The gut microbiome and cardiovascular disease: current knowledge and clinical potential

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Abstract

Cardiovascular disease (CVD) is the leading cause of death worldwide. The human body is populated by a diverse community of microbes, dominated by bacteria, but also including viruses and fungi. The largest and most complex of these communities is located in the gastrointestinal system and with their associated genome, are known as the gut microbiome. Gut microbiome perturbations and related dysbiosis have been implicated in the progression and pathogenesis of CVD, including atherosclerosis, hypertension and heart failure. Although there have been advances in the characterisation and analysis of the gut microbiota and associated bacterial metabolites, the exact mechanisms through which they exert their action is not well understood. This review will focus on the role of the gut microbiome and associated functional components in the development and progression of atherosclerosis. Potential treatments to alter the gut microbiome to prevent or treat atherosclerosis and CVD are also discussed.

Keywords: Atherosclerosis; cardiovascular disease, dysbiosis, microbiome, short chain fatty acids, TMAO

List of abbreviations:

AAA: abdominal aortic aneurysm

ACS: acute coronary syndrome

AMPK: adenosine monophosphate-activated protein kinase

$ApoE^{-/-}$: apolipoprotein E knockout

BA: bile acid

CAD: coronary artery disease

CLA: conjugated linoleic acids

CVD: cardiovascular disease

FOS: fructooligosaccharides

FXR: Farnesoid X receptor

GPR: G protein-coupled receptor

LDL-C: low density lipoprotein-c

PAD: peripheral arterial disease

SCFA: short chain fatty acid

TGR: Takeda G protein coupled receptor

TLR: toll-like receptors

TMA: trimethylamine

TMAO: trimethylamine-N- oxide

16S rRNA: 16S ribosomal ribonucleic acid

Introduction

Cardiovascular disease (CVD) is the leading cause of death and disability globally (60). The human gut is populated by a diverse and complex community of bacteria, viruses, fungi and protozoa, which along with their genome, are collectively known as the gut microbiome (90). An alteration in gut microbiome composition and linked functionality is known as microbial dysbiosis. Studies have shown that gut microbiota interact with the host, contributing to host health and disease status. Although there is a large interindividual variation in terms of microbial composition, healthy individuals share core functional capabilities (146). However, several disease states, including CVD, are associated with microbial dysbiosis (78). There is increasing evidence in CVD demonstrating an alteration in diversity and composition of the gut microbiome and its associated metabolites which may influence the pathogenesis and progression of diseases such as atherosclerosis [acute coronary syndrome (ACS) and stroke], heart failure and hypertension. The complex host-microbiome interaction influences the synthesis and release of several metabolites including trimethylamine-N-oxide (TMAO), bile acids (BA) and short chain fatty acids (SCFA), which impact host homeostasis (139). While recent advances in genomic and metabolomics have allowed the accurate characterisation and quantification of these microbes and their metabolites, the exact mechanisms of their action remain unclear. Interventions targeting the microbiome could provide new avenues for the management and treatment of CVD patients (138). In this review we will discuss the development and progression of atherosclerosis, the contribution of the microbiota, specifically the gut bacteria, and the potential for targeting the microbiome and its associated components as novel therapeutic options.

The gut microbiome

A complex and dynamic community of microbes live in the human gastrointestinal tract, a nutrient rich microenvironment conducive for the growth of microorganisms. (146). There is a dominance of bacteria in this environment belonging to five main phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and Verrucomicrobia (Figure 1) (177). There is large interindividual variation in the composition, diversity and abundance of bacteria and microbes, predominantly due to host genome factors as well as environmental parameters like lifestyle, diet, hygiene, health, medication such as antibiotics and the use of prebiotics and probiotics (18). There is a symbiotic relationship between the host and the microbiota,

developing from birth through to adulthood (76). The symbiotic relationship of the microbiome and the host promote the growth of beneficial commensal bacteria while inhibiting the overgrowth of pathogenic ones (102).

The normal physiological functions of the gut microbiome include fermentation of indigestible dietary substances (87), synthesis of certain vitamins (122), energy metabolism via SCFA production (13) and the regulation of the intestinal epithelial mucosal barrier (73). If disrupted, this barrier will result in 'leakage' allowing bacterial translocation and an increase in exposure to bacterial derived products such as circulating endotoxins.

The gut microbiome has a very diverse role and can have distal effects in the body. There is a complex interplay between the gut microbiome and the health and function of the host via production and release of important metabolites including SCFA, BAs and TMAO as well as influencing various host metabolic pathways (139). These metabolites can be absorbed into the bloodstream via the intestinal epithelium, thus influencing the function of several distal organs and body systems (127).

Atherosclerosis

Atherosclerosis is a chronic inflammatory condition resulting from initial endothelial injury followed by lipid build-up and plaque formation in the arteries. Risk factors for atherosclerosis include hypercholesterolaemia, hypertension, diabetes mellitus, obesity, and a sedentary lifestyle (113). Cellular events that contribute to the initiation and progression of atherosclerosis involve; lipid migration to the inner layer of the arterial wall – the endothelium, adhesion of circulating monocytes and lymphocytes to the endothelial cells, migration of monocytes into the sub-endothelial space and acquiring macrophage characteristics and converting into foam cells (113). Low density lipoprotein (LDL) particles undergo modification, primarily through oxidation, becoming strong chemo attractants (113). These processes, which involve cell adhesion, migration, differentiation, further proliferation and interaction with the extra cellular matrix, are controlled and regulated by a cascade of cytokines and growth regulatory peptides and ultimately lead to the accumulation of cholesterol and the formation of fatty streaks (113). Vascular smooth muscle cells also migrate to the endothelium and proliferate, with additional accumulation of macrophages,

modified LDL and inflammatory cells, together forming the atherosclerotic plaque (11). The plaque consists of various cells (endothelial cells, foam cells, macrophages and leukocytes), accumulated lipids (cholesterol, triglycerides), calcified regions and a necrotic core (113). Vascular dysfunction and hypertension contribute to vessel damage via mechanisms involving an increase in inflammation and LDL, causing plaque formation and build up in arterial walls progressing to atherosclerosis (11). Atherosclerosis progresses to various cardiovascular diseases such as ACS, stroke, AAA and ultimately heart failure.

Microbiota and their metabolites profoundly modulate the progression of atherosclerosis, the most common cause of ACS, stroke and peripheral vascular disease (Figure 2) (74). However, despite this, the mechanisms of action as to how the gut microbiota contribute to atherosclerosis remain to be elucidated. Recent reviews have highlighted the potential role of dysbiosis in several disease states, including atherosclerosis, heart failure, hypertension and diabetes mellitus (3, 145). In these states the gut bacteria diversity, composition and associated metabolic functions can change, leading to disruption of vital physiological processes, including inflammation, lipid metabolism, bacterial translocation and glucose homeostasis which may all contribute to disease development and progression.

Changes in the microbiome composition may also contribute to the development and progression of CVD, with alterations in the ratio of the key phyla of Firmicutes to Bacteroidetes proposed as a potential risk factor of CVD (20, 32, 135). Similarly, there is a correlation between various gut microbiome metabolite levels, including TMAO, SCFA and BA and the pathogenesis of CVD. Finally, risk factors and associated symptoms of CVD may cause further microbial dysbiosis, including accumulation of toxic metabolites (such as TMAO) and pathogenic bacterial species (73, 146, 177).

In the gut, symptomatic atherosclerotic patients had a greater abundance of *Collinsella* compared to healthy controls (48, 68). However, several of these studies were limited by their small sample sizes (<30) and thus further research is required. A recent metagenome wide association study from 218 individuals with atherosclerosis and 187 healthy controls observed that the gut microbiome deviates from a healthy status by an increase in the relative abundance of Enterobacteriaceae (including *Escherichia coli*, *Enterobacter aerogenes* and *Klebsiella* spp.) and *Streptococcus* spp (Table 1). Functionally, this impacts metabolism and

transport of molecules such as TMAO (64). These data suggest that the gut microbiome may be more proinflammatory in patients with CVD (64).

Carotid arteries

Carotid atherosclerosis is linked to an increased risk of cardiovascular events such as stroke and ACS (40). Stroke is divided into two categories; ischemic stroke which occurs as a result of a blockage in a blood vessel supplying blood to the brain and haemorrhagic stroke, occurring from the rupture of a weakened blood vessel in the brain (6). Ischemic stroke accounts for 70 – 80% of all strokes (6). There is a bidirectional relationship between the brain and gut microbiota via vagus nerve activity and the enteric nervous system (26). During ischemic stroke, the brain produces molecules which signal damage, including damage associated molecular proteins and cytokines. The damage associated molecular proteins and cytokines produced in ischemic brain tissue are released into the circulation and communicate with immune and lymphoid organs leading to systemic inflammatory and immune responses (26). The gut will also release cytokines and gut inflammatory and immune cells will travel to the site of brain injury.

