

Role of gut microbiota and their metabolites on atherosclerosis, hypertension and human blood platelet function: A review

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Abstract

Emerging data have demonstrated a strong association between the gut microbiota and the development of cardiovascular disease (CVD) risk factors such as atherosclerosis, inflammation, obesity, insulin resistance, platelet hyperactivity, and plasma lipid abnormalities. Several studies in humans and animal models have demonstrated an association between gut microbial metabolites, such as trimethylamine-*N*-oxide (TMAO), short-chain fatty acids, and bile acid metabolites, amino acid breakdown products, with CVD. Human blood platelets are a critical contributor to the hemostatic process. Besides, these blood cells play a crucial role in developing atherosclerosis and, finally, contribute to cardiac events. Since the TMAO, and other metabolites of the gut microbiota, are associated with platelet hyperactivity, lipid disorders, and oxidative stress, the diet-gut microbiota interactions have become an important research area in the cardiovascular field. Platelets became hyperactive in people with diabetes mellitus, sedentary lifestyle, obesity, and insulin resistance and exhibited increased sensitivity at a baseline level and in response to agonists, ultimately contributing to increased aggregation plaque development. In addition to these factors, TMAO also contributes to platelet hyperactivity. Several approaches are now suggested to reduce plasma TMAO levels, such as microbiota modulation using probiotics, prebiotics, and oral broad-spectrum antibiotics. This review describes the association between microbiota-derived metabolites and CVD development.

Keywords: Platelet, Microbiota, CVD, TMAO, TMA, Platelet hyperactivity, GPIIb-IIIa, Atherosclerosis, Hypertension, Polyphenols, Short-chain fatty acids

Abbreviations used: Cardiovascular disease, CVD; Trimethylamine-*N*-oxide, TMO; Glycoprotein IIb/IIIa, GPIIb/IIIa; von Willebrand factor, vWF; Tissue factor, TF; P-Selectin, CD62P; Short-chain fatty acids, SCFA, Peptidoglycan, PG

Introduction

Cardiovascular disease (CVD) is the single most contributor to global mortality[1]. CVD encompasses multiple disorders, including atherosclerosis, hypertension, platelet hyperactivity, stroke, hyperlipidemia, and heart failure[2]. Although genetic and other health conditions are intimately involved, the diet-gut microbiome interactions are increasingly recognized for their contribution to CVD development and progression. Several studies showed an association between gut microbiota and their metabolites with the CVD risk factors such as hyperlipidemia, overweight, inflammation, hypertension, and platelet hyperactivity[3], highlighting the intricate relationship between diet, gut microbiota, and CVD[3,4]. In addition to their roles in hemostasis and thrombosis, hyperactive platelets are also important mediators of atherosclerosis. There is strong evidence of platelet hyperactivity in conditions like diabetes, smoking, sedentary lifestyles, aging, obesity, certain gut metabolites, and an unhealthy diet[5-7].

Within the human body resides trillions of different microbial species, collectively referred to as the human microbiota. The largest microbe population is found in the gut, containing 100 trillion microbes of at least 1,000 different bacterial species. Sufficient data indicates that the gut microbiome regulates numerous physiology, immune system, cardiovascular system, intestinal function, and absorption and metabolism of nutrients and their metabolites. Several studies have implicated gut dysbiosis in CVD pathology, including atherosclerosis, hypertension, platelet hyperactivity, abnormal lipid metabolism, and vascular dysfunction[8]. Gut dysbiosis is an essential factor responsible for the critical CVD risk factors such as atherosclerosis, hypertension, platelet hyperactivity [9].

Emerging evidence suggests that targeting the gut microbiota and their metabolites can be an effective strategy in the treatment and prevention of CVD[9-11]. Numerous metabolites are produced by different gut microbiota species, depending on the diet and the microbiome

composition, that affect human health. Among the metabolites of the gut microbiota, short-chain fatty acids (SCFAs), secondary metabolites of bile acid, and trimethyl-N-oxide (TMAO) are important modulatory factors for various diseases. Plasma levels of TMAO significantly contribute to platelet hyperactivity, abnormal plasma lipids, obesity, and insulin resistance [3,8]. TMAO increases CVD risk factors by altering cholesterol and bile acid metabolism, activating inflammatory pathways, promoting foam cell formation, and platelet hyperactivation. Whereas SCFAs contribute to atherosclerosis and hypertension process by different mechanisms. Thus, it is important to investigate cellular signaling involving the gut microbiota metabolites in physiology and pathological states to understand their roles in humans' health and disease.

This review describes the current evidence linking gut microbiota and their metabolites with various CVD risk factors.

Roles of the gut microbiota in the atherosclerosis process

Inflammation plays a major role in the development of atherosclerosis. The atherosclerosis process involves fibrosis of the intima, the formation of fatty plaque, the proliferation of smooth muscle cells, migration of monocytes and T lymphocytes, hyperactive platelets, and cholesterol accumulation [12]. Emerging data suggested that gut dysbiosis can also contribute to atherosclerosis development by increasing systemic inflammation [13-15].

Inflammation is commonly involved in many diseases, including CVD[12,16,17]. Accumulated evidence indicated that gut microbiota and their metabolites play an important role in systemic inflammation and modulate various CVD risk factors [18].

