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REVIEW ARTICLE

A gender perspective on diet, microbiome, and sex hormone interplay in cardiovascular disease

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Abstract

A unique interplay between body and environment embeds and reflects host–microbiome interactions that contribute to sex-differential disease susceptibility, symptomatology, and treatment outcomes. These differences derive from individual biological factors, such as sex hormone action, sex-divergent immune processes, X-linked gene dosage effects, and epigenetics, as well as from their interaction across the lifespan. The gut microbiome is increasingly recognized as a moderator of several body systems that are thus impacted by its function and composition. In humans, biological sex components further interact with gender-specific exposures such as dietary preferences, stressors, and life experiences to form a complex whole, requiring innovative methodologies to disentangle. Here, we summarize current knowledge of the interactions among sex hormones, gut microbiota, immune system, and vascular health and their relevance for sex-differential epidemiology of cardiovascular diseases. We outline clinical implications, identify knowledge gaps, and place emphasis on required future studies to address these gaps. In addition, we provide an overview of the caveats associated with conducting cardiovascular research that require consideration of sex/gender differences. While previous work has inspected several of these components separately, here we call attention to further translational utility of a combined perspective from cardiovascular translational research, gender medicine, and microbiome systems biology.

KEYWORDS

cardiovascular disease, gender medicine, gut microbiome, sex differences

1 | INTRODUCTION

Cardiovascular disease (CVD) encompasses multifactorial slowly developing conditions with both heritable and environmental risk factors, and is widely and complexly associated with other health conditions. In early stages, CVD is often asymptomatic, while in the later stages, symptoms can be non-specific leading to late diagnosis and allowing for irreversible damage in the meantime.[1] In recent years, the role of gender and sex aspects of CVD has been increasingly recognized due to differential disease development, presentation, progression, outcomes, and treatment responses between men and women.[2–5] Men tend to develop CVD earlier in life and present different symptoms than women.[6] Despite these differences, diagnostic criteria and proposed treatments are usually the same between sexes, which, together with historically male-biased clinical trial populations, might explain the higher rate of adverse drug reactions in women.[7] Moreover, gender norms and expectations have been noted to impact how healthcare providers receive, witness, and diagnose patients, more often failing to correctly attribute women's symptoms to CVD.[6,8]

To summarize current knowledge on the CVD gender disparity, we here focus on aspects of host–microbiome homeostasis as it links to sex differences in CVD, as a dynamic system influenced by internal and external parameters and a central pivot point for both preventive medicine and an integrator of risk factors throughout the lifespan (Figure 1). We outline the current state of the art of the relevant clinical cardiovascular background and provide detailed mechanistic insight into the roles of genetics and epigenetics from both human and animal studies as well as into sex-divergent biological processes salient for cardiac cells. All the above approaches serve to highlight the relevance of host–microbiome homeostasis in cardiovascular gender medicine, and as a basis to identify knowledge gaps given these insights.

2 | BOX: NOTE ON TERMINOLOGY

In this review, by sex we reference broad context-dependent summaries of phenotype and genotype clusters that follow directly or indirectly from evolutionary adaptations to sexual reproduction. By gender, we reference social or psychological constructs surrounding or relating to sex, potentially modulating its effects; in line with the latter, we use "gender medicine" as per the previous literature to reflect the separate and interacting salience of either in medicine. As we summarize the literature spanning many model systems and clinical cohorts each different in design, how sex and gender were operationalized in each may differ. When we describe results from the human setting, we assume study participants are cisgender/cissexual and endosex such that their status as men/women or male/female (with these terms used interchangeably to reflect most common usage also in the literature) defaults to birth-assigned sex, except where otherwise stated. When relating research on animals, the terms male or female reflect how animals were designated in each study, typically in order to most accurately represent corresponding human patient groups. For research on samples derived from humans such as cell lines, we speak of the sex of these samples as that of the specific human donor (in turn considered as above). When claims are related or hypotheses made on male/female biology or medicine, these should be understood as statistically intended, without the assumption that they apply without exception to every male or female patient or instance of a model but that they have broad generalizability for translational purposes. We have sought to discuss broad or narrow such exceptions as well, but such discussion is nowhere near exhaustive.

3 | SEX DIMORPHISM IN CVD EPIDEMIOLOGY

A recent study examined nationally representative samples from emergency department visits in the US and observed sex differences in the distribution of various CVD presentations, hospitalization rates, and risk of death.[9] Certain conditions such as hypertension, ischaemic stroke, hypertensive crises, supraventricular tachycardia, and pulmonary embolism were more common in women, while men were more likely to present with cardiac arrest and acute myocardial infarction (MI) (Figure 1).[9] Furthermore, systolic blood pressure and hypertension, smoking status and intensity, and diabetes are all associated with relatively higher hazard ratios for MI in women than in matched men.[10] This illustrates how known risk factors can affect men and women differently. Similar observations emerge regarding drug response due to differences in body composition, pharmacokinetic/pharmacodynamic (PK/PD) properties of some drugs, and fluctuations in endogenous sex hormone levels (menstrual cycle, pregnancy), or the administration of oral contraceptives (OCs) or hormone replacement therapy (HRT).[11] These differences are rarely considered when prescribing dosages, contributing to more frequent adverse drug reactions in women. It has thus been proposed that sex differences should be considered in dosing, efficacy, and safety of cardiovascular drugs as an essential first step in personalizing treatment, alongside PK/PD sex differences.[11] Furthermore, while the reporting by sex, race, and

