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REVIEW ARTICLE

Intestinal Microbiota and Cardiovascular Diseases

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Abstract

Recently, gut microbiota has emerged as an important mediator of several diseases such as diabetes, atherosclerosis, arterial hypertension, obesity, cancers and neuropsychiatric diseases including Alzheimer, autism and depression. Intestinal microbiota is formed by bacteria, fungi and viruses and its main function is to facilitate the absorption and metabolism of foods (protein, fat and carbohydrate). One example of the multiple actions of the gut microbiota is the bidirectional relationship between the intestine and the brain, the so-called "gut/brain axis". Furthermore, metabolites produced by gut microbiota can induce effects locally or at distance, which suggests that the intestine is an endocrine organ.

Given the participation of the gut microbiota in several diseases, there is great interest in strategies that may positively affect the gut flora and prevent or even treat diseases. Among these strategies, lifestyle change, but specially diet modulation has gained importance. In this article, we review the mechanisms through which intestinal microbiota participates in cardiovascular diseases and possible therapeutic interventions.

Introduction

For long time traditional risk factors such as hypertension, diabetes, smoking and hypercholesterolemia have been considered the main promoters of atherosclerosis, and their control has been the cornerstone treatment for cardiovascular diseases. More recently, a new independent risk factor has emerged: the gut microbiota.[1,2,3]

Intestinal microbiota is made up of trillions of cells – about 10 times more than all the cells of the human organism – consisting of bacteria, viruses, fungi and archea. The phyla Firmicutes (mainly Clostridia species) and Bacteroidetes represent about 90% of gut microbiota, which is also composed of Actinobacteria, Proteobacteria and Verrucomicrobia.[2]

Until recently, studies on intestinal microbiota relied on culture of bacteria, providing limited information regarding a small fraction of the gut microbiota. Lately, culture-independent techniques, such as the marker gene analysis (16 S rRNA gene sequences), metagenome and metatranscriptone enabled the identification of previously unculturable bacteria.[1]

Gut flora remains relatively constant during an individual´s lifetime. However, it changes considerably from childhood to adult life and then during aging (Figure 1).[4] Thus, total gut microbiota is estimated to be small during childhood, increases considerably during adult life and decreases in old age. Infants have unstable, distinct and heterogeneous microbiota, characterized by low levels of total bacteria. On the other hand, elderly subjects have high levels of E-coli and Bacteroidetes. In the study by Maritat et al.[4] the measured ratio of Firmicutes to Bacteroides was 0.4, 10.9 and 0.6 for children, adults and elderly, respectively. It is tempting to speculate that these two extremes may be related to vulnerability of children and old people.

More recently, clusters or enterotypes in intestinal microbiota have been identified (Figura 2). Arumugan et al.[5] studied fecal metagenomes of 39 individuals from France, Italy, Spain and Denmark by 16S ribosomal RNA-encoding gene. They identified three clusters that are not nation or continent specific. They also found

Keywords

Cardiovascular Diseases; Gastrointestinal Microbiome/physiology; Risk Factors; Hypertension; Diabetes Mellitus; Obesity; Neoplasms; Alzheimer Disease; Metabolism; Atherosclerosis; Diet, Mediterranean.

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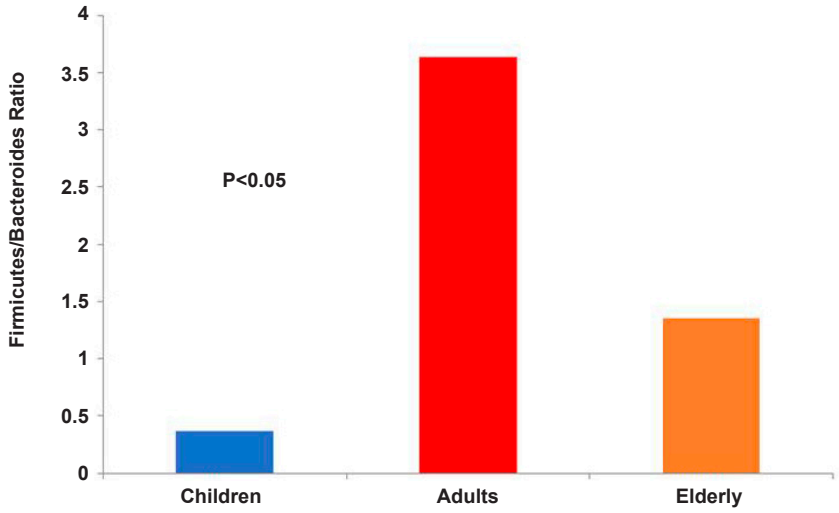