The gut barrier's integrity is essential for maintaining the host's health and preventing inflammation and atherosclerosis processes. Intestinal permeability is impaired by the reduced expression of tight junction proteins such as zonula occludens-1, claudin-1, occludin, and creating an imbalance between intestinal epithelial cell death and regeneration[19,20]. *Akkermansia muciniphila* exert protective effects against atherosclerosis by improving gut

barrier functions [21]. The gut metagenome analysis showed a relatively lower abundance of *Roseburia* and *Eubacterium*, while *Collinsella* was higher in CVD patients than in healthy individuals [22]. The meta-analysis demonstrated that antibiotic treatment had no significant beneficial effect in CVD [23]; even though the gut microbiota plays a vital role in inflammation and CVD risk factors [18]. The integrity of the gut epithelium protects against the pathogens' invasion in the systemic circulation and consequently immune and inflammatory disorders [24]. When the epithelial integrity is compromised, the invasion of pathogen-associated molecular patterns (PAMPs) leads to an immune response and produce systemic and tissue-specific inflammation. Several PAMPs can stimulate inflammatory processes involving host pattern recognition receptors (PRRs), such as CpG oligodeoxynucleotides flagellin, lipopeptides[25].

Impaired gut barrier integrity induced by gut dysbiosis is a significant risk factor for chronic inflammation observed in several diseases, including atherosclerosis: the microbial component, lipopolysaccharide, one of the PAMPs involved in the development of CVD. The association between lipopolysaccharide and CVD risk was first observed in 1999, determined by the endotoxin levels in the patients [26] and, later, the association was confirmed by several studies[27,28].

The dysbiosis increases the intestinal permeability by suppressing tight junction proteins, allowing the translocation of lipopolysaccharide into the circulation [29,30]. Gut dysbiosis-derived lipopolysaccharide binds Toll-like receptors (TLRs) and activates downstream immune reaction [31]. Lipopolysaccharide binds TLR4 complexed with its co-receptors cluster of differentiation 14 (CD14). The upregulation of TLRs initiates the inflammation-driven atherosclerosis process [32,33]. The interaction between lipopolysaccharide and TLR4 activates MYD88 and NF κ B pathways that lead to an enhanced synthesis of pro-inflammatory cytokines such as IL-6, IL-1, IL-27, and TNF- α . These inflammatory cytokines are involved in atherosclerosis and CVD development [34,35]. Another

bacterial PAMP, peptidoglycan, increases the CVD risk by disturbing the intestinal epithelial barrier. Metagenomic sequencing showed that patients with atherosclerosis had enrichment of genes that encoded peptidoglycan synthesis[22]. The presence of bacterial peptidoglycan was detected in atherosclerotic plaques[36]. The nucleotide-binding oligomerization domain (NOD) proteins, NOD1 and NOD2, drive clearance of the intracellular bacteria or bacterial debris through peptidoglycan recognition by involving NF κ B and MAP Kinase [37]. NOD2 is a critical regulator of bacterial immunity and the barrier integrity of the gut. The compositional changes of the gut microflora can modulate the CVD risk. Despite numerous data demonstrating the pathogenic bacteria's contribution to the development of CVD, antibiotic trials produced mixed results[38].

The gut microbial metabolites such as methylamines, polyamines, short-chain fatty acids (SCFAs), trimethylamine (TMA), and secondary bile acids play important roles in host's physiology. The gut microbial metabolites play significant roles in the development of CVD[39,40]. SCFAs, a group of microbial products (such as propionic acid, acetic acid, and butyric acid) are critically involved in the onset and maintenance of various diseases[41]. A correlation between elevated plasma TMAO levels and atherosclerosis was reported[42-45]. These microbial metabolites' involvement in CVD risks in both human and animal models has been extensively reviewed[46]. **Table-1** shows the effects of microflora on CVD risk factors.

Contribution of the gut microbiota to hypertension

Hypertension is the most prevalent but modifiable risk factor for CVD [47]. The convincing data on the gut microbiota's involvement in metabolic diseases[22,48-50], suggested

Table-1: Impacts of the gut microbiota and metabolites on CVD risk factors

Impact on atherosclerosis	Hypertension	Obesity, type 2 diabetes mellitus
↑Lactobacillus [50] ↓Roseburia [22]	↑Firmicutes / Bacteroides ratio [51]	↑Firmicutes [52] [53-57] Bacteroides [54]
↑TMAO [8,44]	↑SCFA[58]	↑BAs, SCFA, LPS and TMAO[59-63]
Dietary choline/ carnitine ↑TMAO [22] DMB suppress TMA/TMAO [43]. ↑TMAO is associated with unstable plaque and MACE[64-66].	Infusion of AngII/TMAO associated with ↑blood pressure[67]	↑insulin sensitivity related to vancomycin treatment [68] ↑Leptin, GLP1, [69-72] PYY [71,73] ↑TMAO was associated with glycemic control[74].
Diet intervention: ↑Bacteroides and Proteobacteria, ↓Firmicutes[75] Probiotics: ↑LV hypertrophy[76] Probiotics: ↑SCFA[77]	high fiber diet resulted in ↓blood pressure[78].	FMT: improve insulin resistance[68,79] and Roseburia.[53,55] Bariatric surgery changed ↑BAs and GLP-1[80-82] Probiotics: ↑insulin secretion [83] ↓body weight and adipose tissue mass [84-86]

T2DM = type 2 diabetes mellitus, TMAO = trimethylamine N-oxide, SCFA = short chain fatty acid, BA = bile acid, LPS = lipopolysaccharide, DMB = 1,3 dimethylbutanol, MACE = major adverse cardiac event, AngII = angiotensin II, LV = left ventricular, GLP-1 = glucagon-like peptide 1, PYY = peptide YY, FMT = fecal microbiota transplantation. Upward ↑ denotes increase, Downward ↓ denotes decrease .

the association between gut microbiota and hypertension [87]. Recently, studies have found an association of gut microbiota with hypertension. The animal studies demonstrated that germ-free rats had elevated blood pressure, underscoring gut microbiota role in blood pressure regulation.

