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Review

Roles of Sex Hormones and Gender in the Gut Microbiota

Kichul Yoon[1] **and Nayoung Kim**[2,3]*

[1]Department of Internal Medicine, Wonkwang University Sanbon Medical Center, Gunpo, Gyeonggi-do, Korea; [2]Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, Gyeonggi-do, Korea; and [3]Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, Korea

The distribution of gut microbiota varies according to age (childhood, puberty, pregnancy, menopause, and old age) and sex. Gut microbiota are known to contribute to gastrointestinal (GI) diseases such as irritable bowel syndrome, inflammatory bowel disease, and colon cancer; however, the exact etiology remains elusive. Recently, sex and gender differences in GI diseases and their relation to gut microbiota has been suggested. Furthermore, the metabolism of estrogen and androgen was reported to be related to the gut microbiome. As gut microbiome is involved in the excretion and circulation process of sex hormones, the concept of "microgenderome" indicating the role of sex hormone on the gut microbiota has been suggested. However, further research is needed for this concept to be universally accepted. In this review, we summarize sex- and gender-differences in gut microbiota and the interplay of microbiota and GI diseases, focusing on sex hormones. We also describe the metabolic role of the microbiota in this regard. Finally, current subjects, such as medication including probiotics, are briefly discussed.
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Key Words
Gastrointestinal diseases; Gender; Gut; Microbiota; Sex hormones

Introduction

Gut microbiota consists of as many as 10^{13} to 10^{14} microorganisms.[1] The collective genome of microbiota (microbiome) covers various genes needed for many biologic processes; it is thus regarded as a separate organ for metabolism, like the human liver.[2,3] Imbalance of the gastrointestinal microbiome could impact many diseases, such as neoplasm and autoimmune or cardiovascular conditions.[4-6] There are several factors that affect the gut microbiome. Among them, gender and sex hormones play an important role after puberty.[7] Interestingly, the bacteria-to-human cell ratio is different between genders, that is, higher in women than in men. The bacteria-to-human cell ratio is 1.3 in men and 2.2 in women.[8] As the gut microbiome is involved in the excretion and circulation process, the "microgenderome" indicating the interaction between sex hormones and the gut microbiome is an emerging research topic.[9,10] In functional gastrointestinal disorders (FGID), microbiota and inflammation can affect gastrointestinal (GI) function and sensation.[11] There is emerging evidence that sex hormones can play a role in the interplay between the microbiome and GI symptoms.[7] We hereby review the interaction between sex hormones, gender, and microbiota.

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*Correspondence: Nayoung Kim, MD, PhD
Department of Internal Medicine, Seoul National University Bundang Hospital, 82, Gumi-ro 173 beon-gil, Bundang-gu, Seongnam, Gyeonggi-do 13620, Korea
Tel: +82-31-787-7008, Fax: +82-31-787-4051, E-mail: nayoungkim49@empas.com

Estrogen and Microbiota

Reproductive endocrine function involves a variety of hormones controlled by intricate feedback mechanisms (Fig. 1).[12] The ovaries, adrenal glands, and adipose tissue produce estrogens. Estrogens produced in the body or ingested as food can be metabolized by gut microbes. The resultant metabolites again influence the host.[13] Sex hormones directly modulate the metabolism of bacteria through steroid receptors, including estrogen receptor beta.[14]

Meanwhile, the gut microbiome with β-glucuronidase activity deconjugates the conjugated circulating estrogens excreted in the bile (Fig. 2).[10] Deconjugation enables the estrogen reabsorption process to the system.[10] The deconjugated estrogens circulate and affect multiple organs not only reproductive, but also skeletal, cardiovascular, and central nervous systems via estrogen receptors.[15] Typically, estrogen binds to nuclear receptors, causing conformational change (Fig. 3).[16] Central and peripheral alterations have been linked to irritable bowel syndrome (IBS) through intestinal barrier permeability and immune system modulation.[9] Epidemiological studies suggest a female predominance of IBS, implying the effect of sex hormones on its pathogenesis.[9,17] GI transit duration also has been reported to vary according to menstrual cycle, pregnancy, and postpartum.[17]

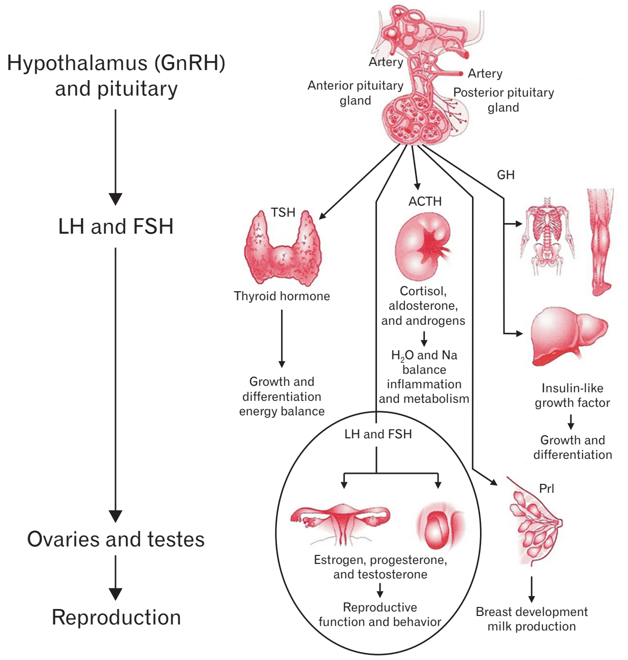

Figure 1. Overview of endocrine physiology. Gonadotropin-releasing hormone (GnRH). LH, luteinizing hormone; FSH, follicle-stimulating hormone; TSH, thyroid-stimulating hormone; ACTH, adrenocorticotropic hormone; GH, growth hormone; Prl, prolactin. Adapted from Molina.[12]

