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REVIEW

The Gut Microbiota and Atherosclerosis: The State of the Art and Novel Perspectives

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Abstract

The human gut microbiota is composed of more than 100 trillion microbes. Most communities are dominated by species belonging to the phyla *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*. Microflora-derived short-chain fatty acids play a pivotal role in the framework of insulin resistance, obesity, and metabolic syndrome. They are an important energy source and are involved in several pathways, with proatherogenic and antiatherogenic effects. The increased gut microbiota lipopolysaccharide levels (defined as "metabolic endotoxemia") induce a state of low-grade inflammation and are involved in atherosclerotic disease through Toll-like receptor 4. Another important inflammatory trigger in gut microbiota–mediated atherosclerotic promotion is trimethylamine *N*-oxide. On the other hand, protocatechuic acid was found to promote cholesterol efflux from macrophages, showing an antiatherogenic effect. Further studies to clarify specific gut composition involved in cardiometabolic syndrome and atherogenesis are needed for greater use of targeted approaches.

Keywords: gut microbiota; atherosclerosis; metabolic syndrome

Introduction

The human gut microbiota is composed of more than 100 trillion microbes, grouped into more than 1000 species, with approximately 5 million genes and an average weight of 1.5 kg [1]. It has recently been investigated as one of the major contributors to host metabolism, and its imbalance and alterations are linked to several intestinal and metabolic diseases, such as metabolic syndrome.

In this review our aim is to describe the specific mechanisms involved in the relationship between the gut microbiota and atherosclerosis and possible novel therapeutic targets to reduce the burden of cardiovascular disease.

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The Human Gut Microbiota

Recent development of technology has made available high-throughput sequencing to analyze the collective genome of the gut microbiota derived from stool samples, known as the "metagenome," giving a remarkable contribution to our understanding of the gut microbiota composition. The combination of metagenomic analysis with clinical phenotypic data is known as a "metagenome-wide association study" [2].

Despite most of the microbiome being constituted of bacterial species, several viruses are present, although their function is almost unknown [3]. It is speculated that intestinal bacterial infections contribute to inflammation and atherosclerosis [4].

The bacterial composition of the intestinal microflora reflects the influence of several factors: diet, bacterial composition of the environment, and host genetics [5]. Although there is considerable diversity between individuals, different groups of scientists have analyzed serial stool collections and have shown that most communities are dominated by species belonging to the phyla *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* [6] and the unique core gut microbiota composition of an individual remains remarkably stable over time [7, 8].

In the last few years, wide investigation of the crosstalk between the gut microbiota and the host has shown a wide range of functions:

- It plays a pivotal role in the homeostasis of the gut immune system as well as in protection against pathogens.
- It contributes to several metabolic functions, such as Ca^{2+} absorption and bone metabolism, and chemical reactions of xenobiotics.
- It is somehow involved in both angiogenesis and the intestinal epithelial barrier [9, 10].

Data are being accumulated that indicate cross talk between the gut microbiota and intestinal disease may be involved in the development of different metabolic disorders, such as type 2 diabetes mellitus and obesity, and subsequent cardiovascular diseases [11]. In particular, interesting findings from a Finnish study suggest a role of inflammatory bowel disease in the development of coronary artery disease in patients who did not have cardiovascular risk factors other than hypertension [12].

Moreover, imbalance in metabolism of different cardiovascular drugs (such as statins), for example, resulting from a reduction of cytochrome P450 3A levels, has been found in untreated celiac disease [13].

The Gut Microbiota in Obesity and Type 2 Diabetes Mellitus: A Driver for Inflammation

It is well established that metabolic syndrome, obesity, and diabetes are major risk factors for coronary artery disease and a primary public health concern. However, only in the past 10 years an explicit link with the specific composition, relative proportions, and functional capacity of bacteria in the intestinal microbiome was recognized [14–16].

Metagenomic studies in humans and mice comparing the diversity of the gut microflora between diabetic or obese individuals and healthy controls underline a considerable variation:

- enrichment in *Firmicutes* and a corresponding decrease in *Bacteroidetes* levels in the microbiota of obese individuals, normalized to the level observed in the healthy controls after weight loss [17, 18];
- lower levels of *Faecalibacterium prausnitzii* in the microbiome of diabetic patients [19].

However, these results have to be verified because discordant data have recently emerged [20, 21].