Generally, blood pressure is regulated by the amplitude of vasoconstriction and vasodilation of blood vessels [88]. In spontaneously hypertensive rats, a significant decrease in

the composition of microflora in the gut was reported, associated with an increase in the ratio of *Firmicutes/Bacteroidetes*[51]. Infusion of angiotensin II(AngII) attenuated the blood pressure increase in germ-free mice compared with conventionally raised mice, indicating that gut microbiota involves blood pressure regulation [89]. The gut microbiota is probably engaged in developing hypertension, though the mechanism is not yet fully elucidated. The gut microbial metabolite, SCFAs, and its effect on ox-LDL levels and other pathways may develop hypertension.

SCFAs such as acetate, propionate, and butyrate play crucial roles in maintaining the gut microbiome's homeostasis and host immunity[90-92]. Interestingly, some gut bacteria but not all produce SCFAs using polysaccharides as substrate [93]. The predominant acetic acid-producing bacteria are *Streptococcus* spp., *Prevotella* spp., *Bifidobacterium* spp., *Clostridium* pp., *A. muciniphila*, and so on[93] whereas propionic acid is produced by *Bacteroides* spp., *Salmonella* spp., *Dialister* spp., *Veillonella*spp., *Roseburia*inulinivorans, *Coprococcus catus*, *Blautia obeum*.[93]. *Lachnospiraceae*, *Ruminococcaceae*, and *Acidaminococcaceae* families mainly produce butyric acid in the gut [94]. The high abundance of butyrate-producing bacteria is associated with lower blood pressure in pregnant women with obesity[95]. Supplementation of the fiber and acetate improved gut dysbiosis by increasing the abundance of *Bacteroides acidifaciens*, which was shown to play a beneficial role in hypertension in a mice model [78].

The role of various G-protein-coupled receptors (GPRs) in hypertension was reviewed[96]. The gut microbial metabolites, SCFAs, modulate the activity of GPRs, including GPR41, GPR43, and GPR109A[97]. SCFAs, regulate blood pressure by the synthesis of renin of the angiotensin-renin system via GPRs-regulated pathways [98,99]. GPR41 knockout mice had high systolic blood pressure compared with wild-type mice, and that SCFAs lowered blood

pressure by activating endothelial GPR41[100]. SCFAs produce hypotensive effect via vasodilation in mice through modulation of olfactory receptor 78 (Olfr78) activity[97,101]. The antibiotic treatment altered gut microbiota composition resulting in increased blood pressure in Olfr78 knockout mice[99]. Overall, these studies demonstrated that gut microbiota might play significant roles in host blood pressure by SCFAs-mediated mechanisms. However, the potential for SCFAs to use as a therapeutic target needs further in-depth investigations.

Dysbiosis can promote oxidation of LDL to oxLDL[102,103]. Thus, gut dysbiosis also contributes to hypertension through vasoconstriction mediated by oxLDL [104]. The higher levels of oxLDL contribute to hypertension by inhibiting nitric oxide synthesis (NO) and endothelin-1[105]. NO, an important vasodilator, is produced from l-arginine by NO synthase. oxLDL may increase blood pressure by decreasing the production of NO and thus reduces vasodilation[104]. Endothelin-1 plays a crucial role in the maintenance of vascular tension and cardiovascular system homeostasis. Endothelin-1 produces vasodilation at low levels by activating the endothelial receptor B and NO production but induces vasoconstriction at high levels by increasing oxLDL level via activating the endothelial receptor A[106]. However, the association of gut dysbiosis and hypertension [11,107], however, still requires further studies for definitive conclusions. The mechanisms associated with the effects of the gut microbiota in hypertension are depicted in **Figure-1**.