In a human study, estradiol, estrone, 13 estrogen metabolites, and total estrogens were measured in urine and feces.[18] In male and post-menopausal females, the total urinary estrogen levels were associated with fecal microbiota abundance and α-diversity.[18] Non-ovarian estrogens were also associated with fecal Clostridia taxa and some Ruminococcaceae, which produce β-glucuronidase. Fecal β-glucuronidase was inversely correlated with fecal estrogens.[18] In contrast, pre-menopausal female estrogen levels were not related to the fecal microbiome or enzymes.[18] From the results they concluded that non-ovarian estrogens were affected by the intestinal microbiome and enzymes such as β-glucuronidase.[18] A recent animal study showed that increasing age was associated with a significant reduction in fecal β-glucuronidase activity in females.[19] Male C57BL/6 mice showed significantly higher β-glucuronidase activity than did females.[19] In this study, fecalase, a cell-free extract of feces, was employed in a colorimetric-based assay to quantify enzymatic activity.[19] This approach could be employed to investigate gender differences in human samples.

Estrogen and testosterone have been shown to directly affect the gut microbiome and immune cells. β-estradiol affects the transformation of dendritic cells to produce IL-12 and IFN-γ.[20] This, in turn, activates pathways for pro-inflammatory cytokines.[20] Estradiol prolongs the survival of B cells and activates polyclonal B cells. The resulting pro-inflammatory environment with altered intestinal gut permeability causes the migration of gut microbiota into the lamina propria, which again promotes inflammation processes.[20]

Androgen and Microbiota

The relationship between androgen and microbiota has been reported. An animal study measured unconjugated and glucuronidated androgen levels. In young adult males, unconjugated dihydrotestosterone (DHT) was 70-fold higher in the feces than in the serum. The distal intestine of germ-free mice showed high glucuronidated testosterone and DHT, but very low free DHT levels. They concluded that the gut microbiome affects the intestinal metabolism and deglucuronidation of androgens.[21]

Another study investigated the gut microbes and serum levels of testosterone in men and estradiol in women.[22] Male and female patients with high testosterone or estradiol levels showed a more diverse gut microbiome.[22]

An animal study showed that antibiotic exposure inhibited

Genus	β-glucuronidase	β-galactosidase
Collinsella	+	−
Edwardsiella	+	−
Alistipes	+	+
Bacteroides	+	+
Bifidobacterium	+	+
Citrobacter	+	+
Clostridium	+	+
Dermabacter	+	+
Escherichia	+	+
Faecalibacterium	+	+
Lactobacillus	+	+
Marvinbryantia	+	+
Propionibacterium	+	+
Roseburia	+	+
Tannerella	+	+

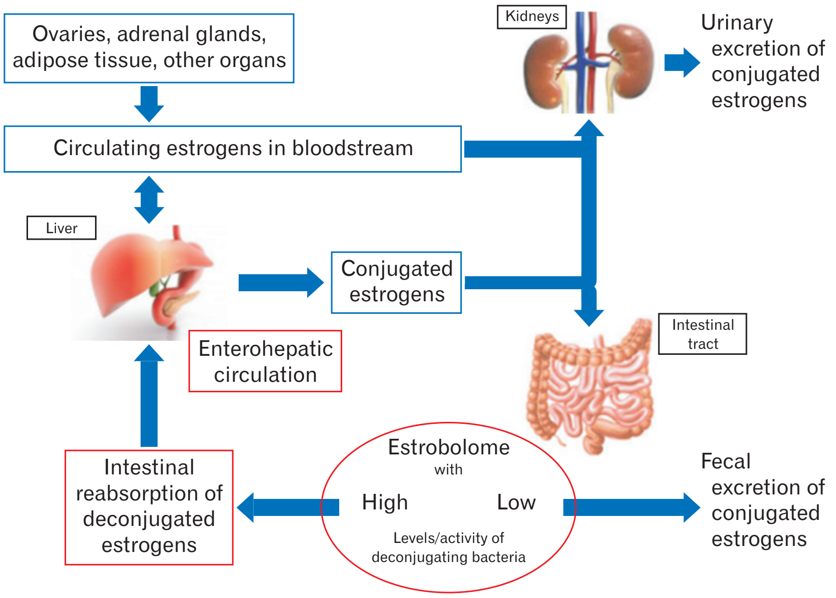

Figure 2. Estrobolome: gene repertoire of intestinal microbiota with the ability of estrogen metabolism. β-glucuronidase from microbiota deconjugates estrogen. The "active" deconjugated and unbound estrogens act on estrogen receptor alpha (ERα) and ERβ. Adapted from Kwa et al.[10]

