Chapter 4

Role of Gut Microbiota in Cardiovascular Disease that Links to Host Genotype and Diet

Hein Min Tun, Frederick C. Leung and
Kimberly M. Cheng

Additional information is available at the end of the chapter

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Abstract

Cardiovascular diseases (CVDs) are major outcomes of metabolic impairments in humans, which result from several genetic and environmental factors. In recent years, a 'microbiome hypothesis' has been proposed as a result of several studies that have attempted to understand underlying mechanisms of CVDs. Similar to CVDs, both genetic and environmental factors, especially diets, have a major impact on shaping gut microbiota and their functions. In the past decade, strong evidence has emerged to confirm the role of gut microbiota in contributing to the onset of CVDs. However, a comprehensive understanding of interactions among diet, host genotype, gut microbiota and CVDs is still facing challenges due to the complicated nature of CVDs. In this chapter, we review the present state of our knowledge about the contributory role of gut microbiota in CVDs and discuss the knowledge gaps that warrant further investigations. Moreover, we review the potential intervention strategies that may target the microbiota-driven pathology in CVDs and discuss the strength and weakness of animal models in studying the roles of gut microbiota in CVDs.

Keywords: gut microbiome, cardiovascular disease, host genotype, diet

1. Introduction

Cardiovascular disease (CVD) is a leading cause of death in industrialized societies, with increasing incidence in developing countries [1]. A combination of genetic and environmental factors contributes to risk for developing CVD [2]. A significant portion of CVDs can be attributed to ischemic heart disease, often a result of underlying coronary arterial diseases

such as atherosclerosis. Risk factors for atherosclerosis include dyslipidaemia, hypertension, obesity, smoking, and diabetes [3, 4]. Extensive searching in recent years for causal genetic variants found less than one-fifth of CVD risk is accounted for by genetic determinants [5, 6]. Excluding tobacco exposure, dietary intake is our largest environmental risk, as we consume kilogram quantities into our bodies daily. However, specific dietary composition and precise quantification of dietary intake of a given individual are often difficult to assess.

Over the past decade, there has been a growing body of knowledge on the ecological diversity of microbes living symbiotically within us, especially in our gastrointestinal tract. More than 100 trillion microbial cells reside in the human gut, which is far outnumbering the host cells of the human body [7]. Microbial symbionts in our gastrointestinal system have coevolved with us and critically contribute to a variety of physiologic and metabolic processes of our body. Undeniably, human DNA is estimated to represent less than one-tenth of the total DNA within our bodies due to the remarkably large number of microorganisms in and on us, mainly within our gastrointestinal tract [8]. The composition of the microbial community in our gut can be largely affected not only by dietary exposures but also by genetic variants of the host, as well as any changes that impaired host's physiology and homeostasis. In recent years, although there is increasing evidence supporting an association between gut microbiota and diseases in human and animals [9, 10], the participatory roles of gut microbiota in our health, immune function, and disease initiation and progression have just begun to be explored. There has been an established understating of the role of microbial dysbiosis in the pathogenesis of some diseases of altered intestinal health [11]. The alteration of gut microbiota may contribute enormously to the digestion of food and absorption of metabolites, which further contribute to the development of a range of CVDs from atherosclerosis to cardiorenal dysfunction [12].

The gastrointestinal ecosystem is arguably the largest endocrine as well as paracrine organ in the body, producing a variety of biologically active compounds that may be transported in the systemic circulation and distributed to other organ systems within the host, thereby influencing diverse essential biochemical processes [12]. This chapter summarizes recent developments in our knowledge of the contributory role of gut microbiota on the initial onset and development of CVDs, and how diets and genetics of the host participate in their development. Potential strategies that can modulate gut microbiota for prevention and therapeutic interventions for CVDs will also be discussed.