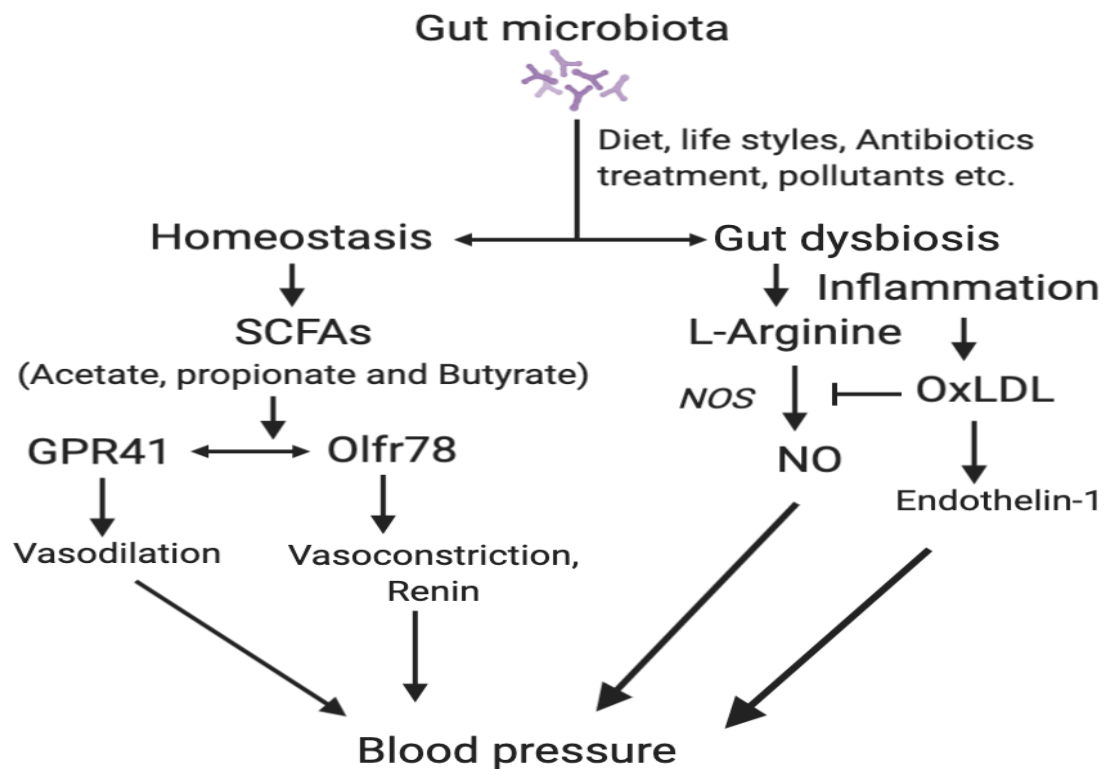


Figure-1: The main mechanisms associated with gut microbiota and hypertension

SCFAs, short-chain fatty acids; GPRs, G-protein-coupled receptors; Olfr78, olfactory receptor 78; NO, nitric oxide; OxLDL, oxidized low-density lipoprotein; ETA, endothelin receptor A. For details, please see the text.

Human blood platelets and their roles in the cardiovascular system

The non-nucleated human blood platelets are produced by cytoplasmic fragmentation of megakaryocytes[108]. The blood platelets are circulated in an inactive state and are activated only when they contact the damaged site of the blood vessel[5,108]. Platelets' main physiological role is to monitor and act on the damaged vessel endothelium and rapidly rush at its damaged site. They initiate the blood coagulation process to stop the bleeding. The fast interactions between activated platelets, their secreted several intracellular components, or thrombin and endothelium at sites of damaged vessels ensure the stable hemostatic plug[5]. Two different

pathways regulate vascular hemostasis and thrombosis, depending on vascular damage or vessel structure. One is the intrinsic pathway mediated by collagen, and the other is the extrinsic pathway, also known as the tissue factor (TF)-factor-VII pathway [109]. The tissue damage or plaque rupture leads to TF release from smooth muscle, adventitial cells, and pericytes. TF complexed with the activated factor VII (VIIa) catalyzes prothrombin conversion to thrombin, fibrin generation, and initiates the blood clotting cascade. Activated platelets also accelerate the prothrombinase complex activity to cleave prothrombin to thrombin.

During hemostasis, damage to the endothelium exposes collagen from the sub-endothelial space. The exposed collagen activates blood platelets. Platelets interact with collagen through their glycoproteins (GP) GPIIb/IIIa and GPIb/IX, and von Willebrand factor (vWF)[110]. Since vWF is an important part of the complex, GPIb, the absence of the vWF prevents hemostasis. Several collagen receptors, such as GPIIb/IIIa and GPIa, are present in the platelet membrane surface [110]. Once the platelets attach with the damaged vessel sites, the autocrine and paracrine mediators' rapid involvement, including ADP, thrombin, epinephrine, and TxA₂, are required [111].

Activated platelets then release granular contents into the extracellular environment, contributing to further platelet activation and engage in thrombus formation[112]. Several intracellular signaling pathways involving collagen, thrombin, TxA₂, and ADP become functional for platelet activation and aggregation. These agonists amplify and sustain the initial platelet aggregation response and recruit additional circulating platelets from the flowing blood for developing the hemostatic plug. Platelets form a three-dimensional structure by binding each other via fibrinogen receptors, activated GPIIb/IIIa complex. Activated platelets aggregate with other circulating platelets by secreting platelet aggregatory/activating agents such as thrombin, ADP, collagen, TxA₂, adrenaline; all these lead to the expression of fibrinogen receptors. TxA₂ mediates expression of platelet membrane surface GPIIb/IIIa complexes

induced by different agonists produced from arachidonic acid, 20:4n-6 (ARA), liberated from platelet membrane phospholipids. Activated platelets release ADP, serotonin, P-selectin, fibrinogen, Ca^{2+} , and TxA_2 , and further amplify platelet activation and thrombus formation[113].

Platelet activation is initiated with the activation of one of the phospholipase C (PLC) isoforms. Different agonists activate different PLC isoforms. Collagen activates $\text{PLC}\gamma_2$, whereas thrombin, ADP, and TxA_2 activate $\text{PLC}\beta$. PLC cleaves the phosphatidylinositol-4,5-bisphosphate to produce inositol-1,4,5-trisphosphate (IP_3), which raises the cytosolic Ca^{2+} levels [114]. The increased level of Ca^{2+} activates integrin and thus initiates the activation of platelets. The rise in the cytosolic Ca^{2+} levels by most platelet-activating agents and critical for platelet activation/aggregation.

