

Role of gut microbiota in cardiovascular diseases

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Abstract

The human gut is colonized by a community of microbiota, primarily bacteria, that exist in a symbiotic relationship with the host. Intestinal microbiota-host interactions play a critical role in the regulation of human physiology. Deleterious changes to the composition of gut microbiota, referred to as gut dysbiosis, has been linked to the development and progression of numerous diseases, including cardiovascular disease (CVD). Imbalances in host-microbial interaction impair homeostatic mechanisms that regulate health and can activate multiple pathways leading to CVD risk factor progression. Most CVD risk factors, including aging, obesity, dietary patterns, and a sedentary lifestyle, have been shown to induce gut dysbiosis. Dysbiosis is associated with intestinal inflammation and reduced integrity of the gut barrier, which in turn increases circulating levels of bacterial structural components and microbial metabolites, including trimethylamine-N-oxide and short-chain fatty acids, that may facilitate the development of CVD. This article reviews the normal function and composition of the gut microbiome, mechanisms leading to the leaky gut syndrome, its mechanistic link to CVD and potential novel therapeutic approaches aimed towards restoring gut microbiome and CVD prevention. As CVD is the leading cause of deaths globally, investigating the gut microbiota as a locus of intervention presents a novel and clinically relevant avenue for future research.

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Core tip: As cardiovascular diseases (CVD) remain the leading cause of mortality, this article reviews the current literature dysbiosis and its role in CVD progression to present a novel therapeutic avenue. In this paper, we provide a comprehensive review on the composition and development of gut microbiota, its changes (dysbiosis) due to endogenous and exogenous factors and the mechanistic association of dysbiosis with development of CVD. Additionally, we explore the potential therapeutic approaches focused at restoring gut microbiota and their impact on CVD.

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INTRODUCTION

The human body hosts trillions of microorganisms, and together they form an interactive ecosystem within and without outside world. The changes and interactions within this ecosystem affect the human body in health and diseases. The entourage of the associated microflora in the host is referred to as the microbiome. Majority of the microflora colonizing human body are found in the gastrointestinal tract, especially in the colon. The gut microbiota plays a major role in maintaining nutrition and immune system, which, in turn, affects the host's susceptibility and response to pathologic conditions. Imbalance in the intestinal microbiome, also known as gut dysbiosis, is associated with several conditions including gastrointestinal disorders, asthma, allergies, central nervous system disorders, metabolic syndrome, cancers and cardiovascular disease (CVD)^[1,2].

CVD, a leading cause of death worldwide, stems from risk factors like smoking, lipid metabolism, diabetes and unregulated blood pressure. Atherosclerosis, the key pathophysiologic mechanism underlying the development of CVD, involves a complex interaction of vasculature, immune system and lipid metabolism. The gut microbiome affects all the component risk factors of atherosclerosis - both directly and indirectly, thus playing an important, albeit poorly understood role, in CVD^[2]. In this review, we outline the role of gut microbiota in CVD and areas of future research and potential interventions.

HUMAN GUT MICROBIOTA

Composition, development and function

It is estimated that the human gut is home to approximately 1000 to 1150 microbial species^[3]. The microbial gene pool has been shown to exceed the size of the human genome and is termed as metagenome^[4]. The international Metagenomics of the Human Intestinal Tract Project identified the gene database of the human gut microbiome, from stool samples of 124 individuals who were healthy, overweight and obese and patients with inflammatory bowel disease. This study found 3.3 million non-redundant microbial genes, derived from 576.7 gigabases of sequence, which is approximately 150 times larger than the human genome size^[3]. The two major phyla, *Bacteroidetes* and *Firmicutes* accounted for 90% of microbial species inhabiting human gut, with the rest comprised of *Actinobacteria*, *Cyanobacteria*, *Fusobacteria*, *Proteobacteria* and *Verrucomicrobia*^[5,6].

