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High-fat Diet Promotes Atherosclerosis Through the Microecological-immunological Axis

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Abstract: Cardiovascular and cerebrovascular diseases (CVDs) have become one of the leading causes of death in China. Atherosclerosis (AS) is the common pathological basis, and intestinal flora dysbiosis and its metabolites are deeply involved in the development of AS through the "microecology-immunity axis". High-fat diet leads to imbalance of flora structure, decrease of beneficial bacteria and increase of pathogenic bacteria, damage the intestinal barrier, promote the entry of endotoxins (e.g., LPS) and harmful metabolites (e.g., TMAO, lmP, PAGIn, BCAAs) into the bloodstream, activate immune-inflammation (e.g., NF-KB, NLRP3 pathway), platelet activation and thrombosis, and disrupt the lipid metabolism. Reduction of protective metabolites (e.g., SCFAs) impairs anti-inflammatory and endothelial protective effects. Current intervention studies have shifted from traditional flora regulation to targeting metabolites and signaling pathways (e.g., ADR, FXR, I1R inhibition) and exploring multimodal therapies, such as exercise and traditional Chinese medicine. In the future, this article focus on individualized precision intervention, multi-target synergistic strategy, drug delivery system and reliable biomarker validation to promote the clinical translation of AS prevention and treatment.

Keywords: Component; High-fat Die; Gut Flora; Metabolites; Cardiovascular Disease.

1. Introduction

Cardiovascular and cerebrovascular diseases (CVDs), which traditionally are highly prevalent in the middle-aged and elderly groups, are currently showing a significant trend of rejuvenation, increasing the threat to the health of young and middle-aged people and making prevention and control a matter of great urgency. The burden of cardiovascular disease in China continues to increase, with the total number of patients reaching 330 million, ranking first among the causes of death for urban and rural residents. Of particular concern is that the incidence of the disease has shown a marked trend of youthfulness, with 44.3% of the population aged 20-39 years in a sick or high-risk state, of which 15.3% are aged 20-29 years, indicating that cardiovascular and cerebrovascular diseases are spreading to the younger age groups, and have become a major public health challenge. The burden of cardiovascular disease in China continues to increase, and the urgency of prevention and treatment is extremely strong. Its common pathological basis is atherosclerosis, which will lead to narrowing of blood vessels, insufficient blood supply to organs and induce diseases. In recent years, studies have found that the association between intestinal flora and cardiovascular disease is a new direction [1]. Long-term high-fat diet will damage the intestinal barrier, cause imbalance of the flora, interfere with metabolism and exacerbate inflammation, and ultimately promote atherosclerosis. In conclusion, it is a fact that the burden of cardiovascular diseases is increasing at a younger age, and the public needs to maintain gut health through a healthy lifestyle to reduce the risk of the disease.

2. Effects of a High-fat Diet on Intestinal Flora

A high-fat diet (HF) is a key factor contributing to intestinal dysbiosis, which significantly reduces flora diversity and disrupts the structural balance of the flora. The abundance of beneficial flora (e.g., butyric acid-producing and anti-inflammatory B. pumilus, Bifidobacterium bifidum, and Lactobacillus lactis) generally decreases; while the abundance of harmful flora (e.g., LPS-producing E. coli, Bacteroides spp.) significantly increases [2–5]. This dysbiosis disrupts the intestinal barrier and promotes endotoxin (e.g., LPS) entry into the bloodstream and systemic low-grade inflammation, while decreasing the production of beneficial metabolites (e.g., butyric acid), which collectively exacerbate oxidative stress, lipid metabolism disorders, and immune imbalances. These changes constitute the core mechanism of the “microecology-immunity axis” pathway, which directly contributes to the development of atherosclerosis (AS). The characteristics of the intestinal flora observed in AS patients (decrease in butyric acid-producing bacteria and increase in specific pathogenic bacteria) are highly consistent with the effects of the HF diet, and the more specific alterations in flora structure (e.g., changes in Roussboulia, Klebsiella, Clostridium IV, and Ruminalococcaceae) with the progression of CVD further confirm the important role of the intestinal flora in the pathogenesis of AS [6].

In conclusion, chronic intake of a high-fat diet causes changes in the gut microbiota, fecal metabolites, and pro-inflammatory factors in healthy adults, which can result in adverse health effects, particularly predisposing to cardiovascular disease.