Figure 1 – Firmicutes/Bacteroides ratio in different phases of life. Modified from Mariat, et al.[4]

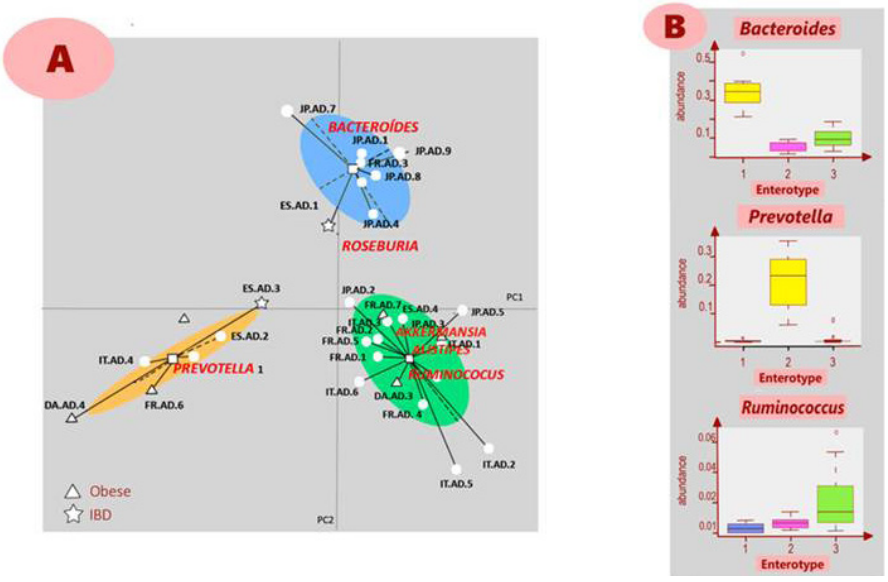

Figure 2 – Human enterotypes as identified by Arumugam et al. (Ref.5). Enterotypes are identified based on predominant bacteria namely Bacteroides, Prevotella and Ruminococcus (A,B). See text Modified from Arumugan et al.[5]

that 12 genes correlated significantly with age and three functional modules with body mass index. There were three main enterotypes –Bacteroidetes/Roseburia, Akkermansia/Alistipes/Ruminococcus and Prevotella. The authors concluded that intestinal microbiota variation is generally stratified, not continuous. Wu et al.[6] also described the link between dietary habits and gut microbial enterotypes (see ahead).

Gut microbiota varies individually and in populations as well, mainly due to different cultures and diets. Diet is a major element; for instance, vegans and vegetarians have higher counts of certain Bacteroidetes compared to omnivores.[7-9] Ayenic et al.[9] compared gut microbiome in rural Bassa with urban individuals from Nigeria. In rural Bassa they documented a predominance of bacteria with high capacity for fiber degradation and almost absence of common members of urban/industrial microbiomes. They also observed an adaptation of intestinal microbiota to urbanization.

Intestinal microbiota also varies in different intestinal regions as in the upper and lower small intestine and the colon.[10] This distribution explains the preferential absorption and metabolization of proteins, lipids and carbohydrates throughout the gut. The question regarding the "normal flora" is still open. Probably there are different enterotypes depending on diet, geographic location and genetic background.[2] On the other hand, the term "dysbiosis" describes a primary imbalance of gut microbiota. Some gut microbiota metabolites detected in plasma correlate directly with plasma trimethylamine-N-oxide (TMAO)[11] indicating the influence of gut microbiota on the pathogenesis of atherosclerotic disease.

Expansion of the knowledge in this area, both in mechanisms and identification of bacteria is expected in the near future. Understanding the functional role of bacteria and their relationship with plasma metabolome is pivotal issues for new research. However, our present understanding in this area is still superficial.