Starting from birth, multiple factors (both intrinsic and extrinsic) affect the development of human gut microbiota pool including host genome, geography and lifestyle factors (*e.g.* diet, disease, antibiotic exposure, *etc.*) (Figure 1)^[7]. In the perinatal life, maternal flora, delivery method, breastfeeding, and weaning off breastmilk affects the development of microbiome. Notably, the gut microbiota of infants

delivered vaginally consists of *Lactobacillus*, *Prevotella*, and *Atopobium*, whereas babies delivered by caesarean section predominantly carry maternal skin microflora in their guts, consisting mainly of *Staphylococcus*^[8]. As the infant matures, the dominant aerobic microbiome diversifies to form an anaerobic environment, as evidenced by a high abundance of Bifidobacteria and *Clostridia* in adolescents compared to adults^[9]. Interestingly, the metabolic environment of the gut changes as the microbiota evolves with age. The composition of core gut microbiota has been shown to be essentially stable throughout adulthood^[9]. Changes occur with old age in accordance with the decline of physiological functions (Figure 2). As the immune system declines, an increase in facultative anaerobes, a shift in the ratio of *Bacteroidetes* to *Firmicutes* phyla, and a marked decrease in Bifidobacteria have been noted^[9].

The gut microbiome plays an important function in both healthy and diseased individuals. It protects the host from epithelial cell injury and enteropathogens, regulates fat metabolism, affects the absorption of various nutrients and optimizes digestion^[10,11]. The immune system is continuously modified by the introduction of components of the microbiome through the leaks in the intestinal wall. This interaction shapes the immune system, which in turn also changes the gut microbiota^[7,12].

Leaky gut syndrome

Intestinal mucosal epithelial barrier, which protects the internal milieu from the hostile external environment, is maintained by the formation of tight junctions (TJs, a complex made of intramembranous proteins, occludin and several molecules from claudin family of proteins) that spread between the epithelial cells, thus creating a semi-permeable seal^[13]. Lipopolysaccharides (LPS, an endotoxin) is a component of Gram-negative bacterial cell wall and is a known inducer of the inflammatory response. LPS, via toll-like receptors (TLRs) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) pathway, induces expression of inflammatory mediators and activates the innate immune system^[14]. Higher levels of bloodstream endotoxins (especially > 50 pg/mL) have been associated with a threefold increased risk of atherosclerosis^[15]. Gut microbiota is a large source of LPS, and under normal conditions with a functional intestinal barrier, it causes no harm and lower levels of LPS have been detected in healthy subjects^[16,17]. In a diseased state, this barrier loses its protective function leading to increased intestinal permeability, especially to the locally produced LPS by the gut bacteria. Earlier, it was thought that leaky gut develops because of specific pathological conditions, but more recently, several studies have indicated a causal role of leaky gut rather than a consequence of the pathologic conditions^[18-20]. In order to understand the role of gut microbiota in CVD, we have first to understand the factors contributing to the leaky gut syndrome.

Nutritional factors

Dyslipidemia is a known risk factor for CVD. High-energy diet and excessive fat intake are associated with significantly increased levels of LPS in blood^[21,22]. Two pathways are proposed to be involved in the increased LPS with such diets - direct and indirect. In the direct pathway, food high in fat content causes an increased accumulation of chylomicrons increasing the local intercellular pressure contributing to loosening of the tight junctions. The loosening of tight junctions allows a generous influx of larger molecules such as LPS^[23,24]. In the indirect pathway, the dietary fat stimulates mast cell activation in the intestinal mucosa with subsequent release of histamine and other inflammatory mediators known to increase intestinal permeability^[25]. Similar to a high-fat diet, high carbohydrate intake can also lead to increased intestinal permeability and endotoxins levels^[26]. With the expansion of industrial food processing, the human gut is increasingly exposed to new food additives such as nanoparticles, emulsifiers, organic solvents, and microbial transglutaminases. These products compromise the integrity of the intestinal barrier and expose the immune system to a number of foreign particles^[27].

Endogenous factors

Genetic susceptibility has been implicated in several autoimmune intestinal diseases that may contribute to the leaky gut such as celiac disease and autoimmune enteropathy^[28]. Zonulin is a physiological modulator of TJs and is activated by intestinal mucosa-microbiota interactions. Zonulin regulates antigen trafficking, and its upregulation in genetically susceptible individuals can lead to inflammatory and autoimmune processes^[29]. Autoimmune disorders have been seen as a consequence of increased intestinal wall permeability; however, the reverse (*i.e.* autoimmune disorder causing increased intestinal wall permeability) has also been suggested in animal studies^[30].

