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Effects of Gut Microbiota on Cardiovascular Diseases

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Abstract **Review Article**

Micro-organisms have always been a part of the ecosystem. In fact, they play a major role in balancing metabolism as they colonize the system. In the past two decades, studies about the human micro biome has been elevating and still continuing as it is perceived as a possible threat to health status or also could be promising and hope filling in novel therapeutics in the mere future. Despite many on-going debates about the relationship of gut bacteria to the physiopathology of cardiovascular diseases, it has been simultaneously established through ample amount of reports and studies conducted both in vivo and in vitro. In this review, we will be stressing on several studies emphasizing on the gut micro biome, their role in influencing the physiopathology of cardiovascular diseases while also reflecting CVD as a global health burden, factors affecting the differences in gut microbiota, gut dysbiosis and its effects on the hosts' metabolism, the intervention of probiotics in balancing gut dysbiosis, bacteriotherapy and the possible hypothesis of SCFAs and TMAOs to be explored on manipulating the gut microbiota in preventing blood pressure, obesity and hypertension which are major risk factors for cardiovascular diseases.

Keywords: Gut microbiota, cardiovascular diseases, Probiotics, SCFAs, TMAO, FMT.

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INTRODUCTION

The gastrointestinal tract harbours diverse species of microorganism which are referred as the gut microbiota. These communities in terms of their collective genomes, activity, size and compositions, and surrounding ecosystems, represent their microbiome [1]. The gut microbiota mainly comprises of obligate anaerobes with lesser numbers of facultative anaerobes, archaea, and yeast involving in activities like digestion which is a major function of the gastrointestinal tract [2]. Besides that, their presence influences pathogen colonisation by competing for attachment sites or nutrient sources, and by producing antimicrobial substances [3]. These bacteria also perform endogenous functions like the transformation of bile acids, breakdown of insoluble fibres and the production of specific vitamins and cofactors during fermentation which is important in a stable functioning of the human body [4]. The production of short chain fatty acids (SCFAs) and trimethylamine N- oxide as the end product of fermentation play a major role obesity, inflammatory bowel diseases [5].

The systemic review was based on PubMed published article which was searched using search engine such as google scholar and Microsoft academic. Qualitative data pertaining to association of gut microbiota with cardiovascular diseases were extracted

from many original research articles. In particular we searched for the following species Fermicutes, Bacteroidetes, Proteobacteria, Fusobateria, Bifidobacterium, Clostridium, Escherichia coli, Klebsiella, Lachnospiraceae, Lactobacillus, Prevotella and Ruminococcus and its relevance to Coronary Artery Disease.

According to the World Health Organisation (WHO), cardiovascular diseases (CVDs) are the number one cause of death globally, taking an estimated 17.9 million lives each year [6] and remains the leading cause of death in the US till 2019. This is a global burden and attracts significant effort for care and research. CVDs are a group of disorders of the heart and blood vessels which include coronary heart disease, cerebrovascular disease, rheumatic heart disease and other conditions. Four out of 5CVD deaths are due to heart attacks and strokes, and one third of these deaths occur prematurely in people under 70 years of age [7-9]. Individuals at risk of CVD may demonstrate raised blood pressure, glucose, and lipids as well as overweight and obesity [10, 11]. At least three quarters of the world's deaths from CVDs occur in low- and middle-income countries. People in low- and middle-income countries often do not have the benefit of integrated primary health care programmes for early detection and treatment of people with risk factors compared to people in high-income countries [9, 12]. The relationship between the gut to the heart and its influence on cardiovascular diseases has been long established. In fact, intestinal microbiota determines cardiovascular health, development and severity of cardiovascular disease [13, 14]. For example, heart failure–associated splanchnic circulation congestion, bowel wall edema, and impaired intestinal barrier function are thought to result in bacterial translocation, the presence of bacterial products in the systemic circulation and heightened inflammatory state [12]. These are thought to also contribute to further progression of atherosclerosis and heart failure [15]. Alterations in gut microbial metabolites like trimethylamine-N-oxide (TMAO) are associated with atherogenesis which will be discussed in later part [16]. A higher ratio of Firmicutes to Bacteroides is associated with obesity and hypertension, and this proportion has been shown to increase with weight loss [17, 18].