Main roles of the gut microbiota

The primary role of gut flora is the promotion and regulation of the absorption and metabolism of what we eat, *i.e.*, proteins, carbohydrates, fibers, nucleic acids, macro and micronutrients. Figure 3 summarizes the main functions of human gut microbiota. For instance, fermentation of non-digestible fibers and starch by microbiota in the colon leads to the production of short chain fatty acids (SCFAs), especially acetate, butyrate and propionate. Fatty acids are essential energetic sources of various organs including the heart, acting in the metabolism of proteins and carbohydrates.[12,13] Although only 5-10% of the energy consumed is from SCFAs, they play fundamental roles as in the signaling of molecules.[14]

The wide range of modulatory effects of SCFAs embrace the nervous system, blood pressure, histone deacetylases, inflammation, production of reactive oxygen species (ROS), inhibition of chemostasis, phagocytosis modulation, maintenance of intestinal barrier integrity and modulation of immune system responses.[2,14] SCFAs act through G-protein receptors, specifically the GRP41 and the olfactory receptor-78 (Olfr78). Olfr78, highly expressed in renal just-glomerular apparatus, mediates renin secretion induced by SCFAs. GPR41 and Olfr78 are also expressed in smooth muscle cells (SMC) of resistance vessels, and studies with KO mice indicate their influence on blood

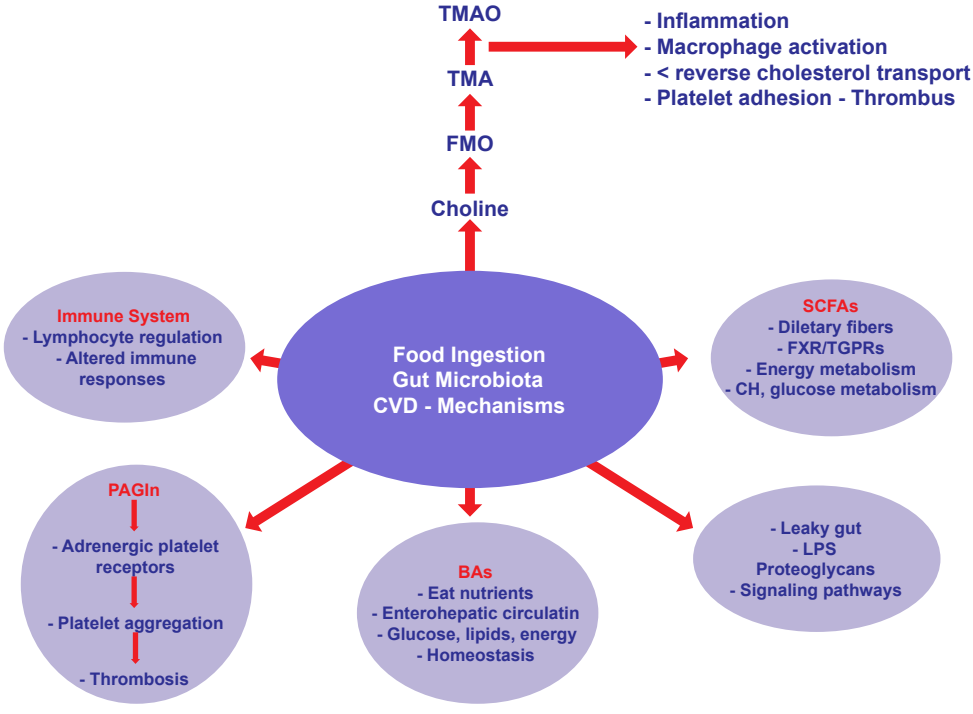

Figure 3 – Schematic summarized representantion of the main mechanisms through which gut microbiota may induce cardiovascular diseases (CVD). PAGIn: phenylacethyl glutamine; BAs: bile acids; SCFAs: short-chain fatty acids; FMO: flavin monooxygenases; TMA: trimethylamine; TMAO: trimethylamine-N-oxide; LPS: lipopolyssacharides.

pressure. Thus, while GRP41 KO-mice are hypertensive, Olfr78 KO-mice are hypotensive.[1] Animal studies also indicate that SCFAs are essential in cardiac repair after myocardial infarction and immune response.[13,14]

Few interventions have focused on SCFAs; diet modulation represents the major tool to alter the gut microbiota. David et al.[15] examined, in 10 normal individuals, the effect of a shift from a plant-based diet to an animal-based diet. The animal-based diet increased bile–tolerant microorganisms, like Bacteroides, and decreased the levels of Firmicutes that metabolize polysaccharides, such as Roseburia. Consequently, there was a reduction in fecal acetate and butyrate when subjects were switched from plant to animal-based diets. This occurred within just a few days, indicating that human intestinal microbiota can be manipulated very rapidly.