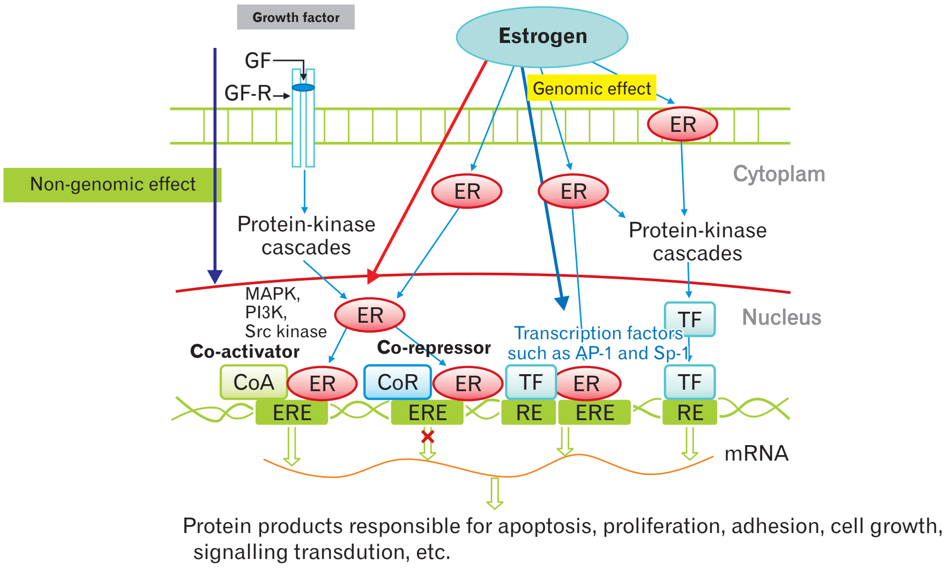

Figure 3. Molecular pathway of estrogen receptor (ER) action. Typically, estrogen binds to the ligand-binding domain of receptors in the nucleus, which causes a conformational change. Estrogen-responsive elements (ERE) mediate genomic effects. RE, response element; TF, transcription factor; GF, growth factor; GF-R, growth factor receptor; CoA, co-activator; CoR, co-repressor; MAPK, mictogen-activated protein kinase; PI3K, phophoinositide 3-kinase; Src, proto-oncogene tyrosine-protein kinase; AP-1, activating protein-1; Sp-1, stimulating protein-1. Adapted from Nie et al.[16]

mitochondrial dysfunction in a mouse Leydig tumor cell line (MLTC-1), inducing reduced steroidogenesis.[23] Mice exposed to doxycycline subchronically showed a reduction in microbiome diversity and changes in composition.[23]

In males, testosterone shows an inhibitory effect on T-cell proliferation. In contrast to estradiol, testosterone does not alter the intestinal barrier.[20] Blood testosterone levels are correlated with gut microbiota. Furthermore, the sex difference was reversed by male

Table 1. Studies Focused on Specific Sex Hormones

Author, year	Human/ animal	Specific hormone	Sex/age	Specific microorganism	Interaction with microbiota
Colldén et al,[21] 2019	Mice	T, DHT	Both	Germ-free mice study	High glucuronidated T and DHT, low DHT
Shin et al,[22] 2019	Human	T, E2	Male (37.45 ± 1.91)	*Acinetobacter, Dorea, Ruminococcus*, and *Megamonas*	Correlated with T level
			Female (46.15 ± 2.26)	Bacteroidetes, Firmicutes	High E group had more Bacteroidetes and less Firmicutes
				Slackia and *Butyricimonas*	Correlated with E2 level
Hou et al,[23] 2019	Mice/cell	T	Male	Doxycycline study	Doxycycline induced T deficiency and sperm quality
Sherman et al,[30] 2018	Rat	Prenatal T	Female offspring	Nocardiaceae, Clostridiaceae, *Akkermansia, Bacteroides, Lactobacillus, Clostridium*	High Nocardiaceae and Clostridiaceae, low *Akkermansia, Bacteroides, Lactobacillus*, and *Clostridium*
Yurkovetskiy et al,[24] 2013	Mice	T	Both/castration	Porphyromonadaceae, Veillonellaceae and Kineosporiaceae, Lactobacillaceae, etc	T level correlated with microbiota
Zheng et al,[25] 2020	Rat	DHT	Female	Bacteroidetes, diversity analyses	DHT associated with microbiota disturbance
Liu et al,[26] 2017	Human	T/LH/FSH	Pre-menopausal female	*Bacteroides, Escherichia/Shigella, Streptococcus, Akkermansia*, Ruminococcaceae	Associated with the clinical parameters of PCOS
Liang et al,[27] 2020	Human	T/LH/FSH/ E2/DHEA	Pre-menopausal female	Bacteroidetes, Firmicutes, and Verrucomicrobia	PCOS showed lower SVs and alpha diversity than did the control
Chu et al,[31] 2019	Rat	T, AMH	Female	*Parasutterella, Corynebacterium, Odoribacter, Acinetobacter*	*Parasutterella* was correlated with T
Jobira et al,[28] 2020	Human	T	Adolescent female	Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Porphyromonadaceae, Streptococcaceae	PCOS showed difference in microbiota. α-Diversity was associated with T
Arroyo et al,[29] 2019	Mice	T/LH	Pubertal female	*Lactobacillus, Lachnospiraceae* spp., *Ruminococcus, Roseburia, Sutterella, Bifidobacterium, Parabacteroides, Blautia*	Letrozole treatment resulted in restoration of α/β diversity
Torres et al,[32] 2018	Human	T	Pre-menopausal female	*Porphyromonas* spp., *Bacteroides coprophilus Blautia* spp., *Faecalibacterium prausnitzii, Anaerococcus* spp., *Odoribacter* spp., *Roseburia* spp., and *Ruminococcus bromii*	PCOS was associated with reduced microbiome biodiversity
Xue et al,[33] 2019	Mice	T/E2	Female	*Bifidobacterium, Proteobacteria, Helicobacter, Parasutterella*	Inulin and metformin alleviated PCOS by modulating gut microbiota
Org et al,[34] 2016	Mice	DHT	Both/GDX	Ruminococcacea, *Akkermansia*	Effect of GDX/hormone replacement on microbiota
Markle et al,[35] 2013	Mice	T	Both, young and old	*Roseburia, Blautia, Coprococcus* 1, *Parabacteroides*, and *Bilophilia*	Microbes protected autoimmune condition
Harada et al,[36] 2020	Mice	T (ArKO)	Both	*Turicibacter, Lactobacillus*	HFD-dependent metabolic disorders in ArKO males were abolished after antibiotic treatment
Harada et al,[37] 2016	Mice	T	Male/castration	Firmicutes, Bacteroidetes, *Lactobacillus*	Castration affected microbiome and obesity
Poutahidis et al,[38] 2014	Mice	T	Male	*Lactobacillus reuteri*	Probiotics improved testis function
Song et al,[39] 2020	Mice	E2	Both/OVX	Verrucomicrobia, Cyanobacteria, Firmicutes, Bacteroidetes	E2 supplement affected AOM/DSS-treated mice