Generally the bacterial population in the oral cavity and the stomach include Gemella (e.g., G. haemolysans), Granulicatella, Streptococcus (e.g., S. mitis), Veillonella, Prevotella, Porphyromonas, Rothia, Neisseria, Fusobacterium, Lactobacillus and Helicobacter pylori where the Allochthonous microbes are generally outnumbered by autochthonous microbes. The small and large intestines are colonized by Escherichia coli, Klebsiella, Enterococcus, Bacteroides, Ruminococcus, Dorea, Clostridium, Coprococcus, Weissella, Lactobacillus (some species) Granulicatella, Streptococcus (e.g., S. mitis), Veillonella, Lactobacillus and species of Firmicutes, Bacteroidetes, Actionobacteria, Verrucomicrobia, and Proteobacteria [19, 20]. However, this composition may vary from one individual to another according to their lifestyle, diet, genetic arrangement and age. Diet influences the variation of gut microbiota composition in different individual [21]. This could be due to the interaction between the microbe and the substrate according to its need for example a survey was done in the U.S to some adults showing that Prevotella was found to be high in the faecal micro biota composition of those consuming more of fibrous foods whereas Bacteroides favours those consuming foods that are high in protein and fats [21, 22]. The gut environment of an individual such as pH, bile salt concentrations or low micronutrient (e.g. Fe) concentrations also play a role in favouring and limiting microbial growth [23-26]. The gut microbiota also varies with age for example, the KOALA birth cohort study about the gut microflora of infants showed that faecal samples were colonised with Escherichia coli (88.6%) and members of the Bacteroides fragilis group (81.6%), whereas colonisation with lactobacilli (32.2%) and Clostridium difficile (25.1%) was less common. A Gram- positive Actinobacteria i.e., Bifidobacteria were detected the highest, followed by E. coli and Bacteroides fragilis group species [27]. The relative abundance of Actinobacteria substantially decreased after weaning and continued to decrease with age. However, Firmicutes predominates after weaning but was less abundant in children younger than 4 years compared with subjects older than 4 years [28]. Increases in the relative abundance of Bacteroidetes and Proteobacteria were observed in subjects over 70 years old. The relative abundance of Bacteroidetes did not change instantly, but a gradual increase was observed beyond 70 years of age. The change in the relative abundance of Proteobacteria was opposite that of Firmicutes [29]. Analysis of faecal microbiota composition in pregnant women resulted in the presence of Bifidobacterium, Lactobacillus, Clostridium coccoides, Clostridium leptum, Bacteroides, Enterobacteriaceae, Escherichia coli, Staphylococcus and Akkermansia muciniphila [30]. However, generally the gut of a healthy individual would highly favour the abundance of Fermicutes, followed Bacteroides, Proteobacteria and an equal proportion of Verrucomicrobia and Fusobacteria with least in abundance also depicted in figure 1 [20, 23-27, 29].

