicutes	*Erysipelotrichaceae*	*Allobaculum*	↑	[18]
Firmicutes	*Erysipelotrichaceae*	*Coprobacillus*	↓	[31]
Firmicutes	*Erysipelotrichaceae*	*Turicibacter*	↑	[31]
Firmicutes	*Eubacteriaceae*	*Eubacterium*	↑	[32]
Firmicutes	*Hungateiclostridiaceae*	*Ruminiclostridium*	↓	[49]
Firmicutes	*Lachnospiraceae*	*Blautia*	↑	[18]
Firmicutes	*Lachnospiraceae*	*Blautia*	↑	[29]
Firmicutes	*Lachnospiraceae*	*Butyrivibrio*	↓	[49]
Firmicutes	*Lachnospiraceae*	*Coprococcus*	↑	[18]
Firmicutes	*Lachnospiraceae*	*Dorea*	↓	[29]
Firmicutes	*Lachnospiraceae*	*Dorea*	↓	[31]
Firmicutes	*Lachnospiraceae*	*Roseburia*	↑	[18]
Firmicutes	*Lachnospiraceae*	*Roseburia*	↑	[29]
Firmicutes	*Lachnospiraceae*	*Roseburia*	↓	[31]
Firmicutes	*Lachnospiraceae*	*Tyzzerella*	↑	[32]
Firmicutes	*Lachnospiraceae*		↓	[29]
Firmicutes	*Lactobacillaceae*	*Lactobacillus*	↑	[31]
Firmicutes	*Lactobacillaceae*	*Lactobacillus*	↓	[29]
Firmicutes	*Lactobacillaceae*	*Lactobacillus*	↓	[30]
Firmicutes	*Ruminococcaceae*	*Ruminococcus*	↑	[18]
Firmicutes	*Ruminococcaceae*	*Ruminococcus*	↑	[29]
Firmicutes	*Ruminococcaceae*	*Ruminococcus*	↓	[29]
Firmicutes	*Ruminococcaceae*	*Ruminococcus*	↓	[30]
Firmicutes	*Ruminococcaceae*	*Ruminococcus_1*	↑	[32]
Firmicutes	*Ruminococcaceae*		↓	[18]

Table 4. Continued

Phylum	Family	Genus	Change	Ref
Firmicutes			↑	[32]
Proteobacteria	*Desulfovibrionaceae*	*Desulfovibrio*	↓	[49]
Proteobacteria	*Sutterellaceae*	*Sutterella*	↑	[29]
Saccharibacteria			↑	[32]
Spirochaetes	*Spirochaetaceae*		↓	[50]
Verrucomicrobia	*Akkermansiaceae*	*Akkermansia*	↑	[31]

Abbreviations: PCOS, polycystic ovary syndrome; Ref, reference number.

these microbes influence the development and pathogenesis of PCOS.

Interplay Between Gut Dysbiosis and Hyperandrogenism in PCOS

Gut dysbiosis as a driver for HA in PCOS

While the etiology of PCOS remains unknown, there are 2 distinct but related hypotheses that could connect the development of hyperandrogenic PCOS phenotypes to changes in the gut microbiome. As discussed previously, 1 hypothesis proposed by Tremellen and Pearce is that gut dysbiosis, influenced specifically by a high-fat diet (HFD) and a high-carbohydrate diet, leads to inflammation through disruption of the gut barrier, which in turn leads to IR, HA, and ovarian dysfunction [17]. This hypothesis places a strong emphasis on diet and gut dysbiosis as driving factors from which pathogenic features of PCOS, such as HA, emerge. However, it also suggests that obesity and IR are prerequisites for PCOS, although it is well documented that not all women diagnosed with PCOS are obese or insulin resistant [52, 53]. In addition, this hypothesis does not take into account that the incidence of PCOS is relatively similar in countries world wide despite differences in diet [2] and that many animal models of PCOS have been recreated independent of diet [54–58].