T, testosterone; DHT, dihydrotestosterone; E2, estradiol; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PCOS, polycystic ovary disease; DHEA, dehydroepiandrosterone; SVs, structural variations; AMH, anti-Müllerian hormone; GDX, gonadectomy; HFD, high-fat diet; ArKO, aromatase-deficient mice; OVX, ovariectomy; AOM, azoxymethane; DSS, dextran sodium sulfate.

castration, which confirmed the effect of androgens on microbiota.[24]

Androgen excess represented by polycystic ovary syndrome (PCOS) is associated with gut microbiota disturbance. 5α-Dihydrotestosterone (DHT) with a high-fat diet (HFD) could lower gut microbiota diversity.[25] In a human study with 33 patients, the co-abundance bacterial groups increased in PCOS were *Bacteroides*, *Escherichia/Shigella*, and *Streptococcus*.[26] They showed a negative correlation with ghrelin, while positive relationships with testosterone and body mass index were noted. In contrast, *Akkermansia* and Ruminococcaceae decreased with PCOS, showed opposite results with body weight, sex hormone, and brain-gut peptides.[26]

A recent human study showed that butyrate producers decreased in a PCOS group.[27] Another human study with 58 obese female adolescents showed that gut microbiota was altered in obese adolescent PCOS patients.[28] The aromatase inhibitor letrozole treatment of PCOS mice showed a positive effect on reproduction, metabolism, and the gut microbial community.[29]

In androgen metabolism, the gut microbiota has been reported to be an important regulator. In the small intestine content of mice, glucuronidated testosterone and DHT were found at high levels. The distal intestine had high levels of free DHT.[21] Markedly high levels of unconjugated DHT were observed in the feces of young adult males.[21] In germ-free mice, glucuronidated testosterone and DHT were high; however, very low free DHT levels were noted in the distal intestine. This implies that gut microbiota affects the intestinal metabolism of DHT and testosterone.

We summarize the relevant studies focused on specific sex hormone in Table 1.

Gender Difference/Gonadectomy Status

Important shifts in the maturation of the gut and sex hormones occur simultaneously. Sex differences within these systems occur at similar ages, implying communication between gender and gut microbiota.[40] We summarize relevant studies focused on gender differences in Table 2.

Females showed greater gut microbial diversity in a study of 1135 individuals.[41] In particular, *Akkermansia muciniphila* was abundant in females.[41] In pre-menopausal women, compared to in post-menopausal female, the Firmicutes:Bacteroidetes ratio was higher, *Lachnospira* and *Roseburia* were relatively more abundant, and plasma levels of glucagon-like peptide-1, one of the intestinal incretins released from enteroendocrine cells in the gut to potentiate glucose clearance in response to the ingestion of food, were also higher.[42] The relative abundances of the *Prevotella*, *Parabacteroides*, and *Bilophila* genera were lower in pre-menopausal than post-menopausal women. The plasma levels of IL-6 and monocyte chemoattractant protein-1 representing inflammatory levels were also lower than those in post-menopausal women.[42]