2. Intestinal microbiota in cardiovascular disease—the good, the bad, and the ugly

The understanding of the link between gut microbiota and CVD was limited until the late 1990s. The fact that axenic (germ-free) ApoE knockout mice were not protected from the development of atherosclerosis suggested that the gut microbiota is not important in the pathogenesis of atherosclerosis [13]. A meta-analysis of clinical trials revealed that the modification of gut microbiota by antibiotics failed to demonstrate any benefit with regard to mortality due to cardiovascular events in coronary artery disease patients [14]. Furthermore,

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in an extensive study involving 4012 patients with stable coronary artery disease, the administration of azithromycin showed no effect on the risk of cardiac events [15]. However, the composition of the microbiota was shown to increase the severity of myocardial infarction in a Dahl S rat model of ischaemia/reperfusion injury of the heart, in which the authors indicated that vancomycin, a poorly absorbable antibiotic, reduced 27% of myocardial infarctions and increased 35% postischaemic mechanical function recovery [16]. This effect was associated with a change in the gut microbiota (both bacteria and fungi) and a reduction of plasma leptin, which was later confirmed by administration of the leptin-suppressing probiotic *Lactobacillus plantarum* 299v [16]. These earliest contradictory findings of antibiotic utilization (azithromycin vs. vancomycin) explained the complexity of gut microbiota-based intervention in terms of efficacy and properties of the applied protocol.

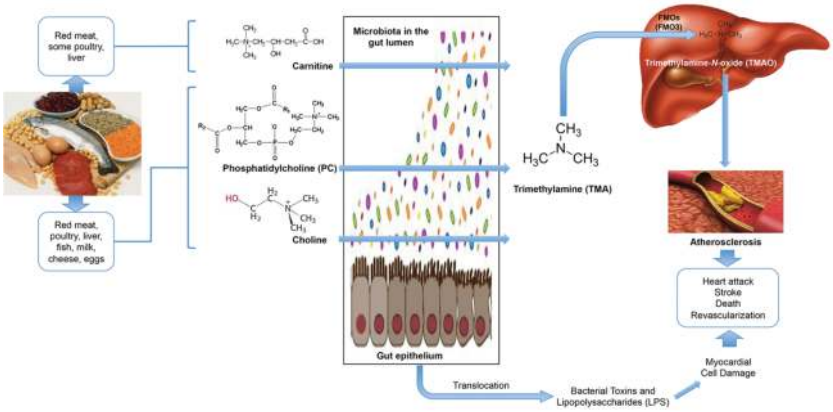

Figure 1. Gut microbiota and its impacts on atherosclerosis and major cardiovascular events through both nutrient/meta-organismal pathways that contribute TMAO formation and translocation of bacterial toxins that cause myocardial cell damage.

Invasion of indigenous and/or pathogenic oral and intestinal bacteria, as well as their metabolites and toxins into the vascular system, has been demonstrated in association with several CVD events [17, 18], although a causal association between periodontal infection and atherosclerotic CVD or its sequel has not been demonstrated. Periodontitis, also known as periodontal disease (PD), is an inflammatory disease of the oral cavity due to chronic bacterial infection of soft and hard tissues of the gum, mainly by Gram-negative bacteria [19]. A high-fat diet can induce not only metabolic alteration but also increased systolic and diastolic pressure in diabetic mice after longer term colonization with periodontal pathogens, such as *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum* [20]. The molecular mechanisms underlying this pathogenic phenotype is linked to bacterial lipopolysaccharide (LPS), which may increase oxidative stress and mitochondrial dysfunction that are responsible for inflammation-induced CVD (**Figure 1**) [21]. Endotoxin levels were shown to be higher in the hepatic veins compared with the left ventricle (LV) or pulmonary artery, suggesting possible endotoxin translocation from the gut into the circulation [22].