In order to begin to mechanistically understand the role of the gut microbiome in the development of PCOS, fecal microbiome transplant (FMT) experiments using stool from women with PCOS are informative. Qi et al performed an FMT from women with PCOS that are normal weight into antibiotic-treated mice and observed reproductive and metabolic changes in the recipient mice [23]. Significant increases in testosterone and luteinizing hormone (LH) levels were observed in mice receiving FMT from women with PCOS (trans-PCOS) compared with mice receiving FMT from healthy women (trans-Control) [23]. In addition to HA, trans-PCOS mice had disrupted estrous cyclicity, decreased ovulation as indicated by a decreased number of corpora lutea in the ovaries, the appearance of ovarian cysts, and a decrease in fertility [23]. Additionally, trans-PCOS mice developed IR as assessed by glucose and insulin

tolerance tests and the homeostatic model assessment for IR calculated based on fasting glucose and insulin levels [23]. The researchers also transplanted one of the bacteria, *Bacteroides vulgatus*, that was positively associated with PCOS into antibiotic-treated mice [23]. Similar reproductive and metabolic phenotypes were observed with *B. vulgatus* compared with trans-PCOS [23]. This potentially ground-breaking study suggests that transplantation of a dysbiotic gut microbiome from women with PCOS or *B. vulgatus* is sufficient to induce a PCOS-like phenotype in mice and supports the idea that changes in the gut microbiome may play a causal role in this disorder (Fig. 2).

Caveats for this study include the use of antibiotics to deplete the gut microbiome instead of the use of germ-free mice. Antibiotics have been shown to affect metabolism in mice [59], and no data were provided to demonstrate that the gut microbiome was actually depleted by antibiotic treatment prior to the FMT or that the FMT resulted in the establishment of gut microbes after the FMT. In addition, no data were provided on whether the mice gained weight or not; thus, it is unclear if a dysbiotic gut microbiome from women with PCOS and normal weight could induce obesity in mice. Future studies where the gut microbiome of recipient mice is sampled and sequenced pre- and post-FMT or bacterial transplantation will provide more comprehensive information about the role of the gut microbiome in the emergence of PCOS-like symptoms in mice. Additionally, using germ-free mice as recipients will clarify the direct effects of gut microbiota on the development of PCOS and whether obesity and IR precede HA [60]. Finally, FMT from women with PCOS that are normal weight and obese as well as non-IR versus IR will help parse out the role of different gut microbiota on the development of the different metabolic phenotypes associated with PCOS.

