Microbiota-associated mechanisms underlying sexual dimorphism in hypertension

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Abstract Word Count: 143

Total Word Count: 5,919

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Abstract

Consistent research over the last 20 years has shown that there are clear sex differences in the pathogenesis of hypertension, the leading risk factor for the development of cardiovascular diseases. More recently, there is evidence in both humans and experimental animal models that causally implicates the gut microbiota in hypertension. It therefore follows that sex differences in the gut microbiota may mediate the extent of disease between sexes. This new field is rapidly changing and advancing, and the purpose of this review is to cover the most up-to-date evidence regarding the sexual dimorphism of the gut microbiota and its potential influence on the differential manifestation of hypertension in males versus females. Emphasis will be placed on the mechanisms thought to contribute to these sex differences in both the gut microbiota and hypertension, including sex steroid hormones, gut-derived metabolites, the immune system, and pregnancy.

Keywords

sex differences, gut microbiota, hypertension, sex hormones, metabolites
Gut Microbiota in Hypertension

Cardiovascular disease (CVD) continues to be the leading cause of mortality worldwide, and hypertension is the primary modifiable risk factor for CVD (Poulter et al., 2015). With the updated 2017 American College of Cardiology (ACC)/American Heart Association (AHA) guidelines defining hypertension as blood pressure of \( \geq 130/80 \) mmHg, 1 in every 3 adults in the world are hypertensive (Whelton et al., 2018). Of the hypertensive population, it is reported that 30-50% of hypertensive subjects are salt-sensitive individuals who exhibit an increase in blood pressure in response to a high salt intake (Weinberger et al., 2001). Salt-sensitivity is associated with a three-fold greater risk of CVD and end organ damage versus salt-resistant hypertension (Weinberger et al., 2001, Bigazzi et al., 1994). While it is known that pathogenesis of essential and salt-sensitive hypertension is multifactorial, compelling data in both humans and pre-clinical animal models indicate that the microbiota is an emerging, previously unrecognized novel factor regulating blood pressure.

Microbiota is composed of commensal including bacteria, viruses, fungi and other microorganisms colonizing on the host. Estimates of the numbers of microorganisms vary largely in the literature ranging from \( 10^{14} \) microorganisms, outweighing the number of human cells in the body by approximately 10-fold (Backhed et al., 2005), to more recent estimates of 1:1 (Walker and Hoyles, 2023). Nonetheless, it is still an impressive number and microbiota can be influenced by multiple factors such as genetic background, diet, lifestyle choices, and early life events (Ursell et al., 2012). It is thought that the triggering colonization event of the gut microbiota is birth, and is dependent on the mode of delivery as well as the species present in the local environment (Ferretti et al., 2018, Yassour et al., 2018). The diversity of the microbiota composition greatly increases through the first few years of life; however, following these early years of development,
the microbiota resembles the diversity, composition and functionality of an adult (Koenig et al., 2011, Rodriguez et al., 2015).

The most studied microbiota in the context of their influence on human health is the microbiota residing in the gut. The gut microbiota has many functions in the overall health of an individual including maintenance of mucosal barrier integrity, provision of nutrients to the host, and protection against foreign pathogens (Thursby and Juge, 2017). Over the last decade, the role of the gut microbiota in contributing to multiple disease states, including cardiovascular diseases and hypertension has been a growing area of research. All of these studies point to microbiota compositional shifts as being linked to the development of cardiovascular diseases and hypertension and are reviewed in depth elsewhere (Galla et al., 2017, Yang et al., 2021, Masenga et al., 2022, Mattson et al., 2022, O'Donnell et al., 2023). However, what remains largely uninvestigated is how these type of findings are affected by the consideration of sex as a biological variable. The importance of studying sex differences in any disease cannot be underscored enough, and these findings will undoubtedly have implications for hypertension diagnosis, treatment, and prevention between men and women. The sex-specific role of the gut microbiota in hypertension is a novel field of research that is rapidly moving, as evidenced by the studies referenced in Table 1, and the intention of this review is to summarize the most recent evidence on sexually dimorphic microbiota while also providing impetus for its study in hypertension research.

Sex Differences in Hypertension

Hypertension impacts both men and women, but the rates of hypertension amongst each sex varies throughout the lifespan. Men have a greater prevalence of hypertension in early adulthood compared to age-matched women until the prevalence of hypertension is either equal or greater
in women starting around the sixth decade of life (Whelton et al., 2018). This fluctuation in prevalence of hypertension is associated with the onset of menopause in women, and it has been shown in a community-based US cohort study that women have steeper increases in BP with age than men (Ji et al., 2020). In addition to sex-specific factors that contribute to hypertension, there are race-specific implications as well with Black individuals having an increase in hypertension prevalence relative to White counterparts (Petruski-Ivleva et al., 2016, Thomas et al., 2018). Follow-up data (~30 years) from the Coronary Artery Risk Development in Young Adults (CARDIA) study demonstrated that Black men and women had a greater increase in blood pressure per decade than their White counterparts (mean difference between Black and White women: 3 mmHg/decade, Black and White men: 4.7 mmHg/decade) (Reges et al., 2023).