Gonadectomy (GDX) model was used in animal studies to investigate the function of sex hormones. A research showed that GDX showed that the abundance of Ruminococcacea was markedly different between GDX males and the control on a high-fat/high-sucrose diet.[34] In females, 2 strains showed differences between the sham control and GDX mice. *Akkermansia* was more abundant in the control than in GDX female mice.[34] In addition, an interesting animal study with low capacity running (LCR) and high running capacity (HCR) rats was reported to mimic the post-menopausal phenotype.[44] A decreased Firmicutes:Bacteroidetes ratio was observed in LCR—but not HCR—mice subjected to ovariectomy (OVX). LCR OVX was associated with increased microbial diversity and Bacteroidetes. HCR OVX did not show any changes in specific phyla.[44]

Age

Sex differences in childhood gut microbiota have not been reported owing to gonadal hormone quiescence. Approximate mean sex steroid levels in plasma shows pubertal surge and difference between sexes.[40,65] The microbiota undergoes changes according to age, represented by a human study showing the core microbiota of elderly subjects was different from that of younger adults, with higher proportion of *Bacteroides* species (spp.) and distinct abundance patterns of *Clostridium* groups.[66] A recent study reported that early-life adverse events such as maternal lipopolysaccharide injection, maternal separation, and unpredictable chronic stress affected gut microbiota according to gender.[67]

One germ-free mice study reported the sex-specific effects of the early-life microbiota status on serotonin production.[44] The reduced sex differences in serotonin concentration in germ-free mice were reversed after microbial colonization. Sex differences in the hippocampal serotonergic neurocircuit also reappeared.[40,68] Pubertal gonadal hormone alteration and its effect on sexually dimorphic brain development could not be reversed by hormone replacement in adulthood.[69]

The core microbiota of the elderly has a greater proportion of *Bacteroides* species and abundant *Clostridium* groups than does that of younger subjects.[66] In centenarians, rearrangement in the Firmicutes population and an enrichment in facultative anaerobes

Table 2. Studies Focused on Gender Difference

Author, year	Human/animal	Sex/age	Specific microorganism	Result in sex difference/effect of microbiota
Sinha et al,[41] 2019	Human	Both	*Akkermansia, Bacteroides caccae, Coprobacillus, Rothia mucilaginosa, Clostridium bolteae,* etc.	Females had higher antibiotic resistance genes
Santos-Marcos et al,[42] 2018	Human	Pre-/post-menopausal female	*Lachnospira, Roseburia, Prevotella, Parabacteroides, Bilophila*	Difference between pre- and post-menopausal women
Koren et al,[43] 2012	Human	Pregnant women	Proteobacteria, Actinobacteria	Microbiota changed from T1 to T3
Cox-York et al,[44] 2015	Rat	Female	Bacteroidetes	Relationship of LCR/HCR with OVX
Lee et al,[45] 2018	Rat	Both young and old	*Akkermansia muciniphila*, Desulfovibrionaceae	Different response to HFD
Johnson et al,[4] 2020	Mice	Both juvenile and adult	*Bacteroides*, Verrucomicrobia, Proteobacteria, Firmicutes, Cyanobacteria, etc.	Microbial depletion affected differently according to sex
Sha et al,[46] 2013	Human	Both	*Bacteroides, Bifidobacterium, Helicobacter, Lactobacillus, Enterococcus,* etc.	No sex difference was reported in IBD
Halfvarson et al,[47] 2017	Human	Both	Enterobacteriaceae, Ruminococcaceae, *Prevotella, Faecalibacterium*	Genetic load scores (GLS) were more predictive than was sex
Frank et al,[48] 2007	Human	Both	*Bacteroides*, Proteobacteria, Fusobacteria, Actinobacteria, Firmicutes, etc.	No sex difference was reported in IBD
Kozik et al,[49] 2017	Mice	Both	Ruminococcaceae, Anaerostipes, Peptostreptococcaceae, *Adlercreutzia*, Coriobacteria, *Streptococcus*, etc.	Sex and age difference in colitis model
Shastri et al,[50] 2015	Rat	Both	Bacteroidetes, Lactobacillaceae, Porphyromonadaceae, Prevotellaceae	Response to oligofructose supplementation was different between sex
Takagi et al,[51] 2019	Human	Both	*Prevotella, Megamonas, Fusobacterium, Megasphaera, Bifidobacterium, Ruminococcus, Akkermansia*	Sex difference in Japanese subjects
Dewulf et al,[52] 2013	Human	Female	*Bifidobacterium, Faecalibacterium prausnitzii, Bacteroides, Propionibacterium*	Effect of inulin diet on obese women
Feng et al,[53] 2014	Human	Both	*Faecalibacterium prausnitzii*	Difference between sex
Aguirre et al,[54] 2011	Human	Both	*Faecalibacterium prausnitzii*	Difference between sex
Mueller et al,[55] 2006	Human	Both	*Eubacterium rectale, Clostridium coccoides, Bacteroides, Prevotella, Bifidobacterium*	Difference between sex
Walsh et al,[19] 2020	Mice	Both adolescent and adult	Antibiotic study	Difference between age and sex
Sheng et al,[56] 2017	Mice	Both	Desulfovibrionaceae, Deferribacteraceae, Helicobacteraceae	Sex difference in WD-induced steatosis
Mu et al,[57] 2017	Mice	Both/castration	*Lactobacillus* strains	Sex difference in probiotic administration effect
Lee et al,[58] 2017	Rat	Both	*Lactobacillus farciminis*	Sex difference in probiotic administration effect
Zhang et al,[59] 2018	Mice	Both	Bacteroidetes, Firmicutes, Proteobacteria, *Barnesiella*, *Clostridium* XIVa, etc.	Sex difference in anti-aging intervention
Karunasena et al,[60] 2014	Mice	Both	*Mycobacterium avium* subsp. *paratuberculosis*, *Lactobacillus animalis* NP-51	Sex difference to introduced microorganisms
Mahnic et al,[61] 2018	Human	Both	Fungal community	No sex difference
Strati et al,[62] 2016	Human	Both	Fungal richness/*Aspergillus*, Tremellomycetes	Fungal richness; difference in culture-based analysis; no difference in metagenomics
Edogawa et al,[63] 2018	Human	Both	*Prevotella*, etc.	Sex difference to NSAID
Son et al,[64] 2019	Mice	Both	*Lactobacillus murinus, Bacteroides acidifaciens,* and *Helicobacter hepaticus*	Sex difference during development of IBD