Turnbaugh et al. [22] reported successful transmission of the obesity trait through cecal microbiome transplant from obese *ob/ob* mice into germ-free mice (which have sterile intestines and less total body fat content) compared with conventionally caged mice fed with a high fat-sugar–rich diet.

To explain the underlying mechanism for gut microbiome–mediated obesity, Bäckhed et al. [23] proposed the role of microflora-derived short-chain fatty acids (SCFAs) in the resistance to diet-induced obesity in germ-free mice. SCFAs derive from fermentation of nondigestible carbohydrates (depending on daily fiber intake) [11] to yield energy, leading to a more efficient harvest from dietary intake. In healthy individuals, SCFAs are mainly composed of acetate (60%), propionate (25%), and butyrate (15%) [22], and these play a complex role in the relationship between the gut microbiome and host metabolism [23]. Once absorbed in the plasma of the host via the intestinal epithelium, they are not only an important energy source but are also involved in several pathways, with proatherogenic and antiatherogenic effects according to their composition.

Using a metagenomic approach, Cani et al. [19] described a reduction of the abundance of *F. prausnitzii,* a butyrate SCFA–producing species, in diabetic patients compared with lean controls, while Karlsson et al. [24] described an increase in the abundance of *Lactobacillus gasseri* and *Streptococcus mutans* and a reduction in the number of *Roseburia* species (another butyrate

SCFA–producing species) and *F. prausnitzii* in patients with type 2 diabetes. These studies outlined a road map for the hypothesis of SCFAs as possible regulators of host metabolic state, showing how butyrate SCFA–producing species may decrease plasma glucose levels by enhancing glucagon-like peptide 1 release and leptin synthesis and reducing insulin resistance [24]. On the other hand, Kootte et al. [11] described a potential role for SCFAs in promoting a chronic inflammatory state subsequent to an impairment of intestinal permeability.

In a randomized trial Vrieze et al. [25] evaluated treatment-naive male participants with metabolic syndrome–derived insulin resistance randomized to receive either an allogenic gut microbiota transplant (from lean male donors with a body mass index <23 kg/m^2) or an autologous gut microbiota transplant (reinfusion of own collected feces). They reported a reduction of the level of overall SCFAs and especially butyrate SCFA, with a concomitant reduced excreted energy content in the feces that was restored when patients received a lean-donor gut microbiota transplant.

Another elegant contribution to the establishment of SCFAs as pivotal mediators in gut metabolism comes from bariatric surgery studies. The importance of a gastric bypass procedure (Roux-en-Y gastric bypass) in the treatment of obese and metabolic syndrome patients is well recognized but recent findings underline a therapeutic effect beyond weight reduction, that is a significant increase in insulin sensitivity before weight loss and an enrichment of the beneficial microbe *F. prausnitzii*. [26–29]. As explained already, this species of bacteria may exert a protective role through butyrate SCFA secretion, since the levels are negatively correlated with inflammatory markers, suggesting a potential role in modulating systemic inflammation [29].

Another mechanism by which the gut microbiota may induce the development of obesity, type 2 diabetes mellitus, and subsequent metabolic syndrome could be macrophage activation by bacterial endotoxins (e.g., lipopolysaccharide, LPS) and intestinal bacteria translocation. Macrophage infiltration in visceral adipose tissue has been highlighted as a probable link because of recent studies showing an association between an altered intestinal microbiota and proinflammatory changes in adipose gene expression [30–32].

Different mechanisms are involved in intestinal bacteria translocation:

- a cotransport on dietary fat–derived chylomicrons [33, 34];
- decreased production of glucagon-like peptide 2 in intestinal neuroendocrine L cells, caused by LPS, which leads to weakened colon epithelial integrity [35].

The increased gut microbiota LPS levels (defined as "metabolic endotoxemia") induce a state of low-grade inflammation in mice fed a high-fat diet. In particular, an association with reduced levels of *Bifidobacterium* and *Eubacterium rectale/Clostridium coccoides* was found to be significant [36].

Sanz et al. [37] described a similar phenotype in murine models comparing mice fed a high-fat diet with those that received long-term infusion of LPS to reach the same plasma LPS levels measured in the first group, showing a specific link between translocation of intestinal bacteria products and activation of inflammatory response by interaction with Toll-like receptor 4(TLR4). The pivotal role of TLR4 in this complex interplay was clarified with mice fed a high-fat-diet that had CD14 knocked out, a key molecule in TLR4 signaling, characterized by strong resistance to the development of obesity.

