Role of Biological Sex in the Cardiovascular-Gut Microbiome Axis

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There has been a recent, unprecedented interest in the role of gut microbiota in host health and disease. Technological advances have dramatically expanded our knowledge of the gut microbiome. Increasing evidence has indicated a strong link between gut microbiota and the development of cardiovascular diseases (CVD). In the present article, we discuss the contribution of gut microbiota in the development and progression of CVD. We further discuss how the gut microbiome may differ between the sexes and how it may be influenced by sex hormones. We put forward that regulation of microbial composition and function by sex might lead to sex-biased disease susceptibility, thereby offering a mechanistic insight into sex differences in CVD. A better understanding of this could identify novel targets, ultimately contributing to the development of innovative preventive, diagnostic and therapeutic strategies for men and women.

Keywords: cardiovascular, gut microbiota, heart failure, sex differences, vasculature

INTRODUCTION

Despite advances in prevention strategies, as well as pharmacological and technology-based cardiovascular (CV) therapies, CV diseases (CVD) remain a major health burden, as they are the leading cause of morbidity and mortality (1). Notably, the development, progression and outcome of CVD, as well as the response to CV pharmacotherapies differ significantly between the sexes (2, 3). However, the contributing mechanisms are incompletely understood. Important modifiable risk factors for CVD include diabetes, hyperlipidemia, hypertension and obesity (4). These factors are linked to nutrition, and interventions aiming at modifying dietary patterns are expected to be beneficial in their successful management and the prevention of CVD (5–8). Interestingly, the old Chinese proverb “disease enters from the mouth” appears to be of relevance. The gut microbiome and its involvement in the development and outcome of CVD have recently attracted wide interest (9, 10), thereby emerging as a key modulator of CV health and disease. Of note, there are several factors that can alter the gut microbiome in a sex-specific manner (Figure 1A). In the present article, we highlight how the gut microbiome can affect the CV system and we explore how biological sex and sex hormones may influence this interaction, thereby contributing to sex differences in CVD. It is not our purpose to provide an exhaustive analysis of the interplay between gut microbiota and CVD, but rather we identify important examples, where biological sex may be of relevance.

GUT MICROBIOME

The human gut hosts tens of thousands of microorganisms, up to 100 trillion microbes, which are collectively referred to as the gut microbiome (11, 12). The four dominant bacterial phyla...
in the human gut are Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria (13), and the Bacteroidetes and Firmicutes phyla constitute the vast majority of the dominant human gut microbiota (14). There is considerable microbial diversity among different individuals because of variations in age, genetics, geography, hygiene, nutrition and social behaviors (15, 16). The gut microbes have an important role in human health and disease, affecting body weight and digestion, the protection against infection, the risk of autoimmune diseases, as well as the body’s response to drugs (17–21). In this context, perturbations...
of the intestinal microbial community through dietary and environmental factors, as well as genetic factors, among others, can lead to the development of various diseases, including autism, autoimmune diseases, cancer, CVD, diabetes, obesity and other diseases (10, 18, 22–30). Technological advances in the genomic and metabonomic fields, as well as in the assessment of the composition of the intestinal microbiome, have improved our knowledge and understanding of the complex interaction of the gut microbiome with the CV system. Recent findings highlight the potential benefit of developing microbiome-targeted therapies for CVD prevention and treatment, thereby pointing to possible applications in personalized medicine (31, 32).

OVERVIEW OF THE INTERPLAY BETWEEN THE GUT MICROBIOME AND THE CV SYSTEM

Alterations in gut microbiota influence the CV system and can lead to the development of CVD (Figure 1B). The intestinal microbiota produce a large number of metabolites, some of which are absorbed into the systemic circulation and play a bioactive role, while others are further metabolized by host enzymes and thus become agents of microbial influence on the host CV system (9, 10, 33). As a result, the intestinal tract with its microbes can act as an “endocrine-like” organ, impacting the distal CV target organs through a variety of metabolism-related processes, thereby affecting the onset and progression of CVD. In this section, we highlight important examples with particular emphasis on how this interaction might impinge on the development of CVD.