LCR/HCR, high (HCR) and low (LCR) aerobic capacity; OVX, ovariectomy; HFD, high-fat diet; IBD, inflammatory bowel disease; WD, western diet; NSAID, non-steroidal anti-inflammatory drugs.

are characteristic.[70] The old rats showed a higher abundance of Ruminococcaceae and Lachnospiraceae, which are butyrate producers.[45] In the cecum of rats, the sulfate-producing Deltaproteobacteria increased with age.[45] Recently, we reported the microbial changes and host response of F344 rat colon, focusing on sex and age.[71] A HFD-induced low species richness and high abundance of *Desulfovibrio* spp. and *Clostridium lavalense* were noted with colon mucosal cell proliferation.[71] Only in young rats and female aged rats, the abundance ratio of *A. muciniphila* and *Desulfovibrio* spp. increased with a HFD.[71]

In people aged over 70 years, changes in gut physiological function can affect the gut microbiome composition.[7,72,73] In a German study with 35 292 adults, total colony-forming units did not show age- or sex-related changes.[52] However, individual bacterial species differed according to age: *Escherichia coli* and *Enterococci* spp. both increased, and *Bacteroides* spp. decreased. *Lactobacillus* and *Bifidobacterium* were stable throughout life.[72,74]

Effect of Diet

Diet has sex differential effects on gut microbiota.[75] In the study of dietary intake of phytonutrients in relation to fruit and vegetable consumption in Korea, a higher proportion of women than men met the recommended intake of fruits and vegetables.[76] In addition, the Korean national health and nutrition examination surveys among 3 groups, men, menopausal women, and postmenopausal women, showed that men's dietary carotenoid intake was the lowest.[77] Since fruits and vegetables are the main source of dietary carotenoids, men consume less dietary fruits and vegetables than women. However, diet study is very difficult to perform in human especially in terms of sex differential effect of diet on the gut microbiota because sex- and gender-difference has many underlying conditions including age, race and culture. Usually diet studies are performed in animals, in which diet can be easily controlled. There are many studies on the relationship between gut microbiota and fat and/or bile acid regarding sex. HFDs induce an increase in bile secretion.[78] Secondary bile acids are produced by gut bacteria from primary bile acids. Large intestinal anaerobic bacteria deconjugate and dehydroxylate to form secondary bile acids, such as deoxycholic acid and lithocholic acid.[79,80] An animal study on sex differences in bile acids reported that sex differences in Western diet-induced steatosis, insulin sensitivity, and microbiota functions were farnesoid X receptor (FXR)-dependent.[56] Male Western diet-fed FXR knockout mice showed the most severe steatosis and highest liver and serum lipid profiles, and insulin resistance than females. FXR deficiency increased the numbers of Desulfovibrionaceae, Deferribacteraceae, and Helicobacteraceae only in males.[56]

In addition, in a study on laboratory fish, mice, and humans, Bolnick et al[81] showed a gender-specific relationship between diet and microbiota. They first employed principal coordinate analysis and further analyzed the relative abundance according to diet and sex. They found that the mammalian gut microbiome is affected by sex–diet interactions. When males were fed a HFD, they showed an increased abundance of *Lactobacillus, Alistipes,* Lachnospiraceae, and *Clostridium*, whereas females did not.[81]

Another animal study investigated the association between gender, physical activity, and the gut microbiome.[82] High-fat, high-corn starch, and high-sucrose diets were provided. Rats were housed in a sedentary environment or under physically active conditions with wheel-running access (RUN). Males in the RUN environment equally consumed the 3 diet types, with significantly less consuming the HFD than in the other groups.[82] Females preferred a HFD. Regarding microbiota, only males showed an effect of physical activity. Males in the RUN showed more richness than did females in the sedentary environment.[82]

In IBS, the gut microbiota involved in bile acid metabolism may affect the pathophysiology.[83]

A study on human fecal samples showed 2 orthologues, which has a role in secondary bile synthesis, were decreased in IBS patients.[83] The microbiota, primary bile acids, and secondary bile acids (mainly deoxycholic acid and lithocholic acid) could be measured to investigate the sex differences in IBS in future studies.