The state of low-grade inflammation determined by microbiota-derived LPS leads to substantial alterations in both glucidic and lipid metabolism: an increase of insulin resistance and activation of macrophages that infiltrated adipose tissue, promoting fasting hyperglycemia, dyslipidemia, obesity, and hepatic steatosis (Figure 1) [38].

Finally, it is worth noting that, besides LPS, other gut microbiota compounds participate in this proinflammatory scenario, including peptidoglycans, lipoproteins and flagellins, which induce activation of innate inflammatory pathways [39, 40].

The Gut Microbiota and Atherothrombosis

The cross talk between the gut microbiota and the pathogenesis of inflammation in atherosclerosis has

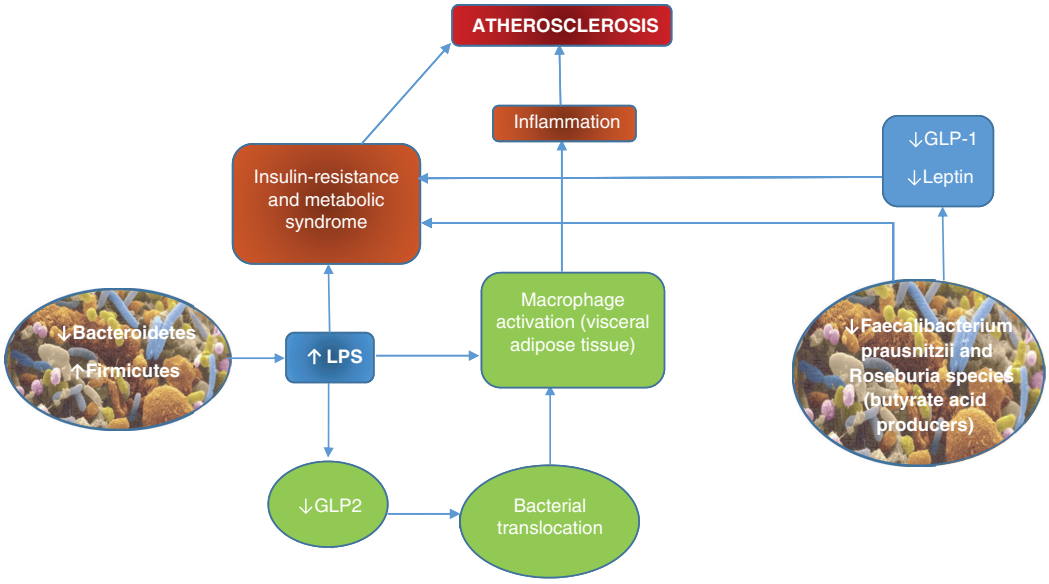

Figure 1 Relationship Between Changes in Gut Microbiota Composition and Atherosclerosis.

been widely investigated in recent years. We can summarize the role of the gut microbiota into two main areas:

- proatherogenic effects;
- antiatherogenic effects.

Proatherogenic Effects

Endotoxemia-derived chronic low-grade inflammation can be considered a potential contributing factor for both metabolic syndrome and atherosclerosis [41].

Bacterial LPSs play a pivotal role in the development of atherosclerosis. They may interact with low-density lipoproteins (LDLs) and influence their metabolism, inducing the production of oxidized LDL through release of superoxide anions (O^{2-}) [42, 43] and endothelial dysfunction [44, 45]. Oxidized LDL is one the major triggers of the inflammatory cascade, promoting transformation of macrophages into foam cells by enhancing the levels of proinflammatory mediators such as interleukin-1 and tumor necrosis factor α [46, 47].

Depending on the concentrations of LPS, different pathways are activated (Figure 2) [48–50]. It is possible to distinguish among:

- high-dose LPS;
- low-dose LPS;
- superlow-dose LPS.

High doses of LPS induce production of several proinflammatory cytokines in macrophages through marked activation of nuclear factor κB and mitogen-activated protein kinases, as well as the negative regulators IκBα (negative regulator of nuclear factor κB), phosphatidylinositol 3-kinases, mitogen-activated protein kinase phosphatase 1, and interleukin-10 as a compensatory mechanism to control excessive inflammation [51–53].