Hypertension

Hypertension is a major risk factor for a variety of CVD (34). Gut dysbiosis—an imbalance of gut microbiota—has been linked to hypertension. Significant changes in the gut microbiome have been reported in human hypertensive patients and different hypertensive animal models, including spontaneously hypertensive rats, Dahl salt-sensitive rats, the chronic angiotensin II infusion rat model and high-salt diet-fed mice (35–38). In particular, a dysbiotic pattern was reported in spontaneously hypertensive rats and the chronic angiotensin II infusion rat model. This included decreased microbial richness and an increased Firmicutes:Bacteroidetes ratio, thereby leading to decreases in bacteria that produce the short-chain fatty acids (SCFA) acetate and butyrate (35). SCFA are major bioactive metabolites of gut microbiota, by which the host's biological processes and distal organs can be influenced. In this context, SCFA can regulate blood pressure by acting on various G protein coupled receptors (39). Transplantation studies have indicated a causal relationship between gut dysbiosis and hypertension. Transplant of cecal contents from hypertensive rats into normotensive rats led to increased systolic blood pressure, as well as decreased microbial richness and an increased Firmicutes:Bacteroidetes ratio (40, 41). Furthermore, fecal transplantation from hypertensive human patients to germ-free mice led to increased blood pressure in the mice (38). Mouse studies showed that a diet high in fiber increased the prevalence of Bacteroides acidifaciens compared with mice fed a control diet (42). This was associated with increased levels of one of the main metabolites of the gut microbiota, the SCFA acetate, which may confer protection against the development of hypertension (42). In contrast, high salt intake, which can lead to hypertension, depleted Lactobacillus murinus in mice, and treatment of mice with L. murinus prevented salt-sensitive hypertension by modulating T helper 17 cells (36). Along this line, a moderate high-salt challenge in a pilot study in humans reduced intestinal survival of Lactobacillus spp., increased T helper 17 cells and increased blood pressure (36). Collectively, these data reveal the gut microbiota as a crucial regulator of blood pressure and indicate that dietary interventions may be useful in manipulating intestinal microbial composition and function to protect against hypertension.

Atherosclerosis

Atherosclerosis is a leading cause of various CVD, including coronary artery disease, stroke and peripheral artery disease. Earlier studies suggested that bacteria from the gut may correlate with disease markers of atherosclerosis (43). Since then, a number of studies has shown that the gut microbiome plays an important role in a variety of atherosclerotic conditions (44–48). A mechanistic link between gut microbiota and the development of atherosclerosis has been established. In particular, bacteria in the gut metabolize dietary phosphatidylcholine to trimethylamine, which crosses the gut-epithelial barrier and is then carried to the liver, where it is subsequently metabolized to the proatherogenic molecule trimethylamine-N-oxide (TMAO) (49–51). Importantly, plasma TMAO levels have been associated with mortality in patients with stable coronary artery disease, as well as in patients with peripheral artery disease, independent of traditional risk factors (52, 53). In an experimental model of myocardial infarction, decreased left ventricular function was underlain by increased gut permeability, mediated by tight junction protein suppression, and microbial translocation, thereby triggering systemic inflammation, ultimately contributing to the pathogenesis of CVD (54). Notably, probiotic administration conferred cardioprotective effects, including improved left ventricular function (55, 56). Collectively, these data demonstrate a key role of gut microbiota in the development of atherosclerosis and suggest that the use of probiotics, in addition to standard drug therapies, may provide additional benefits for patients with coronary artery disease, myocardial infarction and other atherosclerotic conditions.

Heart Failure

Heart failure (HF) is a devastating syndrome with poor prognosis. Gut microbiota and their metabolites produced from dietary metabolism have been linked to HF. To this extent, gut dysbiosis with decreased microbial richness and increased gut permeability has been reported in patients with HF (57, 58), as well as in mice with pressure overload-induced HF (59).
Further studies in humans have shown that the gut microbiome-derived metabolite TMAO is increased in HF and that its levels are associated with poor prognosis (60–64). Studies with experimental animals have suggested a causal role of TMAO in the development of HF. In particular, treatment of rodents with TMAO led to myocardial hypertrophy and maladaptive remodeling, including ventricular dilation and wall thinning, systolic dysfunction and increased fibrosis (65, 66). Collectively, these data suggest a key role of gut microbiota and their metabolites in the pathogenesis and progression of HF.