Other endogenous factors include the role of alterations in the enteric nervous

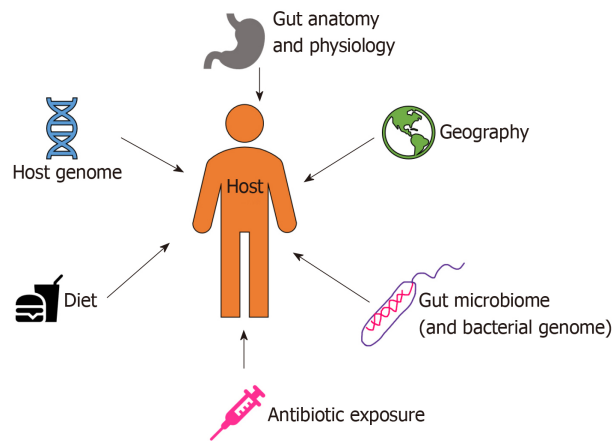


Figure 1 Factors affecting gut microbiome development.

system, and conditions compromising intestinal integrity. The enteric nervous system is a collection of neurons in the gastrointestinal tract, which functions independently from the central nervous system secreting various neurotransmitters including serotonin and histamine. In murine models, the downregulation of serotonin reuptake transporter has been associated with increased proinflammatory bowel response, increased intestinal permeability and increased fructose-induced endotoxin translocation leading to liver steatosis^[31-33]. Studies have reported intestinal insult like major abdominal surgeries, shock and trauma compromises intestinal integrity as a cause or as a consequence of systemic inflammation^[34].

Intestinal infections

The integrity of the intestinal barrier is also prone to many pathogen microorganisms and toxins. *Helicobacter pylori* can cause interruption of TJs by delivering cytotoxin-associated gene A, which results in loss of polarity of epithelial cells^[35,36]. Enteropathogenic *Escherichia coli* secretes EspM and NleA proteins which can induce TJ mislocalization^[37,38]. *Clostridium difficile* toxin A increases paracellular permeability and translocation of zonula occludens-1 protein leading to degradation of filamentous actin^[39]. TJ disruption was also implicated in cases of infection with *Vibrio parahaemolyticus* and *Salmonella enterica*^[40,41].

Lifestyle factors

Chronic stress and alcohol consumption can also affect the gut microbiome. Studies suggest a key role of corticotropin-releasing factor (CRF) and its receptors (CRFR1 and CRFR2) in the pathophysiological mechanism of development of the leaky gut^[42,43]. Acetaldehyde, a product of alcohol metabolism, promotes phosphorylation of tight junction proteins in the intestinal epithelium causing direct damage in addition to indirect damage by an increase in nitric oxide which damages microtubules^[44]. Alcohol also alters the composition of gut microbiota with an increase in Gram-negative bacteria^[44].

ROLE GUT MICROBIOTA IN CARDIOVASCULAR DISEASES

Atherosclerosis is an inflammatory disease with a growing body of evidence supporting a potential autoimmune background^[45]. Infection is one of the major contributors to inflammation in the body and is a proposed mechanism of atherosclerosis. A large number of microorganisms such as *Chlamydia pneumoniae*, *Porphyromonas gingivalis*, *Helicobacter pylori*, Influenza A virus, Hepatitis C virus, cytomegalovirus, and human immunodeficiency virus have been associated with an increased risk of cardiovascular diseases^[46]. Infections contribute towards atherosclerosis *via* two predominant mechanisms: direct infection of the blood vessel wall (making it prone to plaque formation), or indirectly with an infection at a distant site by promoting proinflammatory mediators from a systemic immune response affect plaque growth (Figure 3)^[47]. Additionally, dysbiosis also contributes to the production of atherosclerotic metabolites in the gut like trimethylamine N-oxide (TMAO) and can alter bile acid metabolism^[48]. In this section, we will discuss the role and the evidence for each of the proposed mechanisms.

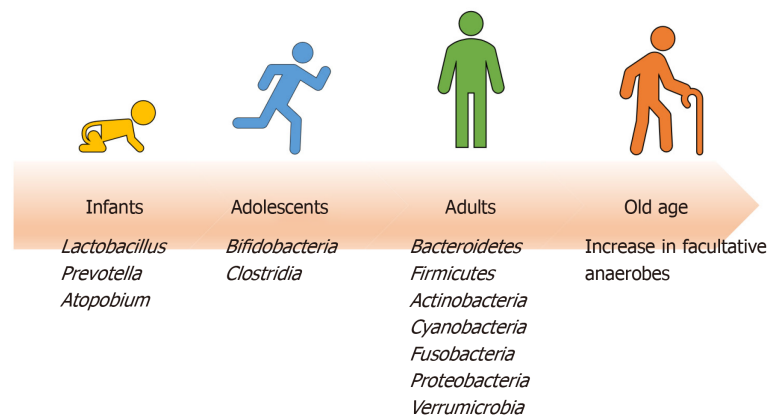


Figure 2 Evolution of gut microbiome with age and host's immune function.