In recent years, more studies have highlighted the contributory role of gut microbiota in CVD. Initial hypothesis-generating studies using untargeted metabolomics analyses of plasma samples identified three metabolites, including phosphatidylcholine (PC; lecithin) metabolism-choline, betaine, and trimethylamine-N-oxide (TMAO) that are potentially associated with cardiovascular risk [23]. Another study also found increased concentration of the metabolite TMAO in patients with atherosclerosis and their correlation with this pathology [24]. Gut microbiota has been demonstrated to be responsible for TMAO synthesis by converting choline, an essential nutrient, into TMA. Subsequent oxidation of TMA through flavin monooxygenase 3 (FMO3) from the liver formed TMAO [25–27]. As an example, the bacteria belonging to *Erysipelotrichia* under the phylum Firmicutes can metabolize choline to TMA [24]. TMA is subsequently absorbed and rapidly oxidized by hepatic cells to form TMAO [28], which is responsible for macrophage foam cell formation by reducing reverse cholesterol transport and consequently promoting cholesterol accumulation in the foam cells of atheroma (**Figure 1**) [29]. However, the molecular mechanisms by which TMAO reduces reverse cholesterol transport are not well understood. These bacteria probably promote not only atherosclerosis through TMA-TMAO production but also non-alcoholic fatty live disease (NAFLD) by reducing choline availability for the synthesis of very low-density lipoprotein in the liver, resulting in triglyceride accumulation in the hepatocytes [30]. Furthermore, the abundance of such bacteria is also associated with an iron-rich diet. Such a diet promotes gut epithelial cell stress through iron accumulation in the enterocytes and consequently inflammation-induced dysbiosis of the gut microbiota in favour of *Erysipelotrichia* bacteria. Thus, an iron-rich diet may promote the development of NAFLD and atherosclerosis through alteration of the gut microbiota [31]. The dysbiosis of gut microbiota has been found in several metabolic diseases, including CVD. However, in different situations, dysbiosis can either be a cause or an effect of the disease or a spiralling cycle. In the case of CVDs, the dysbiosis of gut microbiota needs further investigation to determine whether it is cause or effect or both. Beside TMAO, intestinal bacteria produce certain toxins, such as indoxyl sulphate, p-cresyl sulphate, amines, and ammonia, which can later be eliminated by the kidneys in healthy individuals. In chronic kidney disease patients, however, these toxins may accumulate in the body of the patients.

In addition to the three bacterial metabolites described previously, L-carnitine has also been shown to accelerate atherosclerosis in mouse models, but only in the presence of intact gut microbiota and TMA/TMAO generation. High carnitine levels significantly increased the risk of myocardial infarction (MI), stroke, or death in experimental subjects with concurrently high TMAO levels. Similar to PC/choline, L-carnitine is a TMA-containing compound that releases TMA through the gut microbiota and consequently converted into TMAO by hepatic FMO (**Figure 1**) [29]. Thus, intestinal microbiota may play an obligatory role in generating TMAO from multiple dietary nutrients, and TMAO is the proatherogenic species probably promoting the associations noted between plasma levels and both prevalent and incident CVD risks.

Recent studies reveal that the potential pathogenic contribution of gut microbiota-dependent generation of TMAO may extend beyond the development of progression of atherosclerosis and its adverse complications (MI, stroke, or death). A recent observation also indicated increased TMAO levels in heart failure patients [32]. In these patients, intestinal ischaemia can

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be demonstrated by a decrease in intestinal mucosal pH [33] or reduced passive carrier-mediated transport of D-xylose [34]. Due to the consequences of intestinal ischaemia and congestion, the morphology, permeability, and function of the intestinal mucosa may substantially altered in congestive heart failure (CHF), especially in advanced stages with cardiac cachexia [35]. Our knowledge on the mechanistic associations between gut microbiota and CHF is improving. Although evidence is still accruing, higher concentrations of adherent bacteria have been identified in the intestinal mucosal biofilm of patients with CHF [35]. The composition of intestinal microbiota may alter rapidly during intestinal ischaemia and reperfusion or following an increase in portal vein pressure because of the activation on bacterial virulence in microbiota by gut liminal hypoxia, hypercapnia, changes in local pH, redox state, and norepinephrine [36]. Hypoperfusion and congestion in the intestine may reduce cardiac output and further disrupt the barrier function of the intestine and promote systemic inflammation through bacterial translocation, potentially leading to further CHF exacerbations (**Figure 2**). However, major changes in the gut microbial composition have not been observed in a rat model of CHF induced by coronary artery ligation [37]. In this regard, the role of gut microbiota is possibly unique to human CHF.

