

Review

Sex, Microbes, and Polycystic Ovary Syndrome

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Recent studies have shown that sex and sex steroids influence the composition of the gut microbiome. These studies also indicate that steroid regulation of the gut microbiome may play a role in pathological situations of hormonal excess, such as PCOS. Indeed, studies demonstrated that PCOS is associated with decreased alpha diversity and changes in specific Bacteroidetes and Firmicutes, previously associated with metabolic dysregulation. These studies suggest that androgens may regulate the gut microbiome in females and that hyperandrogenism may be linked with a gut 'dysbiosis' in PCOS. Future mechanistic studies will be required to elucidate how sex steroids regulate the composition and function of the gut microbial community and what the consequences of this regulation are for the host.

Sex-Dependent Interactions with the Gut Microbiome

The microbial community in the mammalian gastrointestinal tract (gut), comprised of bacteria, archaea, fungi, and viruses, rapidly diversifies after birth until it reaches a stable state in adulthood [1] (Box 1). Numerous host and environmental factors are associated with variation in the gut microbiome, including diet, host genetics, and hormones [14,15]. An intriguing example of how hormones interact with the gut microbiome came from investigating the effects of sex on the development of type 1 diabetes in a non-obese diabetic mouse model. Studies showed that exposure of female mice to androgens or the gut microbiome of male mice conferred protection from type 1 diabetes [16–18], indicating that sex-dependent interactions with the gut microbiome can influence the development of pathological states. Given the vast

Box 1. Introduction to the Gut Microbiome

Coevolution of mammals and the gut microbial community resulted in microbial regulation of many processes critical for the host, including energy biogenesis due to the fermentation of dietary fiber into short-chain fatty acids; synthesis of specific vitamins such as folic acid and B12; metabolism of bile acids, neurotransmitters, and hormones; elimination of specific toxins; defense against pathogens; and modulation of immunity and metabolism [2]. Studies showed that a diverse gut microbiome develops after birth and is influenced by mode of delivery (vaginal versus C-section), breast-feeding, and weaning [3,4]. While the gut microbiome of breast-fed infants is dominated by bifidobacteria, the community shifts to one composed predominantly of bacteria from the Bacteroidetes and Firmicutes phyla after the introduction of solid food.

Over the past 15 years, many studies have investigated the relationship between the gut microbiome and host metabolism. Studies demonstrated that the gut microbiome of individuals with metabolic disorders, such as obesity and type 2 diabetes, differed from healthy individuals [5–8]. In addition, mouse models of obesity, such as leptin-deficient *ob/ob* mice and mice fed a high-fat diet, were associated with changes in gut microbes [9,10]. Meta-analyses of the human obesity studies demonstrated that the alpha diversity of the gut microbiome was consistently lower in obese individuals compared with controls but did not observe consistent changes in the Bacteroidetes:Firmicutes ratio or specific bacterial abundances [11]. Studies showed that fecal microbiome transplantation from obese humans into germ-free mice resulted in an obese phenotype [12,13], indicating a potential causative role of the gut microbiome in the development of metabolic disorders.

Highlights

16S rRNA gene sequencing studies revealed that sex influences the taxonomic composition of gut bacteria in humans and rodents.

The idea that sex-dependent differences in gut microbes are driven by sex steroid levels is supported by studies demonstrating that the gut microbiome diverges after puberty and that gonadectomy results in an altered gut microbiome.

Recent studies demonstrated that changes in the gut microbiome are linked with androgen excess in women with PCOS and in female rodent models of the disorder.

Studies reported that PCOS was associated with decreased alpha diversity and changes in the relative abundance of specific bacteria from the Bacteroidaceae, Clostridiaceae, Erysipelotrichidae, Lachnospiraceae, Lactobacillaceae, Porphyromonadaceae, Prevotellaceae, Ruminococcaceae, and S24-7 families previously linked with metabolic dysregulation.

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potential for crosstalk among hormones and gut microbes, it is worth reviewing what is currently known about the interactions of sex and sex steroids with the gut microbiome and whether these interactions have implications for disorders with altered steroid levels, such as polycystic ovary syndrome (PCOS) (Box 2).

While initial **16S rRNA gene sequencing** (see Glossary) studies did not identify significant sex-dependent differences in the gut microbiome [37], subsequent studies reported an important influence of sex on the taxonomic composition of gut microbes [38,39]. Compared with men, women were associated with greater **alpha diversity** of the gut microbiome [39] (see Figure 1 for an explanation of alpha and **beta diversity**). Studies also reported that men had a higher relative abundance of **Bacteroidetes** (including *Prevotella* and *Bacteroides thetaiotaomicron*) than women [38,40,41]. In addition, men had a lower abundance of Clostridia, *Methanobrevibacter*, and *Desulfovibrio* compared with women [39].