3. Mechanisms of Regulation of The Immune System by Metabolites of Bacterial Colonies

These products play a key role in maintaining immune homeostasis and physiological health in the body. However, when the intestinal flora is imbalanced, this delicate regulatory system is disrupted and works together to accelerate the process of atherosclerosis (AS) through

multiple mechanisms**:**(1) Loss of the beneficial barrier: Imbalance of the flora first leads to a decrease in beneficial metabolites (e.g., short-chain fatty acids, SCFAs), which in turn disrupts the integrity of the intestinal epithelial barrier.(2) Invasion of hazardous substances A compromised intestinal barrier allows a large number of hazardous substances (such as lipopolysaccharide LPS, trimethylamine oxide TMAO, and toxic amino acid metabolites) to enter the circulatory system.(3) Triggering of systemic effects: These substances then trigger a systemic chronic inflammatory response and directly interfere with cholesterol metabolism. Ultimately, the two core components of increased systemic inflammation and disruption of cholesterol metabolism promote each other, and together they contribute to the onset and development of atherosclerosis.

3.1. Abbreviations and Acronyms

Short-chain fatty acids (SCFA) have a complex and seemingly bidirectional modulation of inflammation, and their specific effects depend on the context and target of action: In the context of atherosclerosis, SCFA reduce the release of pro-inflammatory factors (e.g., IFN-y, IL-13, IL-2, TNF-a, and IL-12) mainly through inhibition of the NF-KB pathway and induce regulatory T cell (Treg) differentiation through epigenetic mechanisms (e.g. histone deacetylase inhibition) induces regulatory T cell (Treg) differentiation, which further up-regulates anti-inflammatory factors such as IL-10, IL-4 and TGF-β1 to synergistically suppress inflammation and promote immune tolerance [1,7]. In contrast, in specific immune defense scenarios, SCFA play a pro-inflammatory role by activating epithelial NF-KB signaling and enhancing neutrophil function , in order to strengthen the body's clearance of pathogens [8]. Thus, SCFA can maintain immune homeostasis and suppress chronic inflammation in different environments, as well as activate and amplify immune responses during acute infections.

3.2. Atherosclerosis- promoting Metabolites

3.2.1. Imidazole Propionate (ImP)

lmP significantly promotes the progression of atherosclerosis (AS) by interfering with key cell signaling pathways and activating immune-inflammatory response.

(1)Inhibits endothelial repair and impairs the vascular barrier

lmP specifically inhibits the PI3K/AKT signaling pathway in endothelial cells (EC), especially by downregulating PI3K-C2A and blocking insulin receptor-mediated AKT activation. This leads to: 1) decreased phosphorylation levels of FOXO1 transcription factor and increased accumulation in the nucleus; 2) direct inhibition of endothelial cell proliferation and migration, reducing the regenerative capacity of the vascular endothelium after injury; and 3) disruption of the endothelial integrity, increasing inflammatory cell adhesion and infiltration, and exacerbating vascular wall lesions [9].

(2)Activation of the immune-inflammatory response

Via myeloid cells Imidazoline-1 receptor (I1R) signaling, ImP drives the formation of a pro-inflammatory immune state. 1) expansion of pro-inflammatory monocytes, Th1 and Th17 cells; and 2) promotion of inflammatory cytokine secretion, initiation of the inflammatory cycle, further recruitment of immune cells to accumulate in the vascular wall, and disruption of microenvironmental homeostasis [10].

3.2.2. Amino Acid Metabolite

3.2.2.1 Tryptophan (Trp) Metabolite - Indole Derivative

In atherosclerotic plaques, tryptophan metabolites shape an immunosuppressive microenvironment through a variety of mechanisms, and their central role is reflected at the following four levels: first, tryptophan is consumed in large quantities by the enzyme indoleamine 2,3-dioxygenase (IDO), which leads to cycle arrest and functional inhibition of T cells due to amino acid deprivation, directly weakening cellular immune responses; second, the metabolite kynurenine (Kyn) activates the aryl hydrocarbon receptor (AhR) signaling pathway, which promotes the differentiation of regulatory T cells (Treg) and enhances the secretion of suppressor cytokines; thirdly, the Kyn-AhR axis regulates macrophage polarization, inhibits M1-type and promotes M2-type polarization, and regulates the balance between inflammation and repair; lastly, IDO-overexpressing dendritic cells are transformed into a resistant phenotype, which actively induces T-cell inactivation or Treg differentiation, forming and maintaining a self-reinforcing immunosuppressive microenvironment [11].