The Effect of Microbiota on the Disease Manifestations Depending on Sex Differences

Gut dysbiosis triggers diseases that manifest differently between the sexes. The most investigated disease or condition include autoimmune diseases, obesity, and FGID. However, multifactorial factors including sex, age, and dysbiosis affect these diseases and they are also closely related and interact mutually. We tried to review relevant research to date.

Functional Gastrointestinal Disorder and Microbiome-Gut-Brain Axis

Gut microbiota play key roles in modulating the brain-gut axis and intestinal barrier (Fig. 4).[84-86] Short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate are the most abundant

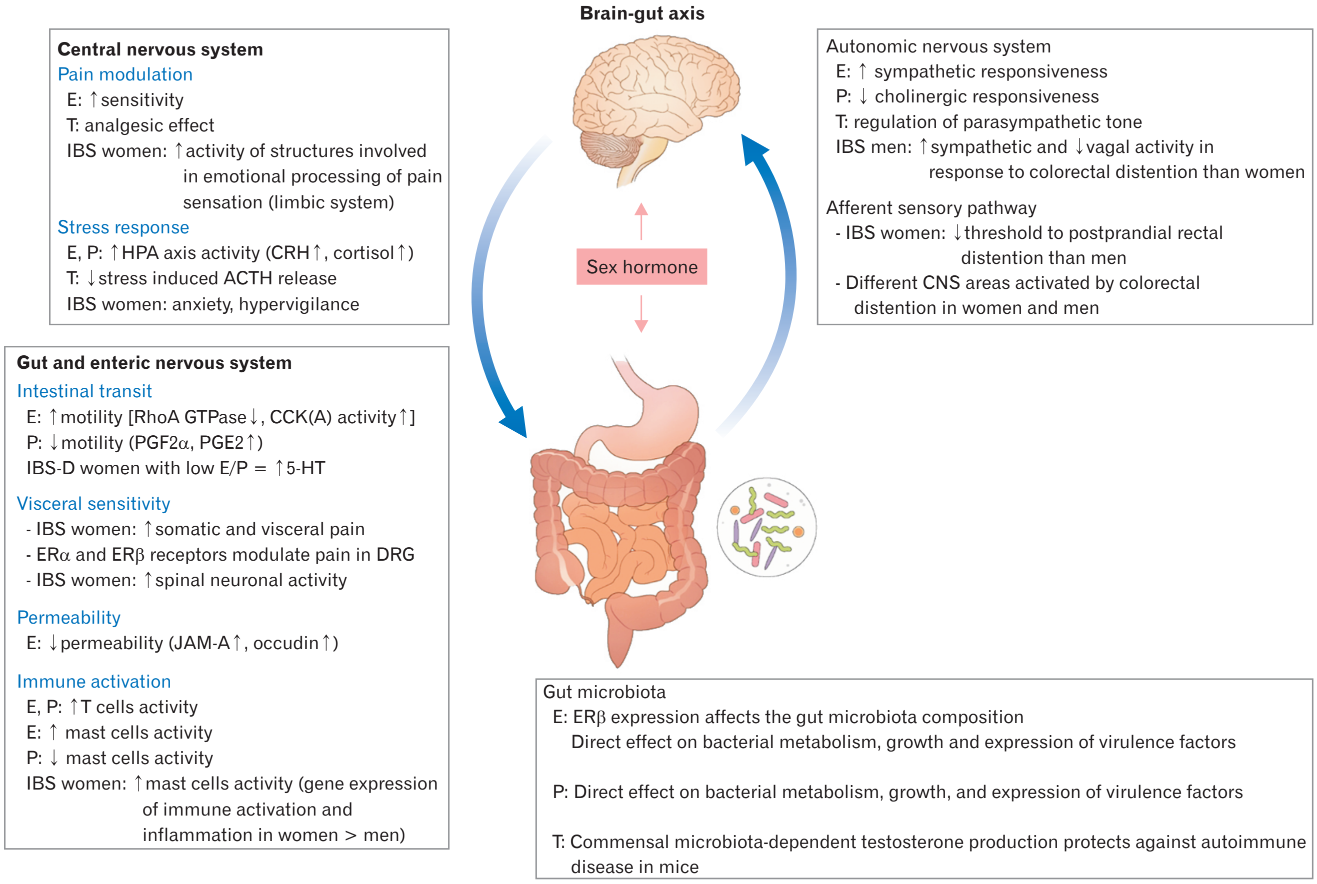

Figure 4. Sex hormone and brain–gut axis in irritable bowel syndrome (IBS). E, estradiol; T, testosterone; P, progesterone; HPA, hypothalamic-pituitary-adrenal; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone; CNS, central nervous system; RhoA, Ras homolog family member A; GTP, guanosine-5'-triphosphate; CCK, cholecystokinin; PGE2, prostaglandin E2; IBS-D, IBS with diarrhea; 5-HT, 5-hydroxytryptamine; ER, estrogen receptor; DRG, dorsal root ganglion; JAM-A, junctional adhesion molecule-A. Adapted from Kim and Kim.[84]