HA as a driver for gut dysbiosis in PCOS

A second hypothesis is that HA leads to gut dysbiosis in association with the development of PCOS (Fig. 1). Potential mechanisms through which testosterone could alter the gut microbiome include a direct effect as a substrate for gut microbial enzymes and an indirect effect via activation of host androgen receptors or modulation of the immune system
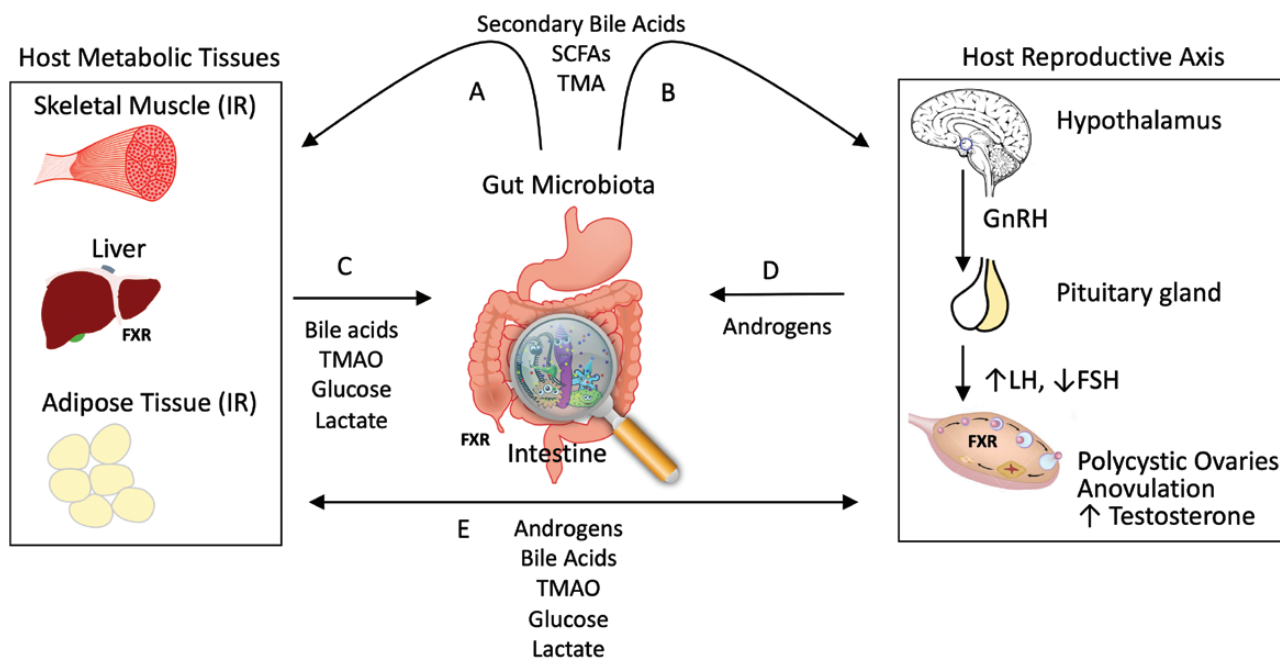
Figure 2. Relationship between the gut microbiome and polycystic ovary syndrome (PCOS). Accumulating evidence, in human studies and rodent models, indicates that there is an association between dysbiosis of the gut microbiome and PCOS. **A–B:** Gut microbes metabolize substrates that enter the gut, from the diet and the host, and produce metabolites that may act directly on the intestines or enter systemic circulation and influence various host tissues whose function is altered in PCOS, such as ovary, skeletal muscle, liver, and adipose tissue. Gut bacterial metabolites reported to be altered in PCOS include secondary bile acids, SCFAs, and TMA. For instance, bile acids bind to receptors, including FXR, in various tissues and activate intracellular signaling. **C:** Metabolic tissues, including skeletal muscle, liver, and adipocytes, produce metabolites (such as conjugated primary and secondary bile acids, TMAO, lactate, and glucose) that enter the gut and may alter the composition of gut bacteria by serving as substrates, thus providing selective advantages to certain strains of bacteria over others. **D:** The host reproductive axis regulates sex steroid hormone production. In PCOS, elevated levels of androgens may alter the composition of the gut microbial community. **E:** Crosstalk between host metabolic tissues and the reproductive axis also occurs independently of the gut microbiome and may be a driver of the pathology and development of PCOS. Further studies are needed to decipher how the interactions outlined in this figure occur mechanistically. Abbreviations: FSH, follicle-stimulating hormone; FXR, farnesoid X receptor; GnRH, gonadotropin-releasing hormone; IR, insulin resistance; LH, luteinizing hormone; SCFAs, short-chain fatty acids; TMA, trimethylamine; TMAO, trimethylamine N-oxide.

(reviewed previously [61, 62]). Although human studies cannot be used to determine causation of gut dysbiosis by HA, it is notable that multiple studies reported correlations between HA and changes in the gut microbiome. Both alpha diversity [22, 28, 37] and beta diversity [28] were associated with HA, indicating that higher testosterone levels are linked with changes in the overall composition of the gut microbial community. Within the phylum *Actinobacteria*, the genus *Collinsella* was positively correlated with testosterone levels in 2 studies [25, 26] (Table 5). Within the phylum *Bacteroidetes*, the genus *Bacteroides* was positively correlated with testosterone levels in 4 studies [20, 22, 24, 25]. Furthermore, the genus *Prevotella* was negatively correlated with the levels of testosterone in 1 study [24] and positively correlated with testosterone in 3 studies [25–27]. Within the phylum *Proteobacteria*, *Enterobacteriaceae* were positively correlated with testosterone [20–22] (Table 5).