Direct infection

Over 50 species of bacterial DNA have been observed in atherosclerotic plaques^[49]. Proteobacteria phylum (*Chryseomonas* and *Helicobacter* genera) is found to be most abundant in atherosclerotic plaques^[49]. Firmicutes phylum (*Anaeroglobus*, *Clostridium*, *Eubacterium*, *Lactobacillales* and *Roseburia* genera) is predominantly found in the oral and gut cavity and is also present in atherosclerotic plaques^[49]. Other bacteria that have been shown to be altered in the gut among patients with atherosclerotic cardiovascular disease includes *Lactobacillales*, *Collinsella* (stenotic atherosclerotic plaques in the carotid artery leading to cerebrovascular events), *Enterobacteriaceae* and *Streptococcus* spp (Table 1)^[50,51]. In fact, it has been suggested that gut microbiota, especially *Bacteroides*, *Clostridium* and *Lactobacillales* could be considered as diagnostic markers in patients suffering from coronary artery disease^[52].

Indirect infection

Microorganisms, through inflammatory cytokine production and stimulation of acute-phase reactants, contribute to the development of atherosclerosis by further adding to the chronic inflammation within the atheromatous plaques^[46]. In murine models, the use of antibiotics has shown an alteration in the gut microbiome, which affects carbohydrate and lipid metabolism. Initial studies investigating the role of pathogens in the development of atherosclerotic plaques had accounted for single microorganisms and not the overall microbiome, more recently it is being recognized that the aggregate number of microorganisms which an individual is colonized or infected with correlates more with atherogenesis, a concept referred to as "pathogen burden" or "infectious burden"^[53].

Another possible mechanism for increased inflammation is cross-reactivity or molecular mimicry between self-antigens and bacterial antigens like heat-shock proteins and oxidized low-density lipoproteins^[54]. Human heat-shock protein 60 (hHSP60) is expressed on the arterial endothelium in response to stress such as acute hypertension, hypercholesterolemia and in reperfusion injury. Also, a major antigenic component of bacteria during infection is the bacterial heat-shock protein 60s (HSP60s). Due to the high degree of homology between human and bacterial HSP, it is suggested that the antibodies formed against bacteria can target host cells expressing hHSP60. Indeed, high titres of serum antibody to mycobacterial HSP-65 were found in subjects with coronary or carotid atherosclerosis and post-myocardial infarction state^[55].

As mentioned before, dysbiosis also leads to alteration in the immune system, which causes increased inflammation and atherogenesis. TLRs have been known to play a crucial role in bacterial infection and activation of the innate immune response. Once activated by ligands such as LPS, TLR dimerizes with the interleukin-1 receptor (IL-1R) forming a complex that binds myeloid differentiation primary response protein, MyD88, leading to downstream signalling cascade ultimately activating NF- κ B. This cascade results in stimulation of the synthesis of proinflammatory cytokines, chemokines and costimulatory molecules^[56]. TLR's expression is found in most cardiovascular cells like endothelial cells, cardiomyocytes, adventitial fibroblasts, and macrophages. Among TLRs, TLR4 is best understood. Studies have described activation of TLR4 by saturated fatty acids, acting as a ligand through the same downstream pathways as for LPS resulting in the production of proinflammatory cytokines and chemokines^[57,58]. Additionally, saturated fatty acids contribute to the induction of the inflammation by alternating gut microbiota in favour of Gram-

