Review

Gut Microbiota - Derived Metabolites in Atherosclerosis: Pathways, Biomarkers, and Targets

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Gut Microbiota - Derived Metabolites in Atherosclerosis: Pathways, Biomarkers, and Targets

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Abstract

This review aims to analyze the critical role of gut microbiota-derived metabolites in the pathogenesis of atherosclerosis, a chronic inflammatory disease driven by lipid accumulation and immune dysregulation, and a leading cause of cardiovascular morbidity and mortality. Gut dysbiosis, marked by reduced microbial diversity and overgrowth of pro-inflammatory bacteria, compromises intestinal barrier integrity, allowing translocation of lipopolysaccharides (LPS) and peptidoglycans, which activate TLR/NF-κB pathways and promote systemic inflammation. Trimethylamine N-oxide (TMAO), derived from dietary choline and L-carnitine, exacerbates endothelial dysfunction, foam cell formation, and thrombosis, while secondary bile acids, such as deoxycholic and lithocholic acid, modulate inflammation via FXR/TGR5 signaling, with effects varying by concentration. In contrast, short-chain fatty acids (SCFAs), particularly butyrate, exert anti-atherogenic effects by enhancing gut barrier function, reducing inflammation, and improving lipid and glucose metabolism through GPCR and HDAC pathways. Therapeutic strategies, including dietary interventions (fiber-rich Mediterranean diets), probiotics (Lactobacillus, Bifidobacterium), prebiotics, fecal microbiota transplantation (FMT), and small-molecule inhibitors (DMB, IMC), target these metabolites to mitigate atherosclerosis. This review highlights gut microbiota-derived metabolites as pivotal biomarkers and therapeutic targets, emphasizing the potential of personalized microbiome-based interventions for atherosclerosis prevention and management.

Keywords: gut microbiota; metabolites; atherosclerosis; dysbiosis; biomarkers; probiotics; prebiotics; therapeutic strategies

1. Introduction

The understanding of atherosclerosis has evolved significantly since the early 19th century, beginning with Karl von Rokitansky's observation of arterial wall thickening due to intraluminal deposits. Ludwig Aschoff later introduced the first classification of atherosclerotic plaques, identifying their lipidic and fibrotic nature. In the 1950s, the WHO defined "complicated plaques" with features such as thrombosis and ulceration. The American Heart Association refined classification in 1994 into eight lesion types, and in the early 2000s, Virmani et al. proposed a clinically oriented model with four main lesion categories, highlighting the dynamic progression of atherosclerotic disease.

Atherosclerosis is now widely recognized as a chronic inflammatory disease of the arterial wall, contributing to approximately 50% of all deaths in industrialized societies. It is fundamentally a lipid-driven process, initiated by the subendothelial accumulation of low-density lipoproteins (LDL) and remnant lipoprotein particles, coupled with localized vascular inflammation. This pathophysiological sequence underlies the development of atherosclerotic cardiovascular disease (ASCVD), which encompasses myocardial infarction, ischemic stroke, and peripheral arterial disease [1–3].

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Cardiovascular disease, primarily driven by atherosclerosis, has been the subject of extensive investigation, yielding critical insights into cholesterol biosynthesis, lipoprotein metabolism, and associated risk factors. Atherosclerosis, the predominant cause of ischemic heart disease, affects large and medium-sized arteries, with lesion development favoring regions exposed to low or oscillatory shear stress. It is a multifactorial pathology shaped by both environmental influences and genetic predispositions.

Atherosclerotic lesion progression is characterized by a transition from early lipid-rich fatty streaks to structurally complex fibrous plaques, which may ultimately lead to luminal narrowing, vascular occlusion, and acute cardiovascular events. These lesions arise within the intimal layer of the arterial wall, which together with the media and adventitia comprises the three-layered architecture of susceptible vessels. Atherogenesis is initiated by endothelial dysfunction, which increases vascular permeability and facilitates the subendothelial accumulation of lipids and immune cells.

Lipoproteins play a pivotal role in this process, particularly low-density lipoproteins (LDL). Elevated plasma levels of LDL, and more critically, its oxidized form (oxLDL), promote vascular inflammation and plaque formation. Oxidative stress marked by an imbalance between pro-oxidant species, such as reactive oxygen species (ROS), and endogenous antioxidant systems further exacerbates endothelial injury and lipid peroxidation. ROS not only directly damage vascular cells but also modulate vasoactive molecules and inflammatory pathways.

The immunoinflammatory component of atherosclerosis involves recruitment and activation of monocytes and T lymphocytes, which migrate into the intima and differentiate into foam cells upon uptake of modified lipoproteins. Foam cells, in turn, secrete pro-inflammatory cytokines and matrix-degrading enzymes, contributing to plaque expansion and instability. Adhesion molecules and chemokines amplify leukocyte recruitment, sustaining a chronic inflammatory milieu.

Endothelial cells serve as mechanosensitive regulators of vascular homeostasis and are profoundly influenced by hemodynamic forces. While laminar shear stress exerts a protective effect by maintaining an anti-inflammatory and antithrombotic endothelial phenotype, disturbed flow patterns promote the activation of pro-atherogenic transcription factors such as NF-κB, which orchestrate inflammatory gene expression and endothelial dysfunction.

Foam cell formation remains a hallmark of atherosclerotic pathology, representing the intersection of lipid metabolism and innate immune activation. The accumulation of foam cells and their secretory products alters the composition and stability of the arterial wall, accelerating disease progression.

A comprehensive understanding of the cellular and molecular mechanisms underlying atherosclerosis is essential for the identification of novel therapeutic targets and the development of effective strategies for the prevention and management of cardiovascular disease [4,5].

Atherosclerosis is a highly modifiable condition, with its progression strongly influenced by dietary patterns and lifestyle behaviors. Interventions such as the adoption of a structured lifestyle, regular physical activity, smoking cessation, and adherence to a nutrient-rich diet are fundamental strategies for symptom management and the enhancement of cardiovascular health and quality of life. Among these, diet plays a pivotal role, as nutritional intake significantly impacts lipid metabolism, systemic inflammation, and plaque stability [6].

Excessive intake of saturated fats and dietary cholesterol is associated with elevated plasma cholesterol levels, contributing to atherogenesis. Conversely, the consumption of polyunsaturated fatty acids (PUFAs), particularly n-3 (omega-3) and n-6 (omega-6) fatty acids, has demonstrated cholesterol-lowering effects and is associated with a reduced risk of atherosclerosis. In contrast, trans fatty acids are strongly correlated with adverse cardiovascular outcomes and should be minimized in dietary practices [7].

Cholesterol homeostasis, regulated by a complex interplay of endogenous biosynthesis and dietary absorption, is modifiable through both nutritional strategies and pharmacological interventions. Functional dietary components such as PUFAs, phytosterols, polyphenols, and

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essential vitamins have garnered increasing attention for their cardioprotective properties. These compounds exert anti-inflammatory, antioxidant, and lipid-lowering effects, thereby contributing to atherosclerosis prevention and plaque stabilization [8,9].

The gut microbiota comprises a diverse community of microorganisms including bacteria, viruses, fungi, and archaea with bacteria being the most abundant. These microbes primarily inhabit the gastrointestinal tract, especially the nutrient-rich, anaerobic environment of the ascending colon [10].

In infants, gut microbiota composition is influenced by the mode of delivery. Vaginally delivered infants are initially colonized by Lactobacillus and Prevotella, while those born via cesarean section harbor skin-associated bacteria such as Streptococcus, Corynebacterium, and Propionibacterium, making them potentially more susceptible to infections. For instance, 64–82% of newborns with MRSA infections were delivered by C-section. The gut microbiota evolves during early life, reaching adult-like complexity and stability by age three. Diet (breast milk vs. formula) and antibiotic exposure further shape microbial development, with long-term health implications [11].

In adults, the dominant gut microbiota phyla include Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomicrobia, with Firmicutes and Bacteroidetes comprising over 90% of the community. The Firmicutes/Bacteroidetes (F/B) ratio is often used as a health marker; it is elevated in obesity and cardiovascular disease. Studies have shown an increased Firmicutes and decreased Bacteroidetes abundance in patients with coronary artery disease and hypertension.

Gut microbiota composition also shifts with aging, marked by reduced microbial diversity and a decline in beneficial species. These changes may contribute to the development of age-related diseases such as atherosclerosis [12].

The global rise in immune-mediated, metabolic, and neurological diseases is increasingly linked to gut microbiota dysbiosis an imbalance in microbial composition marked by reduced diversity, loss of beneficial microbes, and overgrowth of harmful ones. Environmental factors such as diet, medications, and food additives play a significant role in disrupting the gut microbiota, leading to impaired gut barrier integrity, immune dysregulation, and metabolic disturbances [13].

Dysbiosis is associated with numerous diseases, including obesity, diabetes, IBD, cardiovascular disease, liver disorders, and COVID-19. Pathogenic changes in microbiota composition often precede disease onset, suggesting a possible causative role. Studies have identified microbial signatures and metabolites (SCFAs, TMA, succinate) as potential diagnostic and prognostic biomarkers [14].

Therapeutic strategies to restore microbiota balance include probiotics, prebiotics, fecal microbiota transplantation (FMT), and targeted metabolic modulation. FMT is highly effective in treating recurrent Clostridioides difficile infections and is being explored for other conditions. Probiotics like Lactobacillus and Faecalibacterium show promise in managing inflammation and improving gut health, but personalized approaches and high-quality clinical trials are needed [15].

Targeting microbial metabolites such as inhibiting TMA production to reduce cardiovascular risk or enhancing SCFA levels to improve liver health represents a growing therapeutic frontier. Further research is needed to clarify whether dysbiosis is a cause or consequence of disease, but mounting evidence supports its central role in pathogenesis and treatment across multiple disease domains [16].

Atherosclerosis is a chronic inflammatory condition strongly linked to gut dysbiosis and increased intestinal permeability. The gut epithelium acts as a critical barrier that prevents the translocation of microbial components into circulation. Disruption of this barrier marked by decreased expression of tight junction proteins allows pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS) and peptidoglycan (PG), to enter the bloodstream and trigger systemic inflammation [17].

LPS, a component of Gram-negative bacteria, is closely associated with cardiovascular disease (CVD). It activates immune signaling via Toll-like receptor 4 (TLR4) and its co-receptors, promoting inflammatory pathways (NF-κB, MyD88) and increasing cytokine production (IL-6, IL-1, TNF-α).