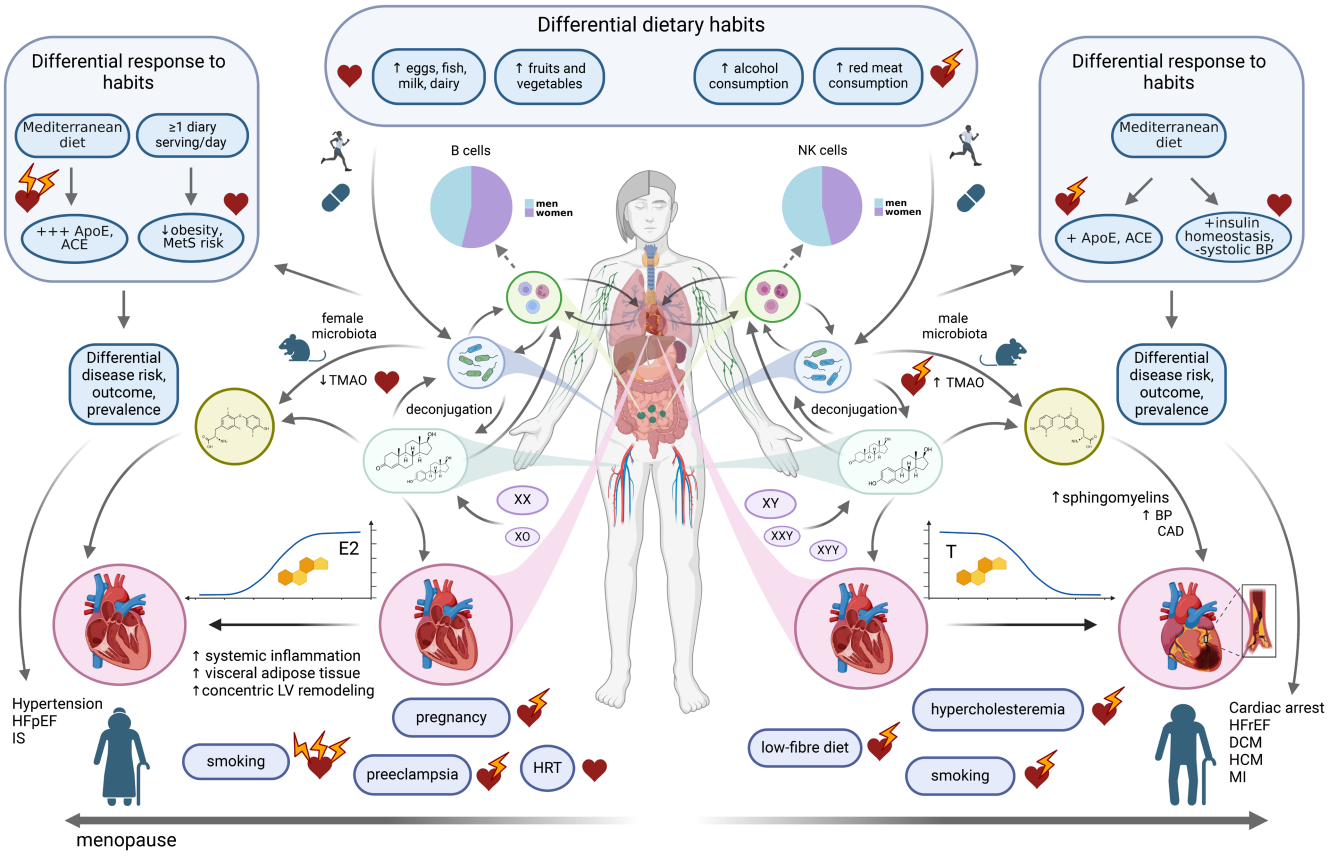

FIGURE 1 Sex-differential exposome effects mediated by gut microbiome through metabolite production and bidirectional interactions with sex hormones and immune system throughout the life course on cardiovascular health and sex-differential disease risk, prevalence, and outcome. Men and women both differ in lifestyle risks and in responses to those risks, impacting cardiovascular health and disease progression. These differences in responses reflect genetic differences largely but not solely mediated by sex hormone effects. These effects in turn impact the gut microbiome, the immune system, levels of circulating and local metabolites, and state and activity of target tissues such as heart and vasculature. The gut microbiome interacts bidirectionally with sex hormone levels via deconjugation and can elicit important pro- or anti-inflammatory immune responses by metabolite production or by providing ligands, culminating in protective or deleterious effects on target tissues. The decline of sex hormone production after reproductive age also affects the gut-microbiome—immune crosstalk in specific ways that are directly or indirectly reflected in the sex-differential disease epidemiology. 2E, estradiol; ACE, angiotensin-converting enzyme; ApoE, apolipoprotein E; B cells, B lymphocytes; BP, blood pressure; CAD, coronary artery disease; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; HRT, hormone replacement therapy; IS, ischaemic stroke; LV, left ventricle; MetS, metabolic syndrome; MI, myocardial infarction; NK cells, natural killer cells; T, testosterone; TMAO, trimethylamine oxide. Generated with BioRender.

ethnicity for NIH-funded randomized controlled trials (RCTs) published in 2021 significantly increased, there is no corresponding increase in enrollment.[12] Women and especially older women are still underrepresented in selected medical field trials in CVDs, neoplasms, endocrine system diseases, respiratory tract diseases, bacterial and fungal infections, viral diseases, digestive system diseases, and immune system diseases according to a recent meta-analysis and systematic review.[13]

Another occurrence of sex-differential disease prevalence has been observed with cardiomyopathies. These conditions stem from gene mutations and should have the same prevalence between genders; however, such disease risk is significantly sex-differential, with both dilated and hypertrophic cardiomyopathy (DCM and HCM) being more prevalent in men than in women (Figure 1).[14–16] This highlights the importance of homeostasis even for inherited disease, as a credible explanation for much of this discrepancy may be the sex-differential availability of compensatory factors that could prevent penetrance of a disease allele. When it comes to disease phenotype, female patients with HCM have less ventricular remodeling,[17] yet more severe diastolic dysfunction[18,19] compared with male patients. Myectomies from female and male patients carrying HCM thick filament (MYH7 or MYBPC3) mutations show cardiac sex differences in tissue and cells, with greater diastolic dysfunction and higher level of fibrosis in women compared with men.[20] Furthermore, diastolic

dysfunction is greater in women with HCM and myectomy onset is later than in men, together with greater left ventricular and left atrial remodeling when corrected for body surface area.[20] At a cellular level, HCM women showed increased compliant titin and a larger degree of interstitial fibrosis.[20] Additionally, compared with male patients, women expressed higher levels of titin N2BA, the more compliant titin isoform expressed in adult human hearts. Different therapeutic approaches for male and female HCM patients might thus be beneficial and more work is needed to fully characterize cellular and molecular mechanisms underlying these differences.

Sex differences in heart failure are reported in western populations with women being more likely to suffer from heart failure with preserved ejection fraction (HFpEF) and men more likely to present with reduced ejection fraction (HFrEF) (Figure 1).[21–23] Among others, the traditional HFpEF risk factors are obesity, hypertension, diabetes, and prior coronary artery disease (CAD).[21] There are also distinct HFpEF risk factors for women that include early menopause, adverse pregnancy outcomes, and other reproductive factors such as parity, infertility, and history of preeclampsia (Figure 1),[24,25] though further exploration is needed to definitely conclude causality. Since HFpEF patients are often older, menopausal women, it was postulated that decline in blood estradiol levels may have a role in the development of HFpEF. Estradiol (E2) is an important regulator of inflammation, reactive oxygen species (ROS) abundance, nitric oxide signaling, and endothelial function.[26] E2 may antagonize the concentric remodeling caused by androgens and downregulate angiotensin receptors, improve vascular endothelium function, protect against vascular injury, and inhibit adverse remodeling processes.[27] Low blood estrogen levels were also associated with systemic inflammation[28] and increase in visceral adipose tissue, which is linked to concentric LV remodeling, a hallmark of HFpEF (Figure 1).[29,30] Moreover, reductions in E2 levels are associated with changes in body fat, blood pressure, and lipids, all of which are implicated in the development of HFpEF.[26] A study of 1941 post-menopausal women and 2221 men found that higher free testosterone and lower sex-hormone-binding globulin were associated with a greater increase in LV mass in both women and men; however, an association with concentric remodeling reached significance only in women,[31] associations with E2 could not be concluded from this study population.

Besides their influence on cardiomyopathies and heart failure, sex differences also figure in the incidence, prognosis, and therapy of cardiac arrhythmias. In atrial fibrillation (AF), representing the most common adult-sustained cardiac arrhythmia, these differences are particularly distinct.[32–35] Several studies and large registries report lower incidence of AF in women than in men.[34–36] However, the general prognosis of women with AF is worse than that for men with an increased risk of stroke, heart failure, and death.[34,37] Furthermore, women are described as more symptomatic with a higher symptom burden and severely reduced quality of life.[34,36] Of note, recent data suggest a potential role of gut microbiota dysbiosis in the development of AF.[38–41] We recently analyzed data from the FINRISK 2002 study linking gut microbiome composition to AF risk.[42] This raises the possibility of particular salience of microbially mediated sex effects in both AF and its sequelae; however, further work is necessary before microbiome can be used for prevention and targeted treatment of AF.[42]

4 | SEX DIFFERENCES IN DIET AND LIFESTYLE RELATED TO CARDIOVASCULAR HEALTH

Research indicates that women exhibit better adherence to healthy diets compared with men, with higher intake of dietary fiber and lower energy foods.[43] While red meat consumption has been identified as a risk factor for CVD regardless of sex,[44] large cohort studies found certain dietary patterns, particularly the consumption of milk and dairy products, protective against CVD, obesity, and metabolic syndrome in women, but not in men (Figure 1).[45,46] Moreover, consistent cross-national associations between gender and certain foods, such as "masculine food habits" (red meat and alcohol) and "feminine food habits" (fish, fruits, and vegetables), have been observed, potentially contributing to gender disparities in cardiovascular health (Figure 1).[47] A recent review proposes that prehistoric gender roles and bodily sexual dimorphisms have been selected for sex differences in energy requirements and metabolic pathways.[48] Indeed, studies reveal sex-differential responses to dietary interventions such as the Mediterranean diet (MD), including greater activation of the apolipoprotein E gene and angiotensin-converting enzyme in women compared with men (Figure 1).[49] Consistent with this, another study indicates that women exhibit greater sensitivity to the metabolic effects of a MD with varying glycemic indices,[50] potentially yielding a more favorable treatment response to blood-glucose targeted diets compared with men. In line with this, women have shown increased conversions from plant-derived alpha-linolenic acid into the bioactive form eicosapentaenoic acid compared with men, during alpha-linolenic acid consumption in a randomized controlled trial,[51] which could have implications for nutritional recommendations in cardiovascular health.[52] Furthermore, the effects of energy-restricted high-protein diets on body composition were shown to differ between men and women.[53]