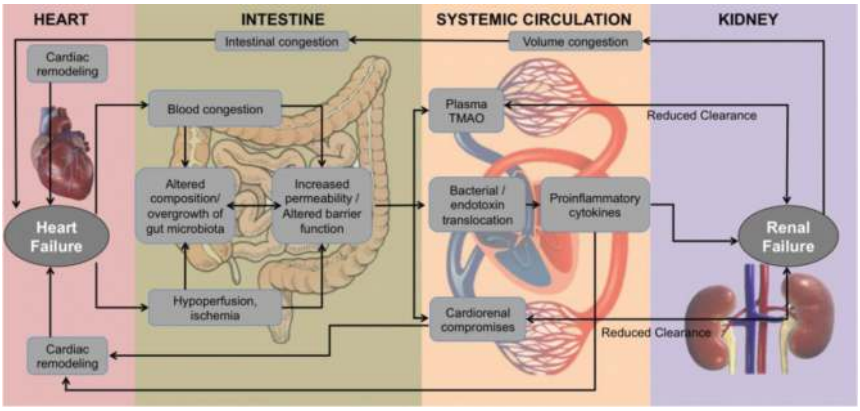

Figure 2. Links between heart failure, gut microbiota, and renal failure. The haemodynamic variations caused by heart failure affect microcirculation in intestinal villi and result in alternations of intestinal permeability and gut microbiota. The increased intestinal permeability favours microbial and endotoxin translocation, TMAO, and cardiorenal compromises can mediate the pathology that leads to further exacerbation of heart failure and renal damage. Reduced clearance of these metabolites due to impaired renal function further promotes this pathology and constitutes a vicious cycle.

The microbial analysis of atherosclerotic plaque has shown that the embedded microbiota is dominated by bacteria of the phylum *Proteobacteria* (e.g. *Escherichia coli*) [38]. *Proteobacteria* are also the most abundant microbiota in the blood of diabetic patients [39]. Hence, the establishment of microbiota might be the first step in the atherosclerotic plaque formation. Another bacterium in the genus *Collinsella* was also found to be dominant in patients with symptomatic atherosclerosis (presence of stenotic atherosclerotic plaques at the level of the carotid artery and leading to cerebrovascular episodes). The same study also indicated that there were more

bacteria belonging to *Roseburia* and *Eubacterium* in the gut microbiota of healthy controls compared with patients [40]. Thus, the changes not only in microbiota composition but also in microbiome functions may be linked with the events of atherosclerosis.

3. Role of diet and host genotype in shaping intestinal microbiota profile associated with cardiovascular diseases

Dietary cholesterol has major effects on gastrointestinal microbiota, which is consequently associated with the onset of CVD. In our recent study, we tested the effect of diet and host genotype on intestinal microbiota using two Japanese quail strains that are atherosclerosis-susceptible (SUS) and atherosclerosis-resistant (RES) [41]. In that study, dietary cholesterol reduced the abundance of *Ruminococcus* and facilitated the abundance of opportunistic pathogens belonging to *Erysipelotrichaceae* in the quail ceca and may have increased the risk of assaults by these opportunistic pathogens. However, both the SUS and the RES strains housed in the same cage and fed the same high cholesterol diet hosted distinctly different ceca microbiomes.