In insulin–resistant patients with metabolic syndrome, fecal transplantation from lean donors led to improved insulin sensitivity and abundance of Roseburia, which is a butyrate–producing bacterium.[2]

Effects of bile acids upon intestinal microbiota

Bile acids (BAs) are synthesized from cholesterol in the liver. This is an important way to eliminate cholesterol from the body. The rate-limiting enzyme is hepatic cholesterol 7 α-hydroxylase ($CYP7A_1$). BAs are conjugated to taurine and glycine, which enhances their water solubility and their secretion into the bile; they facilitate fat digestion.[16] The main conjugated BA are chenodeoxycholic acid and cholic acid (primary BAs); the secondary or deconjugated BAs are lithocholic acid, ursodeoxycholate and deoxycholic acid. About 95% of the bile acids are reabsorbed in the terminal ileum and colon. These molecules are then recirculated to the liver through the portal vein; this process is known as the *enterohepatic circulation*.

BAs regulate energy metabolism through activation of the membrane Takeda G protein-coupled bile acid receptor 1 (TGR5) and the nuclear Farnesoid X receptor (FXR). Both TGR5 and FXR are highly expressed in the intestine and the liver. Humans produce a large conjugate BA pool which is maintained by a feedback mechanism of the FXR in the liver and intestine. BAS act as direct antimicrobial agents because of their detergent properties and hydrophobicity.[2]

BAs exert important effects as hormones dependent on activation of TGR5 and FXR by gut microbiota.[17] These receptors are implicated in lipid and glucose metabolism. In the ileum, FXR activation by BAs induces fibroblast growth factor 19, which circulates to the liver and reduces CYP7A1; such reduction then inhibits the synthesis of BAs, specifically lithocholic acid and deoxycholic acid.

One important observation is that reduced BAs levels in the gut are associated with inflammation and bacterial growth.[17] In this sense, obeticholic acid, a BA analogue and FXR agonist, was approved in the USA for treatment of bacterial translocation and inflammation in steatohepatitis. Also, FXR activation in mice decreased cholesterol absorption by 50%. FXR activation increases apoptosis and reduces inflammation and cell migration; FXR is expressed in endothelial cells, where it increases endothelial nitric oxide synthase (eNOS) expression and reduces endothelin-1 (ET_1). Glucose stimulates FXR and CYP7A1, but insulin inhibits them.[17]

On the other hand, TGR5 is involved in energy metabolism, and its activation has an anti-atherogenic effect. Given these multiple physiological functions, FXR and TGR5 are potential therapeutic targets. Both synthetic agonists and inhibitors have been tested, with conflicting results in animal models and humans. More research is still necessary to establish the role of the intervention on these receptors before clinical application.

Microbiota and Immunity

The immune system, either innate or adaptive, is clearly linked to gut microbiota, which plays a role in modulating the relation regulatory-to-effector T cells.[18,19]

To reach distant organs, microbial signals need to cross the intestinal epithelium. Structural components of the microbiota such as lipopolysaccharides (LPS) and peptidoglycans interact with mucosal surface cells through pattern recognition receptors (PRR). PRR recognize pathogen-associated molecular patterns (PAMPs), which modulate immune responses. LPS and peptidoglycans can trigger a cascade of downstream signaling pathways.[2]

Gut commensal microbiota maintains a balance of immune effectors, to protect the gut against dangerous invaders, and at the same time tolerate innocuous microbial antigens. A thick mucus layer in the intestinal mucosa, together with the epithelial wall, is essential to maintain homeostasis. The contribution of intestinal mononuclear phagocytes (MNPs) has being recognized

as a potential targetable pathway in inflammatory disease.[18] The normal intestinal microbiota can inhibit innate lymphoid cells, which are major producers of interleukin-22 (IL-22), a cytokine that acts in epithelial cells to promote healing during infection. IL-22 also induces antimicrobial peptide production.[18]

In addition, commensals can affect the adaptive immune system by inducing T cell differentiation. Also, Clostridium clusters can induce colonic regulatory T cells (Tregs) that produce anti-inflammatory interleukin-10 (IL-10); to do this, Clostridium provides a transforming growth factor β (TGFβ) and high luminal concentrations of SCFAs, especially butyrate. Thus, SCFAs participate actively in the process called "homeostatic induction", in which bacteria exert immune effects through the differentiation of lymphocytes.[19]

Segmental filamentous bacteria (SFB) induce CD4+T helper cells in the ileal epithelial surface. CD4T helper cells produce IL-17, IL-17f and stimulate Th17 cells. All these cytokines are involved in inflammatory diseases such as inflammatory arthritis, psoriasis and inflammatory bowel disease.[18] These findings suggest that the inflammatory environment of the intestine modulate the differentiation of effector lymphocytes, highlighting the intimate interplay of gut microbiome and immunity.