Platelets have various functions in physiology. Apart from hemostasis, platelets are involved in several cardiovascular processes, such as atherosclerosis, immune system, inflammation, interaction with other cells, and cardiac events [115-117]. Upon activation, platelets secrete more than hundreds of components from their intracellular stores. Platelet α -granules secrete multiple cytokines, mitogens, other components that contribute to the CVD processes. In addition to the several membrane receptors described above, activated platelet also expresses CD62 (P-selectin) and shed membrane particles[7,108].

The platelet membrane microparticles have multiple functions and contribute to thrombus and foam cell formation; they are involved in atherosclerotic processes, blood vessel activation, and inflammation. Thus, inhibiting platelet activation/aggregation can protect against CVD development that affects millions of people worldwide. Though aspirin is still major anti-platelet therapy, it does not benefit all, as evidenced by the phenomenon of aspirin resistance and unwanted side effects. Therefore, aspirin is not recommended for use in the primary prevention of CVD.

Platelet hyperactivity is an important contributor to the development of CVD

Atherosclerosis is a continuous process promoted by several risk factors, including hypertension, hyperlipidemia, cigarette smoking, diabetes mellitus, dyslipidemia, and platelet hyperactivity. Platelet hyperactivity is a major clinical feature observed in hypertension, diabetes mellitus, obesity, and several other metabolic and vascular diseases [118];[119] . Sufficient evidence indicates that hyperactivity of platelets plays an important role in the development of atherosclerosis and the incidence of CVD [118,120]. Blood platelets play a significant role in the development and progression of atherosclerosis[121]. Platelets' pathophysiologic state is the important underlying CVD risk in diabetes, smoking, obesity, sedentary lifestyle, and other conditions. There is clinical evidence that an increased platelet number, platelet activation, and platelet hyperreactivity are associated with cardiovascular events in acute coronary syndromes patients. Platelets showed increased spontaneous activation in patients with diabetes, and hypertension [122,123], promoting thrombus formation. Platelet hyperactivity is associated with the secretion of different components. The shedding of membrane particles plays a vital role in developing atherosclerosis, blood flow, inflammation, immune response, and hypertension. Platelet membrane proteins such as GPIb α , GPV, GPVI, amyloid β A4, TLT-1 (TREM-like transcript-1), P-selectin (CD40L), amyloid-like protein 2, and semaphorin 4D are the most abundantly shed platelet proteins during activation.

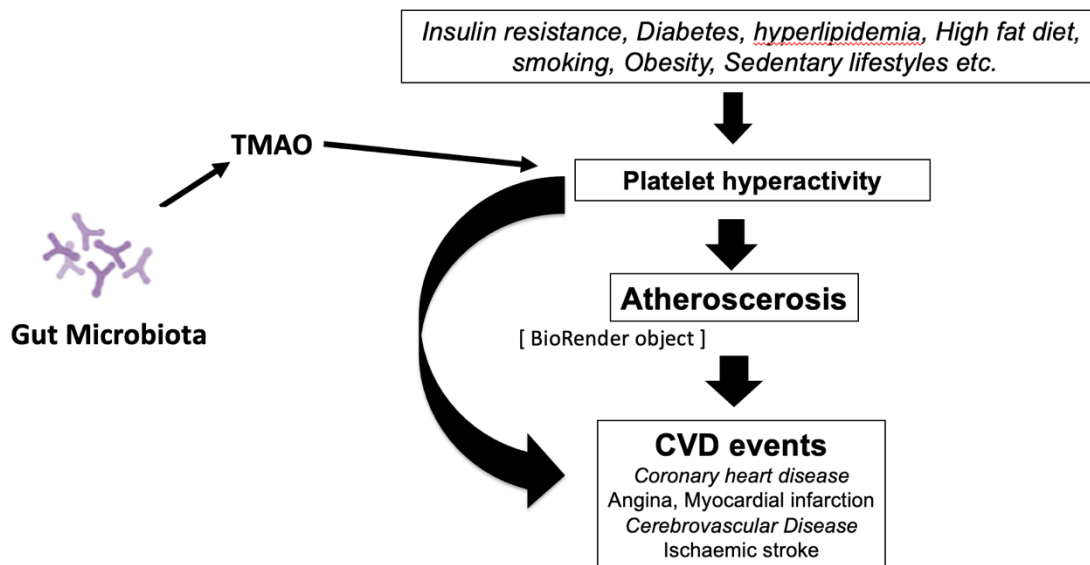


Figure 2. Role of platelets in the development of Cardiovascular disease (CVD)

Platelets play an essential role in the development of atherosclerosis and cardiovascular events. Plasma levels of TMAO can induce the hyper-activity in human blood platelets as observed in diabetes, hyperlipidemic conditions. Different conditions, such as insulin resistance, diabetes, sedentary lifestyle high-fat diet, including TMAO, can induce hyperactivity in blood platelet. Hyperactive platelets contribute to atherosclerosis development by different mechanisms such as membrane shedding, growth factor secretion, and expression of various membrane factors. Besides, hyperactive platelets are involved in the penultimate thrombotic events. *Adapted with permission from O’Kennedy et al. Eur J Nutr. 2017 Mar;56(2):461-482. doi: 10.1007/s00394-016-1265-2. Epub 2016 Jul 7.*

Different pathways platelets such as shedding of membrane particles, cytokines, growth factors activating blood vessels contribute to atherosclerosis, decreased blood flow, and hypertension. In addition to the recruiting platelets at the site of the damaged vessel, vWF is involved atherosclerotic plaque development. P-selectin (CD62P) of platelets stimulates monocytes and macrophages to release chemokines that promote platelet–monocyte aggregates. Activated platelets alter the chemotactic and adhesive properties of endothelial cells by releasing inflammatory molecules. CD40 ligand (CD40L) released from platelets

induces inflammatory responses in the endothelium. Several platelet-derived chemokines and growth factors are detectable in atherosclerotic plaques.