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Elevated LPS levels due to gut dysbiosis have been detected in individuals with higher CVD burden. While some genetic polymorphisms in TLR4 show inconclusive effects on atherosclerosis, TLR4 or MyD88 deficiency in animal models reduces plaque formation [18].

Similarly, peptidoglycan present in both Gram-negative and Gram-positive bacteria activates immune responses via NOD1 and NOD2 receptors, contributing to inflammation and gut barrier dysfunction. NOD1/2 deficiency in mice reduces atherosclerosis, emphasizing the role of innate immunity in disease progression. Other microbial PAMPs (flagellin, CpG DNA, lipopeptides) also contribute to vascular inflammation through host pattern recognition receptors [19].

Collectively, these findings support the view that gut microbiota imbalance contributes to atherosclerosis through microbial translocation and immune activation. However, despite clear links between pathogenic bacteria and CVD, antibiotic trials for atherosclerosis have shown mixed results, highlighting the complexity of host–microbe interactions in cardiovascular disease.

Beyond inflammation, gut microbiota-derived metabolites play a significant role in the development of cardiovascular disease (CVD). These metabolites including short-chain fatty acids (SCFAs), methylamines, polyamines, trimethylamine N-oxide (TMAO), and secondary bile acids (BAs) result from microbial metabolism and co-metabolism with the host. SCFAs are well-known for their roles in metabolic regulation, but growing evidence links TMAO and secondary BAs specifically to the pathogenesis of atherosclerosis [20].

The objective of this review is to investigate the mechanisms by which the gut microbiota contributes to the development and progression of atherosclerosis. Emphasis is placed on the impact of gut dysbiosis in compromising intestinal barrier integrity, facilitating systemic inflammation via microbial components such as lipopolysaccharide (LPS) and peptidoglycan (PG), and activating immune signaling pathways. Additionally, the role of microbiota-derived metabolites particularly trimethylamine N-oxide (TMAO) and secondary bile acids in promoting atherogenesis is examined. By integrating historical perspectives on atherosclerotic plaque classification with recent advances in microbiota research, this review highlights the gut microbiota as a potential source of diagnostic markers and a promising target for therapeutic intervention in cardiovascular disease.

2. Background

A comprehensive literature search was conducted across PubMed, Scopus, and Web of Science using terms such as “gut microbiota,” “atherosclerosis,” “metabolites,” and “therapeutic targets” to identify studies with experimental or clinical data on gut microbiota’s involvement in atherosclerosis development and progression. Articles were selected based on topical relevance, recency, and methodological rigor, excluding those lacking direct evidence of gut microbiota involvement or with incomplete methodologies. Data were extracted regarding experimental models, effects on vascular inflammation, lipid metabolism, and therapeutic interventions. Physio-pathological effects were compared by analyzing animal and human studies.

3. Main Text

3.1. Composition and Functions of Gut Microbiota

3.1.1. Definition and Composition

The human gut microbiota is a dynamic and complex ecological system composed of bacteria, viruses, fungi, and parasites totaling approximately 100 trillion microorganisms. Colonization begins at birth, influenced by delivery mode and maternal microbiota. In healthy individuals, the gut microbiota is dominated by Firmicutes and Bacteroidetes, with key genera including Lactobacillus, Clostridium, and Bifidobacterium. It plays essential roles in gut barrier protection, immune system development, nutrient metabolism, and drug absorption [10].

A healthy gut microbiota is characterized by high alpha diversity, which increases in early life and is associated with metabolic and immune health. Factors such as age, genetics, geography, and especially diet significantly shape microbiota composition. Diets rich in fiber support beneficial

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microbes like Bifidobacterium and Prevotella, while animal-based or Western diets promote bile-tolerant and pro-inflammatory species like Bilophila and Bacteroides, often at the expense of Firmicutes like Roseburia [21].

Unbalanced diets and unhealthy lifestyles are linked to gut dysbiosis and reduced microbial diversity, particularly in obesity. Alterations in the Firmicutes/Bacteroidetes ratio and decreases in beneficial species like Akkermansia muciniphila are common in overweight individuals. Supplementation with A. muciniphila has been shown to improve obesity-related outcomes, including gut barrier integrity and inflammation [22].

Therapeutic dietary interventions can beneficially modulate the microbiota. Evidence supports the use of high-fiber and anti-inflammatory diets in improving both metabolic and inflammatory conditions. Notably, fecal microbiota transplantation (FMT) followed by an anti-inflammatory diet outperformed standard therapies in managing ulcerative colitis. This growing body of evidence highlights the powerful role of diet in shaping the gut microbiome and its potential as a therapeutic tool across a spectrum of diseases [23].

3.1.2. Physiological Functions

The gut microbiome plays a vital role in digesting macronutrients and producing metabolites that can affect human health both locally and systemically. These metabolites ranging from beneficial short-chain fatty acids to potentially harmful compounds are influenced by the type of nutrient metabolized and the microbial species involved. This review explores how microbial metabolism of carbohydrates, proteins, and fats impacts health and disease [24].

The human gut microbiota is a highly complex and essential ecosystem that maintains gastrointestinal homeostasis, primarily through microbial metabolism. Bacteria are the dominant contributors, fermenting both exogenous and endogenous substrates into metabolites that support host nutrition, modulate immune function, and influence gene expression. Most fermentation occurs in the colon due to favorable conditions such as longer transit time and lower pH, but the small intestine also plays a role in regulating nutrient absorption [25].

Gut microbes help prevent pathogen colonization by occupying ecological niches and producing acidic by-products that lower luminal pH. However, some fermentation pathways can yield toxic compounds that damage the epithelium and trigger inflammation.

Macronutrients (carbohydrates, proteins, and fats) that escape or resist primary digestion reach the colon, where they serve as substrates for microbial metabolism. Digestive efficiency and therefore microbial substrate availability is influenced by food structure, nutrient composition, anti-nutrients, and transit time, all of which are affected by diet, activity, genetics, and psychological state [26].

In addition to producing energy-relevant metabolites like short-chain fatty acids, gut bacteria synthesize essential micronutrients (B vitamins) and biotransform plant polyphenols to enhance their bioavailability.

Gut microbiota play a crucial role in digestion and influence distant organs through the gut-brain, gut-liver, gut-kidney, and gut-heart axes via microbial metabolites. While short-chain fatty acids (SCFAs) from carbohydrates are well-studied, protein and fat metabolism by microbes remains less understood. The health impact of microbial activity depends on the balance of metabolic processes and substrate availability, which are influenced by host digestion. Understanding these pathways in health and disease could support the development of targeted therapies for conditions like NAFLD, cardiovascular disease, and neuropsychiatric disorders [27].

The human colonic microbiota is a highly diverse and metabolically active community, comprising over 1,000 bacterial species, with around 160 species found in any given individual. Its collective genome the gut microbiome contains approximately 3 million genes, vastly outnumbering the human genome. This extensive genetic capacity enables the microbiota to perform functions absent in the human host, such as breaking down complex polysaccharides, metabolizing polyphenols, and synthesizing essential vitamins [28].

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The microbiota plays a central role in human metabolism, complementing host enzymatic activity in the liver and gut mucosa. Evidence from germ-free animal models, humanized microbiota experiments, and in vitro systems supports its role in dietary metabolism and disease development. Alterations in gut microbiota composition are associated with gastrointestinal conditions like IBD, IBS, colon cancer, and antibiotic-associated diarrhea, as well as systemic disorders such as obesity and diabetes [29].

The gut microbiota significantly extends the host's metabolic capacity, particularly in processing carbohydrates, proteins, vitamins, and polyphenols. Its metabolites interact dynamically with both host and microbial systems, influencing metabolism, immunity, and energy balance. Due to functional redundancy and cross-feeding among microbes, composition alone cannot predict function. Understanding these complex host–microbiota interactions is essential for linking microbiome activity to health and disease [25].

The gut microbiome plays vital roles in maintaining host health by producing short-chain fatty acids (SCFAs), synthesizing essential nutrients, regulating fat metabolism, and supporting colonocyte function and immune development. It exhibits anti-inflammatory effects through regulatory T cells (Tregs) and G-protein coupled receptor (GPCR) signaling. Germ-free (GF) animal models have shown that the absence of microbiota impairs immune system development, especially T cell differentiation, including Th17 cells which are crucial for mucosal immunity. Specific microbes, such as segmented filamentous bacteria (SFB), are necessary for inducing Th17 responses and enhancing protection against pathogens, though excessive activation may contribute to autoimmune diseases.

Beneficial bacteria like Bifidobacterium and lactic acid bacteria help suppress inflammation and promote Treg-mediated tolerance. Microbial metabolites, particularly SCFAs, influence host immunity through both receptor-mediated signaling and epigenetic regulation, underscoring the complex bidirectional communication between the gut microbiota and the immune system [30–32].

Early microbiota colonization is essential for the development and function of the intestinal barrier, immune system, and enteric nervous system (ENS). Microbiota influence epithelial renewal, gut motility, mucus production, and immune homeostasis. Disruptions in this balance can lead to inflammation and disease, highlighting the critical interplay between microbes, barrier integrity, and host defense [33].

The gastrointestinal tract is the body's largest interface with the external environment and a critical component of immune defense. It processes food, hosts beneficial microbes, and protects against pathogens through a single layer of specialized intestinal epithelial cells (IECs) and tight junctions. IECs, including enterocytes, goblet cells, and Paneth cells, secrete antimicrobial peptides and mucins, support immune signaling, and maintain barrier integrity. The gut also contains a vast immune network, including the gut-associated lymphoid tissue, Peyer's patches, and lamina propria lymphocytes, which help distinguish between harmful and harmless antigens [34].

Tight junctions regulate permeability, while epithelial turnover prevents pathogen attachment. Immune components like dendritic cells and immunoglobulins further defend the mucosa. Importantly, the gut microbiota is essential for nutrient metabolism, immune system development, and maintenance of the intestinal barrier. Its role in shaping host immunity and supporting gut health continues to be a major focus of current research.

Intestinal epithelial cells (IECs) actively interact with the immune system and microbiota, rather than serving as passive barriers. Early microbial colonization shapes immune development, and lifelong microbial diversity influences disease risk. Understanding this complex crosstalk may enable personalized, microbiome-based therapies [35].