Not only bacteria, but viruses can influence immunity; enteric viruses are frequent causes of human gastrointestinal diseases. Recent studies have also suggested interactions between viruses and bacteria – the so called "transkingdom interaction"; an example is the presence of virus-like particles correlated with significant changes in gut microbiome in intestinal bowel disease patients. Also, helminths such as Schistosoma mansoni and Trichinella have been found to modulate immunity.

These inflammatory cytokines can profoundly alter intestinal motility and permeability. One major effect of this phenomenon is the translocation of intestinal bacteria to plasma which can cause bacteremia and sepsis.

Taken together, these data indicate a significant modulatory role of gut microbiota - bacteria, viruses and helminths – in the immune system. Mechanistic studies are needed to further our knowledge in this emerging field.[18,19]

The real impact of gut microbiota in cardiovascular diseases

It has been recognized that gut microbiota is involved in the development of atherosclerosis, diabetes, hypertension, obesity, stroke, heart failure and neuropsychiatric diseases such as depression, autism and Alzheimer.[2] Even birth circumstances affect gut microbiota; in normal deliveries the child is exposed to the vaginal flora of the mother, which is beneficial to the health of the child. On the contrary, cesarean section deprives the baby of such exposure, and asthma and allergies have been encountered more frequently among these children. Furthermore, another gut microbiome metabolite, phenylacetyl glutamine (PAGIn), has been recently associated with cardiovascular disease in humans. PAGIn acts through adrenergic platelet receptors facilitating platelet aggregation and thrombus formation.[20]

A characteristic of the intestine and its microbiota is that they produce substances that act locally and others that act at distance, such as cytokines and noradrenergic products. These features led to the concept that the intestine is an endocrine organ.

Atherosclerosis

The metabolism of phosphatidylcholine, carnitine and choline found in abundance in red meat, milk and eggs has as its final compound trimethylamine-N-oxide (TMAO). These substances enter the intestine and suffer a series of metabolic reactions under the influence of microbiomes. The fundamental reaction is the conversion of choline into trimethylamine (TMA), which is then metabolized by flavin monooxygenases (FMOs), especially FMO3, of the liver into TMAO.[1] TMAO production is entirely dependent on the gut microbiota. In experimental animals fed a choline enriched diet, TMAO production is abolished when animals received broad spectrum antibiotics.[3] The authors also showed that TMAO induced foam cell formation and atherosclerotic plaques in aortic root of rabbits. Seldin et al.[21] observed elevated inflammatory gene expression compared to controls in the aortas of $LDLR^{-/-}$ mice fed a choline diet. Chronic choline feeding led to inflammatory gene expression of cyclooxygenase 2 ($COX-^{2}$), E-selectin, monocyte chemoatractic 1 (MCP-1); macrophage inflammatory

protein2 (MIP-2), TMAO and tumor necrosis factor α (TNF-α). In addition, acute injection of TMAO at physiological levels induced the same inflammatory markers and mitogen activated protein kinase (MAPK), extra-cellular signal related kinase (ECSRK) and nuclear factor kappa beta (NFK-β). To further explore the effects of TMAO, the authors[21] studied human aortic endothelial (HAEC) and vascular smooth muscle cells (VSMC) in culture. Treatment of these two cell lines with TMAO also induced gene expression of inflammatory markers: NFK-β; COX-2, interleukin 6, E-selectin and intercellular adhesion molecule (ICAM). In addition, TMAO enhanced endothelial recruitment of leukocytes.[21] These data indicate that TMAO activates inflammatory pathways in the vasculature, causing recruitment of endothelial cells and leukocytes, and atherosclerosis; these actions are mediated by NFK-β pathway.