After ischemic or haemorrhagic stroke, 50% of patients experience gastro intestinal issues, including gut microbiota dysbiosis, “leaky” gut, increased gut motility, disorganization and gut haemorrhage (169) as well as a reduced diversity and composition of the gut microbiota (173). As a result, intestinal inflammatory and immune responses are triggered (137). These patients have increased mortality rates and worsening neurological function with poor outcomes. This altered immune homeostasis largely occurs due to the gut microbiota’s role in communicating with intestinal epithelial cells, dendritic cells, B and T lymphocytes, mononuclear phagocytes and innate lymphoid cells (67). Zeng and colleagues investigated the association of the gut microbiome with high and low risk stroke and noted enrichment in opportunistic pathogens (e.g. *Enterobacteriaceae* and *Veillonellaceae*) and depleted abundance of butyrate producing bacteria (e.g. *Lachnospiraceae* and *Ruminococcaceae*) with reduced faecal butyrate levels in patients with a high risk of stroke (179). Many aspects of gut microbiota dysbiosis and modulation of inflammatory and immune responses have been implicated in stroke. However, what these effects signify in reality for stroke therapy remains to be investigated.

212 ***Coronary arteries***
213

214 Coronary artery disease (CAD) involves a narrowing of the coronary arteries supplying blood
215 to the heart due to plaque build-up, thereby reducing blood flow. Atherosclerosis is the main
216 pathophysiological basis of CAD. A small study found CAD patients had alterations in the
217 gut microbiota, including an increase in Lactobacillales (from Firmicutes phylum and
218 consisting of the genera *Lactobacillus, Streptococcus and Enterococcus*) whereas the phylum
219 Bacteroidetes (composed of the genera *Bacteroides* and *Prevotella*) was reduced (42).
220 *Bacteroides fragilis* promotes regulatory T-cell function in mucosal T-cell homeostasis (99).
221 A recent study in CAD revealed a decreased microbial diversity and composition, as reflected
222 by enrichment in *Esherichia/Shigella* and *Enterococcus*, while *Faecalibacterium*,
223 *Subdoligranulum, Roseburia* and *Eubacterium rectale* were depleted (180). *Faecalibacterium*
224 has an important anti-inflammatory role (89, 93), while *Roseburia* are important for SCFA
225 production which potentially depletes energy supply for intestinal cells thus negatively
226 affecting the gut barrier (54, 76). In atheromatous plaques of patients with CAD, there was
227 an enhanced bacterial diversity of *Staphylococcus* species*, Proteus vulgaris, Klebsiella*
228 pneumoniae and *Streptococcus* sp. Although, there was a predominant bacterial signature in
229 these plaques, it is not proof that the gut microbiota plays a causal role in atherosclerosis.
230 Instead it has been suggested that the presence of the bacteria could contribute to
231 atherosclerosis development and/or progression (107).
232

233 ACS refers to a group of conditions including, unstable angina and acute myocardial
234 infarction, and is usually characterised by a reduced blood flow to the heart muscle usually
235 due to blockage (44). ACS is associated with inflammation, which plays a role in its
236 pathogenesis. Evidence suggests that microbial dysbiosis may have an impact on several
237 inflammatory or immune disorders via activating proinflammatory responses. Indeed,
238 bacterial DNA was present on epicardial adipose tissue obtained during coronary artery
239 bypass grafting in patients with ACS. This suggests that the epicardial adipose tissue
240 environment is susceptible to microbial colonization, stimulating proinflammatory responses
241 involved in vascular inflammation and plaque formation and instability. However, there is
242 agreement that further research is warranted to provide more clarity to this important area
243 (111).
244

Abdominal aortic aneurysm

Abdominal aortic aneurysm (AAA) is a localised swelling of the walls of the abdominal aorta, the rupture of which can be fatal. Pathogenesis of AAA includes complex inflammatory pathways, extracellular matrix disruption, inflammation, thrombosis, haemodynamic forces and associated signalling molecules (58). Atherosclerosis is an important and independent risk factor for AAA (155) as patients with AAA frequently have atherosclerosis and there is an association of coronary heart disease and peripheral artery disease with AAA (57). An investigation of atherosclerotic plaques from vascular biopsies in patients with vascular diseases (including AAA), with and without chronic periodontitis, detected bacterial DNA in 95%, with most biopsies comprising DNA from multiple bacterial species (5). This reinforces recent findings that suggest more than one bacterial species are involved with CVD (41, 91). Although finding bacterial DNA in atherosclerotic plaques is well established, it is not clear whether an infection initiates or promotes its formation. Animal models have identified microorganisms such as *Aggregatibacter actinomycetemcomitans, Helicobacter pylori, Chlamydophila perfringens* and *Porphyromonas gingivalis,* which may contribute to increasing lesion areas in atherosclerosis (120). However, further research and validation in humans is required. In addition, gut microbiota function is affected by surgical abdominal aortic repair, which involves aortic clamping and can result in negative changes in intestinal permeability, which may also contribute to disease progression (109).

Peripheral artery disease and mesenteric ischemia

Peripheral artery disease (PAD) is characterised by reduced blood flow to the periphery, is a common consequence of systemic atherosclerosis and is associated with significantly higher risks of cardiovascular mortality. In patients with stable PAD, elevated plasma TMAO, a proatherogenic gut microbiota metabolite, was found to be a significant predictor of 5 year all-cause mortality risk, independent of traditional risk factors (131).

Mesenteric ischemia occurs as a result of reduced blood supply to the mesenteries supplying the small intestine. The gut microbiome plays a key role in mesenteric ischemia via Toll-like receptors (TLR) which recognise constituent molecules of bacteria and contribute to maintenance of the intestinal environment by playing a key role in the innate immune system

(103). The TLR signalling pathway, specifically TLR2 and TLR4, activate inflammatory responses, such as the release of tumour necrosis factor-α. Oxidative stress caused by reactive oxygen species and free radicals, the source of which are neutrophils and macrophages, also affect the intestine during ischemia (103). Neutrophils and macrophages release reactive oxygen species and free radicals to sterilize invading bacteria, however, excess production can cause local tissue injury (103). Prevention of oxidative stress is useful for treatment of mesenteric ischemia. Previous studies showed heme oxygenase-1 plays a protective role in mesenteric ischemia and reperfusion, by preventing oxidation (85, 168).

Gut microbiome metabolites

Trimethylamine-N-oxide

The gut microbiota is known to be involved in the synthesis of proatherogenic metabolites, namely TMAO (Figure 3) (100). The breakdown of dietary substances rich in phosphatidylcholine, choline or carnitine (from red meat, fish and eggs) forms trimethylamine (TMA), the precursor to TMAO (84). TMA is absorbed in the intestine and converted via the hepatic enzyme flavin monooxygenase 3 to TMAO, a plasma metabolite released into the bloodstream and eventually cleared by the kidneys (76, 178). TMAO is a biologically active molecule and has been shown to be a predictor of ACS, stroke and death, likely through a proatherogenic pathway (74, 84, 178). When mice were supplemented with chronic dietary L-carnitine, there was an alteration in caecal microbial composition and increased synthesis of TMA and TMAO and enhanced atherosclerosis, however, this was not seen when intestinal microbiota was concurrently suppressed (74). In fact, mice with intact gut microbiota had a reduction in *in vivo* reverse cholesterol transport when supplemented with either carnitine or choline (74), reinforcing the role of the gut microbiota in promoting atherosclerosis via the metabolism of L-carnitine, found in red meat. Evidence shows that individuals at higher risk for stroke and CVD have elevated TMAO levels as well as TMAO precursors (178). This was further reinforced when plaque progression and atherogenesis was promoted in apolipoprotein E knockout ($ApoE^{-/-}$) mice who were administered TMAO precursors or TMAO (55). Similarly, the administration of 3,3-dimethyl-1-butanol, a TMA inhibitor prevented cardiac dysfunction caused by a high sugar and high fat western diet (30).

Studies have demonstrated a correlation between gut microbiota metabolism of TMAO and risk of CVD (74, 84, 147, 148, 165), with a recent meta-analysis revealing a 67% increased

risk of developing CVD with high TMAO levels. Furthermore, for every 10 μmol/L increase in TMAO, there was a 7.6% increase in all-cause mortality (127). TMAO enhances atherosclerosis via vascular inflammation, monocyte endothelial cell interactions and endothelial dysfunction as well as stimulating foam cell formation and atherosclerotic plaque progression (92, 130, 142). TMAO has been shown to activate inflammatory pathways within the vasculature, leading to leukocyte activation and atherosclerosis (130). TMAO levels were also elevated in individuals presenting with ACS and showed a dose-dependent relationship between increased risk of major adverse cardiac events and long-term mortality (84). TMAO has also been shown to play a role in promoting platelet hyperresponsiveness and thrombosis potential (181). TMAO acts to promote platelet reactivity through stimulus dependent calcium signalling, thereby increasing the risk of incident thrombotic events (181, 182). $ApoE^{-/-}$ mice with TMAO supplemented in their diet had enhanced aortic lesions (165). Human studies have also reinforced the role of TMAO in CVD. In a prospective, observational study of heart failure, stable CAD and healthy subjects, plasma TMAO, choline and betaine were elevated in chronic HF patients compared to controls (156). Similarly, in myocardial infarction patients, TMAO independently predicted death at 2 years [292 events, hazard ratio 1.21 (95% CI, 1.03-1.43, P = 0.023)] (143). A study by Tang and colleagues revealed a correlation between elevated TMAO levels and an increased risk of major adverse cardiac events (148).