Platelet-derived CD40L release pro-inflammatory cytokines from vascular cells in the atheroma to stabilize platelet-rich thrombin and inhibit the re-endothelization of damaged vessels. When activated, platelets release different growth factors such as PDGF and VEGF, membrane particles, and cytokines that participate in atherosclerosis development by promoting vascular smooth muscle cell proliferation [124]. Platelets also secrete 5-hydroxytryptamine, ADP, ATP, and lysophosphatidic acid [124]. Hyperactive platelets produce more reactive oxygen species (ROS), enhancing platelet activity by decreasing NO bioavailability and lowering the intracellular concentration of Ca^{2+} [125]. Furthermore, excessive platelet activation is also attributed to the high mechanical shear forces in the circulation, reduced blood flow, and vascular damage, which is observed in patients with hypertension and diabetes [126]. Platelet hyperactivity plays a causal role in triggering and maintaining the pro-inflammatory and pro-thrombotic state of obesity, creating an environment favorable for atherothrombotic vascular events.

Besides these above pathological conditions, the gut metabolite TMAO also activates circulating blood platelets by elevating Ca^{2+} release from intracellular stores, contributing to increased risk of CVD and plaque formation[3]. TMAO produces IP3 by breaking down platelet membrane phospholipids, thus triggering intracellular Ca^{2+} release from internal stores, leading to platelet activation. In several animal models, acute elevation of circulating TMAO by infusion was shown to enhance *in vivo* thrombosis potential [127]. In humans, TMAO increased platelet hyperactivity [128] and altered cholesterol metabolism [129].

Hyperactive blood platelets interact with vessel walls by shedding macro-particles, secreting several adhesive growth factors, and inflammatory agents interrupt the blood flow

and produce a pro-thrombotic state in people with obesity, diabetes, a sedentary lifestyle or hypertension, and dysbiosis leads to the production of TMAO (**Figure-2**).

Therefore, the maintenance of regular platelet activity is critical to maintaining hemostasis. The most predominant anti-platelet agent, aspirin, has a number of severe side effects, making it unsuitable for the primary prevention of CVD. Moreover, aspirin-treated platelets don't have the potential to be involved in other physiological functions apart from aggregation in their lifetime. The use of anti-platelet drugs is not recommended as a primary preventive measure. Therefore, it is imperative to find alternative and safe anti-platelet agents to tame hyperactive platelets to reduce the risk of CVD development. However, only a limited number of food-derived compounds have been investigated in depth for their anti-platelet effects clinically as of yet. High intakes of fruit and vegetables and moderate intake of marine fish can lower platelet hyperactivity to some extent [130,131]. The most studied reversible anti-platelet regime is derived from tomatoes that contain several polyphenols, nucleoside derivatives, phenolic compounds [132]. The potent anti-platelet compounds were isolated in water-soluble tomato extract (known as Fruitflow®) as that significantly inhibited platelet aggregation both in vivo and ex vivo [132-134]. Human volunteer studies demonstrated the potency and bioavailability of active compounds in Fruitflow®. Fruitflow® is a functional product approved by the European Food Safety Authority (EFSA) [133]. Fruitflow® can serve as a safe anti-platelet prophylactic treatment for those at high risk or as an alternative to pharmacological compounds with side effects g[132]. Fruitflow® can be beneficial in preventing CVD. The accumulated data indicate that Fruitflow® may be useful in the primary prevention of CVD.

Gut microbial metabolites and their effects on the CVD risk factors

There is now sufficient evidence that gut microbiota participates in intestinal, cardiovascular health, and immune function. The human gut microbiome has predominantly

five phyla, such as *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* [135]. In the healthy gut, the anaerobic groups *Bacteroidetes* and *Firmicutes* account for more than 90% of the total bacterial species[135]. The gut microbiome has many functions in the host [136]. They are involved in the human digestion of food through two main catabolic saccharolytic and proteolytic catabolic pathways. Both pathways lead to the production of SCFAs from polysaccharides. The second catabolic pathway also produces ammonia, various amines, thiols, phenols, and indoles.

The gut microbiota metabolizes dietary phosphatidylcholine or l-carnitine into TMA [39]. TMA is then taken up the liver and oxidized by hepatic flavin monooxygenase 3 (FMO3), leading to the production of TMAO[8]. One of them is TMA derived from dietary sources of phosphatidylcholine, choline, and L-carnitine. Synthesis of TMAO is secondary to the ingestion of food components containing a TMA moiety, such as choline, phosphatidylcholine, and L-carnitine, all of which are present in high amounts in animal products such as red meat, fish, milk, and eggs. Microbial TMA lyases metabolize these compounds produce TMA. TMA is then transported to the liver via portal circulation and is oxidized in the liver to TMAO by hepatic FMO, primarily FMO3 [8,127]. TMAO is unique from traditional CVD risk factors in that it is a product of the gut microbial metabolism. The plasma level of TMAO is contributed by different factors such as diet, gut microflora, drug administration, and liver flavin monooxygenase activity. TMAO enters the systemic circulation and contributes to atherosclerosis development by altering lipid metabolism, platelet activity, obesity, and vascularity

(Figure-3).