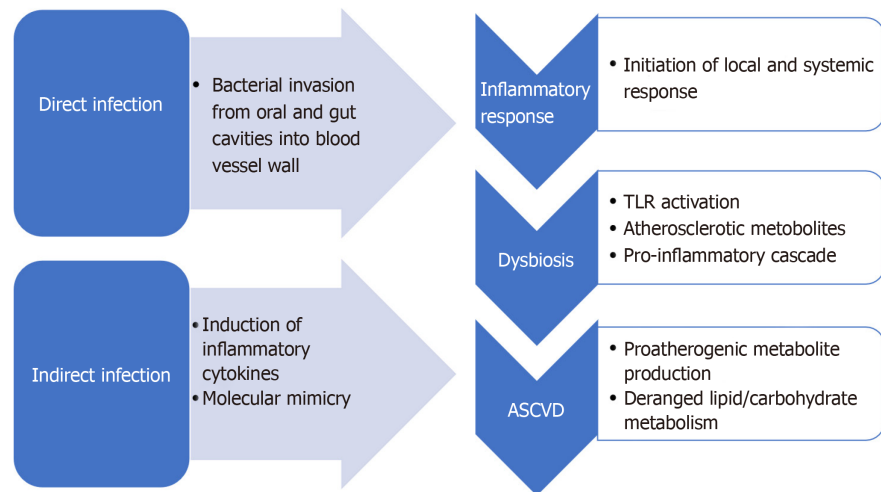


Figure 3 Proposed mechanisms of micro pathogen mediated atherosclerotic cardiovascular diseases. ASCVD: Atherosclerotic cardiovascular diseases.

negative bacteria, thus, increasing LPS levels. These processes promote translocation of bacteria and endotoxins into the bloodstream from the intestinal lumen due to an increase in intestinal permeability, further adding to the activation of TLR4^[59]. In the animal models with a genetic deficiency of TLR4 and MyD88 genes, reduced proinflammatory cytokines and decreased plaque lipid content and aortic atherosclerosis were observed^[60]. Human studies have also shown increased expression of TLR1, TLR2 and TLR4 in atherosclerotic plaques, suggesting a potential role in pathogenesis^[61].

Production of proatherogenic metabolites

TMAO is an intestinal microbiota metabolite of choline and phosphatidylcholine. Dietary components such as choline, phosphatidylcholine, and carnitine, found in various animal-based products and energy drinks, are metabolized by gut microbiota to trimethylamine (TMA), and then oxidized by flavin monooxidases 3 in the liver to TMAO^[62,63]. Flavin monooxidases 3 is an important regulator of TMAO synthesis and is regulated by farnesoid X receptor (FXR) whose expression can be upregulated by bile acids. TMAO can lead to atherogenesis *via* multiple mechanisms, though the underlying pathway is not completely understood. It inhibits reverse cholesterol transport causing reduced cholesterol removal from peripheral macrophages, and also affects atheroprotective effects of high-density lipoprotein thus promotes atherosclerosis^[64]. TMAO also acts on platelets and increases platelet hyperresponsiveness by enhancing the stimulus-dependent release of Ca^{2+} from intracellular Ca^{2+} stores leading to increased thrombotic risk^[63]. The effects of TMAO have also been observed in vascular cells promoting proinflammatory protein activation such as interleukin-6, cyclooxygenase-2, intercellular adhesion molecule-1 and E-cadherin – through the NF- κ B signalling pathway^[65]. Tang *et al*^[62] showed elevated TMAO levels were associated with increased risk of major adverse cardiovascular events, including death, myocardial infarction and stroke over a 3-year follow-up period involving more than 4000 human subjects. A strong correlation between TMAO levels and CVD was noted even after adjustments of traditional risk factors. Also, an increased risk was associated with a graded increase in TMAO levels with a significant risk of major adverse cardiovascular events seen in the highest quartile.

There are an increasing number of studies explaining the complex interplay between intestinal microflora, bile acids and metabolic disease. Bile acids affect cardiac function and play a significant, yet poorly understood, role in CVD^[66]. Direct and indirect pathways have been proposed to explain their effects in CVD. In the direct pathway, bile acids have been shown to interact with cardiac myocytes affecting muscle contractility and electrical excitation. In the indirect pathway, bile acids play a significant role in lipid metabolism, plaque formation, endothelial vasodilation and neovascularization of injured organs^[66]. Having been metabolized by intestinal microflora, bile acid metabolites affect different metabolic pathways through FXR-induced signalling^[67]. FXR is an endogenous bile acid sensor, a member of the nuclear receptor family with chenodeoxycholic acid being its most potent ligand. FXR acts as a receptor-transcription factor which, after being bound by ligand, regulates promoter activity in a coordinated manner. In adult human tissues, FXR