Evidence from rodent models also indicates that HA may drive dysbiosis of the gut microbiome (Fig. 1). Two early studies used letrozole, a nonsteroidal inhibitor of aromatase, to induce HA and PCOS-like symptoms in mice and rats.

These studies showed that dysbiosis of the gut microbiome occurred as a consequence of treatment with letrozole, most likely due to the resulting HA rather than a direct effect of letrozole [18, 30]. In addition to reproductive and metabolic phenotypes similar to women with PCOS [58, 63], pubertal mice treated with letrozole had diet-independent reductions in alpha diversity, changes in beta diversity of the gut microbiome, and changes in the RA of specific bacteria [18] (Table 4). *Bifidobacteriaceae* was negatively correlated with testosterone while *Bacteroides*, *Streptococcus* [49], and *Prevotella* were positively correlated with testosterone [30], consistent with the human studies (Table 5). Two rodent studies showed that a proteobacteria called *Desulfovibrio* was negatively correlated with testosterone [32, 49], while this genus was not observed to be correlated with testosterone in humans (Table 5). Interestingly, these letrozole-induced changes in the gut microbiome appear to be activational rather than organizational changes; removal of letrozole treatment after the establishment of gut dysbiosis restored gut bacterial diversity [29]. Exogenous treatment of rats with DHT, which cannot be converted to estradiol, led to significant reductions in alpha

Table 5. Changes in gut bacteria correlated with testosterone levels in women and PCOS rodent models

Phylum	Family	Genus	Correlation	Host	Ref
Actinobacteria	*Bifidobacteriaceae*	*Bifidobacterium*	Negative	Human	[25]
Actinobacteria	*Bifidobacteriaceae*	*Bifidobacterium*	Negative	Rodent	[49]
Actinobacteria	*Coriobacteriaceae*	*Collinsella*	Positive	Human	[25]
Actinobacteria	*Coriobacteriaceae*	*Collinsella*	Positive	Human	[26]
Actinobacteria			Positive	Rodent	[49]
Bacteroidetes	*Bacteroidaceae*	*Bacteroides*	Positive	Human	[20]
Bacteroidetes	*Bacteroidaceae*	*Bacteroides*	Positive	Human	[22]
Bacteroidetes	*Bacteroidaceae*	*Bacteroides*	Positive	Human	[24]
Bacteroidetes	*Bacteroidaceae*	*Bacteroides*	Positive	Human	[25]
Bacteroidetes	*Bacteroidaceae*	*Bacteroides*	Positive	Rodent	[49]
Bacteroidetes	*Bacteroidaceae*		Positive	Human	[24]
Bacteroidetes	*Porphyromonadaceae*	*Parabacteroides*	Positive	Human	[20]
Bacteroidetes	*Porphyromonadaceae*		Negative	Human	[37]
Bacteroidetes	*Prevotellaceae*	*Prevotella*	Negative	Human	[24]
Bacteroidetes	*Prevotellaceae*	*Prevotella*	Positive	Human	[25]
Bacteroidetes	*Prevotellaceae*	*Prevotella*	Positive	Human	[26]
Bacteroidetes	*Prevotellaceae*	*Prevotella*	Positive	Human	[27]
Bacteroidetes	*Prevotellaceae*	*Prevotella*	Positive	Rodent	[30]
Bacteroidetes	*Prevotellaceae*		Negative	Human	[24]
Bacteroidetes	*S24-7*		Negative	Human	[19]
Firmicutes	*Clostridiaceae*	*Anaerotruncus*	Positive	Rodent	[32]
Firmicutes	*Clostridiaceae*	*Clostridium*	Positive	Rodent	[32]
Firmicutes	*Hungateiclostridiaceae*	*Ruminiclostridium*	Negative	Human	[27]
Firmicutes	*Lachnospiraceae*	*Butyrivibrio*	Negative	Rodent	[49]
Firmicutes	*Lachnospiraceae*	*Coprococcus*	Positive	Human	[25]
Firmicutes	*Lachnospiraceae*	*Fusicatenibacter*	Negative	Human	[27]
Firmicutes	*Lachnospiraceae*	*Tyzzerella*	Negative	Human	[27]
Firmicutes	*Lachnospiraceae*	*Tyzzerella*	Positive	Rodent	[32]
Firmicutes	*Lachnospiraceae-ND3007*		Negative	Human	[27]
Firmicutes	*Lactobacillaceae*	*Lactobacillus*	Negative	Rodent	[49]
Firmicutes	*Rumiacoccaceae-UCG-003*		Negative	Human	[27]
Firmicutes	*Ruminococcaceae*	*Faecalibacterium*	Negative	Human	[25]
Firmicutes	*Ruminococcaceae*	*Ruminococcus*	Positive	Human	[26]
Firmicutes	*Ruminococcaceae*	*Ruminococcus*	Positive	Rodent	[32]
Firmicutes	*Ruminococcaceae*	*Subdoligranulum*	Negative	Human	[27]
Firmicutes	*Ruminococcaceae*		Negative	Human	[22]
Firmicutes	*Ruminococcaceae*		Positive	Human	[34]
Firmicutes	*Streptococcaceae*	*Streptococcus*	Positive	Human	[22]
Firmicutes	*Streptococcaceae*	*Streptococcus*	Positive	Rodent	[49]
Firmicutes	*Veillonellaceae*	*Megasphaera*	Positive	Human	[26]
Firmicutes			Negative	Rodent	[49]
Proteobacteria	*Desulfovibrionaceae*	*Desulfovibrio*	Negative	Rodent	[32]
Proteobacteria	*Desulfovibrionaceae*	*Desulfovibrio*	Negative	Rodent	[49]
Proteobacteria	*Enterobacteriaceae*	*Escherichia*	Positive	Human	[22]
Proteobacteria	*Enterobacteriaceae*	*Raoultella*	Positive	Human	[21]
Proteobacteria	*Enterobacteriaceae*	*Shigella*	Positive	Human	[22]
Proteobacteria	*Enterobacteriaceae*		Positive	Human	[20]
Tenericutes			Negative	Human	[19]
Verrucomicrobia	*Akkermansiaceae*	*Akkermansia*	Negative	Human	[22]