3.2.2.2 Phenylalanine Metabolite - phenylacetylglutamine (PAGln)

Phenylalanine, an essential amino acid, is metabolized by gut microbes to produce phenylacetylglutamine (PAGIn). This metabolite is significantly present in the human body and has been established as an independent risk factor for atherosclerosis (AS) both at the clinical study and mechanistic levels. Its pathogenic mechanism focuses on a direct potentiation of platelet activation and thrombogenicity through the following pathways.

(1) Receptor activation: PAGIn initiates downstream signaling by specifically binding to and activating specific subtypes of the G protein-coupled receptor (GPCR) family, including the a2A-adrenergic receptor (a2A-AR), a2B-AR, and the beta2-AR.

(2) Platelet hyperfunction:

Enhances platelet adhesion to collagen matrix;

Promotes agonist-induced significant elevation of intraplatelet calcium ion concentration ([Ca2+]i);

Enhances sensitivity and aggregation response to multiple platelet agonists.

(3) Acceleration of thrombosis: In an in vivo model, PAGIn treatment significantly accelerated the kinetics of platelet clot formation and enhanced the overall potential for thrombosis.

Therefore, PAGIn, as an intestinal flora-dependent metabolic messenger, clearly promotes thrombosis and atherosclerosis through the “receptor-calcium-signaling-platelet-activation” axis, providing a mechanistic rationale for its use as a new therapeutic target [12].

3.2.2.3 (Branched Chain Amino Acids (BCAA)

Disturbed metabolism of branched-chain amino acids (BCAAs) synergistically contributes to the progression of atherosclerosis (AS) through multilevel mechanisms. The main pathways include: activation of the Sirt1/NLRP3 inflammasome axis, which drives monocyte-macrophage activation and vascular inflammation; alteration of platelet signaling through proteolysis modification, which enhances aggregation and thrombosis risk; antagonism of IRS-1 and activation of the mTORC1 pathway, which induces insulin resistance and lipid metabolism abnormalities; and dysbiosis of intestinal bacteria, which leads to decreased catabolic capacity and reduced beneficial metabolites of BCAAs, further exacerbating the metabolic-inflammatory vicious

cycle. metabolites, further exacerbating the metabolic-inflammatory vicious cycle. These mechanisms form a positive feedback network, promoting the development of AS and cardiovascular events through the "metabolism-immunity-thrombosis" triple coupling effect, which provides a new target for metabolic immune intervention [13].

3.2.3. Trimethylamine (TMA) and Trimethylamine Oxide (TMAO)

Trimethylamine oxide (TMAO) synergistically promotes the onset and progression of atherosclerosis (AS) through multiple mechanisms.

TMAO activates signaling pathways such as MAPK/ERK/NF-KB, enhances oxidative stress and promotes the release of pro-inflammatory factors, driving sustained inflammatory responses and monocyte-macrophage activation and accelerating plaque formation. It also synergizes with angiotensin-II (Ang-II) to prolong its pathological effects, causing vasospasm, activating MMP-2 and endothelin-1, and promoting vascular fibrosis and remodeling through the TGF-3/Smad pathway. In terms of thrombosis, TMAO enhanced platelet reactivity and aggregation sensitivity by affecting intraplatelet calcium release and promoting ERK/JNK phosphorylation, while disrupting cholesterol metabolism, inhibiting reverse transporter, and increasing lipids and blood viscosity, which significantly elevated the risk of thrombosis. In addition, TMAO induces endoplasmic reticulum stress, causing lipid metabolism disorders and cellular dysfunction.

In summary, TMAO systematically drives atherosclerosis progression through a multilevel mechanism of inflammatory activation, vascular fibrosis promotion, lipid homeostasis dysregulation and thrombosis [8].

3.2.4. Endotoxin (lipopolysaccharide, LPS)

Lipopolysaccharide (LPS) is an important pathogenic factor in atherosclerosis, and its pathogenic process begins in the gut and affects vascular health via systemic inflammation. First, factors such as a high-fat diet disrupt the integrity of the intestinal barrier and increase its permeability; subsequently, LPS translocates into the circulation, causing metabolic endotoxemia. LPS entering the bloodstream is recognized by TLR4 on the macrophage surface, which activates downstream proinflammatory signaling pathways, driving macrophage polarization to a proinflammatory phenotype and the release of multiple cytokines (e.g., IL-13, TNF-a, IL-6) and chemokines. These inflammatory mediators trigger systemic low-grade inflammation, which damages the vascular endothelium, promotes monocyte/macrophage infiltration and foam cell formation, and ultimately accelerates atherosclerosis development [1].