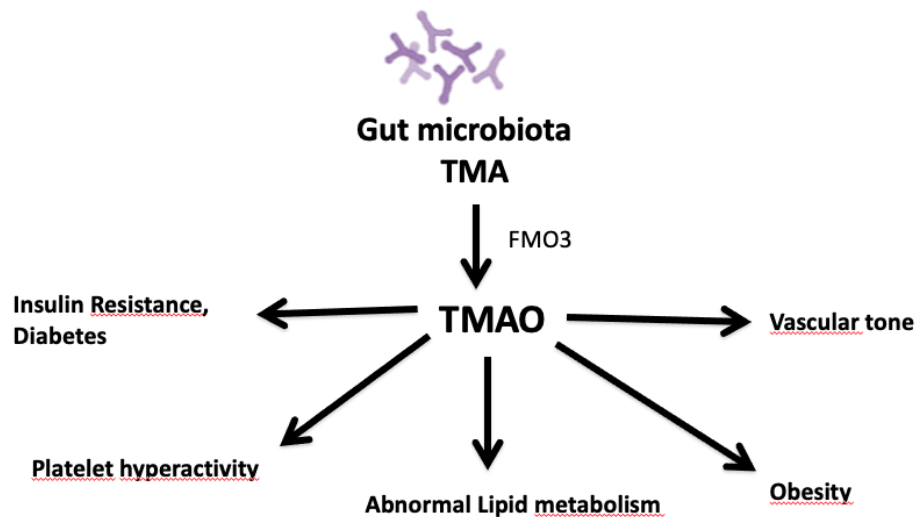


Figure-3: Impact of plasma levels of TMAO on atherosclerosis

TMAO affects platelet activity, lipid metabolism, obesity, insulin resistance, vascular tone, and diabetes, thus stimulates the atherosclerosis process

Use of both prebiotics and probiotics in combination or alone impacts gut microbiota composition. Prebiotics include galactooligosaccharides, fructooligosaccharides, inulin, etc., stimulate the growth of beneficial microflora, while probiotics have specific beneficial bacterial strains. These interventions can help modulate bacteria to transform precursors into TMA and increase bacteria's ability to deplete it or bacteria devoid of the genes responsible for converting carnitine or choline to TMA. A majority of bacteria belong to the *methanobacteriales* that reside in the human gut. The methanogenic bacteria have been shown to deplete both TMA and TMAO [137] [138]. Resveratrol can significantly also modulate the growth of specific gut microbiota *in vivo*, including increasing the *Bacteroidetes*-to-*Firmicutes* ratio and the growth of *Bacteroides*, *Lactobacillus*, and *Bifidobacterium*[139,140]. These changes have been shown to reduce the levels of TMAO. Reducing L-carnitine or choline levels in the diet is not a good alternative, as these are important nutrients.

TMAO is associated with obesity, insulin resistance, and renal disease[141]. A link between TMAO and CVD has been established [142]. The circulating levels of TMAO were correlated well with atherosclerotic plaque size and cardiovascular events [143]. Several meta-analyses have found the association of the plasma levels of TMAO with CVD and mortality risks [141] [144]. Patients with the highest quartile of circulating TMAO level exhibited a higher risk of major adverse cardiovascular events than patients in the lowest quartile [64]. TMAO levels were also associated with the vulnerability and plaque formation and the long-term risks of cardiovascular events in patients, and poor prognosis [65] [66,128].

A high choline diet caused increased TMAO levels and atherosclerosis in animal studies. TMAO mediates, at least in part, the established link between red meat consumption and CVD risk. Therefore, the low blood levels of TMAO as a result of fruits and vegetable intake may account for their cardioprotective effects. Work is going on to find novel therapeutic approaches that decrease plasma TMAO levels. The available data indicate modulating the gut microbial TMAO generating pathway that attenuates atherosclerosis and platelet hyperactivity and in vivo thrombosis potential in animal models; however, the effective treatment modality yet to be established. Lifestyle modification, including exercise, diet, functional foods, and changing microbiota, could be useful for lowering TMAO levels [145].

TMAO exacerbates the vascular wall's inflammatory reactions, induces ROS production, and prevents cholesterol reverse transport [146]. TMAO modulated cholesterol and sterol metabolism help to develop atherosclerosis[44]. The FMO3 knockdown mice had decreased circulating TMAO levels and attenuated atherosclerosis plaque formation despite activating macrophage reverse cholesterol transport[147-149]. The plasma levels of gut microbial dietary phosphatidylcholine metabolites and TMAO that produced l-carnitine and γ -butyrobetaine were associated with CVD risk [150-152]. The plasma level of TMAO was directly related to atherosclerotic plaque formation [8]. A prospective and observational clinical study on patients

with or without chronic heart failure consistently showed that plasma levels of TMAO were positively correlated with the risk of heart failure[153].

The role of TMAO in atherosclerosis was investigated using a dietary choline supplement in ApoE^{-/-} mice. The CD36, steroid receptor RNA activator 1 (SR-A1), and 2 macrophage scavenger receptors were measured. The levels of CD36 and SR-A1 in the macrophages of TMAO-treated mice were increased compared with controls, and antibiotic intervention reduced foam cells' formation by decreasing TMA production[8]. No significant impact of TMAO on foam cell formation was observed.