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3.1.3. Dysbiosis

The concept of dysbiosis an imbalance in the gut microbiota has evolved over more than a century. While often linked to Élie Metchnikoff, who emphasized the importance of gut microbes for health, the term itself was likely coined by C.A. Scheunert in 1920. Helmut Haenel later popularized its modern use, defining dysbiosis as a disturbance in gut microbial composition, contrasting with the healthy state he called eubiosis. Despite its frequent use in contemporary microbiome research, the definition of dysbiosis remains vague and inconsistent, typically referring to changes, imbalance, or loss of diversity often linked to disease, but with limited mechanistic explanation [36].

Studies show that definitions of dysbiosis fall into three categories: general change, imbalance (often defined as a loss of homeostasis), and specific taxonomic shifts. However, without standardized criteria or time-series data, distinguishing normal from abnormal microbiota remains challenging. Some researchers argue for a shift toward functional rather than purely compositional definitions of dysbiosis. Alternative terms like eubiosis, normobiosis, and homeostasis have been introduced but are often poorly defined themselves.

The main critique is that dysbiosis is used too broadly, often as a placeholder for unknown mechanisms, and risks becoming circular if any deviation from a loosely defined "healthy" microbiota is labeled pathological. More precise, functional, and longitudinal studies are needed to clarify dysbiosis' role in health and disease [37].

The adult human gut harbors approximately 10^{14} bacterial cells representing over 1,000 species, primarily from the Bacteroidetes and Firmicutes phyla. In healthy individuals, a balanced interaction between the host and gut microbiota maintains intestinal homeostasis and prevents pathogenic overgrowth. This beneficial relationship is disrupted in dysbiosis, defined as an imbalance in microbial composition, function, or distribution. Dysbiosis can involve loss of beneficial microbes, overgrowth of harmful ones, and reduced microbial diversity often occurring simultaneously. It has been linked to various conditions, including cardiovascular disease, inflammatory bowel disease (IBD), obesity, type 1 diabetes, allergies, autism, and colorectal cancer [38].

Recent advances in microbiome research and molecular techniques have enabled targeted interventions to counteract dysbiosis-associated diseases. Traditional methods include diet and antibiotics, while newer approaches involve probiotics, fecal microbiota transplantation (FMT), and environmental manipulation of bacterial flora. These strategies can enhance biofilm formation, antimicrobial production, immune stimulation, and gut barrier integrity. Dysbiosis is increasingly recognized as a key factor in many diseases. Future studies should consider genetic, dietary, and environmental influences to develop effective, personalized therapies [39]. Table 1 compares the gut microbiota composition in healthy versus dysbiotic states, illustrating microbial shifts linked to atherosclerosis.

Table 1. Gut Microbiota Composition in Healthy vs. Dysbiotic States.

Phyla/Genera	Healthy State	Dysbiotic State (Atherosclerosis)	Impact on Atherosclerosis
Firmicutes	High abundance (e.g., Lactobacillus, Clostridium)	Increased (elevated F/B ratio)	Promotes inflammation, TMAO production
Bacteroidetes	High abundance (e.g., Bacteroides, Prevotella)	Decreased	Reduced SCFA production, impaired barrier function
Actinobacteria	Present (e.g., Bifidobacterium)	Decreased	Reduced anti-inflammatory effects

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Proteobacteria	Low abundance	Increased (e.g., Escherichia, Klebsiella)	Enhances LPS-mediated inflammation
Akkermansia muciniphila	High abundance	Decreased	Impaired gut barrier, increased inflammation

3.1.4. Factors Influencing the Gut Microbiota

The gut microbiota, a dynamic ecosystem shaped by factors like antibiotics, diet, genetics, smoking, and physical activity, plays a critical role in host health, including atherosclerosis (AS) risk. Antibiotics disrupt microbiota composition by reducing diversity, eliminating susceptible bacteria (Bacillota), and promoting resistant species (Pseudomonadota), impairing short-chain fatty acid (SCFA) production and immune regulation, which can increase inflammation and infection susceptibility, as seen in Clostridioides difficile-associated diarrhea.

Diet profoundly influences microbiota: Western diets high in animal proteins and fats increase pro-atherogenic metabolites like trimethylamine N-oxide (TMAO), favor bile-tolerant bacteria (Bacteroides, Bilophila), and reduce beneficial Lactobacillus and Bifidobacterium due to nitric oxide and bile acid effects, while fiber-rich diets promote Bacteroidota and SCFA production, supporting gut homeostasis.

Genetics influence microbial composition, with variants like APOA5 rs651821 linked to higher Lactobacillus and metabolic disease risk. Smoking alters microbiota by increasing Bacteroidota and intestinal pH, favoring pathogens like Peptococcaceae. Physical exercise enhances microbial diversity, promotes beneficial bacteria (Akkermansia, Faecalibacterium), increases SCFA production, and reduces inflammation, supporting gut barrier function. These factors collectively impact AS by modulating inflammation, lipid metabolism, and gut barrier integrity, highlighting the potential of targeted interventions like dietary adjustments and exercise to mitigate dysbiosis-related AS risk [40–42].

3.1.5. Study Methods

The human microbiome, comprising the diverse microorganisms residing on and within the human body, plays a crucial role in maintaining health and modulating disease. Historically, microbes have co-evolved with humans, and the term "microbiome" now encapsulates not only these organisms but also their genetic material. Scientific interest in the microbiome emerged centuries ago with early microscopy, and evolved significantly with the advent of 16S rRNA gene sequencing and metagenomics. The former allows for taxonomic profiling using conserved genetic regions, while the latter provides functional and strain-level resolution by sequencing entire microbial genomes [43].

Technologies such as metabolomics and metaproteomics reveal the biochemical impact of microbes through metabolite and protein profiling, while host responses to the microbiome are studied via immune and epithelial barrier functions. Pattern-recognition receptors like Toll-like receptors (TLRs) detect microbial components and regulate immune responses, playing both protective and pathogenic roles in health and autoimmunity. The gut barrier integrity and bacterial metabolites like short-chain fatty acids (SCFAs) also shape immune homeostasis and systemic effects, including T regulatory (Treg) cell differentiation and modulation of inflammation [44].

Advanced immunologic tools such as IgA-Seq and IgG-Seq help identify bacteria targeted by the host's immune system and have shed light on potentially pathogenic or protective microbial taxa. IgA coating often reflects bacterial localization rather than strict pathogenicity, while IgG responses may indicate microbial translocation or systemic exposure, especially in autoimmune conditions [45].

Microbiome diversity is vast, with individual variability influenced by genetics, diet, geography, and early-life exposures. Despite this variability, a functionally redundant core microbiome has been proposed. Environmental factors particularly diet, cohabitation, and antibiotic use play a more

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substantial role than genetics in shaping the microbiome. Delivery mode, age, and sex also influence microbial colonization and development over the human lifespan [46].

While association studies have identified links between specific taxa and disease, mechanistic studies are now essential to establish causality. Emerging tools such as microbial functional profiling, immune-targeted sequencing, and advanced in vivo and in vitro models are poised to bridge the gap from correlation to understanding the microbiome's therapeutic potential in human disease [47].

3.2. Mechanisms by Which Microbiota Influences Atherosclerosis

Gut dysbiosis, defined as a disruption in the composition and function of the gut microbiota, promotes systemic inflammation a key driver of atherosclerosis through multiple interconnected mechanisms. Characterized by reduced microbial diversity, loss of beneficial species (Akkermansia, Faecalibacterium), and overgrowth of pathogenic bacteria (Proteobacteria), dysbiosis compromises intestinal barrier integrity by downregulating tight junction proteins, resulting in increased gut permeability. This allows the translocation of microbial components such as lipopolysaccharides (LPS) and peptidoglycans (PGs) into the systemic circulation, where they activate innate immune receptors like TLRs and NODs, triggering NF-κB signaling and the release of pro-inflammatory cytokines (IL-6, TNF-α, IL-1β).

Concurrently, gut-derived metabolites such as trimethylamine N-oxide (TMAO) and secondary bile acids (DCA, LCA) further amplify inflammation by promoting oxidative stress, adhesion molecule expression, monocyte recruitment, and endothelial dysfunction. TMAO also contributes to foam cell formation, platelet activation, and vascular permeability.

In contrast, short-chain fatty acids (SCFAs), particularly butyrate, which normally exert anti-inflammatory and barrier-protective effects, are reduced in dysbiosis, diminishing their role in regulating immune tolerance, oxidative stress, and vascular health. The cumulative effect is a chronic low-grade systemic inflammation that fuels atherogenesis by enhancing immune cell infiltration, endothelial injury, and plaque instability. Thus, targeting gut microbiota composition and function through dietary modulation, probiotics, or inhibition of pathogenic microbial metabolites may represent a promising therapeutic strategy in reducing vascular inflammation and preventing atherosclerosis [48,49]. Table 2 summarizes the key gut microbiota-derived metabolites, their sources, mechanisms, and effects on atherosclerosis, highlighting their dual roles in disease progression.

Table 2. Key Gut Microbiota-Derived Metabolites and Their Roles in Atherosclerosis.

Metabolite	Source	Mechanisms in Atherosclerosis	Effect
Lipopolysaccharides (LPS)	Gram-negative bacteria	Activates TLR4/NF-κB, promotes cytokine production (IL-6, TNF-α), induces endothelial dysfunction	Pro-atherogenic
Trimethylamine N-oxide (TMAO)	Dietary choline, L-carnitine metabolism	Enhances foam cell formation, platelet activation, oxidative stress, and vascular inflammation	Pro-atherogenic
Short-Chain Fatty Acids (SCFAs)	Fermentation of dietary fibers	Inhibits NF-κB, promotes Treg cells, enhances NO production, reduces foam cell formation	Anti-atherogenic
Secondary Bile Acids (DCA, LCA)	Microbial transformation of primary BAs	Modulates FXR/TGR5 signaling, promotes inflammation at high levels, anti-inflammatory at low levels	Context-dependent (pro- or anti-atherogenic)

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3.2.1. Lipopolysaccharides (LPS)

Lipopolysaccharides (LPS), key components of the outer membrane of gram-negative bacteria, consist of lipid A (the toxic moiety), a core oligosaccharide, and a variable O-antigen. LPS is essential for bacterial membrane integrity and virulence and acts as a potent endotoxin. Lipid A is recognized by host immune receptors like TLR4, triggering strong innate immune responses including cytokine release, inflammation, and potentially septic shock. While lipid A is conserved, its structural variability among bacterial strains influences immune recognition. O-antigens confer antigenic specificity and help bacteria evade host immunity and form biofilms, enhancing antibiotic resistance [50].