Salt-sensitive hypertension is a specific form of hypertension where individuals exert a chronic increase in blood pressure in response to an increase in sodium intake. Salt-sensitive hypertension is observed in both males and females with a greater susceptibility of salt-sensitivity occurring in Black individuals than White counterparts (Morris et al., 1999). In a meta-analysis of over 100 studies, a reduction in 24-hour sodium excretion (as a marker of sodium consumption) was associated a greater lowering of both systolic and diastolic blood pressure in Black individuals than White (-4.07/-2.37 vs -1.60/-0.82, SBP/DBP) (Huang et al., 2020). These findings were observed in both sexes, demonstrating that both males and females can exhibit salt-sensitive hypertension.

To better understand the pathogenesis of hypertension in both men and women, preclinical models have been utilized to investigate the underlying mechanisms. Similar to humans, there are sex differences in the development of hypertension in animal models. A more thorough review
of sex differences in hypertension in preclinical models have been reported by others (Mariconda et al., 2022, Drury et al., 2023). Focusing specifically on rodent models, sex differences are present in experimental models of hypertension in either pharmacologically-induced or genetic models of hypertension. Chronic infusion of angiotensin II (Ang II) into normotensive animals such as the Sprague Dawley rat or C57BL/6 mouse exerts a greater increase in blood pressure in males compared to female animals (Tatchum-Talom et al., 2005, Xue et al., 2005). In both models, gonadectomy of these animals eliminates the sex difference, highlighting the important contribution of sex hormones in the development of hypertension. An example of a genetic model, the Spontaneously Hypertensive Rat (SHR) closely mimics essential hypertension seen in humans with hypertension, where males have higher blood pressure relative to females (Reckelhoff et al., 1997). The sexual dimorphism in the SHR has been linked to hormones, oxidative stress, and the immune system (Forteppiani and Reckelhoff, 2005, Sartori-Valinotti et al., 2007, Brinson et al., 2013). The Dahl Salt-Sensitive (SS) rat, which closely mimics human salt-sensitive hypertension and accompanying kidney damage, also displays sexual dimorphism in salt-induced elevations in blood pressure (Hinojosa-Laborde et al., 2000, Mattson et al., 2008). This model exhibits overactivation of the immune system in response to a high salt diet, and this occurs in both male and female rats (Fehrenbach et al., 2021, Rudemiller et al., 2014). Even with multiple laboratories invested in studying sex differences in hypertension in these various preclinical models over the last 20+ years, much still remains unknown regarding the underlying mechanisms and potential for sex-specific therapeutics, and perhaps the examination of the gut microbiome as a novel mediator of hypertension will help elucidate innovative targets.

**Sex Differences in Gut Microbiota**

Though sex differences in hypertension and cardiovascular diseases have been clearly established for decades, the intentional investigation of sex differences in the gut microbiota is a
much more recent endeavor. Of all the examinations into the human gut microbiota between men
and women, most studies have found sex differences in composition, with greater microbiota
diversity in women. In 2006, one of the earliest investigations was a cross-sectional study
performed in 230 healthy men and women born and raised across four locations in Europe
(Stockholm, Potsdam, Paris, and Camerino) (Mueller et al., 2006), which identified sex differences
in the total study cohort, with the bacterial group *Bacteroides-Prevotella* being specifically
elevated in males. More than a decade later, Dominianni et al utilized fecal samples collected
from 82 control group participants (51 men, 31 women) from a gut microbiome-colorectal cancer
study in the United States, and found sex to be associated with overall gut microbiome
composition, with a specific reduction in Bacteroidetes abundance in women (Dominianni et al.,
2015). However, these differences appear to be age-dependent, since age-matched men and
women with a mean age of 60 lose the sex differences in α-diversity that is observed in younger
adults (Haro et al., 2016). This sexual dimorphism was later confirmed by two separate studies in
the Netherlands (Sinha et al., 2019) and Slovenia (Mahnic and Rupnik, 2018), showing significant
differences in the overall gut microbiome composition and greater bacterial diversity in women
versus men, all in relation to age. De la Cuesta-Zuluaga et al more recently observed this same
pattern of higher α-diversity in young adult women in a large-scale analyses of adults from the
United States, United Kingdom and Columbia, which was not evident in the adult cohort from
China or in middle-aged women from any of the four geographical regions (de la Cuesta-Zuluaga
et al., 2019).