Bile Acid

BAs are synthesised from cholesterol in the liver in a multistep processes involving several enzymes and are vital in the absorption of dietary fats in the intestine (78).
Primary BAs are secreted into the duodenum and play a role in emulsifying lipid-soluble dietary substances and vitamins to facilitate digestion and absorption (176). Other features of BAs include their strong microbial activity (176) and their function as signalling molecules, acting as ligands for nuclear receptors, thus effecting metabolism. An example is the activation of the 'Farnesoid-X-receptor' (FXR), which supresses the action of cholesterol 7 alpha-hydroxylase enzyme, which is important in the formation of primary BAs from its cholesterol precursor (78).

In the intestine, gut microbiota modify primary BAs via bacterial salt hydrolase activity, which remove the -OH groups, turning them into secondary BAs (167). Primary BAs are

toxic to bacteria, hence making the BA more insoluble provide bacteria with a mechanism to reduce toxicity. Further modification of BAs by the gut microbiota may occur before being returned to the liver for re-conjugation and return to circulation (122). BAs are a vital pathway for cholesterol elimination via excretion in faeces, thereby decreasing circulating cholesterol levels and risk of plaque build-up (78). The gut microbiome can decrease BA synthesis rate, thus increasing the plasma levels of LDL and increasing the risk of atherosclerosis (126).

Short chain fatty acid

Short chain fatty acids such as acetate, butyrate and propionate are produced as a result of the fermentation of indigestible dietary fibres in the gut by the microbiota (47). They are straight chain saturated fatty acids composed of less than six carbon atoms (13). SCFA can either be absorbed via the gut epithelium to participate in various physiological processes or excreted in faeces (140). Butyrate is usually the primary source of energy for colonocytes (59), however SCFA may also have an anti-inflammatory role as well as effecting various other metabolic pathways including lipid metabolism and gluconeogenesis (13). Other SCFA such as acetate and propionate enter the portal circulation and are metabolised by the liver, or released into systemic circulation where they are able to bind to vascular endothelium SCFA receptors to influence cardiometabolic health (35). To prevent high concentrations of SCFA in blood, butyrate and propionate are cleared through the portal circulation (15). Increased faecal SCFA levels have been shown to be associated with decreased gut microbiota diversity, higher gut permeability, systemic inflammation, plasma glucose levels, dyslipidaemia, obesity and hypertension even after adjustment for confounders including diet, total calories and physical activity (33). Although they have very important regulatory roles, they also have quantitative roles as catabolic or anabolic substrates for the formation of glucose, cholesterol and lipid metabolism (35).

SCFAs have been shown to exert a positive effect on modulating appetite, obesity and colonic inflammation (34, 49, 51). Despite this, elevated faecal SCFA have been associated with the metabolic syndrome, a risk factor for CVD (46, 151). This was demonstrated in TLR5 knock out mice, where microbiota dysbiosis generated uncontrolled prolonged production of SCFA and promoted development of the metabolic syndrome (136). TLR5 is a flagellin receptor required for gut microbiota homeostasis. In a study where rats were fed a

high fat diet, there was a positive correlation between obesity and the altered abundances of *Phascolarctobacterium, Proteus mirabilis* and *Veillonellaceae,* producers of propionate and acetate (79). Dysregulation of SCFA production has recently been suggested to also be a trigger in the aetiology of diabetes in two independent human studies (125, 154). Since SCFA energy is harvested from indigestible fibre, and they can enter the tricarboxylic acid cycle (which also regulates lipogenesis), in hypercaloric diets, an overproduction of SCFAs could result in an undesirable effect.

Gut dysbiosis – links to inflammation and dyslipidaemia

Atherosclerosis is considered a state of chronic inflammation coupled with lipid accumulation in the vessel walls. Systemic activation of the innate immune response as well as within and around the atherosclerotic plaque may enhance plaque progression and rupture. The gut microbiome enhances low grade inflammation in the gut, allowing entry of bacteria and their products into the circulation, triggering chronic inflammation (75). Associations between markers of systemic inflammation including C-reactive protein, interleukin-6 and 8 and tumour necrosis factor-α have been described in humans previously (19). Immune cells within coronary and carotid plaques of patients with ACS, have been shown to produce antibodies against gut microbial produced antigens (*Klebsiella* and *Proteus* strains) (22). Atherosclerotic plaques have their own microbial communities, similar in makeup to the oral cavity and gut (66, 77). However, a recent study has reported no association between atherosclerotic plaque microbial composition and clinical characteristics, contrasting with previous studies (86). These differences in results may be due to several reasons including differing methodologies, differences in population studied as well as population size and diet. As such, further research is highly warranted in this field. Hyperlipidemic $ApoE^{-/-}$ knockout mice exposed to nasal vaccination of outer membrane protein of *Porphyromonas gingivalis*, a gram negative oral cavity bacteria, had reduced atherosclerotic plaques and lower circulating levels of inflammatory cytokines (124).

Dyslipidaemia refers to defects in lipid metabolism, where there is an elevation of plasma cholesterol, low-density lipoprotein-c, triglycerides or both, or a reduction of high-density lipoprotein cholesterol-c levels (97). Lipid levels are in part influenced by genetics, however, are also affected by dietary fat intake, exercise, smoking and alcohol consumption. There is extensive evidence from animal studies, clinical trials and observational epidemiological

studies that outline the causal role of LDL-C in atherosclerosis. Reduction of LDL-C lowers relative risk of major cardiovascular events (116) while HLD-C is inversely associated with risk of coronary heart disease (37). Additionally, the gut microbiome has been positively correlated with lipid and fatty acid metabolism and insulin resistance (14). The human gut is incapable of digesting complex carbohydrates in the form of dietary fibre, thus, it is the specific anaerobic gut bacteria that take on this role via fermentation, to produce SCFA. SCFA are involved in host energy metabolism, regulate intestinal immunity as well as influencing lipid and cholesterol metabolism (36). Host energy homeostasis is mediated by ligand/SCFA interaction with G protein-coupled receptors such as GPR41, expressed primarily in adipose tissue and GPR43, highest levels found in immune cells (17). GPR41 is activated by propionate followed by butyrate and acetate while GPR43 is activated by all three SCFAs at a similar rate (17). Both GPR41 and GPR43 proteins have been found in human colonic tissue (150), white adipose tissue (4), liver and skeletal muscle (17), suggesting that SCFAs may also have an effect on energy metabolism in peripheral tissues. Peptide YY, a gut hormone released postprandially in proportion to the calorie content of a meal and glucagon-like peptide-1 found on beta cells of the pancreas also maintain host energy balance, mainly by appetite reduction and promoting insulin secretion respectively. (9). Thus, it is expected that SCFA overproduction and/or different SCFA profiles can have an impact in metabolic homeostasis in the host (129).

SCFA are also able to mediate fatty acid synthesis and oxidation and lipolysis via peroxisome proliferator-activated receptors, which have roles in carbohydrate, protein and lipid metabolism and tumorigenesis (23). As a result, there is an increase in lipolysis and beta oxidation, thus reducing lipid levels and circulating free fatty acids (24, 34). Apart from SCFA, the gut microbiome can influence lipid levels via the actions of conjugated linoleic acids (CLAs) and BA. Conjugated linoleic acids are produced by *Bifidobacteria, Roseburia* and *Lactobacillus* from omega-3 rich dietary sources (106). CLAs influence lipid metabolism by promoting SCFA producing bacteria, creating a cycle of peroxisome proliferator-activated receptor activation (29). BAs modulate hepatic and systemic lipid and glucose metabolism via the FXR (78, 123) or TGR5 (Takeda G protein coupled receptor) (72) receptors. FXR is an important BA receptor, primarily expressed in the liver and intestines, both it and TGR5 plays a major role in carbohydrate and lipid metabolism, specifically, promoting glycogen synthesis and inhibiting gluconeogenesis (28). TGR5 is expressed in brown adipose tissue and muscle, when activated by BAs it promotes glucagon-like peptide-1 release, improving

liver and pancreatic function and enhanced glucose tolerance (152) as well as attenuating diet induced obesity (152).

Microbiota – targeted therapies

In recent years many studies have shown that the composition and diversity of gut microbiota can be beneficially altered by diet, prebiotics, probiotics, and faecal transplantation, to confer benefit to the host.

Dietary intervention

Protein, lipids and carbohydrates are macronutrients which compose the majority of the human diet (117). Dietary habits can influence gut microbiota composition which in turn modulates host health via digestion and absorption of nutrients (127). Microbiota bio-transform dietary substances and synthesise metabolites which travel in the bloodstream to have distal effects on host metabolism and health (31). Reciprocally, diet composition can modulate the composition and function of the gut microbiome (121). Diet provides one of the simplest and easiest ways to modify the microbiome for therapeutic intervention (171). Components such as macronutrients, fibre, polyphenols, prebiotics and probiotics play a crucial role in shaping the gut microbiome, thereby influencing the production and release of gut microbiome metabolites, such as SCFAs (31).

A study linking long term dietary patterns with gut microbial enterotypes demonstrated that intake of animal protein, amino acids and saturated fats were associated with the *Bacteroides* enterotype (171). In contrast, the *Prevotella* enterotype was low in these groups but high for carbohydrates and simple sugars. Vegetarians had an enrichment in the *Prevotella* enterotype. These dietary associations were similar in another study comparing the microbiome of European and Burkina Faso children (32). The microbiome of the European children was dominated by *Bacteroides* associated with the high protein and fat typical of a Western diet. Conversely, the Burkina Faso children's microbiome was the *Prevotella* enterotype as characterised by their typical high carbohydrate and low animal protein diet.