Sex differences were also observed in the composition of the rodent gut microbiome. Similar to humans, female mice had greater alpha diversity than male mice and male mice were reported to have a higher abundance of Bacteroidetes compared with female mice [18,42]. Several other bacterial taxa were reported to differ between the sexes but these results were not consistent among the studies. For instance, one study found that, at the phyla level, the relative abundances of Actinobacteria and Tenericutes were higher in male mice than in females [43]. Moreover, *Allobaculum*, *Anaeroplasma*, and *Erwinia* were more abundant in males compared with females, while *SMB53*, *Dorea*, *Coprococcus*, and *Ruminococcus* were less abundant [43]. In contrast, another study indicated that Ruminococcaceae and *Anaerostipes* were more abundant in males than females, while Peptostreptococcaceae were less abundant [42]. Altogether, these studies in humans and rodents demonstrated that there are sex-dependent differences in the gut microbiome. Future studies are needed to characterize the comprehensive effects of sex on the composition of gut microbes, including bacteria, archaea, fungi, and viruses. In addition, the

Box 2. The Pathoetiology of PCOS

PCOS is a heterogeneous disorder with an estimated world-wide prevalence of 5%–15%. PCOS is diagnosed using the Rotterdam Consensus criteria that require at least two of the following: hyperandrogenism, oligo- or amenorrhea, and polycystic ovaries [19]. Studies have shown that PCOS can result in profound, long-term health consequences [20]. PCOS is the leading cause of anovulatory infertility and women diagnosed with PCOS also have an increased risk of miscarriage and pregnancy complications. In addition, a majority of women with PCOS have metabolic abnormalities such as obesity, hyperinsulinemia, insulin resistance, and dyslipidemia that increase their risk of developing type 2 diabetes, hypertension, and non-alcoholic fatty liver disease [21–25].

The etiology of PCOS is poorly understood, although PCOS is considered to be a polygenic complex genetic trait [26]. Familial studies found a high rate of PCOS in the mothers and sisters of women diagnosed with PCOS [27]. A large study with monozygotic and dizygotic twins identified the heritability of PCOS as ~70% [28]. Moreover, genome-wide association studies have reported multiple susceptibility loci associated with an increased risk of developing PCOS [29]. In addition to genetics, environmental factors such as prenatal exposure to androgens may also play a role in the etiology of PCOS [30].

Despite the association of PCOS with obesity, there is little evidence to suggest that dietary factors or obesity predispose women to PCOS [31]. Metabolic dysfunction occurs predominantly in women with PCOS diagnosed with hyperandrogenism and ovulatory dysfunction, irrespective of body mass index [32,33]. Although these studies suggest that hyperandrogenism is correlated with metabolic dysregulation, it is not understood why women with PCOS become insulin resistant and why they have a higher incidence of insulin resistance than predicted by their body mass index. Insulin resistance and hyperinsulinemia may contribute to metabolic dysregulation by upregulating ovarian androgen production and increasing androgen bioactivity through decreased sex hormone-binding globulin production [34,35]. In addition, obesity exacerbates insulin resistance, hyperinsulinemia, and androgen production in women with PCOS [36], resulting in a vicious cycle of hyperandrogenism and insulin resistance.

Glossary

Activational effect: steroid hormone signaling which causes transient effects on specific cellular processes that are reversible upon removal of the hormone.

Alpha diversity: represents the number of different species within a specific community or individual sample.

Bacteroidetes: gram-negative bacteria that are one of the two predominant phyla present in the mammalian gut.

Beta diversity: represents how similar one community or individual sample is to another.

Beta-glucuronidase: enzyme synthesized in gut bacteria that deconjugates host-derived molecules such as bilirubin, neurotransmitters, and hormones that were previously conjugated in the liver.