**BRIEF OVERVIEW OF SEX DIFFERENCES IN CVD**

There are pronounced differences between men and women in the epidemiology, manifestation, pathophysiology, treatment and prognosis of CVD. As these have been reviewed recently elsewhere (3, 76, 77), a brief overview is provided here. The incidence of CVD differs significantly between men and women; for example, women have a greater risk of developing Takotsubo cardiomyopathy than men (78). The association of major modifiable risk factors with incident myocardial infarction differs between the sexes and age influences this interaction significantly (79, 80). Consequently, the younger age of onset of acute myocardial infarction in men (≈10 years earlier than women) is largely explained by higher levels of risk factors, including abnormal lipids and smoking (79). In addition, women may have risk factors that are unique to them, such as preeclampsia (high blood pressure during pregnancy) and gestational diabetes, thereby increasing the risk of CVD in female individuals only. Women are also more likely to present with HF with preserved ejection fraction (81, 82), which may be due to sex-biased remodeling of myocardial extracellular matrix (83), and the decline of estrogen at menopause might contribute to its pathogenesis (84). Along this line, under pressure overload, there is a higher proportion of male patients with increased left ventricular mass and end-diastolic diameter, and decreased left ventricular relative wall thickness and function (85–91), associated with greater activation of inflammatory factors (92, 93). Physiological differences between men and women may also lead to sex differences in the response to treatment (2, 94–97). Overall, there are significant sex differences in the outcome of a variety of CV disorders (98–100).

**SEX DIFFERENCES IN THE COMPOSITION OF THE GUT MICROBIOME**

Human and animal studies have shown that biological sex has an impact on gut microbial composition (Table 1). In particular, significant differences in the composition of gut microbiota between healthy men and women have been reported, with women appearing to have lower Bacteroidetes abundance compared with men (67–70). The gut microbe-sex interaction, however, is, not surprisingly, affected by obesity, as the abundance of the Bacteroides genus was reported to be lower in men than in women with a body mass index >33 (71). A study of different strains of mice showed that when considering the data at the single strain level, several taxa exhibited significant differences in abundance between the sexes (101). Among these, Bacteroidetes abundance is lower in female mice compared with male mice (72). Interestingly, sex differences in the gut microbiome appear to be responsible for hormone-dependent regulation of autoimmune disease (19). Along this line, in an experimental model of colitis, sex differences in gut microbiota composition were associated with sex-biased severity of the disease (73). Studies in rodents have shown that the levels of the gut microbiome-derived metabolite TMAO are higher in females than in males (74, 102). Biological sex also influences the interaction between the gut microbiome and environmental factors, such as diet (75, 101, 103, 104). For example, the interaction between dietary habits, such as yogurt consumption, and the gut microbiota is significantly different between healthy young male and female adults (105). Collectively, these data demonstrate that microbial composition and diversity differ significantly between the sexes. In this context, we put forward that differences in microbial composition and function between men and women may be a contributing factor to the observed sex differences in CVD (Figure 1C).

**REGULATION OF THE GUT MICROBIOME BY SEX HORMONES**

A large body of literature demonstrates that sex hormones modulate CV physiology and pathology. Of relevance, among the various factors underlying sex differences in CVD, such as sex hormones and (epi)genetic factors (3, 106), sex hormones appear to be crucial. For example, the steroid hormone 17β-estradiol (E2) and its receptors (ER) are thought to play a major role (107–111). The E2/ER axis has been shown to have vast effects in the CV system, regulating, for example, contractile function (micro), vascular function, metabolic processes, calcium

| TABLE 1 | Sex differences in the gut microbiome. |
| Component | Sex difference | References |
|---|---|---|
| Bacteroides | healthy men > healthy women | (67–70) |
| Prevotella | healthy men > healthy women | (69) |
| Bacteroides | obese men < obese women | (71) |
| Firmicutes | obese men < obese women | (71) |
| Bacteroides | healthy male mice > healthy female mice | (72) |
| Ruminococcaceae | healthy male mice > healthy female mice | (73) |
| Peptostreptococcaceae | healthy male mice < healthy female mice | (73) |
| TMAO | healthy male mice < healthy female mice | (74) |
| SOFA | male > female rats fed an oligofructose-supplemented diet | (75) |

SCFA, short-chain fatty acids; TMAO, trimethylamine-N-oxide.
signaling, gene expression and protein abundance (112–126), which can be sex-dependent (109, 127–132).