A diet high in vegetables, fruits, grains and legumes and low in red meats and processed carbohydrates – known as the Mediterranean diet, is beneficial in the prevention of CVD (39). This is due to the presence of antioxidants, nitrate and fibre as well as low

saturated/trans fatty acids, sodium and phosphate in this diet. This is likely to cause a reduction in oxidative stress and inflammation, enhanced antioxidant properties and nitric oxide bioavailability and thus modulation of gut microbiota to improve vascular and cardiac function (71). In contrast to the Mediterranean diet, the Western diet, high in saturated fat, is known to increase CVD risk by decreasing gut microbiota diversity and commensal bacteria, such as *Bifidobacterium* (10).

Currently, there is a clear link between the diet and gut microbiome, such that the gut microbiota profile is different between various diets and geographic regions. However, there is still a substantial gap in our understanding of exactly how our diet modulates the gut microbiome and in turn the consequence of the gut microbiota in modulating host health. Further investigations are required as diet provides a low cost and easily manageable strategy in the potential prevention, management and treatment of diseases such as CVD.

Polyphenols

Polyphenols are a large class of aromatic compounds found in plant-based beverages and foods such as apples, berries, citrus, cocoa, tea and coffee (170). There is substantial evidence that a polyphenol rich diet has cardioprotective and anti-diabetic properties (2, 65, 80, 110, 174). Once ingested, polyphenols are broken down, metabolised and absorbed through the role of the gut microbiota (170). Some polyphenols are affected by pH and change in chemical structure thus affecting their bioactivity (45). In addition, many polyphenols undergo hydrolysis via intestinal brush border or microbial enzymes. They may also undergo glucuronidation, methylation and sulfation during intestinal absorption and liver passage (94). There is extensive metabolism of polyphenols by the gut bacteria which will affect their bioavailability and bioactivity (132).

Short term human studies, involving the consumption of tart cherry juice rich in polyphenols, namely anthocyanins and flavonoids, demonstrated the ability of the gut microbiota to ferment polyphenols to influence polyphenol metabolites (98). In the group (n=5) of individuals with a high *Bacteroides* group from low carbohydrate and fibre consumption, drinking tart cherry juice decreased the levels of *Bacteroides* and increased fermentative Firmicutes. In the low *Bacteroides* group (n=5), there was an increase in *Bacteroides* and *Bifidobacterium* (due to polyphenol availability) and a decrease in Firmicutes. Anthocyanins

act as antiplatelet agents in atherosclerosis and CVD prevention, while also inducing nitric oxide formation in the blood vessels, promoting vasodilation (53). Furthermore, individuals consuming a more Western influenced diet, may have a reduced ability to metabolise polyphenols, thus reducing their bioavailability, bioactivity and hence any potential health benefits (98). Similarly, the intake of polyphenols via grape and red wine demonstrated the gut microbiota's ability to promote beneficial microbial communities to enhance host health (104).

Quercetin is a member of the flavonoid family, a subclass of polyphenols and is thought to have antioxidant and anti-inflammatory properties (16, 153). Quercetin administered as a treatment to mice fed a high fat sucrose diet, modified the gut microbiota and attenuated the increase of the Firmicutes/Bacteroidetes ratio (43). Generally, gut microbiota associated with obesity has been associated with a reduced Bacteroidetes to Firmicutes ratio (82, 115). Similarly, quercetin reduced the abundance of *Erysiepelotrichaceae* on a family level and at the genus level *Bacillus*, which have been related to the Western diet and weight gain (158). Furthermore, it significantly altered the composition of several bacterial species, increasing the relative abundances of *Bacteroides vulgatus* and *Akkermansia muciniphila* which have been inversely associated with obesity, while reducing *Eubacterium cylindroides* and *Bilophilia wadsworthia*, associated with diet-induced obesity (12, 43, 160). A metagenomic study in mice that developed obesity via a high fat sucrose diet demonstrated that the microbiome of high fat sucrose diet fed mice was rich in genes which are associated with sugar uptake for intestinal bacteria (158). Moreover, quercetin improved basal insulin levels (43). This suggests a modulating role of quercetin in altering bacterial profiles to attenuate or improve obesity.

Specific dietary polyphenols have been shown to have beneficial effects on vascular function and attenuation of atherosclerosis. Quercetin and its metabolites have been shown to upregulate adenosine monophosphate-activated protein kinase (AMPK) expression (133). AMPK is an enzyme that plays a role in cellular energy homeostasis and fatty acid oxidation. Quercetin and its metabolites have been shown to protect blood vessels against endothelial dysfunction in intact mouse aortic rings (133). Inhibition of AMPK diminished the positive effects. In human aortic endothelial cells, quercetin and its metabolites induced AMPK and endothelial nitric oxide synthase activation, leading to an increase in nitric oxide, a potent vasodilator (133). In another study, quercetin and theaflavin significantly attenuated

550 atherosclerotic lesion size in both the aortic sinus and thoracic aorta of ApoE $^{-/-}$ mice through
551 reduction of inflammation, improving nitric oxide bioavailability and inducing heme
552 oxygenase-1, an enzyme that plays a crucial role in prevention of vascular inflammation (88).
553 In mice fed a high fat diet, quercetin protects against oxidant-induced endothelial dysfunction
554 and ApoE $^{-/-}$ mice against atherosclerosis (134). These results provide further reinforcement
555 and support for the cardioprotective effects of quercetin, a dietary polyphenol.
556
557 Dietary polyphenols are increasingly recognised as a novel strategy in the potential
558 prevention and treatment of several diseases including CVD and type 2 diabetes. This is
559 explained by the bidirectional relationship which exists between polyphenols and the gut
560 microbiota to influence factors such as blood pressure, endothelial function, insulin
561 sensitivity, thereby promoting cardioprotective and antidiabetic effects. Polyphenols
562 supplemented in the diet at high bioavailability, may improve gut microbiota modulation of
563 CVD and obesity. However, it is recognised that more targeted research activities are still
564 required to resolve the remaining unanswered issues.
565
566 ***Prebiotics, probiotics and synbiotics***
567
568 Prebiotics are nondigestible food ingredients which promote gut microbiota composition and
569 activity with positive effects on the host (56). They are usually obtained in the diet from
570 fibre rich foods (63) and commonly screened for their ability to increase bifidobacterial
571 numbers. A high fibre diet has been reported to modify the gut microbiota to increase acetate
572 producing bacteria, which contributed to a reduction in gut microbiota dysbiosis and offered
573 cardioprotective effects (95). Acetate is associated with regulating many pathways and genes
574 involved in CVD, such as transcription factor *Egr1*, a regulator of CVD through
575 inflammation, cardiac fibrosis and hypertrophy (95). In contrast, mice fed an atherogenic
576 Paigen diet (mimics inflammation in lean subjects) high in saturated fat and low in fibre, led
577 to the development of the metabolic syndrome including hyperinsulinemia, steatohepatitis,
578 hyperglycaemia and inflammatory infiltration into the aorta, contributing to the development
579 of atherosclerosis via the gut microbiota (112).
580
581 Dietary supplementation with an inulin/fructooligosaccharides (FOS) mixture (10 g/day)
582 stimulated Bifidobacterium increase (114) while another study demonstrated the increase in
583 Bifidobacterium following FOS supplementation (149). Beta glucan is another prebiotic

584 shown to have an influence on lowering cholesterol levels and maintaining blood glucose
585 homeostasis. A study where beta glucan was administered as a dietary intervention over a
586 period of 2 months demonstrated a significant decrease in low density lipoprotein and total
587 cholesterol levels as well as improving endothelial function in healthy individuals thus
588 providing cardioprotective effects, mainly through beneficial SCFA production via the gut
589 microbiome (63). In a recent study involving C57BL6 mice, we demonstrated in a long term
590 dietary supplementation with a combination of isoquercetin, a polyphenol and inulin, a
591 prebiotic, that the development of metabolic syndrome was prevented (144). Compared to
592 mice fed a high fat diet, the mice supplemented with isoquercetin and inulin had attenuated
593 weight gain, improved glucose tolerance, insulin sensitivity, as well as improvements in the
594 gut microbiota composition and functionality and SCFA production (144). Inulin was found
595 to increase faecal SCFA levels of acetate, butyrate and propionate. Animal studies have also
596 demonstrated arabinoxylans as a potential prebiotic due to its role in promoting
597 bifidobacterial and propionate synthesis with beneficial effects on lipid and cholesterol
598 accumulation (105, 159). In humans arabinoxylanoligosaccharides supplemented in the diet
599 increased bacterial populations and butyrate concentrations in faeces (162). Taken together,
600 these findings reinforce the importance of diet in the prevention of CVD and the metabolic
601 syndrome.
602
603 Live bacteria, known as probiotics, can be ingested for maintaining or promoting gut
604 microbiota composition (146). Most probiotic products are likely to contain beneficial
605 *Bifidobacteria, Lactobacilli*, *Lactococci* and *Streptococci* (7). Probiotics depending on their
606 nature, may inhibit the overgrowth of pathogenic bacteria, stimulates the immune system,
607 modulates pH levels and prevent inflammation (177). Probiotics can improve host
608 metabolism by stimulating digestive enzyme activity and inhibiting bacterial enzyme activity
609 and production of ammonia. *Lactobacillus* and *Bifidobacterium* are beneficial as they
610 improve intestinal barrier function and play a protective role in inflammatory disease (163,
611 172) by modulating inflammatory and proinflammatory cytokines (such as tumor necrosis
612 factor-α), which may delay and/or improve CVD (62). Probiotics are designed to exert their
613 beneficial effects by restoring the normal composition of microbiota by producing SCFAs
614 (27). *Akkermansia muciniphila* is also known for its probiotic properties and has been
615 demonstrated to be linked with glucose, insulin and leptin which are involved in lipid and
616 glucose metabolism (128). It is also an important modulator of mucus thickness and gut
617 barrier integrity probably due to its close proximity in the mucus layer and closeness to

epithelial cells (108). In a randomized double-blind clinical trial, *Lactobacillus plantarum*, a probiotic, significantly reduced LDL-C and total cholesterol levels and inhibited atherosclerotic plaque formation in hypercholesterolaemic patients (50).