LPS enters the bloodstream via gut absorption, especially in the context of barrier disruption (gut lesions or high-fat diets), or through contaminated pharmaceuticals. Once systemic, LPS activates inflammatory cascades, complement, and coagulation pathways, contributing to endotoxemia and septic complications. Chronic low-level exposure to LPS is implicated in metabolic syndrome, autoimmune disorders, allergies, and neurodegenerative diseases. Pathogens can modify their LPS to reduce immune detection, aiding persistent infections [51].

Biofilm formation, aided by LPS modifications like palmitoylation, allows gram-negative bacteria to resist host defenses and antibiotic therapy. LPS biosynthesis is tightly regulated at the molecular level and integrated with phospholipid metabolism. Detection of LPS is critical in diagnostics, with methods including the Limulus Amoebocyte Lysate (LAL) assay, immunoassays like ELISA, and biosensor-based technologies, though challenges persist due to LPS's amphipathic nature and serotype variability [52].

Clinically, LPS is a biomarker for infection and a target for immunomodulation. It induces B-cell activation and antibody production, and in low doses, can enhance vaccine efficacy through innate immune stimulation. Research into LPS continues to provide insights into infection mechanisms, immune modulation, and potential therapeutic interventions [53].

Atherosclerosis is a chronic inflammatory condition, and growing evidence implicates lipopolysaccharides (LPS) endotoxins from Gram-negative bacteria as key contributors to its pathogenesis. LPS can be detected in low levels in healthy individuals but becomes proatherogenic when elevated, particularly during chronic bacterial infections. LPS initiates inflammation by binding to pattern recognition receptors such as Toll-like receptor 4 (TLR4) on immune and vascular cells, activating intracellular signaling cascades (MyD88, NF-κB, MAPK) that lead to cytokine, chemokine, and adhesion molecule production. This enhances monocyte recruitment, foam cell formation, and vascular dysfunction [54].

LPS interacts with surface proteins including CD14, MD-2, and LBP, which are expressed not only by immune cells but also by endothelial and smooth muscle cells. Genetic polymorphisms in TLR4 (Asp299Gly) and CD14 modulate LPS signaling and influence cardiovascular disease risk. TLR4 is abundantly expressed in human atherosclerotic plaques, and its ligands including bacterial LPS, saturated fatty acids, and endogenous molecules like fibronectin amplify vascular inflammation [18].

LPS also promotes oxidative stress via NADPH oxidase–mediated ROS production and induces endothelial dysfunction and vascular smooth muscle proliferation. It upregulates inflammatory mediators (TNF-α, IL-1β, IL-6), adhesion molecules (ICAM-1, VCAM-1), and chemoattractants (MCP-1, IL-8), which support immune cell infiltration and plaque progression. Moreover, LPS induces cellular senescence in endothelial cells and macrophages, increasing their proinflammatory SASP phenotype, further enhancing lesion instability [55].

Lipoproteins modulate LPS activity HDL binds and clears LPS via the liver, reducing its bioactivity, while LDL has less capacity for clearance. HDL and ApoA-I also inhibit LPS-induced cytokine release through STAT3 activation and upregulation of anti-inflammatory factors like TTP. Modified LDLs, through scavenger receptors (CD36, CD204), synergize with LPS in foam cell formation [56].

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Experimental studies in ApoE-deficient mice show that LPS aggravates plaque formation and inflammation, while LPS antagonists like Rs-LPS reduce inflammatory cytokines and immune cell infiltration. LPS also enhances expression of PAF receptors, NEU1, and IFIT1 factors linked to leukocyte adhesion, inflammation, and plaque instability. Though the role of complement in LPS-induced atherogenesis remains inconclusive, LPS strongly stimulates T-cell and NKT cell responses and may promote autoimmune processes.

Clinically, serum LPS, LBP, and sCD14 are being investigated as potential biomarkers for atherosclerosis risk and progression. Although antibiotic trials targeting LPS-producing pathogens (C. pneumoniae) yielded mixed results, LPS remains a promising target for diagnostic and therapeutic strategies in atherosclerosis [57].

Recent evidence suggests that cellular senescence characterized by irreversible growth arrest and a proinflammatory secretory phenotype (SASP) is a key contributor to atherogenesis. Senescent ECs, VSMCs, and foam cells accumulate in plaques and secrete cytokines (IL-6, TNF-α), chemokines (MCP-1), and adhesion molecules (ICAM-1), amplifying vascular inflammation, monocyte recruitment, and plaque instability [58].

Bacterial infection, particularly with gram-negative organisms such as Chlamydia pneumoniae, E. coli, H. pylori, and P. gingivalis, is implicated in atherosclerosis. These pathogens release LPS, which enters systemic circulation due to gut dysbiosis or chronic mucosal infections. LPS is detected in atherosclerotic plaques and elevated serum levels correlate with cardiovascular disease risk. Mechanistically, LPS activates toll-like receptors (TLR4, TLR2) on immune and vascular cells, upregulating proinflammatory cytokines, adhesion molecules, and promoting monocyte adhesion, foam cell formation, and VSMC proliferation [59].

Importantly, LPS also induces and enhances cellular senescence. In various cell models including ECs, VSMCs, macrophages, and progenitor cells LPS increases SA-β-Gal activity, cell cycle arrest proteins (p53, p21, p16), and SASP components. Senescent ECs show heightened sensitivity to LPS, with increased NF-κB activation and ICAM-1 expression. These effects are amplified due to elevated TLR4 expression on senescent cells, reinforcing the inflammatory loop in atherosclerotic plaques [60].

LPS not only initiates senescence but also intensifies SASP in pre-existing senescent cells, exacerbating vascular inflammation. LL-37, an antimicrobial peptide that neutralizes LPS, can partially inhibit these effects in senescent ECs. These findings highlight a synergistic role of LPS and senescent cells in atherogenesis. Targeting senescent cells or neutralizing LPS may represent novel strategies for mitigating atherosclerosis progression, especially in aging individuals with chronic infections or gut-derived endotoxemia. Further in vivo studies are needed to elucidate the mechanistic link between LPS, senescence, and vascular pathology.

In conclusion, LPS contributes to vascular inflammation, immune cell recruitment, endothelial dysfunction, and senescence key features of atherosclerosis and targeting its pathways may offer novel preventive or therapeutic approaches [61].

3.2.2. Trimethylamine N-Oxide (TMAO)

The endothelium, a monolayer lining the interior of blood vessels, plays a crucial role in maintaining vascular homeostasis by regulating vascular tone, coagulation, inflammation, and vascular smooth muscle cell (VSMC) proliferation. This is achieved through the balanced secretion of endothelium-derived relaxing and contracting factors. When this balance is disturbed, endothelial dysfunction occurs, increasing the risk of thrombosis, inflammation, and cardio-metabolic diseases such as atherosclerosis, coronary syndromes, hypertension, and diabetes [62].

Endothelial dysfunction can be triggered by various factors including hypertension, hyperlipidemia, genetics, and unhealthy lifestyle habits. Among dietary influences, trimethylamine N-oxide (TMAO) a metabolite derived from red meat, fish, and eggs has emerged as a key contributor to endothelial dysfunction and related cardio-metabolic disorders. Elevated TMAO levels are linked to type 2 diabetes, heart failure, atherosclerosis, and peripheral artery disease. Mechanistic studies

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show that TMAO promotes inflammation and oxidative stress, thereby accelerating disease progression [63].

The biosynthesis and metabolism of trimethylamine N-oxide (TMAO) involve a complex interplay between diet, gut microbiota, liver enzymes, and excretion pathways. TMAO is produced when gut bacteria metabolize dietary precursors such as choline, L-carnitine, lecithin, phosphatidylcholine, and betaine into trimethylamine (TMA), which is then oxidized in the liver by flavin monooxygenase enzymes (mainly FMO3) into TMAO [64].

Specific bacterial strains including Clostridium, Edwardsiella, Proteus, and Providencia species are implicated in TMA production. Individuals with cardio-metabolic diseases often exhibit gut dysbiosis, characterized by increased levels of pro-inflammatory bacteria (Firmicutes, Proteobacteria) and reduced populations of beneficial species (Bifidobacterium, Lactobacillus, Faecalibacterium prausnitzii), contributing to metabolic dysfunction and systemic inflammation [49].

TMA is rapidly absorbed into the portal circulation, converted to TMAO in the liver, and then distributed systemically. Around 50% of TMAO is excreted mainly through urine, with small amounts through feces, sweat, and breath. However, elevated circulating TMAO levels persist in patients with diabetes, hypertension, heart failure, and coronary artery disease.

These findings highlight the crucial role of the gut microbiome in the progression of cardio-metabolic diseases. Monitoring TMAO levels could serve as an early biomarker for disease risk, and targeting TMA-producing microbes may offer new preventive or therapeutic approaches [65].

Endothelial dysfunction defined by impaired vasodilation, oxidative stress, and inflammation is a central mechanism in the development of cardiovascular and cardio-metabolic diseases, with trimethylamine N-oxide (TMAO), a gut microbiota-derived metabolite, playing a significant role through multiple interrelated mechanisms. TMAO promotes oxidative stress by inducing reactive oxygen species (ROS) via pathways such as TXNIP-NLRP3 and SIRT3-SOD2, while downregulating protective factors like SIRT1 and increasing NADPH oxidase activity, especially in aging and metabolic disease contexts. It also drives inflammation by upregulating pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) through NF-κB signaling, enhancing leukocyte adhesion, vascular remodeling, and endothelial injury, with clinical studies confirming correlations between elevated TMAO and inflammation [66,67].

Moreover, TMAO increases the expression of adhesion molecules such as VCAM-1, ICAM-1, and E-selectin, promoting monocyte adhesion via PKC and NF-κB pathways. In parallel, TMAO contributes to foam cell formation and atherosclerosis by enhancing cholesterol uptake in macrophages through upregulation of scavenger receptors like CD36, LOX-1, and SR-A1, thereby accelerating plaque development. It impairs vascular tone by reducing nitric oxide (NO) bioavailability through decreased eNOS activity and also disrupts prostacyclin (PGI2) and endothelium-derived hyperpolarization (EDH) pathways, diminishing vasodilation—though these effects vary with dose and experimental conditions [67].