Despite the compelling evidence for sex differences in the human gut microbiota, there are limited
studies in preclinical models. In a systematic examination of gut microbiota composition and
diversity between sexes of 89 different strains of mice, there were clear sex differences within
each independent strain, which were most evident in the C57BL/6J and C3H/HeJ strains (Org et
al., 2016). However, when the entire population of mice were examined as a whole, these patterns were no longer apparent, indicating that the effects of gender may be dependent on host genetics. The importance of the host in dictating the gut microbiota signature was further emphasized by Wang et al, where inoculation of the same human male donor stool into either male or female germ-free mice resulted in differential bacterial colonization that was dependent on sex of the recipient (Wang et al., 2016). Furthermore, it has been demonstrated that disease (metabolic syndrome) can be mediated by the gut microbiota in a sex-dependent manner and be transferred from one sex to another with fecal transplantation between male and female mice (Kaliannan et al., 2018). The additional use of antibiotics eliminated these sex differences, revealing that the gut microbiota plays a causative role in the observed sexual dimorphism. While there is need for greater study of sex differences in preclinical models to help mimic the human condition, these studies provide evidence that sex differences in the gut microbiota exist and can contribute to sex-specific disease.

How Sex Differences in Gut Microbiota Affect Hypertension

As evidenced in multiple recent reviews (Beale et al., 2019, Razavi et al., 2019, Bardhan and Yang, 2023), there is recognition that sex is an important biological variable in the study of the gut microbiota in hypertension, but there is limited published work on the specific topic. In humans, Lv et al demonstrated gut dysbiosis is observed in both male and female hypertensive subjects (Lv et al., 2023). Most recently, a cross-sectional study examined the gut microbiota profile of 284 participants in Hong Kong to specifically explore the potential associations of sex and the gut microbiota with 24-hour ambulatory blood pressure measurements (Virwani et al., 2023). Interestingly, sex-stratified analysis found gut microbiota dysregulation and significant differences in β-diversity to be associated with hypertension in women, but not men. Furthermore, in a large
African origin cohort of 1,904 individuals from Ghana, South Africa, Jamaica, Seychelles, and the US, the global gut microbiota was found to accurately predict diabetes, glucose state, hypertension, obesity, and sex (Ecklu-Mensah et al., 2023). However, when analyzed by the different countries, only the predictive ability for sex was universally maintained, further highlighting the tight relationship between sex and the gut microbiota. Thus far, these are the only studies to have intentionally explored and demonstrated a relationship between sex, the gut microbiota, and hypertension in humans.

Preclinically, our work in the hypertensive Dahl Salt-Sensitive (SS) rat has shown significant sex differences in gut microbiota composition between male and female SS rats, independent of age or dietary manipulations, which correlate with sex differences in the severity of hypertension and renal injury (Abais-Battad et al., 2021). Given the critical role of the kidney in blood pressure regulation, Moore et al have recently compared germ-free versus conventionalized male and female mice, demonstrating significant changes to renal gene expression that is modulated by the microbiome in a manner that is both sex- and organ-specific (Moore and Pluznick, 2023). These underlying sex differences in gut microbiota may have important implications for the efficacy of various therapies, since fenbendazole treatment in BPH/5 hypertensive/obese mice resulted in sex-specific alterations to the gut microbiota, which appears more notable in males than females (Beckers et al., 2023). Reflected in Table 1, the study of sex differences in gut microbiota-hypertension interplay is an emerging field with currently limited published work, but it is evident that this specific field is rapidly progressing and is actively being investigated.