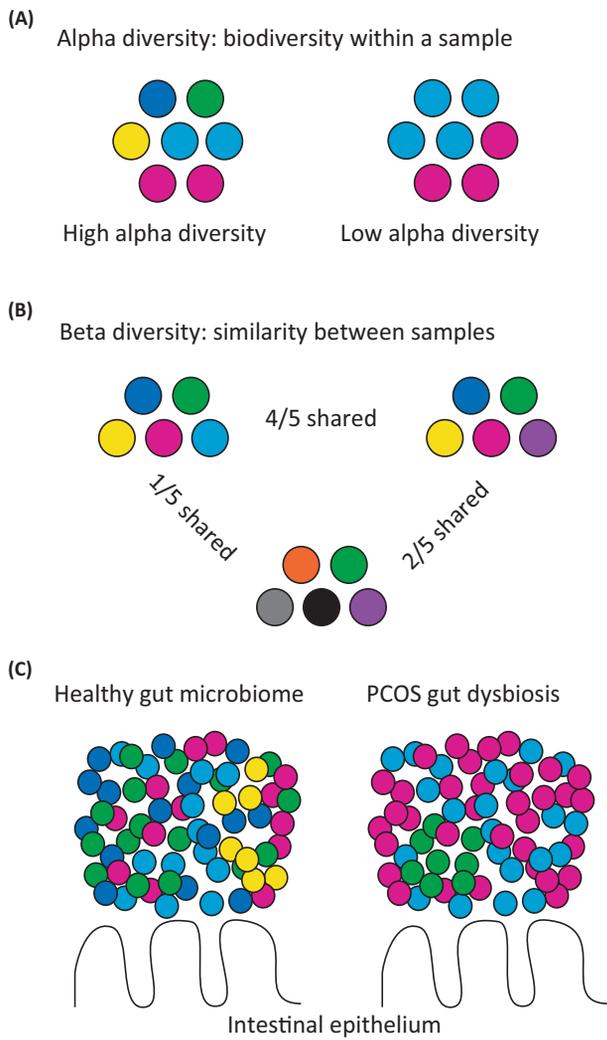
Firmicutes: mostly gram-positive bacteria that are one of the two predominant phyla present in the mammalian gut.

Letrozole: non-steroidal inhibitor of aromatase (the enzyme responsible for the conversion of testosterone to estrogen).

Lipopolysaccharide (LPS): also known as endotoxin, LPS is the major component of the outer membrane of gram-negative bacteria. LPS activation of Toll-like receptor 4 results in cytokine production and activation of the innate immune system.

Organizational effect: exposure to sex steroid hormones during development (e.g., in prenatal or early postnatal periods), which has been shown to result in permanent changes in tissues and organs such as the brain.

16S rRNA gene sequencing: universal primers containing distinct barcodes are used to amplify a region of the bacterial 16S ribosomal RNA gene in fecal or cecum samples and the resulting amplicons are pooled, purified, and sequenced.



Trends in Endocrinology & Metabolism

Figure 1. Polycystic Ovary Syndrome (PCOS) Is Associated with Changes in the Gut Microbiome. Comparisons of alpha and beta diversity between hypothetical, gut microbial communities. Different bacterial taxa are represented by circles with different colors. (A) Alpha diversity represents the biodiversity (species richness) within a specific community or individual sample. The sample on the left has high alpha diversity (five bacterial taxa), while the sample on the right has low alpha diversity (two bacterial taxa). (B) Beta diversity represents how similar one community or individual sample is to another. The samples on the left and right are similar to each other (4/5 shared bacterial taxa), while the sample on the left is not very similar to the sample in the middle (1/5 shared bacterial taxa). (C) PCOS is associated with a microbial imbalance or dysbiosis compared with the healthy state including lower alpha diversity and changes in the relative abundance of bacteria from the Bacteroidaceae, Clostridiaceae, Erysipelotrichidae, Lachnospiraceae, Lactobacillaceae, Porphyromonadaceae, Prevotellaceae, Ruminococcaceae, and S24-7 families.

mechanisms involved in sex-dependent effects on gut microbes and the functional consequences for the gut microbial community and the host need to be elucidated.

Influence of Sex Steroids on the Gut Microbiome

Differences associated with sex in the gut microbiome could be attributable to genetic (sex chromosomes) or hormonal mechanisms. If sex steroid hormones are linked with changes in the gut microbiome, one would expect the gut microbiome to be similar prior to puberty and

then diverge after puberty. Multiple studies in humans and rodents support this idea. For instance, a significant difference was found in the composition of the gut microbiome of fraternal twins of the opposite sex during adolescence compared with twins of the same sex, while no difference was detected between fraternal twins of the same or different sex during infancy [44]. Furthermore, studies in rodents demonstrated that the gut microbiome diverged after puberty in a sex-specific manner. The gut microbiome was found to be similar between male and female mice at 3 weeks of age (prepuberty) but significant differences were observed at 6 weeks of age (postpuberty) [17,18].