Although current smoking and heavy alcohol consumption are more prevalent among men than women,[45] research suggests that the detrimental effects of smoking may be more significant in women than in men (Figure 1).[54,55] Studies indicate that women who smoke have a 22% higher risk of experiencing ST-elevation myocardial infarction (STEMI) as the initial clinical manifestation compared with men, whereas this risk difference is less pronounced in non-smokers.[54] This is concerning as STEMIs are often underdiagnosed and treated with delays in women compared with men.[56] One potential explanation for the gender disparity in smoking effects could be the potential inhibition of protective estrogen activity due to smoking,[57] along with nicotine's possible targeting of mechanisms that drive STEMIs more frequently in women, including vasospasm.[58]

Gender differences in alcohol consumption have been highlighted in a recent review too, revealing intriguing patterns. Notably, for women, alcohol intake shows a J-shaped relationship with hypertension, where moderate consumption of one to two standard drinks per day is associated with a lower risk of hypertension development; conversely, in men, this relationship appears to be more linear.[59] Furthermore, studies indicate a higher prevalence of alcoholic cardiomyopathy[59] and other alcohol-related disorders among men.[60]

Recognizing these sex differences in modifiable lifestyle risk factors for CVDs could serve as crucial targets for improving CVD outcomes. A deeper understanding of these differences can aid in the identification and implementation of targeted intervention measures within specific target groups. Moreover, it has been suggested that the composition of the gut microbiome serves as a significant mediator in potentially impacting cardiovascular risk through various dietary compounds. Specifically, research indicates that the gut microbiome plays a pivotal role in crucial lifestyle-dependent CVD-related factors, including disrupted glucose regulation, dyslipidemia, hypertension, and obesity, with these factors exhibiting sex differences.[61] The gut microbiome is probably bidirectionally influenced by both gendered behavior and by genetic factors influencing cardiovascular health.[61]

5 | PHYSIOLOGICAL BASES OF SEX DIFFERENCES IN CARDIOVASCULAR DISORDERS

Sex differences are seen in baseline cardiac function of healthy adults.[62] Distinct male and female cardiac phenotypes emerge during adolescence,[63] suggesting a predominant role of sex hormones as modifiers of cardiac function. Consistently, lower rates of CVD are reported in premenopausal women compared with age-matched men, while after menopause, the rate of CVD development and mortality in women exceeds that of men.[64,65] Estrogen and associated signaling pathways have long been considered responsible for the cardiac protection observed in premenopausal women compared with men.[66] However, the role of estrogen in cardiac function is not fully understood, nor the extent to which these processes reflect organizational versus activational effects. Recently, work on Turner syndrome (XO) and Klinefelter (XXY) models demonstrated that some cardiac sex disparities occur at the earliest stages of heart formation, before sex hormone production starts, due to X-linked gene dosage effects.[67] These observations suggest cardiac sex dimorphism does not exclusively depend on sex hormones.

Compared with male hearts, female hearts typically exhibit higher beating rates, prolonged action potential duration, preferred usage of lipids as metabolic substrate, and differences in cell population density, gene expression, and epigenetic profiles.[68–75] Although most studies have focused on cardiomyocytes, sex dimorphism is present in other cardiac cell populations.[76] Healthy women's hearts have more fibroblasts,[75] but lower expression of collagen than those of male counterparts, a trend that is reversed with increasing age.[77] The Gene Ontology (GO) gene functional descriptor most strongly differentially expressed between male and female cardiac fibroblasts in humans is "Organization of the extracellular matrix,"[72] including sexually dimorphic regulation of matrix remodelers.[77,78] These studies demonstrate that male and female hearts have different baseline mechanical microenvironments, possibly influencing fibrotic response during disease and injury. This baseline then further influences the extent of response both to external environmental factors and to those of internal environments, such as the gut and other host-associated microbiomes.

6 | THE HUMAN MICROBIOME

The human body hosts millions of microorganisms, including bacteria, archaea, fungi, protozoa, and viruses. These microorganisms inhabit different sites of the human body such as its skin, oral and nasal cavities, lungs, gut, bladder, and vagina.[79,80] This diversity of microorganisms holds about 150 times more genetic information than the human genome and they take part in nutrient extraction, metabolism, and immunity[79]; therefore, they can affect health and disease status, including susceptibility to non-communicable diseases as

well as to pathogen infections, also in a manner relevant for gender medicine. For example, dysbiosis of the oral microbiome can lead to periodontal disease that is associated with a higher risk for CVD.[79,81] Furthermore, the vaginal microbiome can help in fighting pathogen infections, leading to spontaneous clearance.[82] Conversely, vaginal communities with higher species diversity and non-*Lactobacillus* dominance have been associated with increased risk of urogenital infections, pregnancy complications, and preterm birth.[83] It is known that preterm birth is associated with CVD risk for the mother and independently predictive of CVD[84,85]; in this way, vaginal microbiome may indirectly affect the development of CVD. Although the entire human microbiome plays a role in human health, the gut microbiota is often considered most impactful for overall health.[79]

6.1 | Gut microbiome-sex hormone axis

Among the mucosal environments in the human body known to harbor dense communities of symbiotic (commensal) or transient bacteria and other microorganisms, the gastrointestinal tract (gut) has been most studied and most frequently linked to variations in health and disease etiology. During puberty, sex hormone levels shift toward those seen in adults, driving further sex differentiation. These processes also affect the gut microbiome. The interactions of gut bacteria with sex hormones may be crucial for sex-differential disease development and predisposition later in life, including possible shifts during and after the reproductive lifespan. The importance of the gut microbiome in mediating sex differences in disease susceptibility has been demonstrated in several human and animal studies, which we summarize here.

In fecal samples from age-matched mice, a clear sex difference in gut community composition was found at the phylum level; in particular a significantly higher Firmicutes/Bacteroidetes (F/B) ratio in male mice.[86] This ratio generally coincided with higher severity of after-stroke injury, manifested by a larger infarct size, higher neurobehavioral scores, and lower survival rates.[86] Accordingly, the sex differences in these outcomes in the model in question suggest their mediation by gut microbiome sex differences. These results indicate the potential mechanistic role of sex-differential gut microbiota, in the development or outcomes of pathological states, where epidemiology varies between men and women.

Markle and colleagues showed that manifestations of T1D in non-obese diabetic (NOD) mice are greatly diminished when male mice were raised under specific pathogen-free (SPF) conditions.[87] This was neither the case in female SPF NOD counterparts nor in mice raised germ-free.[87] Moreover, transplantation of gut microbiota from protected SPF male NOD mice to female counterparts prior to disease onset conferred upon the latter elevated circulating testosterone and metabolomic changes, reduced islet inflammation and autoantibody production, and robust T1D protection.[87] The degree of T1D protection was linked to testosterone levels and depended on androgen receptor activity.[87] This suggests a potential role of the gut microbiome in T1D manifestation by testosterone regulation and through effects on immunity and metabolome, which may generalize to a wider range of cardiometabolic diseases where these processes are significant.

This importance of gut microbiome in disease manifestation and interaction with sex hormones was also shown in mice with polycystic ovary syndrome (PCOS). Torres and colleagues investigated how the known estrogen inhibitor and PCOS inducer, letrozole affects pubertal compared with adult mice, finding the latter respond differently to this treatment, with less profound changes in the gut microbiome and less severe metabolic phenotype of PCOS.[88] Consistent with this, a similar study showed that female mice displayed metabolic and reproductive dysregulation after receiving stool transplants from women with PCOS, implying mediation of the gut microbiome in PCOS-like phenotypes[89] as well as their potential cardiometabolic sequelae.