TMAO also promotes platelet hyperreactivity and thrombosis by increasing sensitivity to thrombin and collagen via MAPK signaling (ERK1/2, JNK), which elevates intracellular calcium and aggregation potential. Additionally, it contributes to heart failure and fibrosis through activation of NLRP3 inflammasome and Smad3 signaling, leading to cardiac remodeling, apoptosis, and mitochondrial dysfunction.

Metabolically, TMAO impairs insulin signaling, induces hyperglycemia via PERK activation, and is associated with obesity and type II diabetes. It also disrupts bile acid metabolism and stimulates hepatic lipogenesis through FXR signaling, causing hepatic steatosis [68].

TMAO promotes atherogenesis by disrupting cholesterol metabolism, decreasing bile acid synthesis, enhancing foam cell formation, impairing reverse cholesterol transport, increasing vascular inflammation, promoting endothelial dysfunction, and activating platelets and thrombosis pathways. Elevated TMAO levels are associated with increased risk of myocardial infarction, stroke, hypertension, and overall mortality [64].

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TMAO contributes to inflammation and oxidative stress by activating signaling pathways such as MAPK, NF-κB, and NLRP3 inflammasome, while suppressing protective mechanisms like SIRT3-SOD2 antioxidant defense and autophagy (ATG16L1). It upregulates adhesion molecules (VCAM-1, ICAM-1), cytokines (IL-6, TNF-α), and scavenger receptors (CD36, SR-A1), exacerbating macrophage foam cell formation [69].

TMAO also activates endothelial cells and impairs endothelial repair, enhances monocyte and leukocyte recruitment, and promotes vascular permeability and thrombogenicity. Inhibitors of TMAO synthesis, such as DMB (a TMA lyase inhibitor), I3C (an FMO3 inhibitor), IMC and FMC (choline analogs), and trigonelline have shown potential in preclinical studies by reducing TMAO levels, inflammation, platelet aggregation, and plaque progression [70].

However, FMO3 inhibition may risk TMA accumulation and side effects like trimethylaminuria. Combination therapies targeting both microbial and hepatic pathways may provide a balanced approach to mitigate TMAO-induced atherogenesis while minimizing adverse effects, although more clinical evidence is required to confirm their safety and efficacy in humans.

TMAO is a multifaceted disruptor of vascular and metabolic homeostasis, and while preclinical studies provide strong mechanistic insights, further clinical research is essential to confirm its role in human endothelial dysfunction and guide the development of targeted interventions [71].

3.2.3. Short-Chain Fatty Acids (SCFAs)

Short-chain fatty acids (SCFAs) mainly acetate, propionate, and butyrate are microbial metabolites with profound effects on human physiology. They are primarily produced by the gut microbiota through the fermentation of non-digestible polysaccharides, such as dietary fibers and resistant starches, and to a lesser extent from amino acid metabolism. SCFAs mediate their effects via G-protein-coupled receptors (FFAR2 and FFAR3), with acetate predominantly activating FFAR2 and propionate acting on FFAR3, influencing immune modulation, insulin secretion, and neuronal energy balance. They are also transported across the colonic epithelium through MCT1, MCT4, SMCT1, and ABCG2 transporters. SCFAs play a crucial role in maintaining gut barrier integrity by promoting tight junction expression and mucus layer enhancement, particularly through butyrate. They regulate oxidative stress, modulate colonocyte apoptosis and differentiation, and may help prevent colon cancer. Metabolically, SCFAs regulate appetite, suppress hepatic gluconeogenesis, reduce lipogenesis, improve lipid profiles, and lower body weight and fat accumulation in animal studies, although human evidence is limited [32].

Cardiovascular benefits include lowering blood pressure, reducing pro-thrombotic factors, and stabilizing atherosclerotic plaques. Immunologically, SCFAs enhance Treg cell function, reduce neutrophil and macrophage-mediated inflammation, and downregulate NF-κB signaling. The production and proportion of SCFAs vary with age and diet, reflecting changes in microbiota composition such as the dominance of Bifidobacterium in infancy, Firmicutes in adulthood, and Enterobacteriaceae in older age. Notably, acetate-producing Bifidobacterium supports the growth of butyrate producers through microbial cross-feeding. SCFAs are also central to mucus homeostasis, with butyrate enhancing MUC2 expression and promoting beneficial microbial colonization. Imbalances in SCFA production are linked to diseases such as ulcerative colitis (UC), cardiovascular diseases (CVDs), obesity, and type 2 diabetes (T2D). In UC, a loss of butyrate producers and impaired SCFA utilization contributes to chronic inflammation [72,73].

SCFAs regulate blood pressure via GPR41 and Olfr78 receptors, mediate anti-inflammatory effects in atherosclerosis, and modulate satiety hormones (PYY, GLP-1), lipid metabolism, and glucose homeostasis through AMPK and PPARγ pathways. While SCFAs primarily derive from carbohydrate fermentation, minor SCFAs like formate and lactate also play roles in early-life microbiota and host physiology. Advances in genomics and metabolomics continue to uncover the complex roles of SCFA-producing bacteria and their therapeutic potential in maintaining metabolic, immune, and intestinal health [74].

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Atherosclerosis is a multifactorial chronic disease characterized by endothelial dysfunction, lipid accumulation, inflammation, vascular smooth muscle cell (VSMC) proliferation, plaque calcification, and immune cell infiltration, ultimately leading to plaque instability and thrombotic events [5].

Short-chain fatty acids (SCFAs) primarily acetate, propionate, and butyrate are microbiota-derived metabolites produced through the fermentation of dietary fibers and exhibit potent anti-atherogenic properties through multiple mechanisms. SCFAs suppress inflammation by inhibiting NF-κB and MAPK pathways, reducing pro-inflammatory cytokines (TNF-α, IL-6, IL-1β), and promoting anti-inflammatory Tregs, Bregs, and IL-10 production. They mitigate oxidative stress via Nrf2 pathway activation, decrease ROS, and inhibit inflammasome formation (NLRP3), preserving endothelial function [75].

SCFAs improve endothelial cell health by enhancing nitric oxide (NO) production, maintaining tight junctions, reducing permeability, and promoting energy metabolism. Butyrate inhibits VSMC proliferation, migration, apoptosis, and calcification, stabilizing plaques by modulating signaling cascades (PI3K-AKT, HDACs, MAPK). In macrophages, SCFAs block foam cell formation by decreasing ox-LDL uptake (via CD36 inhibition), enhancing cholesterol efflux (through ABCA1 upregulation via Sp1 phosphorylation), and suppressing pro-inflammatory polarization (M1) while promoting anti-inflammatory M2 phenotypes [76].

SCFAs also regulate immune responses by modulating dendritic cells (DCs), mast cells, neutrophils, and natural killer (NK) cells. They reduce DC-mediated antigen presentation, inhibit mast cell degranulation, decrease NET formation and neutrophil migration, and suppress NK cell cytotoxicity by downregulating mTORC1 and inflammatory mediators. SCFAs improve lipid metabolism by inhibiting hepatic lipogenesis (through FASN, ACC, and HMG-CoA reductase), increasing fatty acid oxidation (via AMPK and PPAR-α activation), promoting reverse cholesterol transport (via ABCA1, ApoA-I), reducing LDL, VLDL, and triglycerides, and enhancing bile acid excretion. In the intestine, SCFAs reduce cholesterol absorption by downregulating NPC1L1 and modulating gut-liver signaling [77,78].

SCFAs also regulate glucose metabolism by enhancing insulin secretion (via GLP-1 and PYY), improving insulin sensitivity (via GPR41/43), activating AMPK, and inhibiting hepatic gluconeogenesis (via PEPCK and G6Pase suppression). Through HDAC inhibition, SCFAs boost anti-inflammatory gene expression and suppress pro-inflammatory responses across vascular and immune cells.

Collectively, these findings support the pivotal role of SCFAs in protecting against atherosclerosis by modulating vascular function, immune responses, oxidative stress, and metabolic homeostasis, offering a promising therapeutic avenue for prevention and treatment [79].

3.2.4. Secondary Bile Acids

Secondary bile acids (BAs), including deoxycholic acid (DCA) and lithocholic acid (LCA), are derived from primary Bas cholic acid (CA) and chenodeoxycholic acid (CDCA) which are synthesized from cholesterol in the liver via the classical (CYP7A1) and alternative (CYP27A1, CYP7B1) pathways, then conjugated with taurine or glycine to aid lipid emulsification. Following food intake, cholecystokinin triggers their release into the intestine, where ~95% are reabsorbed in the ileum, while the remainder undergo microbial transformation into secondary BAs through bile salt hydrolase (BSH) and 7α-dehydroxylase (7α-HSDH). DCA is partly reabsorbed, LCA is mostly excreted, and absorption occurs via active transport (ASBT, OSTα/OSTβ) in the ileum and passive diffusion in the colon, with some secondary BAs reaching systemic circulation [80,81].

Their metabolism is tightly regulated by farnesoid X receptor (FXR), which inhibits BA synthesis enzymes (CYP7A1, CYP8B1, CYP27A1) and enhances BA efflux, while external factors like insulin, drugs, fiber, and gut microbiota also modulate BA levels. FXR and TGR5 receptors mediate BA signaling pathways, influencing glucose/lipid metabolism, inflammation, and even cancer risk; FXR suppresses BA synthesis and inflammation, while TGR5 promotes GLP-1 secretion and inhibits NLRP3 inflammasome, but may also contribute to carcinogenesis under dysbiosis [82].

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Gut microbiota, particularly species with BSH and 7α-HSDH activity (Clostridium, Bacteroidetes, Ruminococcus), are key to secondary BA formation, with their abundance influenced by diet, antibiotics, and gut pH. In turn, secondary BAs shape microbial composition by promoting pathogenic bacteria, damaging mucosal barriers, and facilitating tumor development, underscoring their role as both metabolic regulators and modulators of gut-liver health and disease [83].

These secondary BAs, particularly in the context of high-fat diets, reshape gut microbial composition and, at high concentrations, promote DNA damage, oxidative stress, apoptosis, and chronic inflammation.