Evidence of **activational effects** of sex steroids on the gut microbiome comes from studies characterizing the effect of gonadectomy on adult rodents. Studies showed that alpha diversity decreased in ovariectomized mice and rats [45,46]. One study in mice reported a decrease in Bacteroidetes and an increase in **Firmicutes** in ovariectomized mice compared with controls [46]. Specifically, ovariectomy resulted in a decreased abundance of Ruminococcaceae, Rikenellaceae, Clostridia, S24-7, *Bacteroides*, and *Prevotella* and an increase in the abundance of *Lactobacillus* and *Bifidobacterium animalis*. Conversely, another study reported the opposite trend in ovariectomized rats bred for low aerobic capacity, with an increase in Bacteroidetes such as *Bacteroides*, *Barnesiella*, and *Prevotella*, as well as a decrease in Firmicutes, compared with control rats [47]. With regards to males, it is not clear whether any changes in alpha diversity are associated with gonadectomy although castration was reported to result in changes in the beta diversity of the gut microbiome [43]. Similar to ovariectomy, castration was associated with decreased Bacteroidetes compared with control male mice as measured by quantitative PCR [48] and decreased abundance of Ruminococcaceae [43]. Differences in other bacterial taxa were reported to be mouse strain-specific [43].

Given the inherent variation in the gut microbiome amongst individuals and strains of rodents, additional studies will be required to understand how the composition and function of the gut microbiome are regulated by sex steroids and, potentially, sex chromosomes in males and females. Future studies investigating proximal versus long-term effects of gonadectomy and the effects of gonadectomy with or without steroid replacement in rodents will be informative, as well as studies investigating the effects of gonadectomy in other mammalian species. Studies focusing on whether changes in the gut microbiome are associated with sex steroid levels during different developmental stages, such as puberty and menopause, or in pathological states of hormone insufficiency/excess, should also be investigated.

Changes in the Gut Microbiome Are Associated with PCOS

Since steroid hormone levels are linked with changes in the gut microbiome and many women with PCOS have androgen excess that is associated with metabolic dysregulation (Figure 2), it is possible that a microbial imbalance in the gut may contribute to PCOS. Several recent studies investigated whether there was a link between the gut microbiome and PCOS [49–52]. These studies involved women from China and Europe (Austria, Poland, and Spain) diagnosed with PCOS using the Rotterdam criteria [19]. PCOS was associated with a change in the overall composition of the bacteria in the gut, including a decrease in alpha diversity (species richness and phylogenetic diversity) as well as changes in beta diversity [49–51] (Figure 1). One study did not detect a change in alpha or beta diversity in women with PCOS compared with women without PCOS, potentially due to a small sample size and high interindividual variability in the gut microbiome [52]. Alpha diversity has been proposed to correlate with the health of an ecosystem, as diverse communities may increase the stability and productivity of an ecosystem [53]. Lower alpha diversity of the gut microbiome has been associated with human obesity in several meta-analyses [11,54,55]. Thus, it is possible that decreased bacterial diversity results

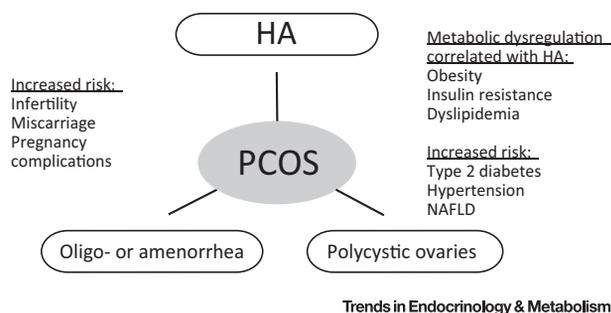


Figure 2. Polycystic Ovary Syndrome (PCOS) Is Correlated with an Increased Risk of Developing Reproductive and Metabolic Dysregulation. PCOS is diagnosed using the Rotterdam Consensus Criteria that require two out of three of the following criteria: hyperandrogenism (HA), oligo- or amenorrhea, and polycystic ovaries. Women diagnosed with PCOS have an increased risk of infertility, miscarriage, and pregnancy complications. Women with PCOS that have HA also have metabolic dysregulation, including obesity, insulin resistance, and dyslipidemia that increases their risk for type 2 diabetes, hypertension, and non-alcoholic fatty liver disease (NAFLD).

in changes in gut function that contribute to metabolic dysfunction in women with PCOS. Future studies will be required to understand how changes in alpha diversity influence the gut microbiome and host physiology.