**Potential Gut Microbiota-associated Mechanisms**

**Sex Hormones**
Perhaps an obvious factor contributing to the sexual dimorphism in the gut microbiota are sex steroid hormones, given their role as determinants in the major differences between males and females. A comparison of pre- versus post-menopausal women demonstrated differences in broad bacterial groups that correlated with estradiol levels (*Gammaproteobacteria* and *Mixococcales*) as well as differences in the predicted functional profile of their gut microbiota (short-chain fatty acid production) (Santos-Marcos et al., 2018). This was one of the first studies to suggest a link between hormonal status (estrogen depletion) and gut microbiota composition and function in humans. Around the same time, Sinha et al investigated the possible influence of female hormonal factors in a Dutch cohort and found that use of oral contraceptives and bilateral ovariectomy in women associated with changes to specific microbial species (*Bacteroides caccae*, *Coprobacillus unclassified*, *Rothia mucilaginosa*, *Clostridium bolteae*) (Sinha et al., 2019). These findings were in agreement with a study by Shin et al, which analyzed the microbiome as a function of steroid hormone with each sex. This group confirmed that sex steroid hormone levels correlate with diversity and gut microbial composition changes in Korean men and women, respectively (Shin et al., 2019). Specifically, *Atopobium*, *Acinetobacter*, *Dorea*, *Megamonas* and *Ruminococcus* bacteria were identified to be testosterone-responsive in men, while *Veillonella*, *Slackia*, *Lactococcus*, *Christensenella*, *Dehalobacterium*, *Adlercreutzia*, *Odoribacter*, and *Butyricimonas* associated with circulating estradiol levels in women. However, it becomes critical to understand how these bacterial differences, due to both sex and hormone status, translate into changes in gut microbiota function. More recently, Mayneris-Perxachs et al showed significant sex differences in metagenome-based functional analyses in pathways related to steroid, amino acid, nucleotide, and carbohydrate biosynthesis and metabolism between pre-menopausal women and men (Mayneris-Perxachs et al., 2020). Most interestingly, these sex differences in microbiota function were lost when comparing postmenopausal women and men (despite having significantly different microbiota compositions), importantly linking the relationship between sex, hormones, microbiota composition, and microbiota function.
While human studies have been able to demonstrate strong, consistent associations between sex hormones and the gut microbiota, it is the use of preclinical models where causation can be established. Multiple animal studies have now found that sex differences in gut microbiota composition do not occur until after puberty, indicating a role for the hormones that change upon sexual maturation (Markle et al., 2013, Yurkovetskiy et al., 2013, Mei et al., 2022). Direct manipulation of sex hormones in mouse and rat models via ovariectomy or castration resulted in overall microbial dysbiosis (Cox-York et al., 2015, Org et al., 2016) and in some cases, reversed originally observed sex differences in gut microbiota composition (Yurkovetskiy et al., 2013). Many of these effects of gonadectomy could be prevented by administration of hormone (Cox-York et al., 2015, Yurkovetskiy et al., 2013, Org et al., 2016). These type of proof-of-principle studies demonstrate how sex hormones causally modulate the gut microbiota. On the other hand, in a nonobese diabetic (NOD) mouse model of autoimmune type I diabetes, Markle et al were the first to show that the relationship between bacteria and sex hormones is bidirectional, where bacteria itself can also affect hormone levels (Markle et al., 2013). Germ-free male NOD mice had lower testosterone compared to conventional male NOD mice harboring gut bacteria, which contrasted germ-free female NOD mice who had higher levels of testosterone compared to conventional female NOD mice. Furthermore, the introduction and colonization of bacteria into the germ-free males affected circulating levels of testosterone, confirming that the bacteria-sex hormone relationship goes both ways. Models that can dissect the question of whether gonadal sex or hormonal sex contributes more to the overall sex differences in microbiota composition are needed. One such model is the “four core genotypes” (FCG) mouse model. The model involves deletion of the testis-determining gene Sry from the Y chromosome and insertion of an Sry transgene onto an autosome. It produces XX and XY mice with testes, and XX and XY mice with ovaries, so that XX and XY mice with the same type of gonad can be compared to assess
phenotypic effects (Arnold and Chen, 2009). More recently, a rat model similar to the Four Core
Genotypes mouse model, allowing comparison of XX and XY rats with the same type of gonad
has been developed (Arnold et al., 2023). In the context of hypertension, the evaluation of gonadal
versus chromosomal sex effects has not yet been performed, but it is certain that in time, these
mechanistic questions will be addressed utilizing these novel mouse and rat preclinical models.

Gut-derived Metabolites

Though the identification of specific bacteria that change in response to sex and disease has been
a large focus of previous studies, metabolite diversity may even surpass microbial diversity
(Dorrestein et al., 2014). Further emphasizing the potential importance of these systemic factors,
sex is a critical determinant of the overall metabolomic profile, with >50% of all metabolites shown
to differ between men and women (Darst et al., 2019). Understanding the systemic, gut-derived
factors that coincide with the changing gut microbiota is an overarching goal of current research,
especially with the consideration of sex differences. Here we focus on the present evidence
surrounding the specific roles of short-chain fatty acids (SCFAs), trimethylamine N-oxide (TMAO),
and bile acids, as they relate to hypertension and, when possible, sex differences.