3.2.5. Bile Acids

Bile acids (BAs) play a central role in lipid metabolism, inflammation and intestinal homeostasis by modulating multiple receptor signaling pathways, and their dysfunction is closely related to atherosclerosis (AS).

(1) Receptor-mediated hormone-like regulation

Bile acids act as signaling molecules that activate two types of key receptors:

- Nuclear receptor FXR: activation regulates gene expression, reduces triglyceride accumulation and promotes fatty acid oxidation.
- Membrane receptor TGR5 (GPCR19): after activation, it inhibits inflammatory signaling pathways and reduces the expression of pro-inflammatory cytokines and chemokines in the aorta [8].

(2) High-fat diet alters bile acid composition and disrupts the intestinal barrier High-fat diet (HFD) significantly alters the composition of the bile acid pool:

- Elevated levels of deoxycholic acid (DCA): disrupts intestinal epithelial cell membrane integrity.
- Decreased ursodeoxycholic acid (UDCA) levels: weakening its protective effect on the intestinal mucosa.

Both together lead to intestinal barrier dysfunction and increased permeability [14].

(3) Promotion of pathogenic bacteria growth and intestinal wall damage

The imbalance of DCA/UDCA ratio further alters the intestinal microecology:promotes the proliferation of pathogenic microorganisms;increases the production of harmful metabolites, such as hydrogen sulfide (HzS), and directly damages the structure and function of the intestinal wall [14].

(4) Core pathological effects

Bile acids systematically regulate: lipid metabolism disorders, insulin sensitivity, immune cell infiltration, and inflammatory states, mainly through FXR and TGR5 receptor-mediated signaling.

Bile acids affect the development of atherosclerosis through the two main lines of "receptor signaling-metabolic/inflammatory regulation" and "compositional changes, intestinal barrier disruption, and bacterial dysbiosis" [8,14].

4. The Main Molecular Mechanism of the "Microecology-Immunity Axis" in the Formation of Atherosclerosis

4.1. Protective Metabolite - Short Chain Fatty Acids (SCFA)

Short-chain fatty acids (SCFAs) play an anti-inflammatory role by coordinating immune homeostasis through a multilevel molecular mechanism.

(1) Inhibit NF-KB pathway and directly reduce the release of pro-inflammatory factors

SCFAs significantly down-regulate the expression and secretion of many key pro-inflammatory cytokines, including IFN-Y, IL-1β, IL-2, TNF-a, IL-12, by inhibiting the activation of NF-KB (Nuclear Factor KB) in immune cells (e.g., macrophages, T-cells).

(2) Modulate epigenetic regulation, and induce regulatory T-cell (Treg) differentiation SCFAs (especially butanediole) have been found to play an important role in the coordination of immune homeostasis. cell (Treg) differentiation

SCFAs (especially butyrate) act as histone deacetylase inhibitors (HDACi), modulating gene expression by altering chromatin conformation by affecting histone acetylation levels:

- inducing dendritic cells (DCs) to assume an immune-tolerant phenotype;
- driving differentiation of the initial T cells into FoxP3-expressing regulatory T cells (Treg);
- further Promote differentiation of Treg subtypes (e.g. Tr1, Th3).

(3) Remodeling cytokine network and strengthening immunosuppressive microenvironment

Treg and other immune cells induced by SCFAs systematically inhibit excessive immune responses by secreting a large number of anti-inflammatory cytokines:

Up-regulate the expression of IL-10, IL-4, and TGF-β1; down-regulate the levels of TNF-a, IFN-y, and IL-12;

The final effect is: to establish and maintain a strong anti-inflammatory environment at the cytokine level. The final effect is to establish and maintain a strong anti-inflammatory environment at the cytokine level.

SCFAs can synergistically inhibit inflammation and promote immunity through the three major mechanisms of "inhibiting NF-KB inflammatory pathway", "epigenetically regulating T-cell fate", and "remodeling cytokine profiles", which are multi-level and multi-targeted. The three mechanisms, namely, the "inflammatory pathway", "epigenetic regulation of T cell fate" and "remodeling of cytokine profiles", can synergistically inhibit inflammation at multiple levels and multiple targets, and promote immune tolerance, thus exerting their protective effects against atherosclerosis [8,15].