and important microbial end-products. They are inflammation modulators that regulate gut motility and wound-healing.[87] They have been also found as a link between the microbiome-gut-brain axis.[6,80,88] Among them, butyrate is considered to be the reason for the health benefit from the fermentation process of indigestible carbohydrate.[89] Interestingly, the sex difference in butyrate-producing gut microbiota has been reported.[90] In addition, a recent study has shown that male and female rat gut microbiomes have different profiles of SCFAs when an oligofructose-containing diet is administered to both.[50] In females, oligofructose supplementation increased the abundance of Bacteroidetes, while no difference was found in males.[50] Furthermore, fecal butyrate, liver IgA, IL-6, and cecal IL-6 levels were increased in males but IL-10 levels were higher in females.[50] Interestingly, Native Africans had remarkably higher abundance in the butyrate-producing bacteria such as *Faecalibacterium prausnitzii, Clostridium* cluster IV, and *Clostridium* cluster XIVa.[91] In contrast, *Bacteroides* was the dominant in African Americans.[91] *Bacteroides-Prevotella* group showed higher levels in men than women.[55]

There have been a few clinical studies regarding the microbiota and FGIDs. For instance, the differences in gut microbiota associated with sex were investigated in 277 Japanese volunteers.[51] Increases in the genera *Prevotella*, *Megamonas*, *Fusobacterium*, and *Megasphaera* in males and increases in *Bifidobacterium*, *Ruminococcus*, and *Akkermansia* in females were reported. In females, 19.4% had hard stools (Bristol stool form scale types 1 and 2), which was higher than that in male subjects. Loose-to-liquid stools (Bristol stool form scale type 6) were more common in male subjects.[51] Tight junction proteins are important for the development of IBS and it contacts with microbiota and SCFA. Our team found that *Helicobacter pylori* was shown to be related to various tight junction proteins, particularly claudin-4 and occludin.[92] Interestingly, an increase in claudin-2 was thought to be involved in male functional dyspepsia but not in females.[92]

Medication and Probiotics

There have been a few reports on medication and sex-difference of microbiota. A human study investigated 23 healthy men and women for differences in NSAID-induced intestinal barrier function and microbiome.[63] The duodenal and fecal microbiota compositions were determined using 16S ribosomal RNA sequencing. Healthy females had lower intestinal permeability, with higher duodenal and fecal microbial diversities than healthy males. Gut permeability increased after indomethacin administration in both sexes.[63] However, only females showed decreased fecal microbial diversity, including an increase in *Prevotella* abundance, after indomethacin ingestion. The duodenal microbiota composition did not show sex-specific changes.[63]

Nowadays probiotics are popular for many reasons and it has been suggested that the effect could be different in terms of sex. Specifically, a probiotic clinical trial suggested an adjustment of the butyrate concentration by administering *Lactobacillus paracasei* DG.[93] They showed that the effect of the probiotic on the microbiota and SFCAs correlated with the initial fecal butyrate concentration.[93] Another study reported that administration of a probiotic mixture of 5 *Lactobacillus* strains to lupus-prone mice improved renal function and showed anti-inflammatory effects in female and castrated male mice, but not in gonadally intact males, suggesting a sex difference in probiotic administration.[57]

In addition, female and male rats responded differently to probiotic treatment for repeated water avoidance stress-induced colonic microinflammation rat model. A 10-day treatment with *Lactobacillus farciminis* effectively treated female rats, but not males.[58]

Furthermore, supplementation of aged obese male mice with the probiotic *Lactobacillus reuteri* helped to restore testosterone levels, increasing seminiferous tubule cross-sectional profiles and spermatogenesis, and Leydig cell numbers, as well as decreasing IL-17 levels. The authors attributed these findings to the anti-inflammatory properties of *L. reuteri*.[38]

Another study reported sex-based differences in the anti-aging effect of a tuna oil and algae oil mixture on the intestinal microbiota.[59] Male mice showed better anti-aging effects than did female mice with a specific oil mixture ratio.[59] The same oil treatment resulted in different intestinal microbiota composition alterations in different sexes. *Lactobacillus* and several butyrate producers were more abundant in males than in females. Some inflammation-related genera, such as *Clostridium* cluster XIVa, were lower in males.[59]

In addition, an animal study investigated the sex difference in the interaction between *Mycobacterium avium* subspecies (subsp.) *paratuberculosis*, probiotic *Lactobacillus animalis* NP-51, and the intestines.[60] Host responses to *M. avium* subsp. *paratuberculosis* and *L. animalis* differed between sexes, especially the cytokines IL-1α/β, IL-17, IL-6, IL-10, and IL-12. *Staphylococcus* and *Roseburia* were consistently overrepresented in females compared to those in males.[60]

Conclusions

There has been significant progress in the research for relationship between gender, sex hormones, and gut microbiota. We tried to summarize recent studies on this subject under various conditions such as androgen excess, aging, GI inflammatory and functional diseases, nutrition, and medication. However, the majority of studies were performed in animals, so far. Further research on the interaction between gender, sex hormones, and gut microbiota may suggest novel preventive measures for relevant diseases.

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