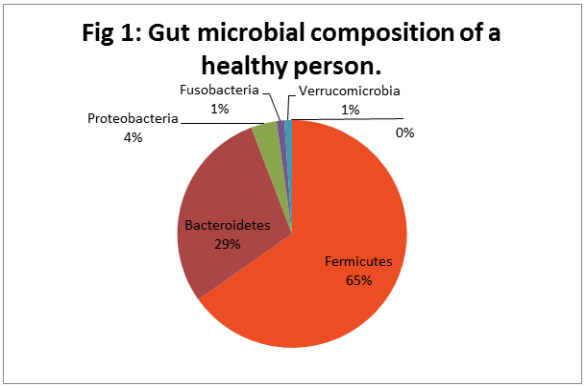

Fig-1: Gut microbiota on coronary artery disease

Coronary artery disease or coronary heart disease results from the building up of plaque in the heart's arteries due to cholesterol deposits which lead to heart attack. It has been documented that the mortality and morbidity rates of CAD are higher in subjects who are overweight and obese [18, 25]. Patients with Acute Coronary Syndrome reported higher levels of Prevotella while healthy controls had higher overall Bacteroides levels. This resulted in increased intestinal permeability being critical in obese patients [30]. Gut dysbiosis in CAD patients reveals lower abundance of Lachnospiraceae NK4B4 group, Lachnospiraceae UCG-004, and Ruminococcus Gauvreauii, and higher abundance of Ruminococcus gnavus [31]. There is also a mechanistic link between the abundance of Ruminococcus gnavus and development of CAD which involves the ability of R. gnavus to use mucin as a carbon source contributing to gut barrier dysfunction and exposure to foreign materials [32]. The increasing abundance in Roseburia, Klebsiella, Clostridium and Ruminococcaceae marked the severity of different stages of CAD [33]. However, oral administration of Lactobacillus plantarum (Lp299v) can reduce systemic inflammation in humans with stable coronary artery disease (CAD) and also improves vascular endothelial function [34]. In a study done by Moludi et al., 2019, where a group of CAD patients were divided in two groups, one group were administered with Lactobacillus rhamnosus GG (LGG) and the other with placebo and it has been observed that LGG supplemented group were seen to be improving in their lipid profile and also a favourable effect on total cholesterol and low density lipoprotein. Simultaneously the same study shows the reduction in anthropometric indices (weight, BMI, and WC) in the probiotic group was more than in the placebo group, but this did not reach to significant levels compared to the placebo group [35]. Another similar study done by Raygan et al., on the effects of probiotic supplementation on metabolic status in diabetic patients with coronary heart disease where one group was administered with a placebo while the other with probiotics Bifidobacterium bifidum, Bifidobacterium lactis, Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus casei, Lactobacillus salivarius, Lactococcus lactis and Lactococcus lactis for 12 weeks. It was found that the probiotics had beneficial effects on glycemic control, HDL-cholesterol, total HDL-cholesterol ratio, biomarkers of inflammation and oxidative stress and also decrease insulin resistance in diabetic patients with CHD [36]. One of the mechanisms on how probiotics lower cholesterol level could be due to their ability to decrease hydroxyl-methyl-glutaryl-Coenzyme-A reductase in liver and also the conversion of cholesterol into bile acids. Furthermore, enzymatic deconjugation of bile acids by bile salt hydrolase produced by probiotics making it easier for the intestines to absorb and eliminate in excretion [37]. Probiotics can also incorporate cholesterol into their growing cellular membranes. This can be observed under the scanning electron microscope [38].