The combination of prebiotics with probiotics for use is known as synbiotics. Synbiotics may further support the growth, survival and persistence of beneficial bacteria as a specific substrate is provided for fermentation by the bacteria (141). Several studies have demonstrated the potential benefits of synbiotics on the gut microbiota and immune functions in chickens (38, 101). In high fat diet mice, symbiotic intervention significantly reduced body weight gain and attenuated features of metabolic syndrome as well as restoring the gut microbial ecosystem structure and function(69).

The studies discussed above have predominately investigated the effects of prebiotics, probiotics and synbiotics on risk factors for cardiovascular disease, such as inflammation, hypertension and effects on glucose and lipid metabolism, as opposed to investigating the direct benefit in atherosclerosis. However, given their beneficial effects on various CVD risk factors, further research investigating how these treatments affect development and progression of atherosclerosis is warranted.

Selective TMAO inhibitors

A recent study was successful in developing a mechanism- based inhibitor drug (3,3-dimethyl-1-butanol), targeting major microbial TMA generating enzymes (CutC/D) where a single oral dose caused a significant reduction in plasma TMAO levels for up to three days as well as reducing enhanced platelet responsiveness and thrombus formation induced by diet (118). This inhibitor is potent, time dependant, irreversible and did not affect other commensals' normal functioning or observable toxicity. When dimethyl-1-butanol was given to atherosclerotic prone ApoE $^{-/-}$ mice on a choline- supplemented diet, plasma TMAO levels significantly reduced, macrophage cholesterol accumulation, foam cell formation and atherosclerotic lesion development were also reduced (166). Microbial protein pairs (CutC and CutD), encoded by genes of the choline utilisation gene cluster (*cut*) have been shown to support choline TMA lyase enzyme activity (96, 119). Current antiplatelet drugs and medication target mammalian enzymes and receptors and have the potential to cause bleeding (52). In contrast, inhibiting microbial generation of TMAO would not supress platelet

function and therefore, increase the risks of bleeding. This study (118) suggests the selective and nonlethal targeting of specific gut microbiome enzymes which are shown to be linked to disease, thus, limiting systemic exposure of the inhibitor in the host. There is growing evidence of the contributory role of the gut microbiota in health and disease, thus 'medicating the microbiome' is at the forefront of clinical potential and could become a common therapy in the future. The development of drugs which inhibit selective gut microbial catabolic pathways mechanistically linked with pathogenesis of disease should be further investigated as a potential therapy for CVD.

Faecal microbiome transplant

Faecal microbiome transplantation refers to the transfer of one's own or a donor's faecal sample to a receiver in order to restore gut microbiome dysbiosis and function (146). With more than 70% of gut bacteria colonising the large intestine, they are commonly represented in a stool sample (127). Currently, faecal transplantation is used for treating gastrointestinal disease, such as *Clostridium difficile* infection and inflammatory bowel diseases such as ulcerative colitis (146, 164). Several studies investigated faecal transplantation as a prospect in altering the gut microbiome as a potential therapy for metabolic syndrome and obesity (8, 157). A study involving gut microbial infusion via a gastroduodenal tube in 9 obese and 9 healthy Caucasian males revealed peripheral insulin sensitivity improved after 6 weeks post allogenic gut microbiota infusion (from lean donors) (161). In obese subjects there was a lower microbial diversity in faecal microbiota, with high levels of *Bacteroidetes* and reduced *Clostridium* levels compared to healthy controls. Upon allogenic gut microbial infusion, gut microbiota diversity significantly increased in the obese group, including butyrate producing bacteria *Roseburia intestinalis* which increased 2.5-fold. This suggests a regulating role of butyrate derived from gut microbiota metabolism causing an improvement in insulin sensitivity in obese Caucasian males. Butyrate has been suggested to prevent translocation of endotoxins derived from the gut microbiota, by promoting intestinal mucosa barrier function (25, 81), which has been associated with insulin resistance (25). Increased gut microbiota diversity has also been linked with improved insulin resistance (25). Recently, faecal transplantation from hypertensive mice to germ free mice have demonstrated that high blood pressure is transferrable (1, 70).

There is currently limited evidence on the role of faecal microbiome transplantation in relation to gut microbiota in human patients with CVD, prompting further research in this area in the future. There are also many methodologies and variation in processing, such as, donor choice and testing, faecal microbiome transplantation via upper gastrointestinal tract, enema or colonoscopy, short and long-term monitoring of patients for adverse effects as well as efficacy of treatment (21). These variations mean that faecal microbiome transplantation needs to be regulated before being offered as a suitable treatment option. The clinical use and consequence of faecal transplantation is further limited by news from a recent case involving the severe sepsis and death of two immunocompromised adults after receiving faecal transplantation containing multi-drug resistant organisms (as reported by the FDA). As such, the future use of faecal microbiome transplantation remains challenging.

Future clinical impact of the gut microbiome on CVD

There is mounting evidence that the gut microbiome contributes to both normal health and disease in the host. This provides a novel perspective with far reaching clinical impact. The approach to disease diagnosis, management, treatment and prevention is expanding. Metagenomic sequencing and analysis along with molecular and biochemical methodologies has advanced, allowing identification and characterisation of specific microbiome taxa and their associated metabolites in various samples. Although, on a mechanistic level, much remains to be elucidated. In the future, microbiome profiling of patients through metabolomic /biomarker analysis may be performed to determine the health of the individual, with possible guidance on dietary and lifestyle changes as an approach to targeted intervention. Furthermore, the use of faecal microbiome transplantation, prebiotics and probiotics to manipulate and alter the gut microbial ecology to produce beneficial metabolites to improve host health may provide an alternate adjunct therapy.

Conclusion

Microbiome-host interactions are now understood to be paramount in health and disease susceptibility. There is a mechanistic link between gut microbiota and physiological processes in the body that affect CVD risk. Furthermore, the gut microbiome is a key player at the intersection of diet and CVD via its role in metabolising dietary components and thus, the synthesis and release of important metabolites such as SCFA and TMAO. Current

literature provides evidence that there is a correlation between atherosclerosis and gut microbiome dysbiosis (Figure 4). This change in gut microbiome may contribute to adverse cardiac function resulting in an increase in morbidity and even death. However, it is not clear whether gut microbiome dysbiosis is a contributor and/or consequence of disease. Further research is required in order to characterise the microbiota in healthy individuals and those with CVD. Research into the roles of various metabolites such as SCFA, TMAO and BA and their mechanism of action is also highly warranted as they have been demonstrated to have a vital role in the modulation and influence of host health. As we gain a more comprehensive understanding of the role of the gut microbiota and their metabolites and exactly how they cause the progression and pathogenesis of disease, we may be able to develop advanced diagnostic strategies and potential therapeutic interventions to cater to a more personalised clinical care of patients in the future. Novel therapies including manipulating the diet and using newly designed prebiotic and probiotic options to treat CVD through modulating the gut microbiota and associated metabolites are all gaining traction.

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Tables, Figures and Figure Legends

Table 1: Summary of studies investigating associations between the gut microbiome and CVD.

Reference	Method	Number of subjects	Disease status	Main findings
(121)	16S rRNA & metagenome sequencing	N =1046	Plasma lipids, Glycaemic traits	Microbiota predominantly shaped by non-genetic factors and explain ≥ % variance in HDL, fasting glucose, obesity
(64)	Metagenome shotgun sequencing	N =218 CVD N =187 controls	Atherosclerosis	In CVD, increased *Enterobacteriaceae & Streptococcus*
(83)	16S rRNA	N =56 pre-hypertensive N =99 hypertensive N =41 controls	Hypertension	Reduced bacterial diversity Overgrowth of *Prevotella/Klebsiella* in prehypertension and hypertension compared to controls
(61)	qPCR	N =179 CAD N =166 ACS N =119 controls	CAD	*Aggregatibacter actinomycetemcomitans* levels linked with risk for CAD
(68)	MEDUSA	N =12 symptomatic atherosclerosis	Atherosclerosis	*Collinsella* genus higher in symptomatic AS

		N=13 controls		*Eubacterium* & *Roseburia* higher in controls
(77)	qPCR 16S rRNA	N =15 CVD N =15 controls	Atherosclerosis	Overgrowth of *Veillonella* and *Streptococcus* in atherosclerotic plaque samples

16S rRNA: 16S ribosomal ribonucleic acid, HDL: High density lipoprotein, CVD: cardiovascular disease, qPCR: quantitative polymerase chain reaction, CAD: coronary artery disease, ACS: acute coronary syndrome, MEDUSA: Metagenomic Data Utilization and Analysis.