TMAO also promotes atherosclerosis by suppressing reverse cholesterol transport and altering cholesterol transporters' activity in macrophages[150]. Besides, TMAO suppresses expression of liver bile acid synthetase Cyp7a1 and Cyp27a1 and bile acid transporters, Oatp1, Oatp4, Mrp2, and Ntcp, leading to deranged bile acid-related pathways and promotes atherosclerosis[152]. Farnesoid X receptor (FXR) also controls bile acids metabolism and TMAO production by regulating the expression of hepatic FMO3 [127]. FXR protected mice against atherosclerosis by inhibiting the expression of CYP7A1 and CYP8B1 in ApoE^{-/-} mice [127,147,154,155].

TMAO accelerates atherosclerosis by several mechanisms such as promoting cholesterol influx, inhibiting cholesterol efflux, blocking the bile acid pathway, causing hyperactivity platelets. TMAO also upregulated the expression of vascular cell adhesion molecule-1 (VCAM-1) and activated protein kinase C (PKC) and NFκB. TMAO thus stimulates atherosclerosis via endothelial cell dysfunction and increasing the adhesion of monocytes [156]. These findings have confirmed the roles of TMAO in CVD. However, further work is required to establish TMAO can be used as a biomarker for CVD risk and atherosclerotic diseases[157-159].

TMAO may be regarded as an independent risk factor for CVD. However, inconsistent results were also observed, especially in broad population observations[160,161]. Choline is

generally considered a dietary source of TMAO; however, there was no substantial evidence of significant associations between choline intake and CVD risk [162]. Administration l-carnitine resulted in a significant increase in circulating TMAO levels in ApoE(-/-)mice, but its status was inversely correlated with aortic lesion size[163]. Several population studies in different countries showed that that dietary choline and betaine intake was not associated with CVD's pathogenesis [160,164]. However, more in-depth studies are required for definitive conclusions on the exact roles of TMAO in atherosclerosis, as well as the validation of its therapeutic potential by targeting TMAO-producing bacteria or enzymes.

Another gut microbiota-derived metabolite, bile acid, is involved in various metabolic diseases[165]. Bile acid is stored in the gallbladder and released into the intestine to aid the absorption of dietary lipids and lipid-soluble vitamins. Primary bile acids are usually metabolized by the gut microbiota-derived enzymes into secondary bile acids, including deoxycholic acid and lithocholic acid, hyodeoxycholic acid, and ursodeoxycholic acid [166]. Suppression of hepatic bile acid biosynthesis may also inhibit the high-fat diet-induced gut microbiome alterations showing the presence of the liver–bile acid–gut microbiome metabolic axis[167]. Recently, the bidirectional relationship between gut microbiota and bile acid metabolism[168] in CVD has been reviewed [46].

Bile acids can accelerate atherosclerosis development through bile-salt hydrolase activity and bile acid receptors[169,170]. Bile-salt hydrolase is present in many bacteria, such as *Methanobrevibacter smithii*, *Clostridium*, *Enterococcus* [48,171]. Bacteria-mediated bile-salt hydrolase activity can promote atherosclerotic progression by stimulating cholesterol accumulation, foam cell formation, and increasing the atherosclerotic plaque size [172].

TGR5, the G protein-coupled receptor, is an important bile acid receptor of the host that mediates the systemic effects of bile acids[173]. TGR5 can inhibit atherosclerosis development

by reducing macrophage-mediated inflammation and lipid loading[174]. Pregnane X receptor (PXR) also regulates the expression of genes involved in the biosynthesis, transport, and metabolism of bile acids. PXR is activated by secondary bile acids [175]. Activation of PXR increases atherogenic lipoproteins VLDL and LDL [176]. Development of atherosclerosis is retarded in PXR and apoE double knockout (PXR^{-/-} and ApoE^{-/-}) mice [177].

The microbiota-derived secondary bile acids play essential roles in atherosclerosis development by modulating various bile acids receptors such as FXR, PXR, TGR5, and VDR, and S1PR2. Favorable modulation of bile acid metabolism by targeting microbiota may also prevent the atherosclerosis process [178].

Conclusions

Emerging data suggest a strong relationship between the microbiota-derived compounds with an increased risk of CVD. Therefore, it is important to investigate further the roles of diets, microbial production of TMAO and SCFAs, and their cellular signaling to determine its effects on cardiovascular physiology. The relationship between platelet pathology and abnormal lipid metabolism with CVD is established now. Given the growing concern over the CVD due to platelet hyperactivity and abnormal lipid metabolism, a new therapy of probiotics and prebiotics may constitute a suitable primary prevention regime without overly reducing the nutritionally important intake of precursors of TMA, such as choline, betaine, and L-carnitine. Although several drugs are available to treat CVD, it is currently the leading cause of death worldwide.

Sufficient convincing data have emerged in the relationship between gut dysbiosis and CVD. However, further work is required for the establishment of gut microbiota-targeted therapy for CVD. Since various experimental and clinical data on the mechanisms of gut microbiota mediated-development of CVD are available, there is a strong possibility of finding new approaches to treat or prevent CVD. SCFAs and some types of bile acids or reducing the

microbial metabolite, TMA can be modulated by diets, prebiotics, and probiotics and with specific TMA inhibitors. The gut microbial composition may also be favorably modified with probiotics, prebiotic, natural components. To this end, well-designed large-scale clinical studies are required to validate the preclinical and other small-sized human trials data. The currently available data allows us to target the gut microbiota and its metabolites to understand CVD mechanisms and develop novel preventative or therapeutic regimes.

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