Gut microbiota on atherosclerosis

Atherosclerosis is a chronic inflammatory disease affecting the endothelial cells of arterial walls layer and is associated with monocyte infiltration [39]. It is mainly caused by hypertension and oxidative stress, smoking, hyperglycemia and hypercholesterolemia, progressive increase in the number of LDLs in blood [39]. A shot gun sequencing of the gut metagenome demonstrate that Actinobacteria spp., specifically Collinsella to be more in numbers in patients with symptomatic atherosclerosis, defined as carotid stenosis leading to cerebrovascular events, where as healthy controls reported to be dominated by Roseburia and Eubacterium [40]. In another study, a structured weight loss program increase gut microbiota phylogenetic diversity and reduces levels of Collinsella in obese type 2 dibetic patients [21]. However, the presence of Akkermansia muciniphila improves gut barrier function and exerting protective effects against atherosclerosis [41]. A comparison of bacterial composition of the oral, gut, and atherosclerotic plaque in patients with atherosclerosis reveals the presence of Chryseomonas, Chlamydia, Rothia, Granulicatella and Propionibacterium in both the oral cavity and the atherosclerotic plaque within the same patients. Commensal existence of Veillonella and Streptococcus is also observed in all atherosclerotic plaque samples which raises the possibility of its association with the pathogenesis of atherosclerosis [42]. In a recent study published by Hassan et al 2020, it was observed that a probiotic strain of Lactobacillus spp. (L. plantarum ATCC 14917), could reduce the formation of atherosclerotic lesion in ApoE−/− mice by modulating the proinflammatory cytokines, blocking the translocation of P65 protein NF-κB of oxidative stress and improving the composition of gut microbiota and ultimately gut health in mice [43]. Another similar result was observed with Lactobacillus acidophilus ATCC 4356 for its ability to inhibit intestinal cholesterol absorption in apolipoprotein E knockout mice [44]. L. acidophilus ATCC 4356 attenuate atherogenesis by reducing serum levels of oxLDL in ApoE−/− mice, showing anti oxidizing properties. Lowering serum and aortic mRNA levels of TNF-α and

IL-10 [45] inhibit the translocation of NF-κB into the nucleus by the IκB proteins, such as NFκBIA and NFκBIB, leading to the inhibition of NF-κB and suppression of pro-inflammatory genes [46]. Another finding suggested the therapeutic potential of Lactobacillus acidophilus K301 as an anti-atherosclerotic agent showing its ability to Induce 24 (S), 25- epoxycholesterol-mediated ABCA1 and ABCG1 production and cholesterol efflux in macrophages and inhibits the accumulation of lipoprotein in atherosclerotic plaques by the induction of squalene reductase (SQLE) and oxidosqualene cyclise (OSC) [47].

Gut microbiota on Myocardial infarction or heart attack.

Myocardial infarction is defined by the demonstration of myocardial cell necrosis due to significant and sustained ischaemia. It is usually, but not always, an acute manifestation of atherosclerosis-related coronary heart disease [13]. The reorganization of gut microbial community such as reduction in Lactobacillus is evidently associated with MI. This could be due to antibiotic supplementation leading to changes across the Lactobacillus, Prevotella, Clostridium, and Oscillospira genera influencing the severity of MI [48]. When mice model are administered with Lactobacillus after being treated with antibiotics before Mi, it restored myeloid cell proportions by altering SCFAs metabolism and positively modulating immune response [49]. Probiotics beneficially affect cardiac remodelling process in patients with myocardial infarction by balancing gut microbiota composition [50]. Moreover, the gut microbiota involve in ventricular remodelling of post myocardial infarction [51]. Studies have shown that the supplementation of probiotic bacterial strains, such as Bifidobacterium breve, Lactobacillus casei, Lactobacillus bulgaricus (Lactobacillus delbrueckii subsp. bulgaricus), and Lactobacillus acidophilus reduces lipid peroxidation and TNF-α level thereby preventing heart injury in rat myocardial infarction model [52]. Addition of L. helveticus R0052 and B. longum R0175 to a low-n-3 PUFA diet reduces apoptosis in the limbic system and to prevent depression-like behaviour in MI patients [53] [54] L. rhamnosus also have anti depression effect relating post MI sympyoms [54]. Pre- administration of Lactobacillus rhamnosus GR-1 to rats with sustained coronary artery occlusion can alter the progression of post infarction heart failure by reducing the increase in the leptin:adiponectin ratio in rats models subjected to coronary artery ligation [55]. A LEfSE analysis performed to determine the differences in gut microbial composition between the exercise-trained and control mice in the non-surgery, sham and MI groups showed the abundance of Butyricimonas, Prevotella, and Akkermansia in exercise-trained mice, while Parasutterella was more in non- surgery control groups. Erysipelotrichaceae, Sphingobacteriales, and Akkermansia were abundant in the exercise-trained mice, while Corynebacterium, Staphylococcus, and Enterobacteriaceae in the control group for the sham groups. Further analysis showed a significant abundance of Phenylobacterium and Roseateles in the exercise-trained MI mice providing evidence that physical exercise affects cardiac function in MI mice and that it can modulate the gut microbiome [56]. Cardiovascular diseases affected by gut dysbiosis is illustrated in figure 2.