Abbreviations: PCOS, polycystic ovary syndrome; Ref, reference number.

diversity and changes in beta diversity compared with placebo-treated rats [32, 50]. Hyperandrogenism driven by dehydroepiandrosterone (DHEA) treatment altered beta diversity of the gut microbiome in mice when combined with an HFD [64]. Taken together, these results suggest a strong role for testosterone as a modulator of the gut

microbiome, although it is unclear whether testosterone exerts an effect on gut microbes directly and/or indirectly through actions in androgen target tissues.

Caveats and future directions

The diagnosis of HA is obtained by measuring hirsutism with the Ferriman-Gallway score and/or biochemically by measuring serum testosterone levels. The "gold standard" method of measuring testosterone is liquid chromatography-mass spectrometry, although methods using radioimmunoassays can provide equivalent results, while other methods such as enzyme-linked immunosorbent assay (ELISA) can overestimate the amount of testosterone in the sample [10, 65]. In the studies reviewed herein, different methods of measuring testosterone were used, including ELISA-based techniques, radioimmunoassays, and liquid chromatography-mass spectrometry. Given that levels of steroid-hormone binding globulin (SHBG) are decreased in women with PCOS [66], it may also be relevant to examine the relationship between changes in the gut microbiome and the free androgen index (ratio of total testosterone to SHBG).