In addition to detecting changes in the overall composition of the gut microbiome, several studies also demonstrated changes in the relative abundances of specific types of gut bacteria of women with PCOS compared with women without the disorder [49–52]. These changes are summarized in Table 1. Notably, changes in the abundance of multiple bacteria from the Bacteroidetes and Firmicutes phyla were associated with PCOS. In particular, Bacteroidetes from the Bacteroidaceae, Porphyromonadaceae, and S24-7 families and Firmicutes from the Clostridiaceae, Erysipelotrichidae, Lachnospiraceae, Lactobacillaceae, and Ruminococcaceae families were higher or lower in women with PCOS. Since many of these bacteria produce butyrate and propionate, changes in the abundance of specific Bacteroidetes and Firmicutes in PCOS may result in altered production of short-chain fatty acids that impact metabolism, gut barrier integrity, and immunity [15]. In addition, one study observed a decrease in bacteria from the Tenericutes phylum (order ML615J) [49], a group of bacteria previously associated with obesity and metabolic dysregulation [56]. Another study observed a decrease in *Akkermansia* from the Verrucomicrobia phylum [50], which has been reported to be decreased in diet-induced obese mice [57].

There are many caveats that need to be considered when comparing studies of the gut microbiome. Factors such as diet, geography, age, prescription drug use, alcohol consumption, and smoking have been reported to influence the composition of the gut microbiome [58,59]. In addition, the use of small sample sizes may limit the ability to detect changes in specific bacteria. Moreover, the use of different methodologies to extract DNA, PCR amplify and sequence 16S rRNA genes, and analyze the resulting data can complicate comparison of gut microbiome studies.

Given the association of hyperandrogenism with metabolic dysregulation in women with PCOS [33], studies also investigated whether hyperandrogenism is linked with changes in the gut microbiome. Torres *et al.* showed that a decrease in alpha diversity was negatively correlated with total testosterone levels and hirsutism [51]. In addition, hyperandrogenism was strongly associated with changes in the overall bacterial community composition of the gut (beta diversity). These results agreed with studies demonstrating that changes in the abundance

Table 1. Changes in Bacteroidetes and Firmicutes Relative Abundances Associated with PCOS

Study	Cohort characteristics	Bacteroidetes	Firmicutes	Refs
Lindheim, 2017 ^a	Austria	↓ S24-7		[49]
	19 controls			
	24 PCOS			
	58% HA			
	Mild IR and not obese			
Liu, 2017 ^a	China	↓ Bacteroidaceae Bacteroides	↓ Lactobacillaceae Lactobacillus	[50]
	15 controls (6 obese)		↓ Ruminococcaceae	
	33 PCOS (21 obese)		↓ Ruminococcaceae <i>Clostridium IV</i>	
	All HA, OA, and PCOM		↓ Ruminococcaceae <i>Oscillibacter</i>	
Torres, 2018 ^a	Poland	↑ Bacteroidaceae <i>Bacteroides coprophilus</i>	↑ Clostridiaceae Faecalibacterium prausnitzii	[51]
	48 controls	↑ Porphyromonadaceae <i>Porphyromonas</i>	↑ Lachnospiraceae Blautia	
	73 PCOS	↓ Porphyromonadaceae Odoribacter	↓ Clostridiales XI <i>Anaerococcus</i>	
	85% HA		↓ Lachnospiraceae <i>Roseburia</i>	
	Average BMI non-obese		↓ Ruminococcaceae Ruminococcus bromii	
Insenser, 2018 ^a	Spain		↑ Erysipelotrichidae <i>Catenibacterium</i>	[52]
	16 control (8 obese)		↑ Erysipelotrichidae <i>Kandleria</i>	
	15 PCOS (8 obese)		↑ Lachnospiraceae Oribacterium	
	All HA			
	Groups age- and BMI-matched			
Kelley, 2016	Letrozole-induced mouse model	↓ Bacteroidaceae Bacteroides	↑ Erysipelotrichidae <i>Allobaculum</i>	[65]
	4-week old, pubertal mice	↓ Porphyromonadaceae Parabacteroides	↑ Lachnospiraceae Blautia	
	pellet implant, 50 μg/day	↓ Rikenellaceae <i>Alistipes</i>	↑ Lachnospiraceae Coprococcus	
		↓ S24-7	↑ Lachnospiraceae Roseburia	
			↑ Ruminococcaceae Ruminococcus	
Guo, 2016	Letrozole-induced rat model		↓ Ruminococcaceae	
		↑ Prevotellaceae Prevotella	↓ Lactobacillaceae Lactobacillus	[66]

Table 1. (continued)