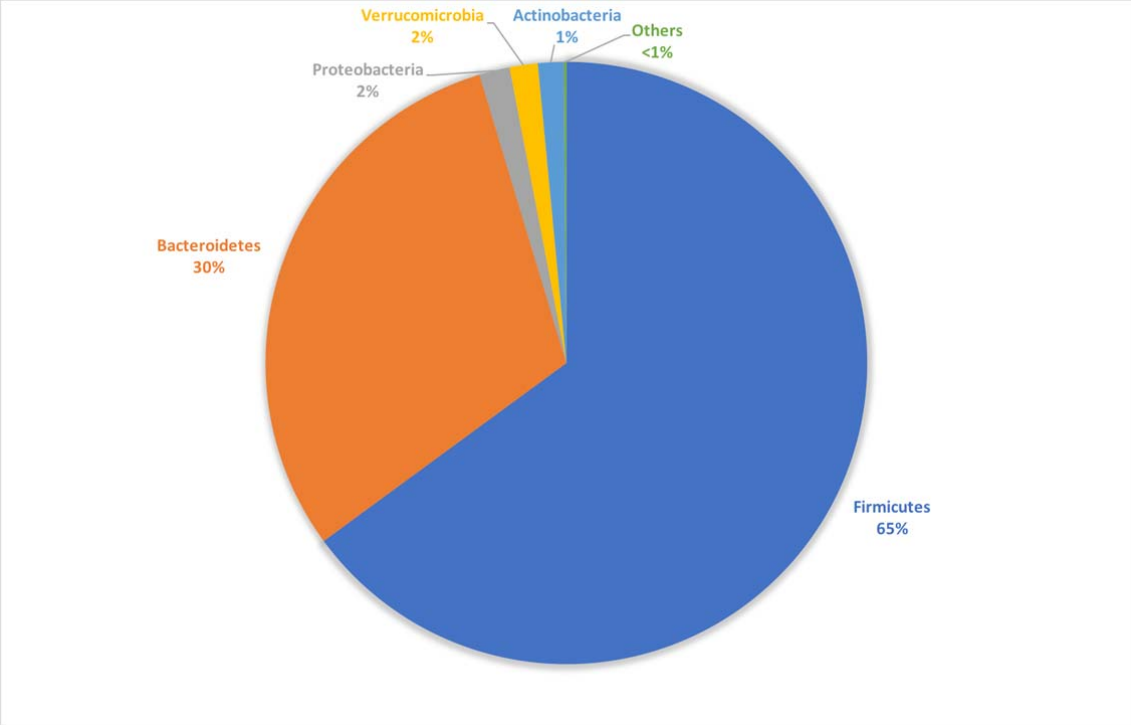
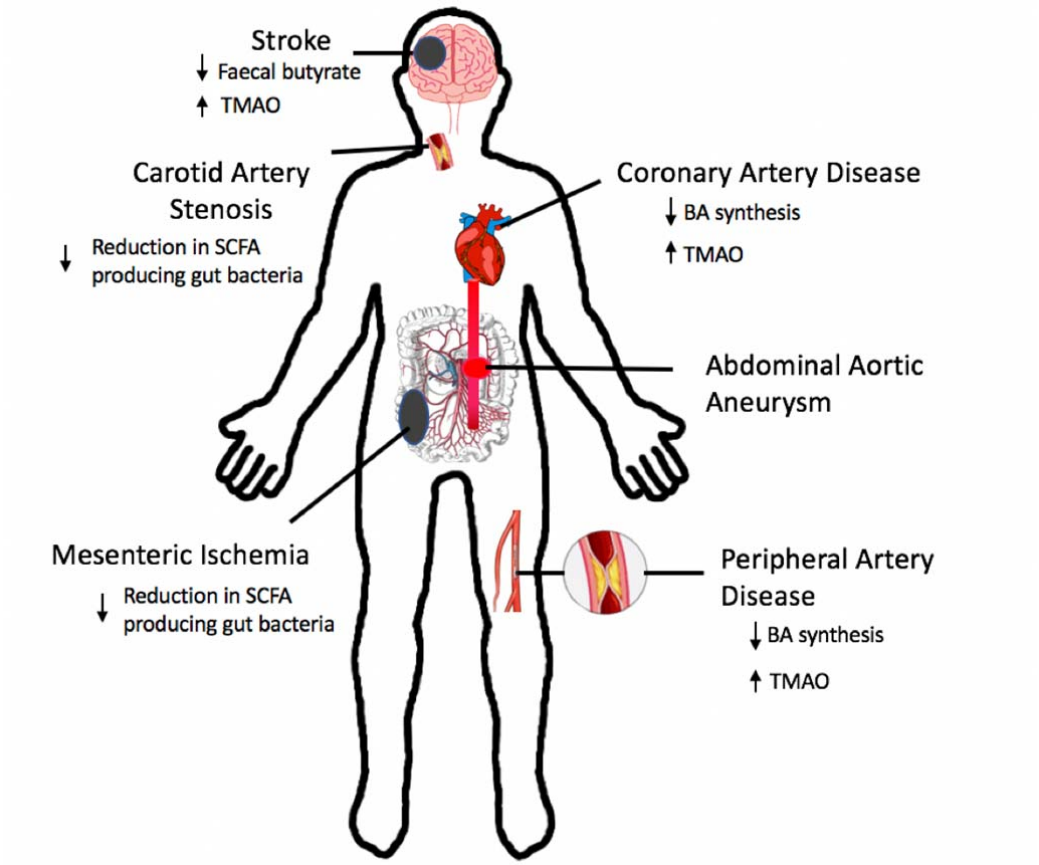


Figure 1: Relative distribution of the five main bacterial phyla found in the gut obtained from the RDP (ribosomal database project) browser. Firmicutes and Bacteroidetes compose 65% and 30% of the gut respectively. *Others* include the remaining phyla: Spirochaetes, Fusobacteria, Deferribacteres, Cyanobacteria, Planctomycetes, Lentisphaerae, TM7 and Tenericutes. Adapted from (175).

1273



1274

1275 **Figure 2:** Cardiovascular disease manifestations in which gut microbiota and their associated
1276 metabolites are altered. BA: bile acid, SCFA: short chain fatty acid, TMAO: Trimethylamine-
1277 N- oxide.

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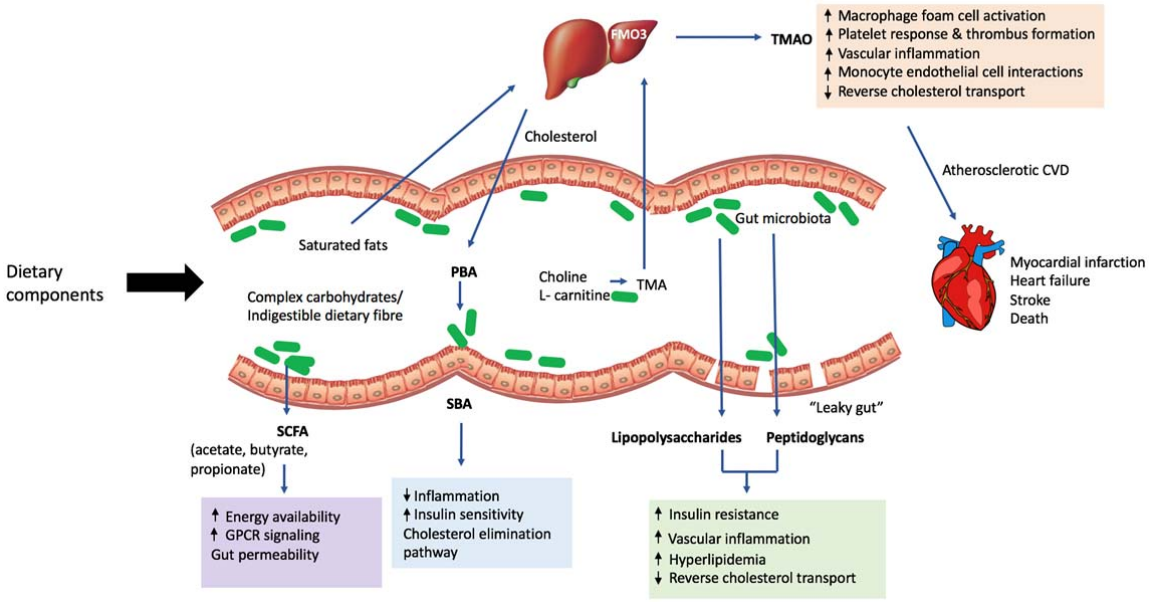

Figure 3: Interactions between diet and the gut microbiota to form metabolites such as SCFA and TMAO, which play a crucial role in host health and cardiovascular risk. CVD: cardiovascular disease, FMO3: Flavin monooxygenase 3, GPCR: G protein coupled receptor, PBA: primary bile acid, SBA: secondary bile acid, SCFA: short chain fatty acid, TMA: Trimethylamine, TMAO: Trimethylamine- N- oxide.