Human studies documented participation of TMAO in atherosclerotic disease. Tang et al.[22] examined the effects of a phosphatidylcholine challenge (two hard-boiled eggs and deuterium-labeled phosphatidylcholine) in 40 normal individuals; they documented a significant increase in plasma and urine TMAO; in six of them, broad-spectrum antibiotics were administered, which completely suppressed TMAO increases. In a second study, 4,007 patients with documented coronary artery disease (CAD) were followed for three years and a graded increase in event risk in relation to TMAO plasma levels was documented, specifically death, non-fatal myocardial infarction or stroke (Figure 4).

In addition, TMAO levels correlate with atherosclerotic burden, as measured by the Syntax score, as well as to early atherosclerosis.[23,24] Furthermore, in a group of patients similar to those of the Courage trial,[25] i.e., with documented CAD and treated medically, higher TMAO levels were associated with worse prognosis due to cardiovascular events.

Emoto et al.[26] compared 39 CAD patients with 30 patients with risk factors and 50 normal controls. They observed that in CAD patients, order Lactobacillales was significantly increased and the phylum Bacteroidetes/ Prevotella was decreased when compared to controls.

On the other hand TMAO is an inductor of atherosclerosis or simply a marker of it. TMAO is clearly dependent on renal function and increases with age. Thus, individuals with even moderately decreased glomerular filtration rates have higher TMAO

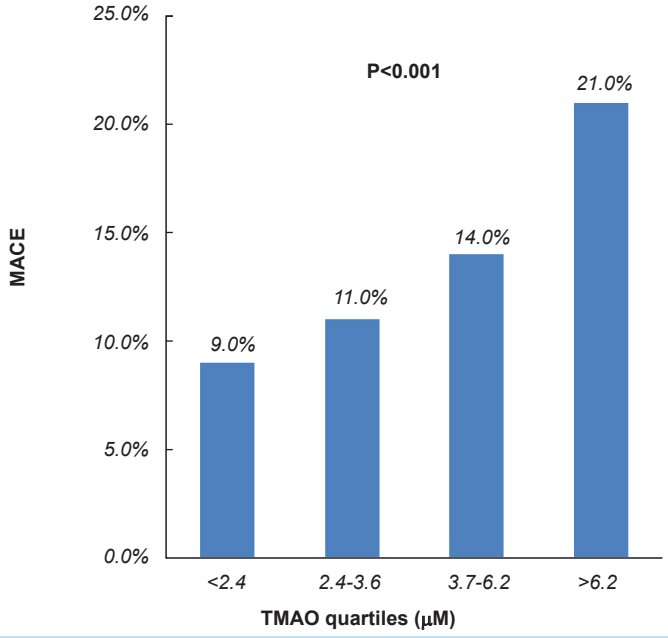

Figure 4 – Incidance of MACE (myocardial infarction, death and stroke) in 4007 CAD patients over 3 years period. Modified from Tang et al.[22]

plasma concentrations.[2] Elderly individuals also have higher TMAO levels compared to middle age persons. One finding that supports the active role of TMAO as an atherogenic molecule is that it induces platelet hyperreactivity and thrombotic risk, both experimentally and in humans.[27,28] Furthermore, the ingestion of deep-sea fish increases urinary TMAO levels.[29]

The mechanisms underlying the atherogenic effects of TMAO include: a. induction of inflammation by expression of inflammatory genes in both vascular SMC and endothelial cells; b. induction of ROS production; c. impairment of bile acids synthesis through interference in the FXR/TGPR5 axis; d. increase in platelet adhesiveness and thrombus formation; e. impairment of reverse cholesterol transport; f. promotion of oxLDL receptors expression in macrophages facilitating foam cells formation[3]

Taken together these experimental and clinical studies indicate that dietary derived TMAO is closely associated with atherosclerosis, is entirely dependent on gut microbiota and is a marker of clinical outcomes; however, it is not yet entirely clear whether it is a marker or a true causative factor of atherosclerosis. It should also be mentioned the physiological functions of TMAO, specifically cell protection against hydrostatic and osmotic stress cells in deep sea fish and humans.[30,31]

Gut microbiota in diabetes and obesity

Patients with type 2 diabetes (DM_2) have typical intestinal flora compared with non-diabetic individuals; lower concentrations of butyrate-producing bacteria, such as Roseburia intestinalis and Faecalisbacterium, and higher concentrations of Lactobacillus gasseri and Streptococus mutans are found in DM2 patients. Also, insulin-resistant patients have increased concentrations of branched-chain amino acids,[32] which are associated with Prevotella copri and Bacteroids vulgatus.[33] In addition, in DM_2 individuals, postprandial glucose in response to diet can be modulated by intestinal microbiota.[34] Also, imidazole propionate, a metabolite produced by microbiota is elevated in DM2 and impairs glucose tolerance.[35]