While low concentrations of secondary BAs may transiently stimulate cell proliferation via EGFR, COX-2, and ERK1/2 signaling, chronic exposure impairs apoptosis and fosters mutagenesis. Secondary BAs also influence immune responses through activation of inflammatory pathways (TLR-NF-κB) and production of ROS and cytokines (IL-6, TNF-α). At lower levels, however, they can exert anti-inflammatory effects by activating TGR5, promoting M2 macrophage polarization, and downregulating pro-inflammatory pathways like TLR4-NF-κB. Key receptors such as FXR, TGR5, PXR, and CAR modulate bile acid signaling and immune function, and their dysregulation increases vulnerability to inflammation and carcinogenesis [84].

Overall, the gut microbiota–bile acid axis plays a pivotal role in colonic health, with secondary BAs acting as double-edged swords beneficial in moderation but harmful in excess, particularly under dietary and microbial imbalances that promote CRC [85].

Beyond coexistence, gut microbes metabolize both dietary and host-derived substances to produce short-chain fatty acids (SCFAs) like acetate, propionate, and butyrate, as well as neurotransmitters such as serotonin, dopamine, and GABA. Unlike these, bile acids (BAs) are synthesized endogenously in the liver from cholesterol, with primary Bas cholic acid (CA) and chenodeoxycholic acid (CDCA) subsequently converted by gut bacteria into secondary BAs like deoxycholic acid (DCA) and lithocholic acid (LCA), which exert potent immunomodulatory effects. Key bacterial taxa such as Lactobacillus, Bacteroides, and Clostridium possess bile salt hydrolases (BSHs), while species like Lachnoclostridium scindens express the bai operon, enabling further biotransformations to derivatives such as isoalloLCA and 3-oxo-LCA [86,87].

These secondary BAs regulate immune function at multiple levels: DCA and LCA reduce the expression of proinflammatory cytokines and co-stimulatory molecules in dendritic cells (DCs) via TGR5 and FXR activation; LCA and its conjugates suppress inflammatory cytokine release from macrophages and shift hepatic macrophages toward an anti-inflammatory M2 phenotype; and T helper cell responses are modulated, with LCA inhibiting Th1 cell differentiation and cytokine production, 3-oxo-LCA and LCA-3-sulfate selectively blocking Th17 cell differentiation through RORγt inhibition, and isoalloLCA promoting Treg development via mtROS generation, epigenetic changes at the FOXP3 locus, and NR4A1 activation [88,89].

LCA, DCA, and their derivatives, produced by the gut microbiota, function as signaling molecules that regulate key immune cells such as dendritic cells (DCs), macrophages, Th1, Th17, and T regulatory (Treg) cells thus influencing immune balance and contributing to the pathogenesis or prevention of autoimmune and metabolic diseases. Notably, centenarians harbor unique gut bacteria capable of generating LCA derivatives like iso-, 3-oxo-, allo-, and isoalloLCA, suggesting a potential link between bile acid metabolism and longevity. Age-related inflammation, driven by senescent cells that evade immune clearance via PD-L1 overexpression, may also be modulated by bile acids: DCA suppresses PD-L1 by inhibiting NF-κB through TGR5 activation, while FXR activation may enhance PD-L1 expression. Since bile acids structurally resemble steroid ligands, they can bind not only to TGR5 and FXR but also to orphan nuclear receptors such as RORγt, NR4A1, and NR4A2. These interactions highlight the importance of reevaluating gut microbiota-derived bile acid metabolites as modulators of immune responses, aging, and disease, reinforcing the need for further research into their signaling roles and therapeutic potential [90,91].

Cardiovascular disease (CVD) remains the leading global cause of death, with atherosclerosis a chronic, inflammatory condition of the vascular wall being its primary pathophysiological driver.

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While traditional risk factors such as hyperlipidemia, hypertension, and chronic inflammation are well established, residual cardiovascular risk persists, prompting growing interest in non-traditional contributors like the gut microbiota and its metabolites, including bile acids (BAs). Synthesized from cholesterol in the liver and metabolized by gut bacteria, BAs serve not only as lipid emulsifiers but also as potent signaling molecules via receptors such as FXR, TGR5, VDR, and PXR, influencing lipid and glucose metabolism, immunity, and cardiovascular function [92].

Alterations in the gut microbiota (dysbiosis) can lead to increased intestinal permeability, systemic inflammation, and CVD progression through modified BA metabolism and composition. Specific bacterial enzymes like BSH, HSDH, and bai genes shape the BA pool, which in turn affects host metabolism and immunity. Studies show that gut microbiota signatures differ in atherosclerosis patients and that BA-modifying bacteria may play causal roles in disease development, as confirmed in animal models. Clinical data reveal that altered serum or fecal BA profiles are associated with CAD severity and outcomes, with low or excessive BA levels linked to cardiac dysfunction [93].

Mechanistically, BAs regulate lipid metabolism by modulating FXR, PXR, and TGR5 pathways, affecting triglyceride levels, cholesterol transport, foam cell formation, and ceramide synthesis. They also modulate immune responses by altering macrophage polarization, T cell differentiation (Th17/Treg balance), and gut barrier integrity. In the heart and vasculature, BAs influence cardiomyocyte apoptosis, endothelial function, vascular tone, and arrhythmogenesis via BARs, muscarinic receptors, and ion channels. Therapeutically, both indirect (prebiotics, probiotics, synbiotics) and direct (BAR modulators like obeticholic acid) approaches targeting BA metabolism show promise in preclinical models. However, species-specific differences in BA metabolism highlight the need for humanized models and clinical validation. Overall, BAs and gut microbiota interactions represent emerging and modifiable contributors to CVD pathogenesis, offering new avenues for diagnosis, risk stratification, and therapy [82,87,94]. Table 3 outlines the key receptors and signaling pathways modulated by gut microbiota-derived metabolites, highlighting their roles in atherosclerosis.

Table 3. Key Receptors and Pathways Modulated by Gut Microbiota-Derived Metabolites.

Metabolite	Receptor/Pathway	Effect on Atherosclerosis	Key Outcomes
LPS	TLR4, NF-κB, MyD88, MAPK	Promotes inflammation, cytokine production, endothelial dysfunction	Increased monocyte recruitment, plaque instability
TMAO	NF-κB, MAPK, NLRP3, SIRT3-SOD2	Enhances oxidative stress, foam cell formation, thrombosis	Increased plaque formation, vascular inflammation
SCFAs	FFAR2, FFAR3, GPR41, Nrf2, HDACs	Reduces inflammation, enhances NO production, stabilizes plaques	Improved lipid metabolism, reduced foam cells
Secondary Bile Acids	FXR, TGR5, PXR, RORγt	Modulates inflammation, lipid metabolism, immune cell balance	Context-dependent (anti- or pro-atherogenic)

3.3. Therapeutic Interventions Targeting Microbiota

The gut microbiome plays a critical role in modulating cardiometabolic risk by acting as a sensor, modulator, and translator of host metabolic changes through its metabolites, including SCFAs, TMAO, secondary bile acids, and phenylacetylglutamine.

Diet is a major determinant of both microbiome composition and cardiometabolic health, with dietary interventions, especially those rich in fiber, shown to influence microbial activity and metabolite production. Clinical studies indicate that over 70% of interventions targeting the gut

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microbiome result in significant improvements in cardiometabolic traits (obesity, type 2 diabetes), though only 63% report changes in microbiome composition [92].

Prebiotic interventions most consistently alter microbiota, followed by dietary changes, while probiotics show the least impact partly due to poor colonization efficiency. No significant difference in efficacy was observed between single- and multi-strain probiotics or synbiotics. Notably, dietary interventions outperformed probiotics in achieving both clinical benefits and microbiome shifts, suggesting that whole-diet strategies may better modulate complex microbial communities. While microbial changes appear to mediate clinical improvements in many studies, methodological inconsistencies hinder definitive conclusions [95,96].

The evidence supports the therapeutic modulation of the gut microbiome to improve cardiometabolic outcomes, yet highlights the need for large, well-controlled trials with standardized biomarkers to identify mechanistic pathways and establish evidence-based guidelines for microbiome-targeted therapies [97].

3.3.1. Diet

Diet is a major determinant of gut microbiota composition and function, capable of modulating microbial diversity, interactions, and resilience. Long-term consumption of plant-based, fiber-rich diets has been shown to enhance SCFA production, strengthen mucosal barriers, and increase beneficial bacteria such as Actinobacteria and Bacteroidetes. Interventions with Mediterranean diets can rapidly shift microbial profiles toward health-promoting genera like Butyricicoccus and Roseburia, while less healthy diets (Canadian-style) favor potentially dysbiotic taxa. Polyphenol- and bioactive-enriched diets, including green tea and orange juice, also enhance microbiota diversity and support SCFA-producing microbes [21,98].

Animal studies highlight that dietary timing is critical: pre-, during, and post-antibiotic consumption of fiber-rich foods (oats) better preserves microbial diversity and mitigates dysbiosis. Similarly, high-fiber diets in humans enhance post-antibiotic microbiome recovery and reduce the abundance of antibiotic resistance genes. Vegan and omnivorous diets, rich in fermentable fibers, increase Firmicutes abundance and butyrate production, contributing to microbial resilience [99]. However, despite these promising findings, the precise mechanisms and key dietary drivers that promote long-term microbiome stability and recovery remain poorly understood, limiting targeted nutritional strategies for microbiome restoration [100].

It was demonstrated that an high-fat diet (HFD), particularly its low fiber content, induces gut microbiota dysbiosis, reducing SCFA production and impairing intestinal immune defenses. This promotes systemic inflammation and atherosclerosis by enhancing gut-derived immune cell trafficking to vascular sites. FMT experiments establish a causal link between HFD-shaped microbiota and atherosclerosis, independent of metabolic alterations. Fiber supplementation (fructooligosaccharide) mitigates these effects by restoring microbial balance and SCFA levels. These findings underscore dietary fiber's therapeutic potential in preventing microbiota-driven CVDs and highlight the gut-immune-vascular axis as a critical pathway in atherosclerosis [101,102].

A recent study in a Southeast Asian population linked healthy plant-based diets rich in dietary fiber to reduced cardiometabolic risk, unlike unhealthy plant-based foods (refined grains, fried snacks, sugar-sweetened beverages). Hutchison et al. demonstrated that dietary fiber attenuates atherosclerosis through gut microbiome modulation, while Sakurai et al. showed that alpha-cyclodextrin, a glucose-based cyclic polymer, reduces atherosclerosis by altering cecal bacteria composition. Additional studies confirm that high intakes of fibers like inulin and pectin similarly mitigate atherosclerosis, suggesting that dietary fiber plays a critical role in halting the onset and progression of atherosclerosis via microbiome-mediated mechanisms [103,104].