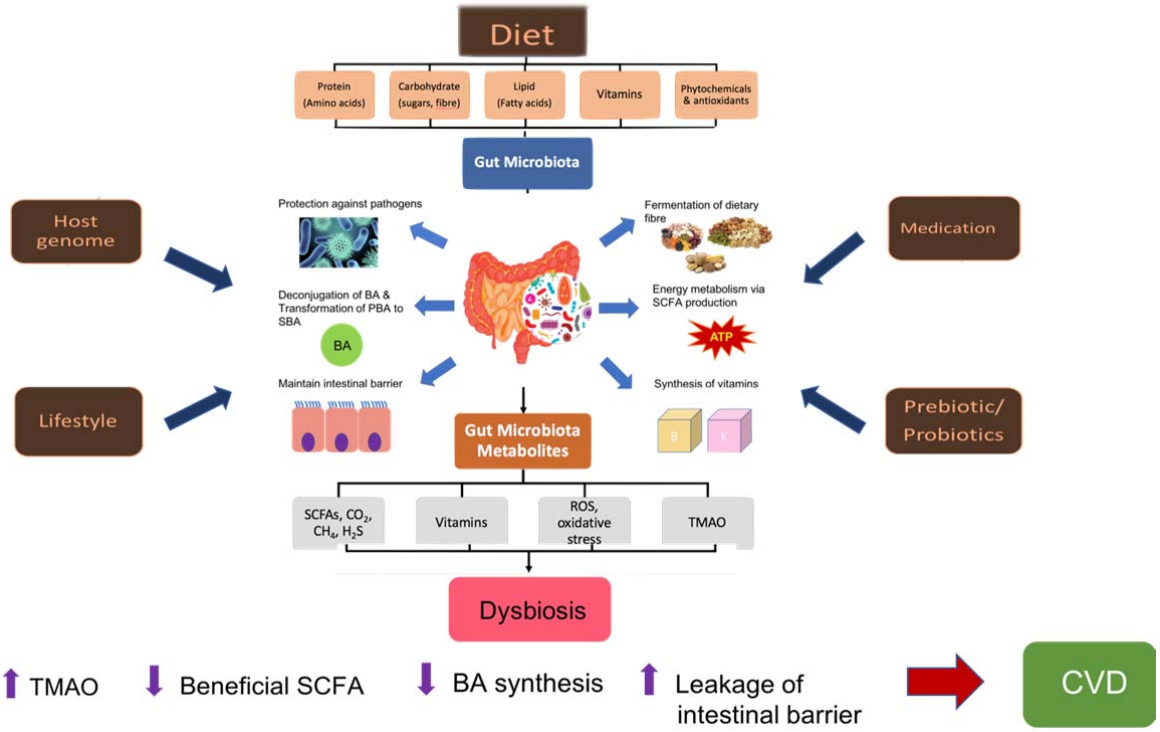

Figure 4: The complex relationship between dietary components ingested and other factors, affecting the gut microbiota, whose composition then influences their functionality and metabolite production and release. A disruption to which leads to dysbiosis, thereby affecting host health and the progression and pathogenesis of various cardiovascular diseases in a vicious cycle. ATP: Adenosine triphosphate, BA: bile acid, CO_2: carbon dioxide, CH_4: methane, CVD: cardiovascular disease, H_2S: hydrogen sulphide, PBA: primary bile acid, ROS: reactive oxygen species, SBA: secondary bile acid, SCFA: short chain fatty acid, TMAO: Trimethylamine- N- oxide.

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(60)	Metagenome shotgun sequencing	N =218 CVD N =187 controls	Atherosclerosis	In CVD, increased *Enterobacteriaceae* & *Streptococcus*
(79)	16S rRNA	N =56 pre-hypertensive N =99 hypertensive N =41 controls	Hypertension	Reduced bacterial diversity Overgrowth of *Prevotella/Klebsiella* in prehypertension and hypertension compared to controls
(57)	qPCR	N =179 CAD N =166 ACS N =119 controls	CAD	*Aggregatibacter actinomycetemcomitans* levels linked with risk for CAD
(64)	MEDUSA	N =12 symptomatic atherosclerosis	Atherosclerosis	*Collinsella* genus higher in symptomatic AS

		N=13 controls		*Eubacterium* & *Roseburia* higher in controls
(73)	qPCR 16S rRNA	N =15 CVD N =15 controls	Atherosclerosis	Overgrowth of *Veillonella* and *Streptococcus* in atherosclerotic plaque samples

16S rRNA: 16S ribosomal ribonucleic acid, HDL: High density lipoprotein, CVD: cardiovascular disease, qPCR: quantitative polymerase chain reaction, CAD: coronary artery disease, ACS: acute coronary syndrome, MEDUSA: Metagenomic Data Utilization and Analysis.

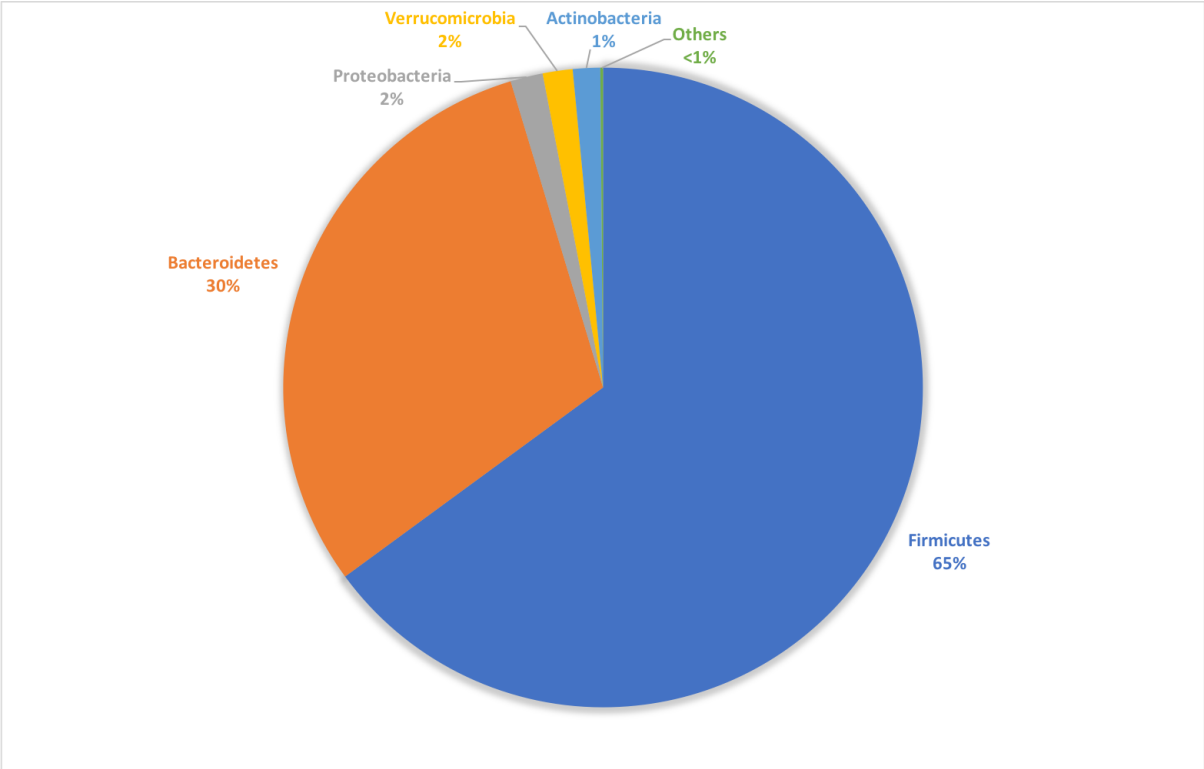

Figure 1: Relative distribution of the five main bacterial phyla found in the gut obtained from the RDP (ribosomal database project) browser. Firmicutes and Bacteroidetes compose 65% and 30% of the gut respectively. *Others* include the remaining phyla: Spirochaetes, Fusobacteria, Deferribacteres, Cyanobacteria, Planctomycetes, Lentisphaerae, TM7 and Tenericutes. Adapted from (170).