The main pathway elicited in this action involves TLR4, which activates the interleukin-1 receptor associated kinases (1, 2, and 4) via the myeloid differentiation primary response 88 (MyD88) adaptor molecule [54]. TLR4 is expressed among other tissues on cardiomyocytes and foam cells. Kiechl et al. [55] described a common polymorphism associated with low levels of circulating inflammatory mediators and reduced risk of atherosclerosis, thus enhancing the importance of TLR4 in atherosclerosis development [56].

Particularly significant are recent data obtained by Maitra et al. [48] showing an important contribution to inflammation by low-dose LPS through hepatocyte nuclear factor 1 homeobox B and downregulation of phosphatidylinositol 3-kinase–dependent negative regulators of inflammatory genes. In this context, an important role is played by generation of mitochondrial reactive oxygen species.

Surprisingly, superlow doses of LPS were associated with mitochondrial fission and cell necroptosis

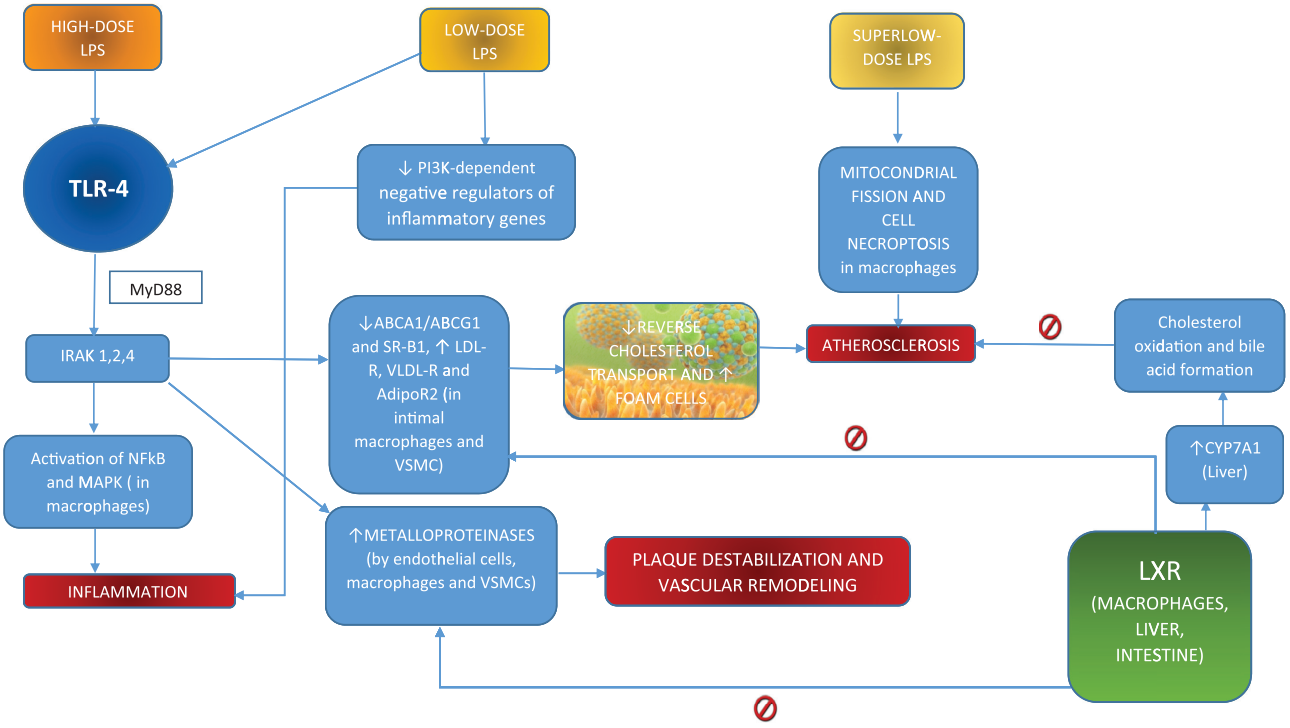

Figure 2 High, Low and Superlow Doses of Lipopolysaccharide and Atherosclerosis.

in murine macrophages [50]. "Necroptosis" refers to a molecular pathway of regulated necrosis induced by inflammatory molecules, such as Toll-like receptors, interferon-γ, death receptors, and intracellular RNA and DNA sensors through receptor-interacting protein kinase 3 (RIPK3)- and mixed-lineage kinase domain-like (MLKL)-dependent molecular cascades, involving degradation of mitofusin 1 and activation of *dynamin-related protein 1* [57].