3.3.2. Prebiotics

Strengthening the stability and resilience of the gut microbiome may be achieved through synbiotic interventions, which combine live microorganisms with substrates selectively utilized by

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host microbes. While prebiotics and probiotics can increase the abundance of beneficial bacteria such as Bifidobacterium and Lactobacillus, their effects on overall microbiota composition are often modest and usually persist only during the intervention [105].

The type, dosage, and duration of prebiotic intake influence the enrichment of specific bacterial groups. Soluble fibers like pectin and inulin are fermented by beneficial microbes to produce SCFAs, which acidify the colon, inhibit pathogens, and contribute to immune modulation, epithelial barrier function, and metabolic health. Insoluble fibers like cellulose and lignin support Prevotella and Ruminococcus, leading to other anti-inflammatory metabolites [106].

Novel prebiotics, such as bifidobacterial-galacto-oligosaccharides (B-GOS), show promise in reducing travelers' diarrhea, while polysaccharides from the mushroom Dictophora indusiata (DIP) have demonstrated the ability to restore microbiota after antibiotic-induced dysbiosis and reduce inflammation. DIP promotes beneficial bacterial families and SCFA production while suppressing harmful taxa, as confirmed by both in vivo mouse studies and in vitro fermentation assays. These findings underscore the potential of targeted synbiotic and fiber-based strategies to foster a resilient, health-promoting gut microbiome [107].

Recent studies underscore the therapeutic potential of polysaccharides/oligosaccharides, particularly when combined with zinc, in addressing obesity-related metabolic disorders and atherosclerosis through gut microbiome modulation. Research indicates that obese individuals have lower zinc levels, linked to metabolic disorders, and zinc supplementation may mitigate obesity. Li et al. demonstrated that a novel Ulva oligosaccharide-based zinc supplement improved intestinal flora, reduced dyslipidemia, and lowered body weight in obese mice. Additionally, Hoving et al. found that mannose oligosaccharides prevented atherosclerosis progression by lowering serum cholesterol, increasing cecal butyrate, and enhancing bile acid excretion via microbiome changes. Li et al. also showed that Laminaria japonica polysaccharides suppressed atherosclerosis by boosting autophagy pathways, while red algal polysaccharides are emerging as a potential treatment. These findings highlight innovative, microbiome-mediated strategies using prebiotic polysaccharides and zinc supplementation for preventing and treating obesity and atherosclerosis [108,109].

3.3.3. Probiotics

Probiotics support gut health by competing with pathogens, enhancing mucosal integrity, modulating immunity, and producing beneficial metabolites and antimicrobial peptides. However, clinical evidence on their role in restoring the human gut microbiome (HGM) post-antibiotics or enhancing its resilience remains inconsistent. Some studies suggest that probiotics may delay microbiome recovery due to competition with native taxa and immune activation, while others report benefits such as reduced antibiotic-associated diarrhea and increased SCFA production [110].

These conflicting results highlight the complexity of host–microbe interactions and the limitations of current probiotic strains. Meta-analyses show limited evidence supporting probiotics in preventing travelers' diarrhea, with only S. boulardii showing consistent efficacy. Most clinical interventions have focused on co- or post-treatment administration, with few exploring pre-antibiotic probiotic use. Some studies show modest improvement in microbial diversity, while others, including Suez et al. (2018), report delayed recovery and increased inflammation with multi-strain probiotic blends [111].

New approaches propose assessing functional rather than compositional shifts and exploring next-generation probiotics such as B. uniformis, A. muciniphila, and F. prausnitzii, which have shown promise in preclinical models for restoring microbiota, reducing inflammation, and promoting mucosal healing. Synergistic combinations of Lactobacillus, Bifidobacterium, Bacteroides, and Akkermansia strains demonstrate superior recovery outcomes in antibiotic-treated mice compared to single-strain therapies. These findings underscore the potential of advanced probiotic formulations and functional assessment metrics in developing effective strategies for gut microbiome resilience and recovery [112].

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Probiotics, live microorganisms found in fermented foods like yogurt, kefir, and sauerkraut, confer health benefits by modulating gut microbiota, lipid profiles, endothelial function, oxidative stress, and inflammation, offering potential for atherosclerosis (AS) prevention and treatment. Strains like Lactobacillus and Bifidobacterium reduce AS risk by lowering cholesterol, trimethylamine N-oxide (TMAO), and inflammatory markers while enhancing gut barrier integrity and short-chain fatty acid (SCFA) production. In ApoE−/− mice, Lactiplantibacillus plantarum ATCC 14917 (10^9 CFU, 12 weeks) prevented plaque formation by improving intestinal integrity, while multi-strain probiotics reduced vascular inflammation. L. plantarum ZDY01 lowered TMAO in choline-fed mice, and L. reuteri and Bifidobacterium species increased fecal SCFAs, correlating with reduced hepatic cholesterol [113,114].

Human trials show Lactobacillus and Bifidobacterium strains improve HDL, lower total cholesterol, and enhance endothelial function in coronary artery disease patients. Probiotics also reduce oxidative stress (lower ox-LDL, MDA) and inflammation (reduced IL-1, TNFα) by inhibiting NF-κB signaling. Emerging Lactobacillus fermentum strains combined with phenolic compounds like quercetin and resveratrol show enhanced antioxidant and anti-inflammatory effects, suggesting novel nutraceutical potential. However, optimal strain combinations, treatment duration, and long-term safety require further clinical validation [115–117].

Targeting gut microbiota through dietary fiber supplementation, probiotics, or prebiotics could reduce systemic inflammation and atherosclerosis risk. Further research into personalized microbiota interventions and immune cell trafficking mechanisms may enhance CVD prevention strategies [36].

3.3.4. Small Molecule Compounds

Recent research highlights innovative methods for regulating the gut microbiome to mitigate atherosclerosis, focusing on probiotics, prebiotics, and small molecules. While probiotics and prebiotics are widely used, their safety, colonization stability, and precise control over microbial composition remain challenging. Scientists at Scripps Research Institute developed a screening method to identify d- and L-α-peptide cyclic molecules that selectively inhibit bacterial growth by disrupting cell membrane function.

In high-fat diet-induced atherosclerotic mice, these peptides reduced cholesterol by 36% after 2 weeks and atherosclerotic plaque area by ~40% after 10 weeks, shifting gut microbiota toward a low-fat diet profile. Additionally, Wang et al. showed that 3,3-dimethyl-1-butanol, a choline analog, attenuated atherosclerosis by inhibiting microbial TMA-lyase activity and TMA/TMAO production. Liu et al. identified a Ganoderma meroterpene derivative that enriched Parabacteroides merdae, enhancing branched-chain amino acid catabolism and improving obesity-related atherosclerosis. These findings reveal novel microbiota-mediated mechanisms of atherosclerosis progression and propose targeted small-molecule interventions for cardiovascular health improvement [70,103,118,119].

3.3.5. Phenolic Compounds

Phenolic compounds, particularly polyphenols like quercetin, resveratrol, curcumin, gallic acid, naringin, procyanidin, geraniin, and protocatechuic acid, show significant potential in preventing and treating atherosclerosis (AS) by modulating gut microbiota and related metabolic pathways [120].

Quercetin, found in foods like apples and red wine, reduces atherosclerotic lesions, cholesterol, and inflammatory markers (TNF-α, IL-6) in ApoE−/− and LDLr−/− mice by increasing gut microbial diversity and beneficial genera like Akkermansia and Bacteroides, while altering bile acid and coprostanol metabolism. Resveratrol, abundant in grapes and berries, lowers TMAO, cholesterol, and LDL, enhances HDL, and improves endothelial function via eNOS and PKA signaling, with gut microbiota modulation increasing Bacteroides and Lactobacillus [121].

Curcumin restores the Firmicutes/Bacteroidetes ratio and reduces TMAO and LPS levels, while gallic acid shows sex-specific reductions in plaque formation. Naringin enhances bile acid excretion

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and shifts microbial composition, favoring Lactobacillus and reducing TMA-producing bacteria. Procyanidin A2 and geraniin increase microbial diversity and beneficial genera like Akkermansia, reducing plaque area, while protocatechuic acid decreases inflammation without affecting TMAO [122].

These compounds collectively improve gut barrier integrity, reduce pro-atherogenic metabolites (TMAO, LPS), and enhance anti-inflammatory and antioxidant effects, suggesting that dietary intake of polyphenol-rich foods could be a promising strategy for AS prevention and treatment, though further studies are needed to explore synergistic effects with probiotics and precise mechanisms [123].

3.3.6. Targeting TMAO

Trimethylamine N-oxide (TMAO), a gut microbiota-derived metabolite, is a recognized risk factor for atherosclerosis (AS), prompting research into strategies to reduce its levels through dietary interventions, gut flora regulation, inhibition of TMAO precursor production, fecal microbiota transplantation (FMT), and pharmacological approaches, including traditional Chinese medicine [124].

Dietary interventions like the Mediterranean diet, vegan diets, and intermittent fasting lower TMAO by modulating gut microbiota, with studies showing reduced TMAO in vegetarians and those on fasting-mimicking diets due to shifts in microbial composition (lower Clostridia, higher Trichospira). However, nutrients like choline and L-carnitine, while essential, can increase TMAO, necessitating a balance to retain their cardiovascular benefits. Regulating intestinal flora with antibiotics suppresses TMAO but risks dysbiosis and resistance, while probiotics like Lactobacillus plantarum ZDY04 and Bifidobacterium species reduce TMAO and improve lipid metabolism, though results vary by strain [125].

Inhibiting TMAO precursor production targets microbial choline TMA lyase (CutC) with inhibitors like 3,3-dimethyl-1-butanol (DMB), iodomethylcholine (IMC), and fluoromethylcholine (FMC), which reduce TMAO and plaque formation without harming gut flora. Inhibiting TMA conversion to TMAO by targeting flavin-containing monooxygenase 3 (FMO3) with compounds like trigonelline or antisense oligonucleotides decreases TMAO and thrombosis risk, though safe inhibitors are needed [64].