Study	Cohort characteristics	Bacteroidetes	Firmicutes	Refs
	6-week old, pubertal rats		↓ Ruminococcaceae <i>Clostridium</i>	
	1 mg/kg daily oral gavage		↓ Ruminococcaceae Ruminococcus	
Sherman, 2018	Prenatal androgenized rat model	↑ Porphyromonadaceae Tannerella	↑ Clostridiaceae F. prausnitzii	[68]
	5 mg/kg testosterone cypionate	↑ Prevotellaceae Paraprevotella	↑ Lachnospiraceae Blautia	
	Gestational day 20	↑ Rikenellaceae <i>Rikenella</i>	↑ Lachnospiraceae Oribacterium	
		↓ S24-7	↑ Lachnospiraceae Roseburia	
		↓ Bacteroidaceae Bacteroides	↓ Erysipelotrichidae <i>Allobaculum</i>	
		↓ Porphyromonadaceae Odoribacter	↓ Lachnospiraceae <i>Coprococcus</i>	
		↓ Porphyromonadaceae Parabacteroides	↓ Lachnospiraceae <i>Dorea</i>	
			↓ Lactobacillaceae Lactobacillus	
			↓ Ruminococcaceae Ruminococcus	
Moreno-Indias, 2016	Neonatal androgenized rat model	↑ Prevotellaceae Paraprevotella	↑ Lachnospiraceae Coprococcus	[45]
	1250 µg testosterone propionate	↑ S24-7	↑ Ruminococcaceae Ruminococcus	
	Postnatal day 1	↓ Bacteroidaceae Bacteroides	↓ Ruminococcaceae <i>Oscillospira</i>	

Bacteria are listed by family, genus, and species (if known); bacteria altered in more than one study are in bold type.

Abbreviations: BMI, body mass index; HA, hyperandrogenemia; IR, insulin resistance; OA, oligo- or amenorrhea; PCOM, polycystic ovarian morphology; PCOS, polycystic ovary syndrome.

^aHuman studies.

of several gut bacteria correlate with total testosterone levels and hirsutism [49,60]. It should be noted that one study detected a positive correlation between alpha diversity and testosterone levels that was opposite to the trend observed in the other studies [52]. While one cannot infer causation from association studies, the accumulating data suggests that androgens may be an important factor in shaping the composition of the gut microbiome and changes in the gut microbiome may influence the development and pathology of PCOS. Future studies should investigate how the composition and function of the gut microbiome is altered in PCOS and whether the gut microbiome of women diagnosed with PCOS using the criteria of oligomenorrhea and polycystic ovaries is distinct from women diagnosed with the other subtypes of PCOS that include hyperandrogenism.

Since many women diagnosed with PCOS are obese, several studies investigated the influence of obesity on the gut microbiome in the context of PCOS. It is important to understand whether

obesity influences the gut microbiome in a similar or distinct manner in women with and without PCOS. It would also be informative to determine whether obesity influences the gut microbiome in a similar or distinct manner from hyperandrogenism. Studies in China and Spain recruited four groups of women (non-obese and obese women with and without PCOS) to address these questions [50,52]. Liu *et al.* demonstrated changes in alpha diversity between non-obese control women and obese women with PCOS, as well as changes in beta diversity between non-obese control women and the other three groups [50]. However, the authors could not differentiate amongst the other groups, making it difficult to conclude whether the patterns that they observed were driven by obesity or PCOS. Insenser *et al.* did show that there was a difference in beta diversity between non-obese and obese women with PCOS [52]. Given that these changes were not observed in non-obese and obese control women, it is possible that obesity has specific effects on the gut microbiome in the context of PCOS. Further sampling of the gut microbiome in large cohorts of obese women with and without PCOS should provide greater insight into whether metabolic factors such as obesity and insulin resistance influence the composition and function of the gut microbial community in women with PCOS.

Using Rodent Models to Study the Role of Gut Microbiome in PCOS

In order to study mechanistically how changes in the gut microbiome influence the pathology of PCOS, model systems need to be employed. Studies have shown that there are broad similarities between the gut microbial communities in humans and rodents at the phylum level [61], suggesting that rodents may be suitable models for studying the interaction between the gut microbiome and PCOS. Approximately 80% of the gut bacteria in humans and mice are composed of bacteria from the Bacteroidetes and Firmicutes phyla, with ~10% composed of Actinobacteria and Proteobacteria. Other phyla such as Tenericutes and Verrucomicrobia are present at much lower abundances. Different approaches have been used to model PCOS in rodents, including models that induce hyperandrogenism during fetal development, early postnatal development, puberty, or adulthood [62–64]. Two studies employed the nonsteroidal aromatase inhibitor, **letrozole**, to increase testosterone levels by limiting the conversion of testosterone to estrogen [65,66]. The letrozole model has many PCOS-like characteristics, including hyperandrogenism, acyclicity, polycystic ovaries, and increased luteinizing hormone, as well as a metabolic phenotype that includes weight gain, hyperinsulinemia, and insulin resistance [67]. However, low estradiol levels and hemorrhagic ovarian cysts in the letrozole model are not characteristic of PCOS. Other models used treatment of testosterone during late gestation or during postnatal day 1 to explore **organizational effects** of androgens on the adult gut microbiome and metabolism [45,68]. A caveat for these models is that treatment with exogenous testosterone may also result in altered estradiol levels, since testosterone can be aromatized to estrogen.