Along this line, among various types of hormones with regulatory effects, sex hormones have a key role in the regulation of the distribution of the gut microbiome. In this context, sex hormones affect gene expression and other processes of gut microbiota, thereby influencing intestinal microbial composition and function. In parallel, the microbial community also alters sex hormone levels, thereby regulating disease development and outcome (19, 72). Therefore, there is a complex interaction between gut microbiota and sex hormones. Interestingly, this is true not only for mammals but also for fish. Treatment of zebrafish with E2 altered the intestinal microbial composition significantly, which may lead to further physiological changes in the host (133). Androgens also appear to influence the composition of the gut microbiome. In fact, in pathological situations of hormonal excess, such as polycystic ovary syndrome, there is decreased microbial diversity and changes in microbial composition that are associated with metabolic dysregulation, thereby indicating that hyperandrogenism may be linked with gut dysbiosis in women with this disorder (134–137). Moreover, testosterone treatment after gonadectomy prevented the significant changes in gut microbiota composition that occurred in untreated males (101). Further studies in humans have shown that the composition of gut microbiota differs significantly among women with different hormonal status (67). The key role of sex hormones in microbial composition is further supported by studies in mice with gonadectomy, which resulted in an altered gut microbiome (101). To this extent, it was shown that the deficiency of androgens alters the intestinal microbiome and induces abdominal obesity in a diet-dependent manner (138). Collectively, these data indicate that sex hormones mediate (at least partly) the differences in gut microbiota composition between the sexes. Currently, it is unclear how sex hormones regulate the composition and function of the gut microbial community. Further research is warranted.

CHALLENGES AND OPPORTUNITIES

Potential differences in microbial composition, diversity and function between humans and animal models might limit the translation of experimental studies with animals. Furthermore, given the major impact that environmental factors have on gut microbiota, it needs to be highlighted that most environmental conditions of humans differ significantly from those of laboratory animals. In addition, estrous cycle-related changes in gut microbiota in rodents might not reflect any changes due to menstruation in women. Nevertheless, the degree of any inconsistencies remains to be determined. Lastly, abundant species do not necessarily confer abundant molecular functions, underscoring the importance of a functional analysis to understand microbial communities (139).

The role of sex has yet underestimated consequences for physiology and pathology (140). A better understanding of the mechanisms accounting for sex differences in microbial composition could provide opportunities for the development of novel sex-specific diagnostic tests and therapeutic approaches for CVD. Given the evidence provided by the current literature, we put forward that the “gut microbiome status” in the context of the patient’s sex should be taken into account in CVD management and we provide the following specific recommendations as a paradigm: (1) a dietary intervention to modulate gut microbiota could be an innovative nutritional therapeutic strategy for hypertension; (2) a new therapeutic intervention by probiotics targeting gut bacteria and protecting gut function may be a potential option to improve CV outcomes post-myocardial infarction; (3) strategies for the modulation of TMAO levels could be beneficial in the prevention of HF; (4) approaches to reduce the levels of TMAO in patients with HF could improve long-term prognosis. In this context, an experimental approach for the directed remodeling of the mouse gut microbiome with peptide treatment was recently shown to inhibit the development of atherosclerosis (141).

Moreover, the gut has been shown to be a target of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In fact, gastrointestinal manifestations of SARS-CoV-2, such as anorexia, nausea, vomiting, diarrhea and hepato-cellular injury (transaminitis), among others, have been widely reported (142, 143). SARS-CoV-2 is primarily considered a respiratory pathogen. Nevertheless, patients with coronavirus disease 2019 (COVID-19) present with gastrointestinal symptoms, given that angiotensin-converting enzyme 2 (ACE2), which SARS-CoV-2 uses as a host cell entry receptor (144), is ubiquitously present in the human gut, including small intestine and colonic enterocytes, hepatocytes and cholangiocytes. However, the effects and impact of SARS-CoV-2 on host microbial flora and gut microbiota composition are unclear. Interestingly, COVID-19 patients appear to have sex-dependent CV risk and complications. However, the underlying mechanisms are incompletely understood (145, 146). To this extent, we put forward the notion that SARS-CoV-2 may lead to significant sex differences in the gut microbiome status of COVID-19 patients, which, in turn, impacts the CV system, thereby contributing to the observed sex-biased CV complications in these patients. This further highlights the opportunity of employing the gut microbiome as a novel target for sex-specific therapeutic interventions for (severe) CV complications in patients with COVID-19. Further research is warranted.

CONCLUSIONS

The mechanisms by which the gut microbiome affects CV (patho) physiology are incompletely understood. From several human and animal studies, it is clear that the gut microbiome exerts sex-biased effects in health and disease. At least in part, sex hormones account for these sex-dependent effects in a complex bi-directional interaction with the microbial community. Gut microbiota may become a novel target for pharmacological or dietary interventions as part of new preventive and therapeutic strategies in CVD. A better understanding of the effects of biological sex and considering its role in such novel approaches will improve clinical care and management via new strategies for the prevention, diagnosis and treatment of disease in both men and women.
AUTHOR CONTRIBUTIONS

GK conceived the work. SL and GK wrote the manuscript. Both authors contributed to the article and approved the submitted version.

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