While the 2 hypotheses about the interaction between HA and gut dysbiosis were discussed separately, they are likely interconnected (Fig. 2). In 2017, vom Steeg and Klein reviewed evidence in support of the crosstalk between sex steroid hormones and the microbiota of the host [62]. However, much remains to be discovered about how sex steroid hormones interact with gut bacteria, especially with regards to how testosterone impacts the gut microbiome in females compared with males. To accomplish this, it will be important to employ metagenomic sequencing to identify gut microbial species/strains and microbial genes that are altered in response to increased levels of testosterone in women and PCOS rodent models. Moreover, transplantation of microbiota from women with PCOS or PCOS-like rodent models into germ-free mice will be crucial to comprehend the temporal changes in testosterone levels relative to other symptoms of PCOS as a result of exposure to PCOS-related microbiota. Finally, pharmacological inhibition of the androgen receptor using antiandrogens such as cyproterone or spironolactone [10] will help elucidate the role of androgen signaling in driving gut dysbiosis in women with PCOS or rodent models. Complementary studies using androgen receptor knockdown within specific host tissues will identify which sites of androgen action are required for gut dysbiosis.

Metabolites Associated With Gut Dysbiosis and PCOS

Although association does not equal causation, examining metabolite profiles in women with PCOS compared with healthy controls may provide insight into host/microbe interactions mediated by metabolites that can influence PCOS pathology. An early study in women showed that elevated levels of serum lactate were associated with PCOS [67], and host-produced lactate has been shown to enter the lumen of the gut and serve as a substrate for lactate-utilizing bacteria [68–70], potentially exerting a selective pressure within the microenvironment of the gut (Fig. 2). Moreover, 2 studies reported an association between increased serum levels of trimethylamine N-oxide (TMAO) and PCOS in women [71, 72] (Fig. 2). Trimethylamine N-oxid is a liver metabolite that originates from trimethylamine, which is produced by gut microbes from dietary precursors. Elevated levels of TMAO have been associated with an increased risk of cardiovascular disease [73]. While measuring microbiota-related metabolites in systemic circulation of women with PCOS may help identify mechanisms of regulation of host physiology by gut bacteria, investigating changes in metabolites in the gut or feces (as a proxy) may also highlight regulatory mechanisms. Along these lines, 1 study of the letrozole-induced PCOS rat model showed significant decreases in fecal SCFAs that are produced by gut bacterial fermentation of fiber and serve as signaling molecules in the host [49].

The relationship among bile acids, gut microbiota, and metabolic diseases (extensively reviewed elsewhere [74–77]) highlights an emerging key role for bile acids in regulating metabolic diseases including, potentially, PCOS. Primary bile acids serve as substrates for gut microbial enzymes that result in secondary bile acids that are recycled between the gut and liver via enterohepatic circulation [77]. An examination of glyco- and tauro-conjugated primary bile acids in systemic circulation showed that they were at higher levels in women with PCOS than in healthy women and were positively associated with HA [78]. On the other hand, another study reported that serum glycocholic acid was lower in women with PCOS than in women without the disorder [79]. Additionally, targeted metabolomics showed that the secondary bile acids, glycodeoxycholic acid (GDCA) and tauroursodeoxycholic acid (TUDCA), were lower in the serum and feces of women with PCOS and normal weight compared with healthy women [23]. It is intriguing that TUDCA was reported to be decreased in mice that received an FMT from women with PCOS or transplantation with *B. vulgatus* [23], suggesting that an altered gut microbiota was sufficient to cause changes in specific bile acid levels. Besides their functions in the absorption of lipids, bile acids act as signaling molecules by binding and activating receptors such as the farnesoid X receptor (FXR) [80–82] (Fig. 2), Takeda G protein receptor 5 [83], vitamin D receptor [84], pregnane X

receptor [85], sphingosine-1-phosphate receptor 2 [86], and muscarinic M2 receptor [87]. One study showed that FXR-knockout mice exhibited glucose intolerance and IR when challenged with glucose or insulin, respectively [88]. This suggests a role for bile acid signaling through FXR in regulating metabolism. However, the contributions of specific bile acids and gut bacteria that deconjugate bile acids (such as *Bacteroides* [89]) in PCOS remain unclear (Fig. 2).