Androgen treatment of rodents resulted in changes in biodiversity and the relative abundance of specific bacterial families that are also altered in women with PCOS, suggesting that these models may be suitable for exploring the effects of androgens on the gut microbiome and host physiology. Alpha diversity decreased in the letrozole-induced PCOS mouse model and the neonatal androgenized rat model compared with controls, while beta diversity changed in three models [45,65,68]. More specifically, Bacteroidaceae, Clostridiaceae, Erysipelotrichidae, Lachnospiraceae, Lactobacillaceae, Porphyromonadaceae, Ruminococcaceae, and S24-7 were altered in rodent PCOS models and in women with PCOS (Table 1). Additionally, the studies in rodents highlighted changes in Prevotellaceae that were not detected in the studies of women with PCOS (Table 1). Interestingly, *Prevotella* was reported to be more abundant in men than women [40] and a *Prevotella*-dominated microbiome was associated with altered short-chain fatty acid production [60]. The prenatal androgenized rat model also had a decrease in *Akkermansia* similar to Chinese women with PCOS [50,68]. Changes in the relative

abundances of additional bacteria from the Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria phyla were also reported in the prenatal androgenized rat model, although the significance of these changes remains to be confirmed, since correction for multiple comparisons does not appear to have been employed in this study. Indeed, more studies in rodent models are needed to define the suite of microbes (bacteria, archaea, fungi, and viruses) that are associated with hyperandrogenism in females. In addition, there is a need to explore the temporal patterns of these changes as well as to determine how these changes impact gut microbial function and host physiology.

If hyperandrogenism modulates the composition of the gut microbiome, one possibility is that androgen treatment of females results in a microbial community more similar to males than females. As discussed previously, males were reported to have a higher abundance of *Bacteroides* than females. Since women with PCOS and female mice treated with androgens had a lower abundance of *Bacteroides* than controls, this suggests that the gut microbiome is not masculinized in PCOS. However, males were reported to have lower abundances of Clostridia and *Ruminococcus* than women and this decrease was observed in women with PCOS and female mice treated with androgens relative to control females. One study investigated whether the gut microbiome of women with and without PCOS could be distinguished from men [52]. Potentially due to a small sample size, the alpha and beta diversity of the gut microbiome of women with or without PCOS could not be differentiated from each other, making it difficult to evaluate whether the gut microbes of women with PCOS were more similar to men than control women. It is interesting to note that *Catenibacterium* and *Kandleria* were observed to be more abundant in women with PCOS and in men compared with women without PCOS. Further studies are needed to clarify whether hyperandrogenism in females results in masculinization of the gut microbiome.

Potential Mechanisms for Sex Steroid Effects on the Gut Microbiome

Currently, there is little understanding of the mechanisms involved in sex steroid regulation of the gut microbiome. Studies have reported that the amount of conjugated versus deconjugated estrogens excreted in urine and feces was altered after antibiotic treatment [69,70]. These results make sense in light of the fact that many gut bacteria synthesize **beta-glucuronidase** enzymes that deconjugate host-derived molecules such as bilirubin, neurotransmitters, and hormones that were previously conjugated in the liver [71]. While conjugation targets the compound for excretion via bile, feces, or urine, deconjugation facilitates reabsorption into enterohepatic circulation. Since deconjugation results in the liberation of a sugar group, glucuronic acid, this process produces energy for the gut bacteria. Thus, it is interesting to speculate that sex steroids could directly affect the composition of the gut microbiome by altering beta-glucuronidase activity and energy production due to changes in substrate levels.