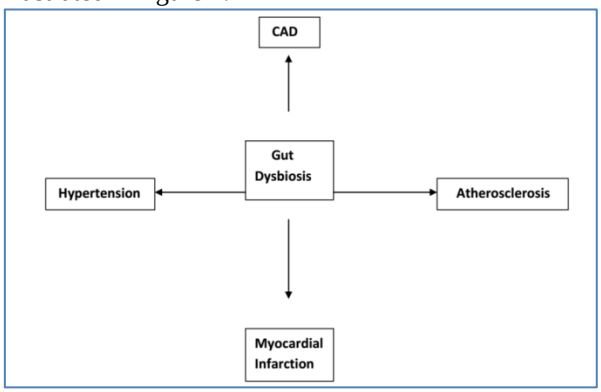

Fig-2: Cardiovascular diseases affected by gut dysbiosis

Short chain fatty acids and CVD

Another way of modulating CVD directly is by our gut microbes that produces short chain fatty acids as end products of fermentation [4]. This is related to diet as it acts as the substrate for the organism. Vegetarian diet is associated with the increase in Streptococcus and Anaerostripes genera promoting the gut barrier integrity of the host while reducing Clostridium which are percieved as pathogens. Mediterranean diet increases the production of propioniate, butyrate and acetate that could intervene with inflammatory cytokines such as VEGF, MCP-1, IL-17, IP-10 and IL-12 and attenuate inflammation [57]. One of the mechanism through which SCFAs regulate gut hormones is via their endogenous receptors free fatty acid receptors 2 (FFAR2) and 3 (FFAR3) [58, 60]. Effects of SCFA administration in mice showed that butyrate, propionate, and acetate all protected against diet-induced obesity and insulin resistance. FFAR3 being a common receptor activated by butyrate and propionate play a minor role in butyrate stimulation of Glucagon-like peptide-1, and is not required for butyrate- and propionate-dependent induction of Glucose-dependent insulinotropic peptide. However, FFAR3-deficient mice show normal body weight and glucose homeostasis. This regulation of gut hormones and food intake inhibition by butyrate and propionate may represent a novel mode of action by which gut microbiota stimulates host metabolism [58, 59]. Other receptors for SCFA include GPR109A and OLFR78. GPR109A was first identified as a receptor for niacin which is activated by β-hydroxybutryate and butyrate which are expressed in the epithelial cells of the colon and are studied for their role in repressing inflammation [60]. OLFR78 which is activated only by acetate and propionate is expressed in blood vessels specifically in renal vessel where it is involved in renin secretion. When gut microbiota-derived SCFAs are bound to

OLFR78, it induces the release of renin, which is involved in the modulation of the blood pressure [61-63].