Hypertension

It is well known that elevated salt intake is implicated in hypertension. In mice, high salt intake induced significant changes in gut microbiota associated with a reduction in Lactobacilus murinus. When this species was added to the diet, hypertension was no longer induced,[36] partially due to modulation of TH17 cells. Another mechanism involves G-protein coupled receptors (GRCRs) that are regulated by SCFAs. SCFAs can stimulate GPCRs, affect renin secretion and hence blood pressure;[37] in this line of evidence, KO mice for $GPCR_{41}$ showed systolic hypertension and SCFAs lowered blood pressure through regulation of $GPR_{41.}$[37]

Furthermore, Olfr78 and GPR41 are expressed in vascular SMC of resistance vessels; interestingly, propionate can cause vasodilation in mice through modulation of Olfr78 and GPR41. Also, high levels of oxLDL contribute to hypertension through inhibition of NO, which is a classic endothelial vasodilator. In summary, the link between diet, microbiota and hypertension includes two branches: a) production of SCFAs that are the final substances of fiber fermentation in the gut and their effects upon GRPs and Olf78 that are present in SMC and control blood pressure; b) increases in oxLDL from diet which inhibits NO and increases endothelin-1, which acts on SMC.

Cheema et al.[38] investigated metabolites associated with infused Ang II in mice. They found four up-regulated and eight down-regulated plasma metabolites; in feces there were 25 unregulated and 71 down regulated. These effects did not occur in germ-free mice. Thus, the relationship between AngII and hypertension is differentially regulated by microbiota-dependent metabolites, by complex mechanisms. Karbach et al.[39] also observed that gut microbiota facilitates AngII- induced vascular dysfunction and hypertension. Clinical observations have indicated that butyrate-producing bacteria is associated with lower blood pressure in pregnant women.[36]

Despite these strong mechanistic studies that support the interaction of diet, gut microbiota and hypertension, the role of human microbiota in hypertension needs further studies.

Heart failure

The participation of intestinal microbiota in heart failure (HF) has been suggested in many studies.[1] For instance a depletion of gut microbiota and its diversity has been observed.[40] Tang et al.[41] also showed that elevated TMAO in HF patients indicates higher long-term mortality risk, independent of traditional risk factors and cardiorenal function.[41] Although mechanisms are not clear, one hypothesis is that bacterial translocation, inflammation and oxidative stress make these patients more vulnerable; that is an explanation well-suited to the

"gut/brain axis hypothesis". In support of this hypothesis is the observation that HF patients are more prone to Clostridium difficile infection.[42]

Intervention on gut microbiota

Diet is the main tool to modulate intestinal microbiota. De Fillipis et al.[7] analyzed gut microbiota in 153 individuals who were omnivorous, vegetarians and vegans. There were significant associations between the consumption of vegetable-based diets and increased levels of fecal SCFAs, Prevotella and some fiber-degrading bacteria. On the contrary, higher urinary TMAO levels were observed among those who did not follow a Mediterranean diet. These data indicate that a Mediterranean type of diet influences gut microbiota and protect against atherosclerosis.[45]

Resveratrol, a polyphenol encountered in grapes, vegetables, berries and red wine may influence gut microbiota. Ingested resveratrol has low bioavailability due to its metabolization in the liver and intestine. Bifidobacteria infantis and Lactobacillus acidophilus are bacteria involved in the metabolism of resveratrol. Chaplin et al.[43] also showed, in animals, potential beneficial effects of resveratrol in fat accumulation, adipose deport extension, hepatic fat accumulation, glucose intolerance and insulin resistance, high blood pressure and lipids; in view of these effects, the authors concluded that resveratrol might be useful in metabolic syndrome.

Chen et al.[44] investigated the effects of resveratrol on TMAO and BA synthesis by gut flora in Apo $E^{-/-}$ mice. Resveratrol attenuated TMAO- induced atherosclerosis in these mice. Resveratrol also increased Lactobacillus and Bifidobacterium levels, which increased bile salt hydrolase activity, thus enhancing BA deconjugation and fecal excretion. In addition, resveratrol suppressed the FXR-TGR_5 axis and increased CYP7A1 and hepatic BAs neosynthesis. In antibiotic- treated mice none of these effects were noted. The authors concluded that resveratrol attenuated TMAO-induced atherosclerosis by decreasing TMAO levels and augmenting hepatic BA neosynthesis via gut microbiota remodeling. As indicated before, BA synthesis is an important pathway to eliminate cholesterol from the body.