In addition to a potential direct effect on gut microbes, sex steroids may indirectly modulate the gut microbiome through activation of steroid receptors in the host. While steroid hormone signaling in the nervous and immune systems has received significant attention, not much is known about the role of estrogen or androgen receptors in the gastrointestinal system. Estrogen receptor beta is expressed at higher levels in intestinal epithelial cells than estrogen receptor alpha and a knockout of estrogen receptor beta was reported to have an altered gut microbiome compared with wild-type mice [72,73]. Androgen receptor was reported to be expressed in human colon mucosa, while another study observed that the androgen receptor was expressed in colon stromal cells [74,75]. Thus, it is possible that changes in estrogens or androgens could result in altered intestinal functions such as contractility, transit, or enteric hormone secretion by regulating steroid receptor signaling within the gastrointestinal system,

the brain, and/or the periphery. Interestingly, stool consistency (which reflects transit time and water availability) has been shown to be an important contributor to individual variation in the human gut microbiome [39].

Since studies have shown that there are sex differences in the immune system and most immune cells express steroid receptors, it is possible that changes in sex steroids could influence the composition of the gut microbiome by modulating systemic or intestinal immunity of the host. Although this is an understudied area, one possible mechanism could involve steroid signaling in dendritic cells, which are antigen-presenting cells important for promoting tolerance of commensal bacteria and bridging innate and adaptive immunity [76]. Studies have shown that estrogen signaling via estrogen receptor alpha is important for the development and function of dendritic cells [77]. However, these studies are complicated by the fact that sex-dependent differences in the gut microbiome may have an effect on immunity. For instance, transplantation of feces from male or female mice into germ-free mice showed that the percentage of specific immune cells in Peyer's patches and mesenteric lymph nodes in the germ-free recipients differed according to the sex of the donor [78]. It is also possible that changes in sex steroids could alter the immune response indirectly by regulating the integrity of the intestinal barrier. Decreased integrity of the intestinal barrier is associated with infiltration of gram-negative bacteria into circulation and activation of a peripheral inflammatory response by **lipopolysaccharide (LPS)**. Studies have shown that ovariectomy resulted in increased colonic epithelial cell permeability associated with decreased expression of tight junction proteins, occludin, and claudin [79,80]. Studies also demonstrated that markers of intestinal barrier dysfunction, including zonulin, a regulator of tight junctions and LPS binding protein, were increased in the serum of women with PCOS compared with healthy women [49].

Summary

The explosion of data arising from the sequencing of bacterial 16S rRNA genes has begun to provide insight into interactions between sex or sex steroids and the gut microbiome. It is now evident that sex plays a role in the maturation of the gut microbiome after puberty. Furthermore, studies have shown that changes in sex steroid levels can impact the composition of the gut microbial community in models of steroid insufficiency (ovariectomy and castration), suggesting that sex-dependent changes in the gut microbiome are driven primarily by circulating steroid hormone levels in females and males. Metagenomic and metabolomic studies are needed to confirm the associations of sex and sex steroids with the gut microbiome and define the full suite of microbes and microbial metabolites that are regulated by estrogens and androgens. Future mechanistic studies are also needed to understand how sex steroids shape the composition and function of the gut microbiome during normal physiological processes and in pathological situations of hormone insufficiency/excess. Regarding PCOS, studies in germ-free mice will be instrumental in determining whether gut microbes are necessary or sufficient for the development of the PCOS reproductive and metabolic phenotypes. Moreover, further studies are required to determine whether manipulation of the gut microbiome can be used to treat PCOS and to identify potential candidates for pre-/probiotic therapies.

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Outstanding Questions

What are the effects of sex and sex steroids on the composition and function of the entire gut microbial community, including bacteria, archaea, fungi, and viruses? In addition to 16S rRNA sequencing, metagenomics and metabolomics could provide exciting insights into hormonal regulation of the gut microbiome.

What are the mechanisms involved in sex-dependent differences in the gut microbiome? How do steroid hormones regulate the gut microbiome in females and males with regards to normal biological functions and in pathological states of hormonal insufficiency or excess? Tissue-specific knockouts of estrogen and androgen receptors in mice could be informative in determining whether steroid receptors in tissues such as the brain, immune cells, intestinal epithelium, or liver are required to mediate sex steroid-dependent differences in the gut microbiome.

What are the consequences of sex and sex steroid-mediated changes in the gut microbiome on host physiology and host-microbe interactions? Do sex-dependent differences in the gut microbiome influence the development and pathology of disease states?

How is the composition and function of the gut microbiome altered in women with PCOS? Are these alterations influenced by androgen excess or obesity? Do women diagnosed with PCOS using different criteria have similar or distinct changes in their gut microbiome? Does hyperandrogenism result in masculinization of the gut microbiome? Can we identify prebiotics, probiotics, or microbial metabolites that could be used to treat PCOS?

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