Besides LPS triggering inflammation, a novel role for low-dose LPS was characterized in cholesterol metabolism, acting at the very first step of atherosclerosis: a reduction of the levels of proteins involved in reverse cholesterol transport, such as the ATP-binding cassette transporters ABCA1 and ABCG1 and scavenger receptor SR-B1 [49].

Reverse cholesterol transport is an established atheroprotective mechanism, determining cholesterol efflux from foam cells accumulated in atherosclerotic lesions [58]. There is a complex interplay in cholesterol systemic balance in and out of intimal macrophages and vascular smooth muscle cells between TLR4 and liver X receptors (LXRs) based on reciprocal inhibition of each other [59, 60].

Higashimori et al. [61] provided further data from aortic plaque analysis in apolipoprotein E/Toll-like receptor 2 (TLR2) and apolipoprotein E/TLR4 double-knockout mice that outlined the already described bacterial LPS-activated TLR2/TLR4/MyD88 pathway promoting intracellular cholesterol accumulation.

LXRs are distributed in macrophages, the liver, and the small intestine, and promote reverse cholesterol transport through expression of the sterol transporters ABCA1 and ABCG1 in macrophages, degradation of LDL receptor, very low density lipoprotein receptor, and adiponectin receptor 2, and enhancement of liver expression of cholesterol-7-α-hydroxylase, thus increasing cholesterol oxidation and bile acid formation, with subsequent reduction of atherosclerotic burden [62–64].

Moreover, the interplay between the bacterial LPS-mediated TLR2/TLR4 pathway and LXRs has recently been described in plaque destabilization and vascular remodeling, involving matrix metalloproteinase 9 production by endothelial cells, macrophages, and vascular smooth muscle cells. While TLR4 mediates activation of metalloproteinases, LXRα blocks TLR2/TLR4-dependent stimulation [65–67].

Another important inflammatory trigger involved in gut microbiota–mediated atherosclerotic promotion is trimethylamine *N*-oxide (TMAO), a proatherogenic compound derived from the metabolism of

phosphatidylcholine by the gut flora [68]. Systemic levels of three metabolites of phosphatidylcholine (choline, TMAO, and betaine) were described as eligible predictors of the risk of cardiovascular disease in large clinical cohorts with use of a metabolomic approach [69, 70].

TMAO is produced in a two-step process, starting with degradation of dietary phosphatidylcholine or carnitine (especially from red meats) by specific intestinal bacterial strains, such as *Prevotella*, into the precursor trimethylamine. The second step occurs in the liver, where trimethylamine is converted into TMAO by flavin monooxygenase 3 [69, 70].

TMAO promotes atherosclerosis through two main pathways (Figure 3):

- accumulation of cholesterol in macrophages by inhibition of reverse cholesterol transport through an increase in the levels of scavenger receptors CD36 and SR-A at the cell surface [68];
- decreased synthesis of bile acids from cholesterol and their transporters in the liver [69].

Hypercoagulability plays a crucial role in obesity-related chronic inflammation, because of upregulation of tissue factor expression in the intestinal microvasculature, increased production of factors II (prothrombin), VII, IX, and X and well-known procoagulant vitamin K–dependent clotting mediators, and reduced fibrinolytic capacity [71, 72].

Antiatherogenic Effects

The gut microbiota may exert atheroprotective effects through suppression of the inflammatory cascade, reduction of cholesterol accumulation in macrophages, and insulin resistance. Protocatechuic acid, a microbiota-derived metabolite of cyanidin 3-*O*-β-glucoside, was found to promote cholesterol efflux from macrophages, thus showing a profound antiatherogenic effect [73]. Protocatechuic acid downregulates miR-10b, whose targets are cholesterol transporter ABCA1 and ABCG1 mRNAs, thus increasing their expression (Figure 4) [70, 74].

Moreover, dietary intake–derived anthocyanin pigment Cy-3-G was described as a regulator of the LXRα/ABCG1 axis, inhibiting TLR4-mediated proinflammatory signaling in macrophages, promoting cholesterol depletion and subsequent disrupting lipid rafts [75].