When mice were fed a 'Western diet', which was high in fat and cholesterol, the overall diversity of their gut microbiota dropped significantly due to a bloom of a class of *Firmicutes* called *Mollicutes*, a member of which is *Eubacterium dolichum* [42]. *E. dolichum* has a number of genomic features that could promote their own fitness in competition with other microbes in the cecal nutrient metabolic milieu created by the host's consumption of the Western diet. Their abundance is associated with obesity in mice [42]. In our study, a similar situation may have occurred in RES quail in their reaction to dietary cholesterol. The ceca of RES quail were dominated by *E. dolichum* [41]. On the other hand, SUS quail fed the cholesterol diet had an abundance of *Lachnospiraceae* in the ceca [41]. At the same time, the abundance of Ruminococcaceae was not compromised [41]. *Lachnospiraceae* and *Ruminococcaceae* have been shown to be associated with the maintenance of gut health [43, 44]. These two families are specialists for degrading cellulose and hemicellulose components of plant materials, which are fermented and converted into short chain fatty acids (SCFAs) readily absorbed and used by the host [45]. SCFAs play an important role in maintaining intestinal homeostasis [43, 44]. Our study indicated that the divergent selection for susceptibility and resistance to diet-induced atherosclerosis may have adversely affected the cecal health of RES, but not SUS quail, through modification of their cecal microbiomes [41]. Whether this change in the cecal environment has effects on the metabolism and absorption of dietary cholesterol remains to be studied.

In the past decade, numerous studies have been published on the relationship between gut microbiota and cardiovascular diseases in human and in animal models. In humans, about 50% of dietary cholesterol is absorbed in the duodenum; consequently, the rest can be metabolized by *Eubacterium* bacteria to coprostanol and minor amounts of coprostanone in the large intestine [46]. Coprostanol, unlike cholesterol, is poorly absorbed by the human intestine, and hence, conversion of cholesterol to coprostanol might be a way to lower serum cholesterol in humans and rodents [47, 48]. However, feeding *Eubacterium coprostanoligenes*

to laying hens did not lower plasma cholesterol levels [49]. In our study using the quail model, *E. dolichum* was found in higher abundance in the cecum of RES but not in SUS quail [41]. Although a negative correlation of *E. dolichum* abundance with plasma HDL level was significant in our study, the ability of *E. dolichum* to convert cholesterol to coprostanol has not been demonstrated. As the primary cholesterol absorption sites are in the small intestine, a comprehensive examination of the microbiota in a complete set of intestinal tract should be done to understand physiological variations at different anatomical locations of the intestinal tract, which will further elaborate the potential targets by therapeutic interventions. In the concurrent analysis on small intestinal microbiota of RES and SUS quail fed the cholesterol diet, high abundance of *Lactobacillus* species were observed in both ileum and duodenum [50] of RES but not in SUS quail. This finding is significant since *Lactobacillus* species have been proposed as an effective probiotic to lower cholesterol in humans [51].

A number of studies including our quail model highlighted the importance of host genotype in responding to diet-induced atherosclerosis. However, further research effort is need to address the underlying biochemical pathways by which host genetics interplay with diets to influence the CVD events through alteration of gut microbiota.

4. Animal models for studying gut microbiome in cardiovascular disease

Cardiovascular diseases (CVDs) involve complicated multifactorial pathologies, in which both genetic and environmental factors are involved. In order to provide us with important insights into the pathophysiology of CVD events, the development of animal models of CVD is essential as tools to evaluate novel therapeutic strategies to predict and to prevent these complications. Until now, there have been numbers of animal models used for CVD, including those implemented in both large (pig and dog) and small (mice and rat) animals, designed for enhancing scope with more precision and to better represent human pathologies. With or without genetic modifications, mouse, rat and rabbit models are more commonly used and less expensive animal models for studying CVDs compared to porcine and canine models, which better represent the human pathology, but are less popular due to the cost and difficulties in handling. For atherosclerosis, mouse models have proven to be useful to study development and progression of atherosclerotic lesions. In particular, knockout and transgenic mouse models have been well developed to study the molecular and cellular mechanisms involved in atherogenesis and to evaluate the effectiveness of new and existing drugs for the prevention and/or treatment of atherosclerosis. The most widely used knockout mouse models include low-density lipoprotein receptor-deficient mice ($LDLR^{-/-}$ mice) and apolipoprotein E-deficient mice ($ApoE^{-/-}$ mice). Mice carrying ApoE mutations such as ApoE3Leiden (E3L) and ApoE (Arg 112→Cys→142) transgenic mice are very useful mouse models to study hyperlipidaemia and atherosclerosis. The high-cholesterol diet rabbit model has been widely used for experimental atherosclerosis [52]. Several porcine models have been employed for closer representation to pathologies in humans [53–56]. However, the extensive application of porcine models is still limited. In heart failure, dog models of myocardial infarction and serial microemboli-