In further support of a microbiome-endocrine interrelationship, several human studies showed correlations between gut microbiome and sex hormones during fluctuations of the latter such as across the menstrual cycle or in menopause. Gut microbiome differences seen between men and women prior to menopausal age was attenuated by the loss of ovarian hormones such that postmenopausal women's microbiota more resembled that of men than that of premenopausal women, with the menopause transition associated with a loss of gut bacterial diversity.[90,91] However, other studies did not observe differences in diversity.[92,93] Furthermore, the short-chain fatty acid (SCFA)-producing genera *Akkermansia* and *Lactococcus* are found to be most abundant during the luteal phase of the menstrual cycle when levels of estradiol and progesterone are higher in contrast to the follicular phase.[94] In support of these findings, another study found an increase in *Lactococcus* in ovariectomized mice undergoing hormone replacement treatment with estradiol[95]; however, *Akkermansia muciniphila* was found to be depleted in postmenopausal women.[91] Studies were conducted in women taking oral combined hormone contraceptives containing 17-β-estradiol and progesterone. These contraceptives decrease serum levels of estradiol and progesterone and have been found to reduce alpha diversity, gut microbial richness, and alter composition when compared to unmedicated controls.[94,96]

6.1.1 | Gut microbiome metabolism of sex hormones

These responses of the gut microbiome to changes in circulating sex hormones follow in part from the ability of numerous bacterial species in the gut to metabolize sex hormones, making a bidirectional interaction possible. Increasing evidence indicates such crosstalk between microbiome and steroid hormones through metabolism and modification of host hormones.[97] After excretion from the bile in their conjugated form, estrogens,[98] androgens,[99] and progestins[100] can undergo bacterial enzymatic deconjugation (Figure 1). The collective genes in the human gut that can metabolize estrogens, called the "estrobolome," can impact endogenous estrogen metabolism and estrogen levels in the circulation.[98] Specifically, β-glucuronidases enable bacteria to metabolize conjugated steroids enabling their reabsorption into enterohepatic circulation. It was previously shown that β-glucuronidase is prevalent in Firmicutes within clostridial clusters XIVa and IV.[101] Moreover, urinary estrogen levels correlate with gut abundance of *Clostridia* and Ruminococcaceae.[102] β-glucuronidases have also been found in a class of Gram-positive Firmicutes gut bacteria with low G + C%, which includes *Bifidobacterium* spp. and *Bacteroides thetaiotaomicron*.[101]

There is therefore reason to expect that changes to the gut microbiota may impact circulating hormone levels, especially after menopause[94] in the absence of strong endogenous production. Studies in humans indicate that the percentage of reabsorbed estrone and estradiol is about 35%–45%, which suggests a substantial role of gut bacteria in estrogen regulation.[103,104] Moreover, gut microbiome diversity correlates with estrogen levels in urine of postmenopausal women and men, but not in premenopausal women which further supports the salience of gut microbiome for estrogen levels where no ovarian source can balance them.[97] Interestingly, there is a positive correlation between functional activity of fecal β-glucuronidase and urinary estrone, and negative correlation with fecal total estrogens, which further indicates microbially dependent reabsorption and thus retention of estrogens.[105] In addition, the gut of postmenopausal women exhibits higher bacterial genetic potential for the sulfate transport system pathway, alongside lower potential for the pathogenic bacterial secretion system and the β-glucuronidase pathways.[91] The two-way interaction between microbiome and sex hormones is further supported by the fact that carriage of β-glucuronidases varies between closely related bacterial species,[106] possibly suggesting host adaptations. Moreover, gut bacteria can either fully or partially metabolize free forms of steroids,[107,108] implying the possible use of these compounds for energy in cases of complete degradation or for detoxification in the latter case, especially in species sensitive to testosterone.[109] Altogether, this further indicates the possibility of variable microbiome impact on circulating steroid levels that in turn may underlie variable risk for sex-differential CVD progression mechanisms.

6.2 | Microbiome-alterable sex-differential homeostasis of metabolism and vascular function

Extending to animal models of host–microbiome interaction, in T1D female mice, fecal SCFA were increased compared with female controls and correlated positively with abundance of *Ruminococcus*, *Odoribacter*, *Parabacteroides*, *Roseburia*, and *Escherichia*, whereas male T1D mice instead presented decreased SCFA compared with wild-type male mice and no correlation with microbiome taxa (Table 1); this suggests possible alleviation of T1D in female mice via gut microbiome modulation.[110] In addition, the increase in serum glucose and decrease in pyruvate and creatine in T1D male mice suggest suppressed glucose metabolism compared with female counterparts (Table 1).[110] Such decreases in pyruvate and creatine levels correlated with higher gut abundances of *Sutterella* and *Desulfovibrio* in male T1D mice (Table 1).[110] On the contrary, the increased levels of citrate and succinate were correlated with abundances of *Roseburia*, *Odoribacter*, *Sutterella*, *Escherichia*, *Parabacteroides*, and *Oscillospira* in T1D female mice (Table 1),[110] suggesting a greater relative shift in the gut microbiota in female mice during the development of T1D.

In addition, male mice may be more likely to exhibit impaired amino acid metabolism than female mice during the development of T1D, particularly concerning BCAA levels which have previously been linked to insulin resistance.[111] The levels of isoleucine and valine in serum were positively associated with gut abundance of *Desulfovibrio* and *Sutterella* in male mice, while tyrosine in feces was positively correlated with gut *Ruminococcus*, *Escherichia*, and *Parabacteroides* in female T1D mice (Table 1).[110] Moreover, hepatic choline level negatively correlated with gut abundance of *Escherichia*, *Oscillospira* and *Parabacteroides*, and positively with *Sutterella* in female mice, implying sexual dimorphism of T1D in choline metabolism (Table 1).[110] Furthermore, impaired ketogenesis activity or mitochondrial dysfunction as indicated by decreased levels of 3-hydroxybutyrate (3-HB) in serum and liver and increase in liver glutathione levels was possible to conclude in male, but not female T1D mice (Table 1).[110] This 3-HB change correlated with gut abundance of *Mucispirillum*, *Bacteroides* (positively), *and Sutterella*

TABLE 1 Overview of microbiome health associations observed differentially in male and female animals and/or humans.

Organism	Model/condition	Effect in male subjects	Effect in female subjects	Associated microbiome feature	Associated biomolecule	Ref.
Mouse	Ischaemic stroke	↑ F/B ratio baseline	↑ Survival rate ↓ The infarct area ↑ Behavioral test performance ↓ TMAO ↑ SCFA and Trp ↓ Inflammatory cytokines	F/B ratio, female microbiota	TMAO, SCFA	[86]
Mouse	SPF T1D non-obese diabetic (NOD)	↓ Autoantibody production ↓ Pancreatic islet inflammation • Robust T1D protection	–	SPF male microbiota	Testosterone, Androgen receptor activity	[87]
Mouse	C57BL/6NHsd female mice + Letrozole	–	Partial PCOS phenotype: ↑ Serum testosterone • Acyclicity • Cystic follicles in ovaries	Shift in beta diversity of gut microbiome	–	[88]
Mouse	T1D	↓ Glu metabolism Serum BCAA (isoleucine and valine) and liver GSH correlated positively and Cre negatively with *Sutterella* and *Desulfovibrio* abundance • Impaired ketogenesis activity or mitochondrial dysfunction, indicated by a decrease in 3-HB in serum and liver, and an increase in GSH in liver • 3-HB correlated positively with gut abundance of *Mucispirillum*, and *Bacteroides* and negatively with *Sutterella*	• Hepatic choline level positively correlated with *Sutterella* • Fecal SCFA positively correlated with abundances of *Odoribacter*, *Parabacteroides*, *Escherichia*, Oscillospira, *Roseburia*, and *Ruminococcus* • Serum citrate positively correlated with *Escherichia*, *Roseburia*, *Odoribacter*, *Parabactoroides*, and *Oscillospira* and negatively with *Suterella* • Tyr in feces positively correlated with gut *Ruminococcus*, *Escherichia*, and *Parabacteroides*	*Sutterella*, *Desulfovibrio*, *Mucispirillum*, *Bacteroides*, *Odoribacter*, *Parabacteroides*, *Escherichia*, Oscillospira, *Roseburia*, *Ruminococcus*	SCFA, Pyr, Cre, BCAA, Tyr, 3-HB, GSH, Succinate, Citrate	[110]
Rat	Wistar	• Aortic relaxation	• Greater effect on aortic relaxation	–	Red wine polyphenols	[129]
Human	Hypertension	• SM38:3, SM42:4, SM40:3, and conjugated bile acids associated with higher SBP • *Bifidobacterium* genus, *Ruminococcaceae* family, *Lachnospiraceae families* associated with sphingomyelin	• Dihomo-lineoylcarnitine, 4-hydroxyphenylacetylglutamine and VLA associated with higher SBP	*Bifidobacterium* genus, *Ruminococcaceae* family, *Lachnospiraceae families*	Sphingomyelins	[116]