Caveats and future studies

Our understanding of how gut metabolites are altered in PCOS is extremely limited. Most of the studies have investigated metabolomic profiles associated with PCOS within systemic circulation, rather than the feces, and these studies have not been performed in a comprehensive manner due to the difficulty of identifying metabolites using untargeted metabolomics. Both liquid and gas chromatography coupled with mass spectrometry could be utilized to begin to comprehend the full array of gut microbial metabolites that are changed in the feces of women with PCOS, and these analyses should be complemented with quantitative, targeted metabolomics focused on specific metabolites such as bile acids and SCFAs (Fig. 2A and 2B). Moreover, correlations between gut metabolites and microbial species/strains obtained with metagenomic sequencing will help provide a more comprehensive picture of the important interactions occurring in the gut of women with PCOS and potentially shed light on how steroid hormones can influence the gut microbiome.

Treatments of Gut Dysbiosis in PCOS

Fecal microbial transplant

Since gut dysbiosis has been proposed as a driver of PCOS symptoms, treatment with an FMT from healthy donors or representative microbes from a healthy gut might serve as a useful therapy to re-diversify the gut microbiome, although there are limited studies to support this idea. One study showed that performing an FMT from healthy rats into a letrozole-induced PCOS rat model resulted in a reduction of androgen levels, improved estrous cycles, normalized ovarian morphology, and increased levels of *Lactobacillus* and *Clostridium* species and decreased *Prevotella* [30]. Additionally, a co-housing experiment showed improved reproductive and metabolic symptoms of PCOS in letrozole-treated mice that were co-housed with healthy, placebo-treated mice in addition to changing the RA of *Coprobacillus* and *Lactobacillus* [31]. Overall, these

studies show promise that adjusting the gut microbial community in women with PCOS may alleviate some of the diet-independent, hyperandrogenic-induced symptoms.

Prebiotics: wheat dextrin and inulin

Resistant dextrin, a glucose polysaccharide that is fermented in the colon by microbes rather than absorbed in the small intestine, was given to women with PCOS and women without the disorder for 3 months [90]. Resistant dextrin lowered levels of free testosterone, hirsutism, the interval between menstrual cycles, fasting blood glucose, and lipid profile [90], but changes in alpha diversity or the RA of specific microbes was not assessed in this study. Additionally, the probiotic inulin was shown to improve gut dysbiosis, lower testosterone, and increase estradiol levels while improving ovarian morphology and weight gain in DHEA-treated mice fed an HFD [64]. However, alpha diversity was not assessed in this study, and thus, no conclusions can be specifically made about the role of inulin on the richness or evenness of the gut microbial community. In addition, it is unclear in mice whether inulin is targeting the effects of HFD or DHEA alone or in combination, so further studies investigating the effects of fermentable, dietary fiber on PCOS are warranted.

Probiotics: *Bifidobacterium lactis*, *Lactobacillus*, and lactic acid bacteria

Consuming probiotic (or beneficial) strains of bacteria has the potential to improve gut dysbiosis either directly through repopulation of the gut with healthy microbes or indirectly through the production of gut metabolites. *Bifidobacterium lactis V9* given as a 10-week treatment for PCOS in 14 women with the disorder decreased LH and increased intestinal SCFAs [25]. *Lactobacillus* given to letrozole-treated rats reduced androgen levels, improved estrous cyclicity, normalized ovarian morphology, as well as increased *Lactobacillus* and *Clostridium* species and decreased *Prevotella* [30].

Several studies also investigated the effects of probiotic combinations on PCOS phenotypes. A combination of *Bifidobacterium*, *Lactobacillus acidophilus*, and *Enterococcus faecalis* in a DHT-induced PCOS rat model improved reproductive and metabolic functions as well as alpha diversity of the gut microbiome [50]. In addition, certain strains of *Lactobacillus* and *Bifidobacterium* (HL2 and HB3) given to letrozole-treated rats protected against pathological changes in the ovaries and restored testosterone levels while upregulating levels of SCFAs [48].