One of the most studied microbial metabolic pathways are the SCFAs butyrate, propionate, and
acetate. Derived as a byproduct of bacterial fiber fermentation, SCFAs are recognized to be
beneficial metabolites mediating metabolism and immunity, and promote overall gut health
(Natarajan and Pluznick, 2014). In 2013, Pluznick et al pioneered the study of SCFAs in the
context of blood pressure and the kidney (Pluznick et al., 2013), and showed that gut microbiota-
derived propionate had both vasodilatory and hypotensive properties, which was modulated by
SCFA receptors Olfr78 and Gpr41. Multiple studies have since confirmed this protective role of
propionate in other animal models as well as in humans. Propionate supplementation exhibited beneficial effects against cardiac hypertrophy, fibrosis, vascular dysfunction, and hypertension in both an angiotensin II-infused model and in apolipoprotein E knockout-deficient mice (Bartolomaeus et al., 2019). In humans, total plasma SCFAs and propionate alone were independent predictors of blood pressure only in women, not men (Virwani et al., 2023), again highlighting the importance of considering sex differences in gut microbiota function in the potential management of hypertension. Other SCFAs may also play a role, since administration of acetate reduced DOCA/salt-induced hypertension, cardiac fibrosis, and left ventricular hypertrophy in mice (Marques et al., 2017) as well as reduced hypertension in a rat model of obstructive sleep apnea (Ganesh et al., 2018). In a recent randomized, double-blind, placebo-controlled crossover trial examining the effects of dietary sodium reduction on hypertension and circulating SCFA levels, sodium reduction significantly increased 5 SCFAs (2-methylbutyrate, butyrate, hexanoate, isobutyrate, and valerate), which associated with decreased BP and improved arterial compliance (Chen et al., 2020a). However, when stratified via sex, there were differences in the SCFA response to sodium reduction, where the increases in butyrate, hexanoate, isobutyrate, isovalerate, and valerate only occurred in women and not men. Though SCFAs have an established mechanistic role in hypertension, these present studies collectively show that the potential sex differences in SCFA levels, receptors, and function is a critical area that necessitates deeper investigation.

While not to the same extent as SCFAs, there is some evidence showing the contribution of other gut-derived metabolites like TMAO and bile acids. Gut microbes can metabolize trimethylamine precursors carnitine, choline, and betaine to produce detrimental metabolites like TMAO, and these metabolites are known predictors of CVD and major adverse cardiac events (Koeth et al., 2013). Plasma TMAO levels is also associated with all-cause mortality, especially in subjects with
reduction kidney function (Gruppen et al., 2017). Interestingly, the same study found that female sex inversely associated with TMAO, and a more recent examination by Rath et al found the concentration of all three precursors to be significantly higher in males (Rath et al., 2021). In the exploration of other microbiota-related metabolites, the Joe laboratory has performed studies on bile acids, which are generated collaboratively between the host liver and gut microbiota. Through the analysis of the bile acid profiles of humans and both hypertensive and germ-free rat models, the Joe laboratory discovered that conjugated bile acids were inversely associated with blood pressure in both hypertensive humans and rats (Chakraborty et al., 2023). Enhancing conjugated bile acids by administration of taurine lowered both systolic and diastolic blood pressure in SS rats, and this occurred in both males and females, indicating that the protective effects of taurine-conjugated bile acids were independent of sex. It is suspected that during hypertension, an enrichment of select microbiota which de-conjugate conjugated bile acids could be responsible for depleting the pool of the anti-hypertensive conjugated bile acids. The identities of such de-conjugating microbiota remain to be revealed. Contrary to the lack of sex effects in rats, in mice it has been shown that there are differences in the bile acid profile between sexes, which significantly changed in response to gonadectomy (Org et al., 2016). Bile acids have also been shown to modulate gut bacterial composition (Islam et al., 2011), and therefore offer another potential mechanism mediating sex differences in the gut microbiota. Interestingly, Verhaar et al have recently shown that different plasma metabolites can predict blood pressure in males and females from the HEalthy Life in an Urban Setting (HELIUS) cohort study conducted in the Netherlands – sphingomyelins, N-formylmethionine and conjugated bile acids were predictive in males, and dihomo-lineoylcarnitine, 4-hydroxyphenylacetateglutamine and vanillactate were predictive in females (Verhaar et al., 2023). Utilizing machine learning prediction models, they further found that several of the most predictive metabolites were specifically associated with gut microbiota composition. Together, these human and pre-clinical studies all signify the importance of sex in understanding the function of these microbiota-related metabolites.
Immune System