TABLE 1 (Continued)

Organism	Model/condition	Effect in male subjects	Effect in female subjects	Associated microbiome feature	Associated biomolecule	Ref.
Human	CVD risk	• MD improved insulin homeostasis and SBP • Favorable redistribution of LDL subclasses from smaller to larger LDL ↓ Adiponectin concentration	–	–	LDL, Adiponectin	[120– 122]
Human	Obesity	–	• High GI MD after low GI MD diet increased plasma Glu concentrations (menopausal women)	–	–	[123]
Human	T2D + low-energy, low GI diet	–	• Reduced body weight • Improved metabolic outcomes including insulin resistance, liver function, and chronic inflammation	–	Endothelin-1	[124]
Human	Obesogenic lifestyle + insulin infusion	↓ Leg blood flow ↓ Skeletal muscle microvascular perfusion ↓ Plasma concentration of adropin and nitrite	• Protection against short-term-diet-induced vascular insulin resistance	–	Adropin, Nitrite	[125]

Abbreviations: 3-HB, 3-hydroxybutyrate; BCAA, branched-chain amino acids; Cre, creatine; CVD, cardiovascular disease; GI, glycemic index; Glu, glucose; GSH, glutathione; LDL, low-density lipoprotein; MD, Mediterranean diet; PCOS, polycystic ovary syndrome; Pyr, pyruvate; SCFA, short-chain fatty acids; SPF, specific-pathogen free; SM, sphingomyelin; T1D, type 1 diabetes; TMAO, trimethylamine oxide; Trp, tryptophan; Tyr, tyrosine; VLA, vanilla acetate.

(negatively) in male mice, whereas the glutathione elevation correlated positively with abundance of *Sutterella* and *Desulfovibrio* (Table 1).[110] This suggests that gut microbiota may modify T1D-related ketone and glutathione metabolism in a sex-differential manner.

Furthermore, a clinical study found that SCFAs in plasma independently predicted systolic and diastolic blood pressure in women, but not in men.[112] Gut bacteria *Ruminococcus gnavus*, *Clostridium bolteae*, and *Bacteroides ovatus* were also significantly more abundant in the hypertensive women when adjusting for several covariates,[112] suggesting a possible pathway crossing microbiome to SCFA production to hypertension risk. However, previous studies showed a negative correlation between BP and the gut abundance of SCFA-producing gut bacteria and fecal SCFA.[113] Findings from several studies have highlighted the role of particular bacterial families, such as Lachnospiraceae and Ruminococcaceae, as main producers of the SCFA butyrate, implicated in modulating inflammation. Specifically, a reduction in SCFA-producers, *Ruminococcus spp*, and *Eubacterium hallii*, was seen in stool samples from heart failure (HF) patients.[114] Moreover, a reduction in *E. hallii* and several other members of Lachnospiraceae was linked to severity markers, that is, elevated levels of soluble CD25 and heart transplant need or death.[115]

Similarly, the microbially produced metabolite phenylacetate has been shown to be a very good predictor of lower heart rate variability, specifically in men. In this subgroup, phenylacetate levels correlate particularly strongly with gut abundance of Ruminococcaceae and anticorrelate with *Blautia*, Lachnospiraceae.[116] Another example is sphingomyelins, which are membrane sphingolipids previously associated with hypertension[117] and CAD[118]; moreover, sphingolipids metabolize to ceramides that are involved in vascular and blood pressure homeostasis.[119] In this study, sphingomyelins were likewise significantly associated with higher systolic BP only in the male subgroup (Figure 1).[116] Gut abundance of the bacterial families Lachnospiraceae and Ruminococcaceae as well as the *Bifidobacterium* genus similarly were significantly correlated with sphingomyelin levels only in men.[116]

6.3 | Sex-differential efficacy of nutritional interventions in cardiovascular disease

Another main pathway for gut microbiome influence on cardiovascular and cardiometabolic health progression lies in the variable impact particular nutritional interventions have—possibly the largest modifiable risk factor for CVD, and in several cases, such impact has also been shown to differ between men and women. One study evaluating the MD for CVD prevention could conclude significant improvement in certain cardiometabolic variables only in men, alongside improvements in insulin homeostasis and systolic blood pressure (Figure 1).[120] A favorable redistribution of LDL subclasses from smaller to larger LDL[121] together with decreased adiponectin concentration following MD achieved significance only in male probands in a similar study.[122]

In contrast, comparing modified MD with high and low glycemic index (GI) in overweight men and menopausal women, another study observed the expected increase in plasma glucose concentrations when switching from low to high glycemic index (GI) diet only in the (menopausal) woman probands.[50] Furthermore, female mice were better protected than male mice against obesity-induced vascular insulin resistance under an obesogenic diet.[123] In line with this, a low-energy, low GI diet trial conducted in type 2 diabetes (T2D) patients concluded significantly reduced body weight and improved metabolic outcomes, including insulin resistance, liver function, and chronic inflammation only in women.[123] The authors suggest these beneficial effects of diet-induced weight loss may be mediated by reduced vasoconstrictor peptide endothelin-1 production, reducing circulatory proinflammatory cytokines.[123] Another study reported that vascular insulin resistance followed a 10-day intervention of reduced ambulatory activity and increased consumption of sugar-sweetened carbonated beverages in male but not female young healthy volunteers.[124]

Various dietary components can have protective effects on CVD by exhibiting antioxidative effects,[125] and thereby affecting ROS balance, inflammation,[126] vascular function, and blood pressure.[127] Beyond differing in habitual diet, the bodies of typical men and women may respond differently to several of these dietary components. For example, red wine-derived polyphenols showed a greater effect on aortic relaxation in female rats.[128] This modulation of beneficial effect partly reflects sex-dependent regulation of enzymes involved in their metabolism such as sulfotransferases that determine the phenol bioavailability being expressed in an estrogen-dependent manner.[129] Importantly, while both mice and rats show a sex difference in this regard, the direction of effect is inverted between the species,[130] underscoring that aspects of sexual phenotype are not conserved even between closely related species by necessity, and may reflect lineage-specific evolutionary adaptations, in analogy to the observation above regarding genes for host sex steroid metabolism rather are lineage-specific than ubiquitous in the gut microbiota, which has coevolved with its host. Another such gene class, β-glucosidases, influences host isoflavone bioavailability through degradation of phenolic glucosides such as

the soybean isoflavones genistein and daidzein.[131] These exert beneficial effects suspected to be mediated via estrogen receptor signaling since phenolic aglycosides such as S-equol can bind to estrogen receptor-β with similar affinity as 17β-estradiol.[132,133] Thus, both species-specific yet variable adaptations in gut microbial taxa and their animal hosts affect homeostatic regulation programs where sex steroid signaling plays a crucial part.