4.2. Atherosclerosis- promoting Metabolites

4.2.1. Imidazole Propionate (ImP)

(1) Activation of the I1R/mTOR pro-inflammatory pathway

lmP binds to the imidazoline-1 receptor (I1R) on the surface of myeloid cells (such as monocytes/macrophages) → activates the downstream mTOR signaling pathway → mTOR, as the central kinase integrating nutritional and energy states, promotes the synthesis and release of pro-inflammatory cytokines (such as TNF-α) upon activation → directly initiates and amplifies the inflammatory response.

(2) Inhibit the PI3K/AKT endothelial protective pathway

lmP downregulates the activity of phosphoinositide 3-kinase (PI3K) → inhibits the phosphorylation and activation of its downstream effector molecule protein kinase B (AKT) → resulting in the transcription factor FOXO1 failing to be phosphorylated by AKT and remaining continuously retained in the cell nucleus while maintaining its activity → activated FOXO1:

· Inhibits endothelial cell migration and angiogenesis;

· Upregulates the expression of inflammatory markers (such as ICAM-1, VCAM-1);

→ Together, these lead to endothelial dysfunction and accelerate atherosclerosis.

lmP drives disease progression through two parallel and synergistic signaling axes: "activation of I1R/mTOR (pro-inflammatory)" and "inhibition of PI3K/AKT (endothelial damage)"—acting on both immune and vascular levels [10].

4.2.2. Amino Acid Metabolite

4.2.2.1 Tryptophan (Trp) Metabolites --- Indole Derivatives

(1) Tryptophan depletion directly inhibits T cell function

Tryptophan deficiency activates the GCN2 kinase within T cells → phosphorylates eukaryotic initiation factor 2a (eIF2a) → globally inhibits protein translation → blocks T cell proliferation and effector molecule synthesis, leading to T cell dysfunction.

Tryptophan deficiency simultaneously inhibits the mTOR signaling pathway (core nutrient-sensing pathway) → exacerbating cell cycle arrest and functional inhibition.

(2) Metabolite kynurenine activates the AhR signaling pathway

Kynanuric acid (Kyn) enters the cytoplasm as a ligand and binds to the aryl hydrocarbon receptor (AhR) → the AhR-ligand complex translocates to the nucleus, dimerizes with the AhR nuclear translocation protein (ARNT) → binds to XREs in the promoter regions of target genes → upregulates a series of genes including:

Foxp3 (drives Treg differentiation);

Multiple immune regulatory cytokines and enzymes;

→Actively induces the formation of an immunosuppressive microenvironment.

(3) Downstream metabolites induce oxidative stress and apoptosis

Downstream metabolites of the Kyn pathway (such as 3-hydroxykynurenine, 3-hydroxyphenylalanine) act as pro-oxidants → Produce reactive oxygen species (ROS) → Triggering mitochondrial apoptosis → Clearing activated T cells and other sensitive immune cells.

Tryptophan metabolism acts through three molecular mechanisms—"amino acid starvation sensing (GCN2/mTOR)", "transcriptional regulation (AhR-XRE)", and "oxidative stress (ROS)"—to synergistically induce immune suppression at three levels: translation inhibition, phenotypic reprogramming, and cell clearance [11].

4.2.2.2 Phenylalanine Metabolite --- Phenylacetylglutamine (PAGln)

Intestinal microbial metabolic enzyme genes precisely influence the host's risk of thrombosis by regulating the production of specific metabolic products.

(1) Key genes regulate metabolite synthesis

porA gene: primarily responsible for regulating the production of phenylacetic acid (PAA).

fldH gene: primarily responsible for regulating the production of phenylpropionic acid (PPA).

(2) Changes in metabolite levels affect thrombosis

Knocking out the fldH gene → leads to elevated PAA levels → which in turn affects the levels of its derivative PAGly → ultimately significantly reducing thrombosis formation capacity.

(3) PAGIn mediates platelet activation through specific adrenergic receptors

The metabolite PAGIn acts as a signaling molecule, specifically activating G protein-coupled receptors (GPCRs) expressed on the platelet surface: · a2A-adrenergic receptor (a2A-AR) · a2B-adrenergic receptor (a2B-AR) · β2-adrenergic receptor (β2-AR)

After binding to these receptors, PAGIn → initiates downstream intracellular signal transduction → ultimately mediates platelet activation and aggregation reactions, promoting thrombus formation.