Table 1 Microorganisms associated with cardiovascular disease

Microorganisms associated with cardiovascular disease
<i>C. pneumoniae</i>
<i>P. gingivalis</i>
<i>H. pylori</i>
Lactobacillales
Influenza A
Cytomegalovirus
Human immunodeficiency virus
Enterobacteriaceae
<i>Streptococcus parasanguinis</i>
<i>Collinsella</i>
<i>Veillonella</i>
<i>Aggregatibacter</i>
Firmicutes
Bacteroidetes
Actinobacteria
Fusobacteria
Proteobacteria
Candidate division TM7 single-cell isolate TM7c
<i>Spirochaetes</i>
SR1
Tenericutes
<i>Deinococcus-Thermus</i>
<i>Gemmatimonadetes</i>
<i>Chloroflexi</i>
<i>Neisseria polysaccharea</i>
<i>Neisseria subflava</i>
<i>Waddlia chondrophila</i>
<i>Prevotella</i>
<i>Beggiatoa</i> sp. P5
<i>Alloprevotella</i> rava
<i>Megasphaera micronuciformis</i>
<i>Acidovorax</i> sp. CF316
<i>Atopobium parvulum</i>
<i>Solobacterium moorei</i>
<i>Clostridium difficile</i>

expression has been found in adrenal glands, colon, liver, small intestine, kidneys and heart whereas no expression detected in brain, lung and skeletal muscles^[68]. *In vitro* studies have recognized the prevention of vascular inflammation and neointimal proliferation as the potential roles FXR activation in the vascular smooth muscle cells^[69].

THERAPEUTIC INTERVENTIONS: IMPROVING GUT MICROBIOME AND PREVENTING CARDIOVASCULAR DISEASE

As our understanding of the gut microbiome and its role in CVD grows, the gut microbiome is emerging as a major potential target for intervention among patients with CVD for improving clinical outcomes. The currently proposed therapeutic interventions are targeted towards the restoration of the intestinal barrier and improvement of gut microbiota. In this section, we will discuss the role of dietary modification and supplementation in the gut microbiome, followed by the possible role of faecal transplantation and targeting microbial enzyme pathways for further

prevention of CVD.

Low-fermentable oligo-, di- and monosaccharides and polyols diet

Fermentable oligo-, di- and monosaccharides and polyols group includes short-chain carbohydrates and sugar alcohols that have poor absorption in the small intestine due to osmotic activity and undergo rapid fermentation by gut microflora^[70]. Studies have shown their potential therapeutic effects in diseases that are associated with increased intestinal permeability, such as non-celiac gluten sensitivity and irritable bowel syndrome^[71-74]. These findings suggest their potential role in dyslipidaemias and atherosclerosis, though further investigations are warranted.

Dietary fibers/prebiotics

Whole-grain intake has been inversely associated with metabolic syndrome and mortality from CVD, independent of demographic, lifestyle and dietary factors^[75]. Epidemiologic studies have also suggested a decreased risk of CVD with adequate dietary fiber intake likely through the reduction of low-density lipoprotein levels^[76]. Prebiotics are fibers, mostly oligosaccharides, that are selectively fermented (mostly *Lactobacilli* and *Bifidobacteria* genera) and exert changes on both the composition and function of the gastrointestinal microflora to confer benefits upon host well-being and health^[77]. Their proposed health benefits were observed in a mouse model, where a diet rich in various inulin-type fructans, was associated with a reduced burden of atherosclerosis^[78].

Probiotics

Probiotics are live viable microorganisms (predominantly *Lactobacilli* and *Bifidobacteria*) that improve microbial balance in the gut, thus exerting positive health effects^[79]. In a randomized trial, consumption of live *Lactobacillus Plantarum* was shown to diversify homogenous gut microbial flora and was associated with a reduction in incident CVD events^[80]. Naruszewicz *et al*^[81], in a study of 36 healthy volunteers who were active smokers showed an inverse correlation between administration of *Lactobacillus Plantarum* and blood pressure levels, fibrinogen levels, degree of adhesion of isolated monocytes and levels of proinflammatory cytokines suggesting its potential role in primary prevention of atherosclerosis. Reduced levels of low-density lipoprotein were noted in women with normal or moderately elevated cholesterol after ingestion of fermented milk containing *Lactobacillus acidophilus* and *Bifidobacterium longum*^[82]. Another study found *Akkermancia muciniphila* to suppress inflammation and atherosclerotic lesion formation in the apolipoprotein E-deficient (ApoE^{-/-}) mice. It was proposed that *A. muciniphila* reduce circulating endotoxins and improve the intestinal barrier by increasing the expression of TJ proteins^[83]. Looking through the prism of intestinal microflora and gut permeability, probiotics appear to be promising protective agents, especially with regards to prophylaxis of atherosclerosis. Larger clinical trials with hard clinical outcomes are awaited for this approach to gain more credibility.