zation of the coronary artery were developed [57]. Like the pig models, dog models are very restricted due to their cost, ethical complications, and difficulties in handling.

Since the 'microbiome hypothesis' has been applied to CVDs and other metabolic diseases, the most common and feasible animal model is the mouse model. As we know, murine models have been extensively applied in biomedical research due to similarities in anatomy, physiology, and genetics, which have allowed numerous inferences about human pathology to be drawn from murine experimentation. In gut microbiota research, mouse models are being increasingly used to study the role and functioning of the gut microbiota and its association with diseases. However, application and direct translation of results obtained from traditional CVD mouse models to study the role of the gut microbiome and its interaction with the host have their limitations for the following reasons: [1] the variation of the gut microbiota of laboratory mice relates to genetic, physiologic, and environmental factors, and those factors also trigger the pathologies of CVD; [2] cross-talk between the gut microbiota and the host is host-specific so observations in mouse models might not be applicable to humans; [3] the inherent genetic variations in the human population cannot be captured by the inbred mouse strains that have genetic homogeneity; and finally [4] differences in multiple factors between mice and humans, such as genetic background, birth mode (caesarean or vaginal), mode of feeding (breast or bottle), diet, age, medical history, and social activities, which all contribute in shaping the gut microbiota of humans.

Existing animal models for CVDs have not yet been fully evaluated in studying the role of the gut microbiome in developing pathologies of CVD events. This should be considered in future investigations, and the most appropriate animal model to study the links between gut microbiota and CVD should be proposed and recommended. Rabbits [58], guinea pig [59], pigeon [60], and quail [61] have been used as models for studying atherosclerosis but not in association with gut microbiota. Recently, we proposed a new quail model that would be useful for studying the interaction of host genotype and diet in affecting the gut flora in association with the development of atherosclerosis [41]. We proposed that our Japanese quail model may have advantages over others because quail are naturally deficient in apolipoprotein E. When we fed a high cholesterol diet, males of the SUS quail developed lesions exhibiting structural features (e.g. focal haemorrhage, calcification, and fibrosis) that closely similar those in the human atherosclerosis [62, 63]. In addition, quail model is easier to be handled, lower costs for larger sample size, and require less laboratory space compared to other porcine or canine models. As a further incentive, our recent microbiome study has provided the baseline understanding for the association between the gut microbiome and the development of atherosclerosis in quail model.

5. The potential of modulation of gut microbiota as novel preventive and therapeutic strategies for cardiovascular disease

During these past few years, several research efforts aimed to modulate both structure and function aspects of the gut microbiome were reported [64, 65]. Faecal transplantation is one of

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the successful stories for restoring impaired gut microbiome into normal gut microbiome, which has shown certain success in applications of certain human diseases especially in *Clostridium difficile* infection [66]. However, several underlying questions still have not been fully resolved and more baseline information is needed. Likewise, therapeutic tools available to modulate the microbiota-driven pathogenesis of CVD remain to be validated. Besides the well-known faecal transplantation, the composition of gut microbiota can be modulated by diet, antibiotics, and prebiotic/probiotics. If we are to modulate the microbiome functions or biochemical pathways involved in microbiota-driven pathology, the crosstalk (detail mechanisms) between host and microbiota becomes a major concern, and pharmacological interventions are needed to target both host and microbiota metabolisms.