Valuable evidence from studies with probiotics showed a key role played by several specific intestinal bacterial strain, such as *Lactobacillus plantarum* DSM9843 and *L. plantarum* 299v: Karlsson et al. [76] outlined in men with carotid atherosclerosis that changes in bacterial diversity promoted production of SCFAs with antiatherogenic effects.

Similarly, other strains used in this approach demonstrated decreased levels of the proinflammatory cytokine interleukin-6, reduced adhesion of monocytes to endothelial cells, and LDL synthesis reduction [77].

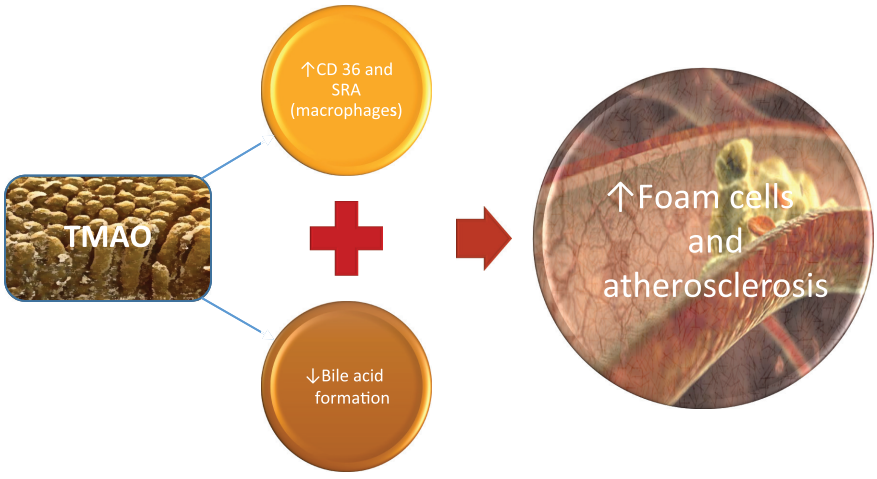

Figure 3 Trimethylamine-N-Oxide(TMAO) Pathways in Atherosclerosis Development.

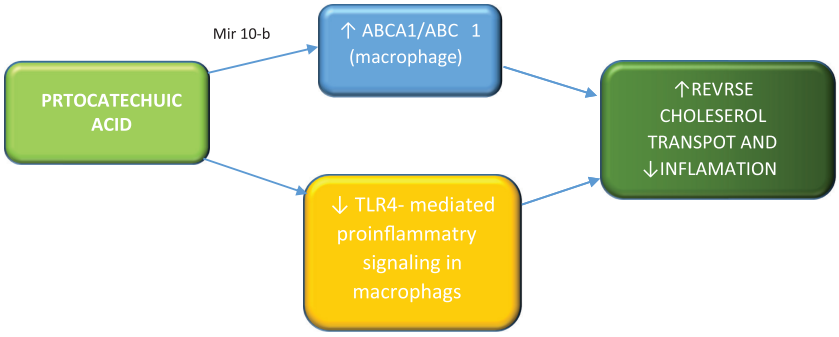

Figure 4 Protocatechuic Acid Antiatherogenic Effects.

The Gut Microbiota: Therapeutic Prospects and a Look to the Future

Studies in humans and mice emphasize the delicate contribution of the gut microbiota in modifying the risk of obesity, insulin resistance and atherosclerosis.

Different bacterial species play a pivotal role in this complex relationship, leading either to metabolic syndrome or a lean phenotype. Recent observational and interventional studies confirm a potent correlation among diets rich in choline and trimethylamines, altered gut microbiota composition, probiotics and anthocyanin metabolism and cardiovascular disease.

Many additional investigations are required to reveal the many hidden aspects of the gut microbiota's role in this complex relationship with the host and the subsequent implications for its role in atherosclerotic inflammation.

Further studies to clarify the specific gut composition involved in cardiometabolic syndrome and atherogenesis are needed for greater use of targeted approaches, such as antibiotics, microbiota transplant, probiotics, or specific immunotherapy. Further exploration of the metabolic pathway leading to TMAO, a wide investigation on probiotics, and investigation of the relationship between the gut microbiota and adipose tissue–associated activated macrophages offer the most promising mechanisms for therapies.

Conflicts of Interest

The authors declare no conflict of interest.

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