Anthocyanin

Anthocyanins represent a group of flavonoids that commonly found in fruits, vegetables, grains, and even red wine. They play a protective role against atherosclerosis after being transformed to various metabolites by gut microbiota^[84,85]. Protocatechuic acid (PCA) is a metabolite derived from human gut microbiota metabolism of anthocyanin called cyanidin-3-O-glucoside^[86]. PCA had been shown to inhibit atherosclerosis by reducing monocyte inflammation and adhesion in ApoE^{-/-} mice^[87,88]. PCA has also shown to decrease miR-10b expression in macrophages, which induces gene expression promoting reverse cholesterol transport contributing to regression of established atherosclerotic plaque in ApoE^{-/-} mouse model^[85]. Human studies are needed to show a clinical benefit of anthocyanin as a food supplement in the prevention of atherosclerosis.

Faecal microbiota transplantation

Faecal microbiota transplantation (FMT) is described as the restoration of “healthy” functional gut microflora by administering a faecal solution from a donor into the intestinal tract of the recipient. The beneficial effect of FMT for recurrent *clostridium difficile* infection has been proven and is now a part of the guidelines for the treatment of recurrent *clostridium difficile*. It has also been explored as a therapeutic intervention in several other pathologies such as irritable bowel syndrome, metabolic syndrome, neurodevelopmental disorders, autoimmune diseases, allergic diseases and chronic fatigue syndrome^[89,90].

In a mouse model, gut microbial transplantation was conducted from the atherosclerosis-prone strain of mice and atherosclerosis-resistant strain of mice to

apolipoprotein e null mice in which resident intestinal microbes were first suppressed with antibiotics. Mice which received FMT from atherosclerosis-prone strain demonstrated choline diet-dependent enhancement in atherosclerotic plaque burden as compared with recipients of atherosclerosis-resistant strain^[91]. In another study with human subjects, allogenic FMT from lean subjects to obese subjects with metabolic syndrome leads to improved insulin sensitivity and glucose metabolism^[92]. The role of FMT as a secondary or primary prevention strategy to improve CVD outcomes remains to be explored with a severe limitation of its delivery method and possible complications of exposing the host to other infections.

Targeting enzyme pathways

Aortic lesions have a positive correlation with TMAO but an inverse correlation with choline levels^[93]. Inhibition of FMO gene expression has been shown to reduce TMAO levels, alteration of lipid and cholesterol metabolism, and reduction in atherosclerotic lesions^[94-96]. A study by Wang *et al*^[97] showed that 3,3-dimethyl-1-butanol, a structural analogue of choline, inhibits microbial TMA lyases resulting in reduced TMAO levels and atherosclerotic lesion development in mice. In 2018, Roberts *et al*^[98] reported the development of choline analogues iodomethylcholine and fluoro-methyl choline which can irreversibly inactivate choline TMA lyase activity. In animal models, these potent inhibitors reduced plasma TMAO levels > 95% after a single dose, for a sustained period and without any reported toxicity. The inhibitor selectively accumulated within intestinal microbes to millimolar levels, a concentration over 1-million-fold higher than needed for a therapeutic effect^[98]. These studies reveal that mechanism-based inhibition of gut microbial TMA and TMAO production reduces thrombosis potential, a critical adverse complication in heart disease. They also offer a generalizable approach for the selective nonlethal targeting of gut microbial enzymes linked to host disease limiting systemic exposure of the inhibitor in the host. Despite holding significant potential, these agents still need to undergo human testing for efficacy and safety evaluation.

CONCLUSION

Gut microbiota represents an inseparable part of the human organism and remains an area of exploration in its role in the development of various pathological conditions. So far, significant progress of acknowledging our co-habitants has been made with respect to discovering its genome, functions, composition differences across different age and cultural groups. In addition, the recognition of the leaky gut syndrome has paved the way to reveal potential pathophysiological mechanisms behind numerous associations between the gut microbiota and CVD. Several factors have been identified, exogenous and endogenous, in the leaky gut and has made gut microbiome alteration a potential therapeutic target in managing several diseases including potentially CVD. However, much needs to be explored to evaluate the translation of benefits observed predominantly in animal studies to human subjects.

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