FMT shows potential to alter microbiota and reduce TMAO, but limited studies and ethical concerns restrict its use. Pharmacological approaches, including metformin and resveratrol, modulate gut microbiota and lower TMAO, while traditional Chinese medicines like berberine and Ganoderma reduce TMAO by adjusting microbial composition and inhibiting TMA synthesis, showing promise but requiring further clinical validation. These strategies highlight the potential of targeting TMAO to mitigate AS, with ongoing research needed to optimize efficacy and safety [126].

3.3.7. Fecal Microbiota Transplantation

Fecal microbiota transplantation (FMT), a method involving the transfer of filtered fecal samples from healthy donors or autologous sources to restore gut microbiota balance, has gained traction as a therapeutic approach for dysbiosis-related conditions, including atherosclerosis. Initially developed in the 1960s, FMT is highly effective for treating Clostridium difficile infections and shows promise in extra-intestinal diseases like cardiovascular diseases (CVDs) by altering bile acid composition and reducing plasma triglycerides, as seen in obese recipients receiving FMT from lean donors [42,127].

These changes suggest anti-atherosclerotic effects by mitigating dyslipidemia and pro-atherogenic metabolites like trimethylamine N-oxide (TMAO). Preclinical studies demonstrate that FMT from healthy donors reduces plaque development and systemic inflammation in atherosclerosis models. However, human evidence is limited, with small-scale studies showing microbiota shifts but no significant improvements in TMAO, lipids, or endothelial function. One ongoing clinical trial (NCT04410003) is investigating FMT for severe atherosclerosis, but larger trials are needed to confirm

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efficacy. Risks, including endotoxin transfer and long-term safety concerns, currently limit FMT's widespread use, necessitating further research to optimize its therapeutic potential in atherosclerosis management [128–130].

Table 4 summarizes therapeutic interventions targeting the gut microbiota, including their mechanisms, evidence from studies, and current challenges.

Table 4. Therapeutic Interventions Targeting Gut Microbiota in Atherosclerosis.

Intervention	Mechanism	Evidence	Challenges
Dietary Interventions	Enhances SCFA production, reduces TMAO, promotes beneficial bacteria (e.g., Mediterranean diet)	Reduced cardiometabolic risk, improved microbial diversity (6, 98)	Requires long-term adherence, variable response
Prebiotics	Promotes SCFA production, enhances gut barrier (e.g., inulin, pectin)	Reduced atherosclerosis in mice, increased butyrate (108, 109)	Modest, temporary effects on microbiota
Probiotics	Reduces cholesterol, TMAO, inflammation (e.g., Lactobacillus, Bifidobacterium)	Improved lipid profiles, reduced plaque in ApoE−/− mice (113, 114)	Inconsistent colonization, strain variability
Small Molecules (e.g., DMB, IMC)	Inhibits TMAO production, reduces cholesterol	Reduced plaque area, cholesterol by 36% in mice (70, 118)	Potential side effects (e.g., trimethylaminuria)
Phenolic Compounds	Reduces TMAO, inflammation, enhances microbial diversity (e.g., quercetin)	Decreased lesions, improved lipid profiles in mice (120, 121)	Limited human data, synergistic effects unclear
Fecal Microbiota Transplantation (FMT)	Restores microbial balance, reduces TMAO, improves lipid metabolism	Reduced plaque in animal models, limited human data (127, 128)	Safety concerns, limited clinical evidence

4. Conclusions

This review consolidates evidence highlighting the gut microbiota's critical role in atherosclerosis, a multifaceted cardiovascular condition with mechanisms still under exploration. A balanced gut microbiota supports cardiovascular health by regulating cholesterol metabolism, immune function, and generating beneficial metabolites like short-chain fatty acids (SCFAs), whereas dysbiosis heightens atherosclerosis risk through microbial translocation into plaques and production of pro-atherogenic compounds like trimethylamine N-oxide (TMAO). Promising therapeutic approaches, including probiotics (Lactobacillus, Bifidobacterium), prebiotics, and fecal microbiota transplantation (FMT), demonstrate potential in atherosclerosis prevention and treatment. Probiotics effectively reduce cholesterol, TMAO, inflammation, and enhance gut barrier function, while prebiotics and FMT improve lipid profiles, boost SCFA production, and optimize bile acid metabolism, contributing to anti-atherosclerotic benefits. Advances in microbiome research confirm the safety and efficacy of these interventions in animal and human studies, but larger clinical trials are essential to establish optimal dosing, safety, and precise mechanisms. The complex interplay among bacterial species drives host effects, necessitating further studies on microbial metabolites, beneficial versus pathogenic bacteria interactions, and tailored microbiome-based therapies.

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Abbreviations

The following abbreviations are used in this manuscript:

- A. muciniphila: Akkermansia muciniphila
- ABCA1: ATP-binding cassette transporter A1
- ABCG2: ATP-binding cassette super-family G member 2
- ACC: Acetyl-CoA carboxylase
- Akt: Protein kinase B (also part of PI3K-Akt pathway)
- AMPK: AMP-activated protein kinase
- ApoA-I: Apolipoprotein A-I
- ApoE: Apolipoprotein E
- AS: Atherosclerosis
- ASBT: Apical sodium-dependent bile acid transporter
- ASCVD: Atherosclerotic cardiovascular disease
- ATG16L1: Autophagy related 16 like 1
- B. uniformis: Bacteroides uniformis
- bai: Bile acid-inducible (operon)
- BARs: Bile acid receptors
- BAs: Bile acids
- B-GOS: Bifidobacterial-galacto-oligosaccharides
- BSH: Bile salt hydrolase
- CA: Cholic acid
- CAR: Constitutive androstane receptor
- CD14: Cluster of differentiation 14
- CD204: Cluster of differentiation 204
- CD36: Cluster of differentiation 36
- CDCA: Chenodeoxycholic acid
- COX-2: Cyclooxygenase-2
- COVID-19: Coronavirus disease 2019
- CRC: Colorectal cancer
- CVD: Cardiovascular disease
- CYP27A1: Cytochrome P450 family 27 subfamily A member 1
- CYP7A1: Cytochrome P450 family 7 subfamily A member 1
- CYP7B1: Cytochrome P450 family 7 subfamily B member 1
- DCA: Deoxycholic acid
- DCs: Dendritic cells
- DIP: Dictophora indusiata polysaccharide
- DMB: 3,3-dimethyl-1-butanol
- E. coli: Escherichia coli
- EDH: Endothelium-derived hyperpolarization
- EGFR: Epidermal growth factor receptor
- ENS: Enteric nervous system
- eNOS: Endothelial nitric oxide synthase
- ERK1/2: Extracellular signal-regulated kinase 1/2
- F. prausnitzii: Faecalibacterium prausnitzii
- F/B: Firmicutes/Bacteroidetes (ratio)
- FASN: Fatty acid synthase

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- FFAR2: Free fatty acid receptor 2
- FFAR3: Free fatty acid receptor 3
- FMC: Fluoromethylcholine
- FMO3: Flavin monooxygenase 3
- FMT: Fecal microbiota transplantation
- FOXP3: Forkhead box P3
- FXR: Farnesoid X receptor
- G6Pase: Glucose-6-phosphatase
- GABA: Gamma-aminobutyric acid
- GF: Germ-free
- GLP-1: Glucagon-like peptide 1
- GPCR: G-protein coupled receptor
- GPR41: G-protein coupled receptor 41
- H. pylori: Helicobacter pylori
- HDAC: Histone deacetylase
- HDACs: Histone deacetylases
- HDL: High-density lipoprotein
- HFD: High-fat diet
- HGM: Human gut microbiome
- HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A
- HSDH: Hydroxysteroid dehydrogenase
- I3C: Indole-3-carbinol
- IBD: Inflammatory bowel disease
- IBS: Irritable bowel syndrome
- ICAM-1: Intercellular adhesion molecule 1
- IEC: Intestinal epithelial cell
- IECs: Intestinal epithelial cells
- IFIT1: Interferon-induced protein with tetratricopeptide repeats 1
- IgA: Immunoglobulin A
- IgG: Immunoglobulin G
- IL-1: Interleukin-1
- IL-1β: Interleukin-1 beta
- IL-6: Interleukin-6
- IL-8: Interleukin-8
- IMC: Iodomethylcholine
- isoalloLCA: Isoallolithocholic acid
- JNK: c-Jun N-terminal kinase
- LAL: Limulus Amoebocyte Lysate
- LBP: Lipopolysaccharide-binding protein
- LCA: Lithocholic acid
- LDL: Low-density lipoprotein
- LDLr: Low-density lipoprotein receptor
- LL-37: Cathelicidin antimicrobial peptide
- LOX-1: Lectin-like oxidized low-density lipoprotein receptor-1
- LPS: Lipopolysaccharides
- M1: Macrophage type 1
- M2: Macrophage type 2
- MAPK: Mitogen-activated protein kinase
- MCP-1: Monocyte chemoattractant protein 1
- MCT1: Monocarboxylate transporter 1
- MCT4: Monocarboxylate transporter 4

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- MD-2: Myeloid differentiation factor 2
- MDA: Malondialdehyde
- MRSA: Methicillin-resistant Staphylococcus aureus
- mtROS: Mitochondrial reactive oxygen species
- MUC2: Mucin 2
- MyD88: Myeloid differentiation primary response 88
- NADPH: Nicotinamide adenine dinucleotide phosphate
- NEU1: Neuraminidase 1
- NET: Neutrophil extracellular trap
- NF-κB: Nuclear factor kappa B
- NK: Natural killer
- NKT: Natural killer T
- NLRP3: NLR family pyrin domain containing 3
- NO: Nitric oxide
- NOD1: Nucleotide-binding oligomerization domain 1
- NOD1/2: Nucleotide-binding oligomerization domain 1/2
- NOD2: Nucleotide-binding oligomerization domain 2
- NPC1L1: Niemann-Pick C1-like 1
- NR4A1: Nuclear receptor subfamily 4 group A member 1
- NR4A2: Nuclear receptor subfamily 4 group A member 2
- Nrf2: Nuclear factor erythroid 2-related factor 2
- Olfr78: Olfactory receptor 78
- OSTα/OSTβ: Organic solute transporter alpha/beta
- oxLDL: Oxidized low-density lipoprotein
- P. gingivalis: Porphyromonas gingivalis
- PAF: Platelet-activating factor
- PAMPs: Pathogen-associated molecular patterns
- PD-L1: Programmed death-ligand 1
- PEPCK: Phosphoenolpyruvate carboxykina

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