5.1. Dietary intervention

As choline, PC, and carnitine are primary sources of gut microbiota-associated TMAO production, dietary modulation is a logical intervention strategy [12]. It has been shown that vegetarians and vegans have markedly reduced production of TMA and TMAO from dietary L-carnitine and have lower plasma TMAO levels than omnivores [29]. Similarly, studies have shown that different gut microbial communities were found in vegetarians and vegans compared with omnivores [29, 67]. In animal model studies, long-term exposure to dietary L-carnitine increased TMA synthetic capacity by 10-fold with a concurrent shift in gut microbial composition [29]. Thus, chronic dietary exposure (e.g., omnivore vs. vegan/vegetarian among humans or normal chow vs. chow plus L-carnitine in mouse studies) shifts gut microbiota, with a selective advantage for certain bacterial species that prefer L-carnitine as a carbon fuel source to increase in proportion within the community and amplify the potential to produce TMA [12].

The elimination of L-carnitine from the diet is a potentially achievable goal that may reduce some TMAO production. But, choline is an essential nutrient and its complete elimination from the diet is unwise. Furthermore, bile has a very high total choline (PC) content, and the rapid turnover and sloughing of intestinal epithelial cells results in significant exposure of distal gut segments (and hence microbes) to choline, independent of dietary intake. Absorbent removal of TMA from the intestines by specific oral binding agents is a challenging but potentially feasible therapeutic approach for reducing TMA and TMAO levels. The details of application of binding reagents will be discussed in the following specific section.

5.2. Antibiotic intervention

The association between certain groups of bacteria and CVD such as atherosclerosis has previously been postulated. However, a number of randomized controlled studies have failed to demonstrate a benefit of antibiotic therapy for secondary prevention of cardiovascular events [15, 68]. On the other hand, antibiotics can influence the pathophysiological outcomes driven by changing the abundance or composition of the gut microbiota. A well-known antibiotic, vancomycin, presented a reduction of myocardial infarct size in a rat model of ischaemia-reperfusion [16]. Interestingly, there was no effect on severity of myocardial infarction by direct infusion of vancomycin into the coronary circulation. Furthermore, the oral

administration of the antibiotic polymyxin B reduced monocyte production of certain proinflammatory cytokines in patients with HF and improved flow-medicated dilation [69]. Although the previous findings reflect the effect of antibiotics in the modulation of gut microbiota on the pathophysiology of various CVD events including HF, the potential adverse effects of antibiotics, such as microbial substitution and generation of antibiotic-resistant microbes, commonly occur in clinical practices. Hence, the extensive application of this strategy is arguable and challenging. Careful considerations are needed to minimize the adverse effects of antibiotic agents. Additional investigations are needed to determine the benefits of proper application of antibiotics in specific circumstances in clinical practices.