6.4 | Sex differences in the microbiome-inflammation axis of cardiovascular diseases

The gut microbiota is necessary for the development and normal functioning of the immune system. Crucial interaction with the immune system takes place at the intestinal barrier, composed of mucus and epithelial cells.[134] Upon disturbance of the intestinal barrier due to various factors such as diet, antibiotic use, or genetic susceptibility, increased permeability allows passage of microbial ligands that can elicit immune response.[134,135] The increased intestinal permeability associated with systemic inflammation and dysbiosis of the gut microbiome has been observed in CVD.[136] It is important to note that the disruption of the intestinal barrier sometimes can be reversed with probiotic treatment or through elevating levels of gut microbiota-produced metabolites such as SCFAs that can reduce local and systemic inflammation.[136,137] On the contrary, capsular lipopolysaccharides (LPS) can induce inflammatory responses, disrupt the gut barrier and induce chronic inflammation[138]; LPS also play a role in cardiac contractility, insulin resistance, and endothelial function.[139–141] High levels of serum LPS, indicative of reduced gut barrier function, have been associated with pathological processes, including diabetes, kidney disease, obesity, and inflammation.[142] LPS consisting of a hydrophobic domain known as lipid A (or endotoxin) are elevated in decompensated HF.[143,144] The neurohormonal activation in HF leads to multiorgan hypoperfusion and dysfunction, while gut ischemia and edema disrupts the gut barrier and allows the transition of bacteria and their products causing inflammation; this is known as the "leaky gut" hypothesis of HF.[144] With sex differences observed in both the microbiome, the immune system, and in the epidemiology of CVDs, the particular homeostatic challenge under gut barrier dysfunction therefore may be an area where sex-differential host–microbiome homeostasis has a major medical impact.

Both epidemiology and outcomes of ischaemic stroke differ between men and women.[145] A recent study examining the role of sex-differential gut microbiota in a mouse model of ischaemic stroke finds that female-to-male gut microbiota supplementation positively modulates poor stroke outcomes, improves prognosis, and reduces systemic inflammation in male mice.[86] In addition, these mice showed an increase in blood circulation and brain tissue anti-inflammatory factors and a decrease in proinflammatory factors and inflammatory cell infiltration in the infarction center. Specifically, the significantly increased level of IFN-γ, IL-1 β, IL-17, and TNF-α in the male group could be reversed by FMT from females, while female mice who received male microbiota showed the opposite trend.[86] The better pre-intervention outcomes in female mice were accompanied by lower levels of circulating trimethylamine oxide (TMAO) (Figure 1), which has been previously linked to cardiovascular health, as well as higher levels of SCFAs and tryptophan compared with male mice. Following cross-sex microbiome transfer these differences were attenuated, consistent with sex-divergent gut microbiota impacting stroke outcomes through metabolic products. These bacterial metabolites have regulatory roles in inflammation response after stroke, implying that elevated inflammatory cytokines in circulation may follow from the combined action of TMAO, SCFAs, and tryptophan after they reach blood circulation.[86] Moreover, TMAO was previously associated with pathogenesis of ischaemic stroke where it exacerbated stroke injury,[146] while tryptophan and SCFAs have been shown to have protective effects.[147–149] In addition, TMAO has been suggested as a biomarker of poorer functional outcome events and mortality of ischaemic stroke[150] and associated with higher risks of major adverse cardiac events and CVD.[151] TMAO also shows a positive correlation with proinflammatory intermediate $CD14^{++}CD16^{+}$ monocytes and with ischaemic stroke incidence.[152] This implies the potential role of sex differences in gut microbiota in relation to ischaemic stroke, the mechanisms of which may extend also to other CVDs with sex-differential epidemiology and a substantial microbiome contribution to the outcome.

7 | INSIGHTS FROM SPECIALIZED CARDIOVASCULAR HOST–MICROBIOME DISEASE MODELS

Increasing work has aimed at building more realistic and sophisticated in vivo models for researching CVDs, including those that explore the role of the gut microbiota. These promise further insights into how for example sensitivity to long-term risk factors or response to treatment may differ meaningfully between men and women. Gut microbiota abnormalities were detected in several models of heart failure and hypertension. A study comparing rat models for spontaneously hypertensive heart failure

(SHHF) and spontaneous hypertension (SHR) with normotensive rats found significant differences in gut alpha diversity, F/B ratio, taxonomic composition, and functional profiles comparing wild type to SHR, even before cardiac differences were present.[153] A weaker signal was seen for SHHF, suggesting a potential role of gut microbiota in the evolution of HF associated with hypertension. Furthermore, the study shows that SHHF rats have an increase in Muribaculaceae and Prevotellaceae families (Bacteroidetes) and depletion of SCFA-producers, Ruminococcaceae and Lachnospiraceae families (Firmicutes) consistent with their decrease in HF patients.[115,154] Muribaculaceae members can potently degrade several complex carbohydrates[155] which may facilitate the translocation of LPS of highly abundant Gram-negative Bacteroidetes members. An increase in Bacteroidetes was previously documented in many other pathological conditions associated with low-grade inflammation such as obesity, diabetes mellitus, or metabolic syndrome[156] again underscoring a role of proinflammatory response and low-grade inflammation. The study further found a decrease in *Akkermansia*, a genus that shows inverse association with LPS plasma levels[157] and *Mucispirillum* (*Deferribacteres*), a genus that transforms iron into absorbable form in the small intestine[158]; this could be related to prevalent iron deficiency in HF patients.[159] On the contrary, an increase in *Prevotella* and *Paraprevotella* found in SHHR rats was also previously associated with inflammatory disorders.[160] Specifically, *Paraprevotella* is known to produce succinic acid, which was found to increase interleukin-1β (IL-1β).[161] Moreover, succinic acid was found increased in plasma and urine from hypertension, diabetes, and metabolic disease rodent models.[162,163] Heart failure is also studied in doxorubicin-induced cardiotoxicity models; a recent study demonstrated how changes in gut microbiota upon HF induction that are consistent with those found in HF patients, including increased abundance of *Escherichia/Shigella* and lower F/B ratio compared with control group.[164–166] In mice, the 2-hit model of HFpEF has recently been established as a combination of high-fat diet simulating metabolic stress and L-NAME (inhibitor of constitutive nitric oxide synthases) simulating the mechanical stress which forms a phenotype that most closely resembles human HFpEF, including cardiac hypertrophy, pulmonary congestion, exercise intolerance, and worsened diastolic function.[167] These mice models show significantly increased blood pressure and impaired diastolic function compared with controls, the standard chow-diet-fed (Control 1) and high-fat-diet-fed mice (Control 2). Microbiome analysis showed significant differences in the gut microbial diversity and composition in obese HFpEF mice compared with controls.[167] Consistent with human findings, the obese HFpEF phenotype is associated with relatively more pronounced gut dysbiosis.[168] Further analysis is needed to reveal mechanistic roles of specific gut microbiota members in these HFpEF models. Taken together, work in these models underscore substantial microbiome involvement in yet another important spectrum of vascular diseases where hypotheses surrounding the sex-differential epidemiology of these conditions may be further explored. Crucially, for many of the bacteria shown to impact disease progression in these newer-generation CVD models, sex-differential carriage or impact were shown previously in earlier models as outlined above, but little testing of such differences has yet been reported in the here described setting. This constitutes an important present knowledge gap where microbiome-moderated CVD sex differences are expected, but so far not comprehensively described.

7.1 | Limitations of available models and resulting discrepancies

As noted, while there are consistent findings showing the salience of host–microbiome homeostasis in the etiology of sex-differential disease risk and progression, there is also frequent discrepancy between even well-powered studies, as well as in the translation between human and animal data. Below we discuss several reasons for this.