The gut microbiota is known to be intricately involved in the development and maintenance of the immune system (Round and Mazmanian, 2009), and there is a well-established relationship between immunity and hypertension (Mattson et al., 2021). As of recent, there is mounting evidence for modulation of the immune system via manipulation of the gut microbiota in various animal models of hypertension. First demonstrated by Wilck et al, a high salt diet raised blood pressure, depleted *Lactobacillus murinus* in the gut, and concurrently increased pro-inflammatory T$_{H17}$ cells in the small intestinal lamina propria of FVB/N mice (Wilck et al., 2017). However, daily gavage of *L. murinus* given concomitantly with high salt diet normalized blood pressure by preventing the generation of T$_{H17}$ cells in intestinal tissue. These results importantly mirrored their observations in humans, where a two-week high salt regiment of 6g NaCl/day resulted in a significant increase in circulating T$_{H17}$ cells and a loss of *Lactobacillus* species in the stool. Demonstrating causality between the gut microbiota and the immune response, Ferguson et al performed a fecal microbiota transfer from high salt-fed donor mice into germ-free recipient mice which led to a predisposition of the germ-free mice to systemic inflammation and hypertension (Ferguson et al., 2019). It has also been shown that the beneficial effects seen with SCFA propionate, like amelioration of vascular dysfunction and hypertension, are dependent on increases in regulatory T cells (Tregs) and reductions in splenic effector memory T cells and T$_{H17}$ cells (Bartolomaeus et al., 2019). SCFA promotion of Treg expansion has also been observed in other diseases such as experimental autoimmune encephalomyelitis and in humans with end-stage renal disease (Haghikia et al., 2015, Meyer et al., 2020). Our own work further confirmed a specific role for T cells in the modulation of hypertension via the gut microbiota, where fecal transfer from donors fed a pro-hypertensive diet into protected recipients worsened salt-sensitive hypertension, renal injury, and inflammation (Abais-Battad et al., 2021). Investigation of the
immune cell subsets in the kidney revealed that transfer of the pro-hypertensive bacteria specifically increased numbers of T cells, and not other cell types, like B cells or myeloid-derived cells. While it is clear that the gut microbiota plays a critical part in modulating the immune response that amplifies hypertensive disease, and though there are known immunologic sex differences in hypertension, studies specifically examining these interrelated mechanisms are sparse. Fransen et al published one of the most comprehensive studies thus far by utilizing sex-specific gut microbiota transfer experiments in C57BL/6J mice to address whether sex differences in gut microbiota composition are a cause or consequence of sex differences in immunity (Fransen et al., 2017). Interestingly, germ-free mice still exhibit sex differences in expression of certain immune-related genes, indicating an aspect of immunity that is microbiota-independent. These baseline differences contribute to the selection of sex-specific bacteria in the gut of males versus females, which further amplifies sex differences in the immune system. With hormones known to affect immune status (Santos-Marcos et al., 2018), it is extremely likely that sex hormones are also involved in the establishment of the gut microbiota-immune relationship.

Pregnancy and Preeclampsia

Preeclampsia is a pregnancy-specific increase in blood pressure and end-organ damage such as proteinuria, liver dysfunction, or thrombocytopenia. While it is hypothesized that the development of preeclampsia is of placental origin due to improper remodeling of the spiral arteries, there are multiple risk factors for the development of preeclampsia which include inflammation, oxidative stress, or genetic predisposition. A new area of interest in the pregnancy/preeclampsia field is the contribution of the gut microbiota in the development of adverse pregnancy outcomes. As observed in essential hypertension, preeclampsia is associated with gut dysbiosis relative to normotensive pregnancies in human patients (Lv et al., 2022, Chen et al., 2020b, Wang et al., 2019, Zhao et al., 2022). This gut dysbiosis is associated with alterations in various mechanisms...
that were previously discussed. In an experimental model of autoimmune encephalomyelitis, treatment of animals with estrogen to levels observed during pregnancy prevented alterations in composition and diversity of gut microbiota relative to control animals (Benedek et al., 2017). This study demonstrates the important cross talk between sex hormones and the microbiota. Work by Koren et al further supported this by showing that the fluctuations in estrogen levels observed in pregnancy are associated with alterations in gut microbiome composition (Koren et al., 2012).

Gut-derived metabolites have also been implicated in the gut dysbiosis that is associated with the pathogenesis of preeclampsia. In a small cohort of preeclamptic women who exhibit gut microbiota dysbiosis, Wang et al demonstrated that there was a significant increase in fecal and plasma levels of bacteria-derived lipopolysaccharide (LPS) and plasma levels of TMAO compared to healthy controls (Wang et al., 2019). In a study out of the Zhang lab, fecal SCFA levels of butyric and valeric acid were significantly reduced and LPS biosynthesis increased in samples collected from preeclamptic women (Chang et al., 2020). Moreover, investigators utilized LPS injection during early and mid-gestation in rats to induce an increase in blood pressure whereas supplementation of butyrate prevented this increase in blood pressure. This work was supported by Yong et al, who administered L-NAME during pregnancy to induce a preeclamptic-like phenotype, and treatment with sodium butyrate alleviated the adverse pregnancy outcomes (Yong et al., 2022). These studies directly link the gut microbiota and SCFA production to the development of preeclampsia.

There is also evidence highlighting the connection between the gut microbiota, SCFA levels and regulation of the immune system in pregnancy. As previously reported, gut dysbiosis was observed in preeclamptic women which was associated with a reduction in SCFA-producing
bacteria and SCFA levels (Jin et al., 2022). These findings were linked to an increase in inflammation, specifically an increase in T\textsubscript{H}17 cells and reduction in Tregs in the preeclamptic women. The authors were able to recapitulate these findings in rats through a fecal transfer approach where preeclamptic fecal microbiota was transplanted into rats treated with L-NAME and administration of propionate and butyrate alleviated these preeclamptic symptoms. Similar findings were reported in a mouse model of preeclampsia (Chen et al., 2020b). Taking all of these reports together demonstrate the interplay between the gut microbiota, metabolites and inflammation in the development of hypertension during pregnancy.