5.3. Prebiotic/probiotic intervention

Prebiotics are non-digestible food ingredients, mainly fibres that beneficially affect the host's health by selectively stimulating the growth and/or activity of some genera of gut microorganisms especially in the hindgut. Probiotics are live microorganisms that confer a health benefit to the host when administered in adequate amounts through improving the intestinal microbial balance [70]. However, the effectiveness of both prebiotics and probiotics varies on their sources, methods of preparation and administration, and the dosage. They have been extensively applied in most gastrointestinal disorders, and recently their applications in metabolic and cardiovascular diseases have been studied due to their potential role to modulate gut microbiota that consequently may diminish the pathophysiology of those diseases. In a study, done in a 'humanized' mouse model (germ-free mice colonized with human gut flora), the probiotic administration alters the production of several metabolites including TMAO through modulation of symbiotic gut microbial-host interactions [71]. Evidence has been provided that demonstrates that intervention with a probiotic product can favourably affect cardiac morphology and function in animal models [16]. A leptin suppressing probiotic bacteria, *Lactobacillus plantarum*, led to the attenuation of ischaemia-reperfusion injury in rats [16]. Additionally, in a rat myocardial infarction model, probiotic administration (*Lactobacillus rhamnosus* GR-1) reduced left ventricle (LV) hypertrophy and improved LV ejection fraction (LVEF), without colonization in the gut [37]. In HF patients, a yeast probiotic, *Saccharomyces boulardii* was shown to be beneficial by improving cardiac systolic function (LVEF) and decreasing serum creatinine and C-reactive protein (CRP) during short-term follow-up [72]. Although probiotics have generated much attention for improving CVD [37, 73], the attention on prebiotics has been limited due to its unclear definition and unfeasible applications [69]. Non-digestible beta-glucans have become one of the popular prebiotics for improving several metabolic diseases and CVD. With limited research, they have shown beneficial effects of non-digestible beta-glucans on CVD and metabolic diseases and their modulatory effect on gut microbiota (reviewed in [74]). However, long-term benefits of prebiotic and probiotic intervention strategies remain to be determined. As we described earlier in this chapter, host genotype significantly influences both the composition and probably the function of the gut microbiome, which may further interact with administered probiotics or prebiotics. Thus, the effectiveness of probiotic/prebiotic treatments may vary depending on the host genotype.

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5.4. Binding agents of key mediators

As the metabolites (e.g. TMAO) and their precursors (e.g. TMA) play important roles in the pathogenesis of CVD, a promising intervention would be to remove such metabolites and their precursors from the gut by oral administration of specific non-absorbent binding agents. Oral charcoal absorbent (AST-120) has been clinically applied to remove uremic toxins, such as indoxyl sulphate, in patients with advanced renal failure [75]. AST-120 has been shown to prevent progression of LV hypertrophy and cardiac fibrosis in rats with chronic kidney disease (CKD) [76] and in a combination model with CKD plus HF [77] without affecting blood pressure. However, the efficacy of binding agents has not yet been demonstrated in human, and more research should explore the potential use of such strategies.

6. Conclusion

Coevolution over millions of years between human and microorganism has led to a mutualistic relationship, in which diverse ecosystems of gastrointestinal microbiota and its metabolic functions contribute to the maintenance of our metabolic homeostasis. The interaction between heart and gut, or the heart-intestine axis, has emerged as a novel concept to provide new insights into the complex mechanisms of CVD. Gut microbiota function as a filter for our largest environmental exposure, our dietary intake, and the microbial community within each of us obviously influences how we experience a diet. We need to appreciate that our gut microbial ecosystem makes up a large and plastic endocrine organ that influences numerous metabolic and physiological processes. Although recent sequencing efforts of gut microbiota provide multiple evidences of its associations with CVD events, simply cataloguing the microbes within is not sufficient and further studies should focus on discovery of the functional aspects of microbiota and its metabolites that contribute to the pathophysiology of CVD and other metabolic diseases that trigger CVD events. Not all currently available animal models are suitable for discovering the role of gut microbiota on CVD and associated diseases, thus, new *in vivo* models need to be developed and/or existing reliable models should be recommended based on their reliability and better representation of the human condition. There is increasing attention towards modulating the gut microbiota as a new target for therapeutic intervention and targeting for treatment and prevention of complex cardiometabolic diseases. However, at present time, the role of gut microbiota-targeted interventions remains ambiguous due to the absence of solid and well-documented clinical evidence. Further advances in this area have enormous potential in the development of novel therapeutic tools for microbiome modulation of CVD.

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Author details

Hein Min Tun[1], Frederick C. Leung[2,3] and Kimberly M. Cheng[4*]

*Address all correspondence to: kmtc@mail.ubc.ca

1 Department of Animal Science, University of Manitoba, Winnipeg, Canada

2 School of Biological Sciences, University of Hong Kong, Hong Kong, China

3 FCL Bioscience (Hong Kong) Limited, Hong Kong Science and Technology Park, Hong Kong, China

4 Faculty of Land and Food Systems, University of British Columbia, Vancouver, Canada

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