Small molecules: metformin and IL22

Previous studies have shown that metformin decreases total testosterone, hirsutism, acne, LH, BMI, waist-to-hip ratio, and fasting insulin, and increases SHBG, follicle-stimulating hormone, and progesterone, while it also improves menstrual cycles [91, 92]. In addition to its role in the inhibition of androgen biosynthesis [93, 94], metformin treatment of individuals with T2D decreased the RA of *Bacteroides fragilis* and FXR signaling, while it increased levels of glycoursodeoxycholic acid in the gut in 1 study [95], as well as the RA of *Akkermansia muciniphila* and SCFA-producing microbes in another study [96]. Although no studies have investigated the effect of metformin on the gut microbiome of women with PCOS, 1 study in DHEA-treated mice showed that it improved dysbiosis of the gut, including increased levels of *Bacteroidetes* and decreased levels of *Helicobacter* and *Proteobacteria* [64]. In this mouse model, metformin also decreased testosterone levels while also improving ovarian function, weight gain, and IR [64].

IL22 is an anti-inflammatory cytokine produced by cells of the lymphoid lineage, including cells in the lamina propria of the intestinal wall called group 3 innate lymphoid cells (ILC3) [97]. IL22 was used to treat symptoms of PCOS in mice that received an FMT from women with PCOS, a transplantation of *B. vulgatus,* or treated with DHEA [23, 98]. In all of these models, IL22 improved reproductive and metabolic symptoms including a decrease in testosterone levels, increased insulin tolerance, and enhanced browning of adipose tissue [23, 98]. However, it is unknown whether IL22 altered the composition or function of gut microbiome or if other mechanisms were responsible for the beneficial effect. Qi et al hypothesized that an increase in the abundance of *B. vulgatus*, and potentially other bacteria in the intestine of women with PCOS, results in increased deconjugation of secondary bile acids and lower levels of GDCA and TUDCA, which normally bind to receptors involved in the production of IL22 [23]. Since there is no current evidence demonstrating that GDCA and TUDCA bind to receptors on ILC3 cells in vivo and that this binding leads to the production of IL22, future studies in PCOS rodent models will be useful to identify specific mechanisms by which changes in gut bacteria regulate secondary bile acids and IL22 production, as well as how IL22 signaling influences reproductive and metabolic phenotypes of PCOS.

Summary

In summary, this review highlights recent progress made in understanding the relationship between PCOS and dysbiosis of the gut microbiome in both humans and rodent models. Although there is considerable variability in the results obtained from 16S rRNA and metagenomic gene sequencing, many studies support the idea that changes in gut microbiota are associated with PCOS, including a decrease in biodiversity as well as changes in specific bacterial taxa. Notably, these changes occur both in adolescent girls and women with this disorder that are normal weight or obese, suggesting that a gut dysbiosis manifests along with other features of PCOS during puberty independently of BMI, although it is unclear whether obesity influences changes in the gut microbiome observed in PCOS. In addition, changes in gut microbiota are correlated with HA, indicating that elevated levels of testosterone may regulate the composition of the gut microbiome in females. We explored hypotheses that HA as well as dysbiosis of the gut microbiome could act as drivers of PCOS through their interaction with each other, although future studies are needed to understand the mechanisms involved. Moreover, we reviewed the limited studies that investigated changes in specific microbial metabolites associated with PCOS. While preliminary, these studies justify further exploration of this understudied area, with the exciting potential to uncover novel targets for small molecule therapeutics focused on PCOS. Finally, we reviewed studies investigating the efficacy of prebiotic, probiotic, and postbiotic treatments that modulate the gut microbiome and, in turn, improve symptoms of PCOS in both human and rodent studies. Although most of these studies need to be reproduced, the positive results on reproductive and metabolic features of PCOS from treatment with dietary fibers, probiotics such as *Bifidobacterium* and *Lactobacillus,* and small molecules including bile acids and anti-inflammatory cytokines indicate that this is an area deserving of future study.

Acknowledgments

Additional Information

Correspondence: Varykina G. Thackray, PhD, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Diego, La Jolla, CA 92093. E-mail: vthackray@health.ucsd.edu.

Disclosure Summary: The authors have nothing to disclose.

Data Availability: Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

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