Enterotypes have been linked to dietary patterns. Thus, the first enterotype described by Arumugan et al.[5] which is high in Bacteroides and low in Prevotella, is found in long-term Western diets, rich in animal proteins, choline and saturated fats; the second enterotype is high in Prevotella, low in Bacteroides and is associated with plant-based diets rich in fibers and simple sugars; the third enterotype has a slightly higher population of the genus Ruminococcus of the phylum Firmicutes.[45] Wu et al.[6] confirmed, in 98 individuals, that enterotypes are strongly associated with long-term diets, especially protein and animal fats with Bacteroides, in contrast to Prevotella which is preferentially linked to carbohydrate metabolism. Taken together, these data suggest that diet modulation, especially the Mediterranean diet, may beneficially influence the gut microbiota. Personalized diets according to the intestinal microbiota is a promising approach for glycemic control, as suggested by Zeevi et al.[34] In our group, we tested the effects of red wine on gut microbiota and plasma metabolomics in CAD patients (Wineflora Study). Preliminary results suggest a potential beneficial effect on gut microbiota by induction of anti-atherosclerotic bacteria.

Another possibility is *enzymatic blockade* of TMA formation by suppressing FMO3. However, this approach leads to TMA accumulation in plasma and consequent fish odor syndrome, which hampers its clinical application.[46]

Also, bacterial enzyme inhibitors, such as choline TMA lyase and carnitine TMA lyase, represent another approach to reduce TMA production.[47] However no human data is yet available. Another approach would be the use of long-term broad-spectrum antibiotics to suppress TMAO formation, as mentioned before. Unfortunately, this is not possible in clinical practice. Further, the use of antibiotics in patients produced no effects in preventing coronary events.[48]

Prebiotics and *probiotics* are potential ways to interfere with gut microbiota. Probiotics are substances that contain live bacteria such as Lactobacillus.[45] Tannock et al.[49] gave a milk compound containing Lactobacillus rhamnosus to 10 normal individuals; they observed transient changes in fecal microbiota, specifically lactobacillus and enterococcus, but no concomitant modifications in biochemical parameters. Experimental clinical studies have offered promising results related to BA metabolism. Prebiotics are foods such as fibers whose metabolism provide the growth of "protective bacteria"; for instance, ingestion of nondigestible fibers may induce the growth of commensals and alter intestinal motility.[47] Prebiotics and probiotics are in early phases of development but will likely constitute valuable alternatives for gut microbiota modulation.

Another intervention that impacts on intestinal microbiota is *bariatric surgery*, in which increased circulating levels of primary and secondary BAs were observed.[2]

Finally, *fecal transplantation* can be employed in especial circumstances.[50] Few experiments have been conducted on humans, showing inconsistent results. A series of technical and ethical problems, such as the definition of healthy donors, still need clarification. However, in special circumstances such as IBD resistant to conventional treatment, fecal transplantation may be a valuable alternative.

Conclusions

Gut microbiota plays a pivotal role in atherosclerosis, heart failure, diabetes, and obesity, acting as an independent risk factor. Gut microbiota is essential for metabolism of nutrients like proteins, carbohydrates, and plant derivates. It interferes directly in the metabolism of SCFA, BAs, inflammation and immune system. It also induces the formation of TMAO, an atherogenic molecule. The intestine is considered today an endocrine organ since it produces substances that act locally or at distance. The intestine and the brain maintains constant and bidirectional influences through the "gut-brains axis". Human intestinal microbiota is profoundly influenced by diet, and for this reason, diet modulation, especially by adopting a Mediterranean type diet, is the most promising approach to beneficially influence gut microbiota. However, there are no clinic studies analyzing the long-term effectiveness of dietary interventions on gut microbiota. Further research is needed to clarify the roles of intestinal microbiota in health and human diseases.

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Author contributions

Conception and design of the research: Luz PL. Statistical analysis: Favarato D. Critical revision of the manuscript for intellectual content: Haas EA, Favarato D.

Potential Conflict of Interest

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