TMAO and CVDs

Trimethyl amine N-oxide is a rising topic of research currently in the field of CVD. TMAO is a metabolite synthesized by the metabolism of gut microbes. Several studies have shown that when red meat which are potential source of choline, lecithin and carnithine are acted upon by gut microbes, they convert L-carnithine to γ-butyrobetaine and then into TMA. In the presence of flavin monooxygenase 3 produced by the host's liver, TMA is converted to TMAO [64, 65]. In addition to red meat like beef, fish and egg are good sources for generating TMAO [66]. Several families of bacteria that are able in producing TMA and TMAO include Deferribacteraceae, Anaeroplasmataceae, Prevotellaceae and Enterobacteriaceae [67-39]. Commensal species of Fermicutes and Proteobacteria are identified for their capability of producing TMA from choline. Other additional genera include Anaerococcus hydrogenalis, Clostridium asparagiforme, C. hathewayi, C. sporogenes, Escherichia fergusonii, Proteus penneri, Providencia rettgeri, and Edwardsiella tarda [70]. Regulation of TMAO levels in the body is important because increasing TMAO accelerates atherosclerosis and cardiac risks [71, 72]. Several researchers have enlightened about the influence of TMAO in responsive and thrombosis [73]. TMAO has also been associated with heart failure [74]. Since microbes are the main catalyst for the generation of TMAO, presumably antibiotic treatment should be able to reduce its circulating levels. However, that is not an ideal solution since reports have shown that TMAO levels increased after stopping antibiotic consumption [75]. Antibiotics might also bring about additional unwanted results like bacterial resistance. The search for an ideal method leads to the discovery of other trials like the use of certain bacteria that could biologically convert TMA into a molecule with a more desirable property. These include methanogenic archaea that can reduce TMA formation in the gut by converting it into methane [75]. Another alternative method is the concept of colonizing the human gut with bacteria that uses TMAO as an alternative terminal electron acceptor of a respiratory transport chain [76]. However, this concept needs further validation. Desulfovibrio desulfuricans has been identified with a gene cluster that is able to convert choline to TMA by utilizing a glycyl radical enzyme [68]. A clinical assessment using an oral carnitine challenge test (OCCT) was conducted by Wu et al., which identified TMAO producer phenotype of gut microbiota. This may serve as a personal guidance in CVD prevention and treatment [77]. Somehow, Huc et al., studied a positive effect about moderate increase in plasma TMAO and its positive effects on hypertensive rats by reducing diastolic dysfunction in pressure overloaded heart [78]. Further investigation on TMAO is still required in order to be able to fully implement it for therapeutic use.

Another method for resetting the gut microbiota is by faecal transplantation or bacteriotherapy. This is an old practise which dates back to the late 1960s [79]. Faecal microbiota transplantation is performed with a concept of displacing intestinal pathogens by introducing faecal contents from a healthy subject into the gastrointestinal tract of patients [79]. It is found to be remarkably effective in treating recurring Clostridium defficile infection in humans which is related with inflammatory bowel disease [80, 81] and also in improving insulin sensitivity [82]. However this practise is limited due to the possibility of some other pathogens and infectious toxins being transferred that could be even more risky [83]. Alongside the controversies that have arisen about FMT emerging as a potential therapy for gut dysbiosis and associated diseases, the need for further reports for supporting its justification is important as it involve a recipient and a donor.

CONCLUSION

Although the speed of knowledge about CVD and gut microbiota intervention has not been equally advanced. Trials and researches are still going on in order to catch up with the speed of the increasing deaths due to this disease. Attempts on establishing the role of diet on gut microbiota composition have been successful. Factors like age, geographical lifestyle have also been influencing in the diversities and differences in gut microbial composition. Several studies on bacterial intervention through probiotics supplementation to correct gut dysbiosis and ultimately attenuate CVD like coronary artery diseases and atherosclerosis are also rising each time. Myocardial infarction, a heart condition which mainly arises due to imbalance in microbial population is mainly treated by Lactobacillus supplementation. Advances in the study of short chain fatty acids and their role in metabolism are also adding up since this field also appear to be of a potential therapeutic ability. Presently, studies on TMAO and its mode of action are greatly rising since it appears to be a dynamic tool in manipulating physiopathology of CVD and how dietary choline or TMAO supplementation disturbs microbial community balance in the gut and enhances the development of atherosclerotic lesion in mice. However with contradicting results simultaneously being reported, the need for further validation for every proposed hypothesis is accountable. Many promising proposal about the manipulation of gut microflora and its use in therapy have also come out remarkably. Even for this, further investigation is a necessity both in vitro and in

vivo in order to be able to implement its therapeutic use in CVD in the mere future. Bacteriotherapy or faecal microbial transplantation a method which is long used in correcting gut dysbiosis is however continuing to be perceived as a method in question due to its limitations.

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