Comparisons between women and female experimental animals are challenging due to the significant species-specific differences in estrous cycle duration and serum sex hormone levels.[169,170] Phytoestrogens are present in standard laboratory rodent chow, such as genistein, and directly affect cardiac contractility and inhibit tyrosine kinase activation in cardiac myocytes.[171–173] CVD has higher prevalence in postmenopausal women, but most experiments to assess CVD in rodent models are performed in females with an active estrous cycle. Additionally, ovariectomized female rodents do not fully recapitulate mechanisms of postmenopausal women,[174] warranting caution when extrapolating animal data to identify mechanisms of sex differences in human CVD.

Moreover, for many years, female animal models and women in studies were often avoided due to a belief in greater variability due to hormonal fluctuations during the estrous cycle.[175] More recent evidence demonstrates that female rodents are no more variable than male, across diverse traits from gene expression to hormone levels, and across multiple species.[176] This historical exclusion led to major knowledge gaps in both basic and clinical research

causing discrepancies in results between animal models and humans including lack of general understanding of female disease processes, the importance of sex as a variable, and more common adverse drug reactions in women.[176] It is also important to note that other factors such as age, duration of disease exposure, sex hormone, and growth hormone profiles, many forms of stressors, likewise differ between animal models and humans,[5] especially with rodents that contain several orders of magnitude fewer cells and mature, live, and die during a much shorter time span.

Especially in CVDs, oxidative stress and its homeostatic balancing through antioxidant activity involves genes that show major expression differences between species,[177] perhaps underlying, for example, poor replication in humans of antioxidant supplementation which worked in animals.[178,179] Menopause and pregnancy-related conditions such as preeclampsia are similarly hard to model well in animal models though new techniques for the former are promising.[180]

Additionally, species divergence of sex-biased cardiac gene expression is another contributing factor; data suggest that GO pathways are enriched in the opposite direction between humans and animals.[181] Rodents and humans have also dramatically different heart rates and express different levels of the two cardiac sarcomeric isoforms of myosin heavy chain: the α-MyHC (MYH6) and β-MyHC (MYH7). In the adult human ventricle, the cardiac myosin composition is approximately 95% MYH7 (β-MyHC) and 5% MYH6 (α-MyHC),[182,183] a ratio that is almost exactly reversed in mice. α-MyHC has a higher ATPase activity but generates less force than β-MyHC,[184] making animal models less appropriate to study myosin mutations. Notably, the myosin isoform ratio changes even more in favor of β-MyHC in CVD[185–187] and expression of myosin heavy chain isoforms has been shown to be sexually dimorphic in humans.[183] As previously noted, also, various processes that are sex-differential may show even opposite effect directions across species, recognizing a role of heterogeneity and possibly quick adaptation in some of these regards.

There is a complicated trade-off between ease of intervention and translatability of findings that challenge the study of gender medicine, precisely because for most patient cohorts, chromosomal, endocrine, anatomical sex all align, alongside gendered environmental risk factors. These can be separately controlled in many animal settings, allowing, for example, gonadectomy and hormone supplementation at any point in the lifespan, as well as prenatally while retaining wild-type sex chromosome complement and X-inactivation through SRY translocation and other genetic modifications. However, the above general limitations of rodent models remain in place.

8 | BEYOND THE BINARY—OPPORTUNITIES FROM AND CHALLENGES IN ATYPICAL PATIENT DEMOGRAPHICS

While most humans carry typical XX or XY sex chromosome complement driving outcomes throughout prenatal, postnatal, pubertal, reproductive, and post-reproductive life through endocrine exposures shaping physiology, anatomy, immune system, and gut microbiota in characteristic ways, there are demographics where some or all of these assumptions do not hold.[188]

Intersex conditions are an umbrella term for genotypes and/or phenotypes that spontaneously diverge from the most common (endosex) male and female biologies in some ways. These include unusual karyotypes (e.g., Turner, X0, and Klinefelder XXY), specific gene mutations or purely phenotypic[189] instances possibly reflecting environmental factors in utero or later causing a more unusual development. While intersex populations have been and remain studied as natural experiments in sex mechanisms and their health implications, it is important to note that these populations historically have been both stigmatized, invisibilized[189] and frequently still today sometimes undergo substantial unconsented surgeries early in childhood,[190] which emerging intersex patient groups often describe in terms of medical abuse aimed to enforce body norms.[191] This context must be borne in mind in any research that draws on intersex probands, as must the relative rarity (compounded further by frequently missed diagnoses, or many cases where an intersex person themselves were not informed) and heterogeneity of these conditions. However, where possible, powerful insights can be gained and work is currently ongoing for example with cell lines from intersex donors, a technique where there is less need for large, balanced cohorts. More crucially for the area of host–microbiome cardiovascular medicine as it pertains to intersex individuals, precisely this rarity and heterogeneity means that very little is known conclusively for a given patient, though a baseline expectation might be in many cases an intermediate phenotype, risk profile, and treatment response pattern between that of endosex men and women. Accountable treatment guidelines for a stigmatized and vulnerable minority population must also recognize the dignity and agency of such patients.

Another (overlapping, as there are both cis and trans intersex persons either of which may seek gender-affirming healthcare) demographic are trans individuals.[192] This is an umbrella term for a heterogeneous group of persons, many of whom reject their birth-assigned sex reflecting a felt inner need, usually relieving lifelong prior distress by doing so.[193] While some research suggests trans modality has a neural substrate representing

atypical brain differentiation,[194] the relevance for the present work is solely that many trans persons seek what is termed gender-affirming healthcare, which aims to alter phenotype away from birth-assigned sex toward the individual's target sex.[195] Aside from surgeries including to the reproductive organs such interventions chiefly involve gender-affirming hormone therapies (GAHT) intending, for example, to shift circulating sex steroid levels in a trans man (assigned female at birth) to that most similar to cis (not trans) men, and vice versa in trans women.[195] In some ways similar to the physiology of intersex persons, those transitioning through GAHT undergo variable degrees of pubertal differentiation, resulting in changes to tissues, organs, and cells that place them likewise in an intermediate stage between most male and female patients.[196]

As in intersex persons, little is as yet known on the extent to which GAHT-induced changes predispose to risk profiles, symptomology, or treatment response characteristic of birth-assigned versus target sex, and to which degree this varies between different disease mechanisms or from individual to individual. Similarly, little is known on either microbiome or immune changes resulting from GAHT, though research is ongoing in several laboratories.[197] Accordingly, persons transitioning under GAHT both represent an opportunity to study endocrine sex impact longitudinally in a way otherwise usually only possible in animal models, and a patient group where healthcare guidelines established from work done in cis populations can be expected to only partially hold. Reflecting genetics alongside both endogenous and exogenous sex hormones, drug responses are presently more challenging to predict in transgender patients.[198] More generally, hormonal contraceptives, menopausal hormone treatments, or over-the-counter performance-enhancing drugs may also influence the metabolism of other pharmaceuticals, and these interactions may vary by dose, formulation, and mode of delivery of the steroid hormones. As yet these complexities are rarely considered either in labeling or prescription. There is so far little research into long-term cardiovascular health in populations undergoing GAHT.[199] Adverse events including lipid profile alterations,[200] venous thromboembolism, stroke, and myocardial infarction[201–203] have been described, but it is unknown to what extent this reflects therapy rather than baseline. Epidemiologically speaking, CVD burdens are higher in trans populations in general, whether or not GAHT is initiated,[204] similar to what is seen in other vulnerable minority groups and likely greatly reflecting marginalization and stigma resulting in elevated allostatic load.[205,206] Higher risks at the population level remain obscure due to heterogeneity of studies, difficulty in defining suitable control groups and small samples, and a recent large-scale systematic review and meta-analysis emphasizes the importance of inclusion of potential socioeconomic and lifestyle factors and cardiovascular risk management.[207] In order to make informed decisions and tailor GAHT to individual needs, understanding the individual variability of both intended and potential side effects is crucial, and likely involves the same mechanisms as underlies variability in sex-differential health progression in cis populations, including contributions from both host and microbiota.