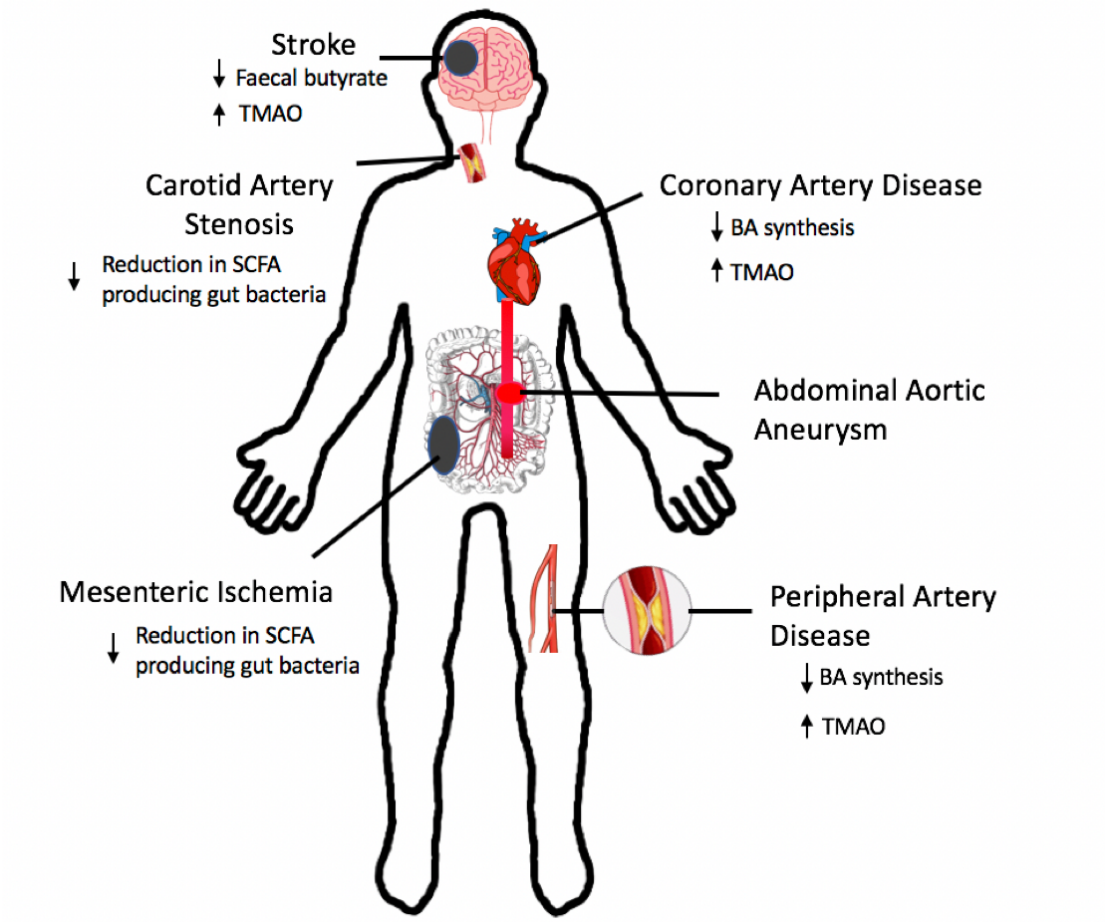

Figure 2: Cardiovascular disease manifestations in which gut microbiota and their associated metabolites are altered. BA: bile acid, SCFA: short chain fatty acid, TMAO: Trimethylamine-N- oxide.

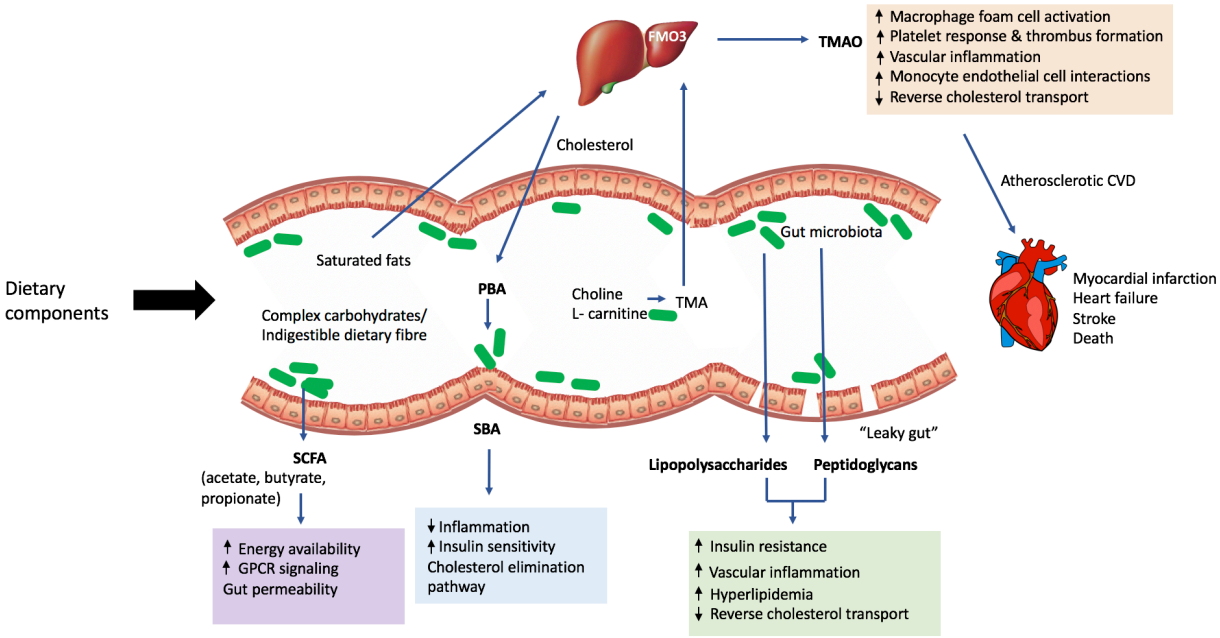

Figure 3: Interactions between diet and the gut microbiota to form metabolites such as SCFA and TMAO, which play a crucial role in host health and cardiovascular risk. CVD: cardiovascular disease, FMO3: Flavin monooxygenase 3, GPCR: G protein coupled receptor, PBA: primary bile acid, SBA: secondary bile acid, SCFA: short chain fatty acid, TMA: Trimethylamine, TMAO: Trimethylamine- N- oxide.

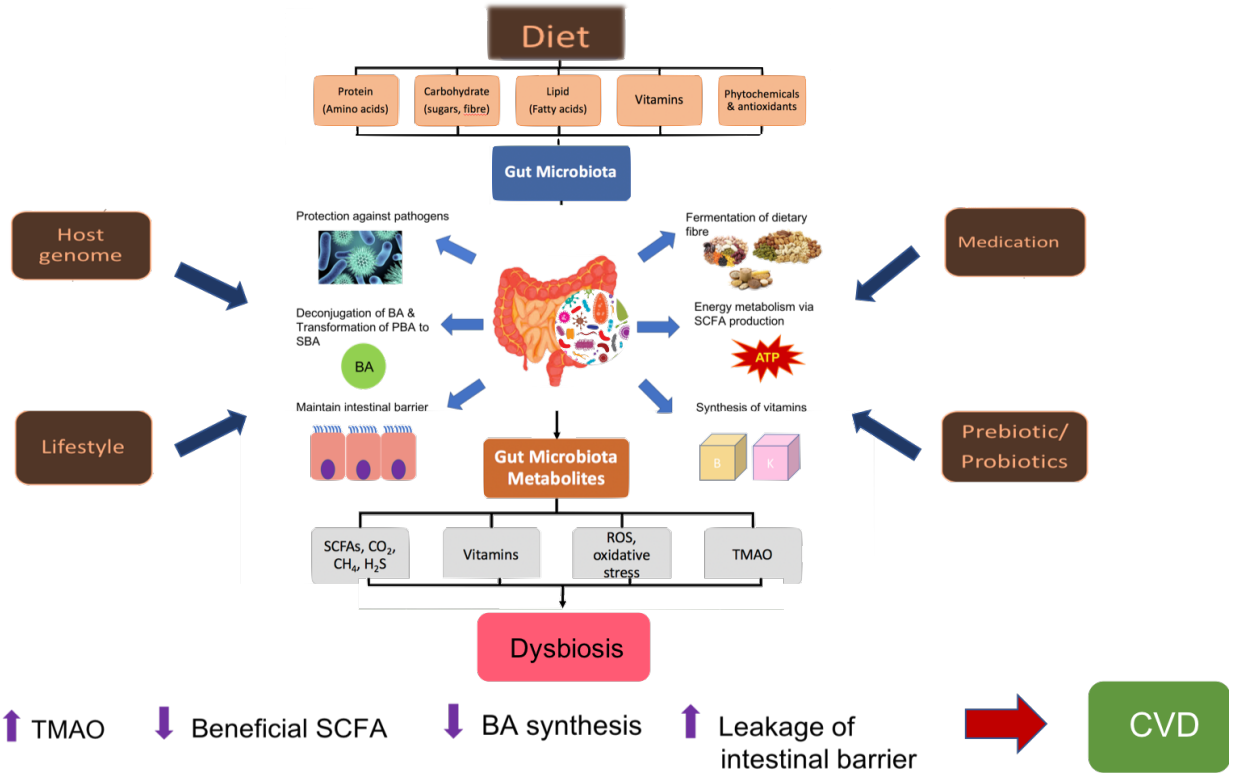

Figure 4: The complex relationship between dietary components ingested and other factors, affecting the gut microbiota, whose composition then influences their functionality and metabolite production and release. A disruption to which leads to dysbiosis, thereby affecting host health and the progression and pathogenesis of various cardiovascular diseases in a vicious cycle. ATP: Adenosine triphosphate, BA: bile acid, CO_2: carbon dioxide, CH_4: methane, CVD: cardiovascular disease, H_2S: hydrogen sulphide, PBA: primary bile acid, ROS: reactive oxygen species, SBA: secondary bile acid, SCFA: short chain fatty acid, TMAO: Trimethylamine- N- oxide.