Finally, there is evidence demonstrating how maternal health during pregnancy can impact the health outcomes of the fetus. Hu et al demonstrated that maternal serum acetate levels were reduced in preeclamptic women, which associated with an impairment in fetal CD4+ and Treg development (Hu et al., 2019). In a study from Dr. Joe's laboratory, Galla et al demonstrated how maternal exposure to antibiotic treatment during pregnancy had long-term implications on blood pressure in the offspring (Galla et al., 2020). The importance of maternal environmental factors were also demonstrated in the Dahl SS rat, where our group has demonstrated that dietary protein source in the rat chow impacts the development of maternal syndrome as well as severity of salt-sensitive hypertension and renal damage in the offspring (Dasinger et al., 2021, Geurts et al., 2015, Abais-Battad et al., 2019). Since treatment options in pregnancy can be limited due to the potential impact on the developing offspring, targeting the gut microbiota through dietary manipulations could be a pursuit for future therapeutics.

Microbiota Beyond the Gut and Potential Therapies for Hypertension
Since the gut is not the sole organ housing microbiota, research on sex differences in hypertension should encompass investigations on microbiota in other locations of the host. To our knowledge, there is only one such report to date which investigated the potential role of both oral and skin microbiota in the development of hypertension. When comparing 4-week old male and female SS rats, sex-specific differences in β-diversity were observed only in skin microbiota (Mei et al., 2022). For the first time, this study demonstrated the contributing effects of bacteria from sites outside of the gut to hypertension in males versus females, which will become an important factor when considering therapeutics against microbiota-related targets.

Another emerging area of exploration as a therapeutic target for hypertension is the gut-brain axis. This bidirectional communication is proposed to be involved in the complex etiology of hypertension through a number of mechanisms that include alterations in the autonomic nervous system and neuroinflammation (Yang and Zubcevic, 2017). While evidence for these connections are still forthcoming, reports in both human patients and pre-clinical models of hypertension demonstrate an imbalance in the autonomic nervous system (ANS) can lead to an increase in sympathetic outflow resulting in elevations in blood pressure and other physiological responses (Narkiewicz et al., 2005, Zubcevic et al., 2014). A recent publication by Dirr et al demonstrated that subdiaphragmatic vagal nerve stimulation in the SHR model resulted in attenuated hypertension without influencing cardiac effects of the vagal nerve (Dirr et al., 2023). In addition to the direct connections between the gut and the nervous system, the ANS can be influenced by short chain fatty acids. Short chain fatty acid receptors are found on the nervous system, including the vagus nerve, and concentrations of SCFAs have been shown to influence retrograde messengers along the ANS to regulate CNS outputs (Soret et al., 2010, Pluznick, 2014). Since SCFAs concentrations are directly associated with microbiota composition, there is a clear need to examine this connection. Investigations into the microbiota-gut-brain axis are very promising.
as a potential therapeutic target for hypertension; however, the majority of the research occurring in this area are performed in male subjects, highlighting the need for more in-depth investigations of these pathways to include both male and females.

Fecal microbiota transplantation (FMT), another alternative therapeutic, has been a valuable preclinical tool used to demonstrate causality between the gut microbiota and hypertension (Li et al., 2017, Wilck et al., 2017, Abais-Battad et al., 2021). Though its clinical use for treating gastrointestinal infections like *Clostridium difficile* is common, the feasibility of FMT as treatment for hypertension in humans is much less understood. In 2021, Zhong et al published their findings using washed microbiota transplantation (WMT – microfiltered fecal suspension for removal of fecal particles, parasite eggs, and fungi) from healthy donors infused into both hypertensive and normotensive patients via nasojejunal (upper GI) or transendoscopic enteral (lower GI) tube (Zhong et al., 2021). The administration of healthy WMT lowered blood pressure in hypertensive patients (-5.09 mmHg SAP, -7.74 mmHg DAP), with even greater protection seen in hypertensive patients not on antihypertensive drugs and who received WMT via the lower GI. This antihypertensive effect of WMT was not observed in normotensive patients, indicating a reduction in blood pressure that may be specifically related to the shifts made to the gut microbiota composition. With FMT considered to be an core therapy for gut microbiota remodeling, its utility as a novel treatment for hypertension will certainly continue to be explored, and there are ongoing clinical trials dedicated to testing the efficacy and safety of FMT and gut microbiota restoration in hypertension (Fan et al., 2022).