9 | CURRENT CLINICAL IMPLICATIONS AND FUTURE FOCUS OF RESEARCH

Thus altogether, research to date indicates microbiota (of the gut, likely other sites as well) contribute to the development and progression of CVDs, including as modulators, mediators, and moderators of environmental factors and in intense bidirectional crosstalk with the host immune system. Processes of growth, repair, metabolism, and defense all respond to host endocrine signaling, including through hormones as one main mechanism of sexual differentiation and maintenance. Sex hormones also form substrates for gut bacteria and experience modification and reuptake variation reflecting the gut ecosystem, resulting in a second route of cross-talk, where different microbiome compositions both follow from hormone exposure and show the ability to drive and maintain altered circulating or excreted hormone levels. These respective dynamics allow for potentially multiple different homeostatic equilibria which may have different consequences for metabolism, inflammation, signaling, tissue remodeling, and progressive diseases of the cardiovascular system that lie downstream of such changes. As epidemiology reveals substantial and clinically relevant differences between men and women in terms of these diseases, there is a major unmet need for better understanding of sources of their variability, which could help select the appropriate preventative or therapeutic measures for each individual. The medical implications of a person's sex shifts across their lifespan in varying ways, including puberty, easy, or difficult pregnancies and menopause in most women, andropause in most men and a variety of possible shifts seen in sex- and gender-minority populations. A truly expansive gender medicine could make use of these factors as a basis for personalized medicine, going beyond choosing between one reference range or another for a diagnostic biomarker, and in doing so also draw on a deeper understanding of dynamic systems like the microbial and immune cell populations in a patient's body.

Aside from the challenges and limitations from both easily accessible study populations and animal models described in this review, there is a general reproducibility

issue within the topic area of host–microbiome gender medicine much as elsewhere in biomedical research. Disagreement between studies can often gradually resolve through systematic reviews, which here are needed, but there are also particular challenges in study design within this subfield that could be addressed through more sophisticated approaches.

One source of apparent study disagreement lies in the choice of statistical methodology. Much of the work we have reported whether in animal models or in humans, operate through separate disaggregated statistics—for example, testing an association between a gut bacterium and a marker of cardiovascular health separately for significance in male and female subjects, achieving nominal significance in the one group but not the other, then reporting this as a sex-specific association. If the study (as frequently) is statistically underpowered in the separate male and female groups, an association may thereby be incorrectly described as sex-specific (concluding evidence of absence from the absence of evidence). On the contrary, pooled rather than disaggregated analyses can miss strong but opposed correlations in male versus female subjects as the overall signals cancel out. One solution, alongside more stringent consideration of statistical power already at the study design stage, is to consistently model associations between biomarkers and outcomes as a cross-product with sex, such that both main and interaction terms are present in all analyses then conducted on pooled data.

A related issue comes from the nature of homeostasis itself where an outcome typically will reflect the compounded impact of many factors. A mechanism by which one factor affects the outcome may be equally responsive in two populations or conditions, yet masked in the one case due to another factor saturating the outcome response (e.g., microbiome contribution to circulating estrogens having less impact in the presence of functioning ovaries). This is particularly important comparing studies of patients at different severity, that is, what may have major impact at early stages of disease progression may be drowned out by other factors later (e.g., how menopausal hormone replacement therapies appear protective in cohorts where they are initiated before much vascular damage versus late initiation where the immune effects of estrogen instead may exacerbate the damage). Similarly, a directional response (such that a shift in one feature will drive a corresponding shift in another) equally strong in two populations may fail to achieve significance in the one population if for another reason the "driver" feature does not vary sufficiently there, for example, the observation that a factor such as the activity of a gene, concentration of a metabolite or hormone, or abundance of a bacterium does not correlate with a particular outcome in women while it does in men may simply reflect the first variable being less variable in the group of women studied. This becomes important as there may be rare conditions where this variation would increase, at which point the overlooked association still would become active. All these challenges are served by more thorough consideration of the statistical power possible within a given setting and a resulting more sophisticated analysis approach.

10 | CONCLUSIONS AND OUTLOOK

As systems medicine matures enough to respond to stakeholder requests to address cardiovascular health sex and gender health inequity, new insights are emerging, both validating (e.g., sometimes substantial sex differences in disease symptoms and progress) and questioning (e.g., whether greater male variability holds as generally as has been claimed) previously recognized ideas. More excitingly, new scopes of knowledge emerge. For instance, the gut microbiome both affects and is affected by the sex steroid signaling system. Studies in some animal models have demonstrated causality, showing that gut bacteria change in response to circulating hormone levels and can also influence these hormone levels, particularly when the body's own hormone production declines. Moreover, not just the heart and blood vessels, but also organs like the liver, kidneys, and pancreas—which indirectly contribute to maintaining cardiovascular homeostasis—exhibit variations that reflect both genetic and endocrine aspects of sex. These variations are further influenced by gender differences in modifiable lifestyle factors. Disentangling these contributions from the human data directly is challenging but ultimately necessary to make the most of such insights. Central processes such as innate (including lipid and amino acid metabolism) and xenobiotic (including rates of drug metabolization) metabolism, stress response, inflammation, and immunity (including the role of modulation by short-chain fatty acids) differ statistically between men and women. These differences influence CVD susceptibility and responses both to nutritional (e.g., during calorie restriction conditions) and pharmaceutical interventions, and can have a substantial impact also on model systems such as cell cultures. Substantial epidemiology link reproductive life course variation to potentially large variation in CVD risk, but while causality remains unclear, our options to address these risks also remain limited, reflecting the deeply complex and entangled roles of components of sex and gender in health and homeostasis that makes it both relevant and difficult to study.

Sex differences relevant for CVD are often not universal either in occurrence or strength, neither within

human populations, across the lifespan, nor across different model systems. Such heterogeneity hinders reproducibility, robust conclusions and effective translation unless accounted for, both in the majority and in sex- and gender-variant populations often already disproportionately affected by healthcare disparities. In many cases, study and analysis designs either fail to detect sex differences or fail to differentiate between differences of degree versus true dimorphisms, or to qualify which contexts respective differences become salient in. While less complex than the human cohort setting, technical variation in experimental systems (such as hormone levels in cell growth media) still may confound such research. Breakthroughs in more comprehensive use of patient or volunteer "big data" (e.g., from electronic health records or femtech applications) may allow better conclusions, as will continued follow-up of deeply phenotyped population cohorts. In some but not all mechanisms, pleiotropy may be limited enough to allow relevant causality inference through the natural genetic experiments viewed in such cohorts through Mendelian randomization (MR) analysis, whereas other insights will follow from observational studies of sex- and gender minority volunteers such as those receiving GAHT treatment. Increasingly sophisticated in vivo and ex vivo model systems may further contribute. Joining microbiome quantification with measurements of exposome, host gene expression, metabolism, and immune action, we gain a more complete view of homeostatic balances that may be maintained or lost in a sex-dependent manner, resulting in different disease progressions and outcomes. Taken together, these approaches (all currently pursued by us and others) will allow increasing clarification of both (1) intermediate mechanisms of sex differences impacting CVD outcomes and (2) the necessary and sufficient contexts wherein each such mechanism is salient. Harnessing these insights will bring not only a more robust understanding of cardiovascular gender medicine from a mechanistic standpoint but also enable translation into personalized cardiovascular gender medicine practices that can account for individual variation in risks and needs across a person's lifespan, upholding a mandate of value-based care.

AUTHOR CONTRIBUTIONS

Nina Jovanovic: Conceptualization; writing – original draft; visualization. **Veronika Zach:** Writing – original draft; writing – review and editing. **Claudia Crocini:** Writing – original draft; writing – review and editing. **Lina Samira Bahr:** Writing – original draft. **Sofia Kirke Forslund-Startceva:** Conceptualization; writing – original draft; writing – review and editing; supervision. **Kristina Franz:** Conceptualization; writing – original draft; writing – review and editing; project administration.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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