Another alternative therapy with great potential is the use of bioengineered, recombinant bacteria. *Lactobacillus*, a beneficial microbe with known blood pressure-lowering properties, has been...
genetically engineered to express human angiotensin converting enzyme 2 (hACE2) that when administered to hypertensive Ace2−/− Dahl SS rats, resulted in a sex-specific lowering of blood pressure in females, but not males (Mei et al., 2023). This study was the first to demonstrate the effectiveness of engineered bacteria to deliver an antihypertensive agent. Together, these diverse strategies to target the gut microbiota by altering its composition or by manipulating its function (vagal nerve stimulation, FMT/WMT, bioengineered bacteria) deliver great promise for the future of gut microbiota-related therapies for hypertension.

Perspectives

The evidence presented in this review exemplarily demonstrate that the interplay between sex differences, the gut microbiota, and all associated mechanisms – hormones, metabolites, immunity – is highly complex (Figure 1), with many of the relationships potentially occurring bidirectionally. However, there is much that remains unknown, especially in the context of hypertension. Given the unfortunately low rate of hypertensive patients that are able to reach blood pressure control with classic therapies, the study of the gut microbiota not only offers a potential explanation for the inefficacies of present therapies, but also offers a fresh avenue of research that holds promising and diverse therapeutic targets personalized for each sex.

Disclosures and Funding

JHD and JMA have no disclosures. BJ is Editor-in-Chief of Microbiota & Host and was not involved in the review or editorial process for this paper, on which she is listed as an author.
We gratefully acknowledge funding from the National Heart Lung and Blood Institute of the National Institutes of Health to BJ (HL1430820) and JHD (HL157549), and the American Heart Association to JMA (CDA34660184).

Author contribution statement

JD, BJ, and JA drafted and revised the manuscript. JA prepared the figures and tables.

Table 1. Summary of recent publications related to sexual dimorphism of the gut microbiota, specifically in the context of hypertension.

Figure 1. Summary of the proposed mechanisms contributing to sex differences in gut microbiota composition and function, immune response, and ultimately in the pathogenesis of hypertension.
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Sex-specific Gut Microbiota Composition & Function

Sex Chromosomes

Pregnancy

Sex Hormones

Intestinal Cells

Sex-specific Immune Response

T\textsubscript{H}17 versus Treg

Intestinal Lumen

Gut Microbiota-derived Metabolites

Protective SCFAs

Pathological carnitine

conjugated bile acids

choline

betaine

TMAO

Blood

Hypertension & Related End-Organ Damage

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### Table 1 Current Evidence on Gut Microbiota Sexual Dimorphism in Hypertension.

<table>
<thead>
<tr>
<th>Study</th>
<th>Major Findings</th>
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<tbody>
<tr>
<td><strong>Clinical Studies</strong></td>
<td></td>
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<tr>
<td>Chen et al. (2020a)</td>
<td>Sodium reduction sex-specifically increased plasma SCFAs, which was associated with decreased blood pressure, in women and not men</td>
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<tr>
<td>Virwani et al. (2023)</td>
<td>Total plasma SCFAs and propionate were independent predictors of blood pressure in women, not men</td>
</tr>
<tr>
<td>Verhaar et al. (2023)</td>
<td>Different plasma metabolites predict blood pressure in males versus females, with ten metabolites demonstrating explained variance by gut microbiota composition</td>
</tr>
<tr>
<td>Lv et al. (2023)</td>
<td>Demonstrated specific alterations in gut microbiota taxa in hypertensive females and males, respectively, in northwestern China</td>
</tr>
<tr>
<td>Ecklu-Mensah et al. (2023)</td>
<td>Diabetes, glucose state, hypertension, obesity, and sex could be accurately predicted from the global microbiota in an African origin cohort across 5 countries, but the capacity of the gut microbiota to predict sex was the only factor universally maintained by all countries</td>
</tr>
<tr>
<td><strong>Pre-Clinical Studies</strong></td>
<td></td>
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<tr>
<td>Abais-Battad et al. (2021)</td>
<td>Gut microbiota mediated the dietary modulation of hypertension and kidney damage in Dahl Salt-Sensitive rats, with significant gut microbiota composition differences in male versus female rats</td>
</tr>
<tr>
<td>Moore et al. (2023)</td>
<td>Kidney gene expression was differentially regulated by the microbiota in a sex-specific manner, and the influence of the gut microbiota on gene expression appears to be tissue specific</td>
</tr>
<tr>
<td>Beckers et al. (2023)</td>
<td>Fenbendazole treatment alters gut microbial communities in the hypertensive BPH/5 mouse, with more notable effects in males than females</td>
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<tr>
<td>Mei et al. (2023)</td>
<td>Sex-specific lowering of blood pressure in hypertensive female Ace2−/− rats with colonization of Lacto-hACE2 (Lactobacillus paracasei, genetically engineered to produce and deliver human ACE 2)</td>
</tr>
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