This mechanism reveals a clear signaling pathway from "microbiota genes (porA/fldH)" to "metabolites (PAA/PPA/PAGly/PAGIn)" to "host receptors (ADR)," elucidating the molecular pathway by which microbial metabolism influences thrombosis risk and providing a potential new target for intervening in thrombotic diseases [12].

4.2.2.3 Branched-chain Amino Acids (BCAA)

(1) Activation of the NLRP3 inflammasome (core inflammatory mechanism)

BCAAs inhibit the activity of the deacetylase Sirt1 → lifting its inhibition of the NLRP3 inflammasome → promoting the assembly and activation of the NLRP3 inflammasome and Caspase-1 → leading to the maturation and release of potent pro-inflammatory factors such as IL-1β

and IL-18, and inducing pyroptosis → directly damaging vascular cells and exacerbating plaque inflammation.

(2) Inducing mitochondrial oxidative stress

BCAA metabolites (such as branched-chain α-keto acids, BCKAs) directly stimulate excessive production of reactive oxygen species (ROS) in mitochondria → leading to oxidative stress and disruption of cellular function → further activating multiple inflammatory pathways, forming a vicious cycle of inflammation-oxidative stress, and continuously damaging vascular homeostasis.

(3) Promoting platelet protein acetylation modification (thrombosis mechanism)

BCAAs (especially valine) are metabolized into propionyl-CoA within platelets → acting as a modification donor, they perform lysine propionylation modification on platelet functional proteins (such as the K255 site of Tmod3) → significantly enhancing platelet activity and aggregation capacity → greatly increasing the risk of occlusive thrombosis formation after plaque rupture.

(4) Activation of the mTORC1 pro-inflammatory signaling pathway

BCAAs act as potent activators, directly activating the mTORC1 signaling pathway within macrophages → driving macrophages toward a pro-inflammatory phenotype → secreting more inflammatory factors, exacerbating local vascular inflammatory responses.

BCAAs drive the progression of atherosclerosis through four molecular pathways: the "Sirt1/NLRP3 axis" (inflammation), "mitochondrial ROS" (oxidative stress), "propionylation modification" (thrombosis), and "mTORC1 signaling" (macrophage polarization), acting on multiple levels including inflammatory activation, oxidative damage, thrombosis formation, and immune regulation [13].

4.2.3. Trimethylamine (TMA) and Trimethylamine Oxide (TMAO)

(1). Exacerbation of oxidative stress and inflammatory response

TMAO directly increases vascular oxidative stress levels → promotes the expression and phosphorylation activation of ERK and NF-κB → regulates the expression of apoptosis-related genes (Bcl-2 mRNA ↓, Bax mRNA ↑) → promotes the release of downstream pro-inflammatory factors → drives sustained inflammatory response.

(2). Synergistic effects with angiotensin II (Ang-II) to prolong pathological effects

TMAO can bind to Ang-II and prolong its action time, leading to sustained pathological changes in blood vessels through the following mechanisms:

(I) Inducing sustained arterial spasm and activating MMP-2 and endothelin-1 (ET-1), promoting vascular remodeling;

·(II) Further increases the expression of inflammatory factors and ET-1 through the MAPK pathway;

·(III) Activates the TGF-β/Smad signaling pathway, mediating vascular fibrosis.

(3). Promotes thrombosis

·TMAO affects platelet surface receptors and activation status → enhances platelet reactivity;

Simultaneously increases lipid levels and blood viscosity → significantly increases the risk of thrombosis.

TMAO synergistically promotes vascular inflammation, fibrosis, remodeling, and hypercoagulability through three major mechanism modules—"oxidative stress/inflammatory activation," "Ang-11 signal amplification," and "thrombogenesis"—thereby comprehensively driving the occurrence and development of atherosclerosis [8].

4.2.4. Endotoxin (lipopolysaccharide, LPS)

(1). Activates TLR4 inflammatory signals, driving plaque progression

LPS binds to Toll-like receptor 4 (TLR4) on the surface of macrophages → activates downstream pro-inflammatory signaling pathways such as NF-κB → promotes the massive release of pro-inflammatory cytokines (including TNF-α, IL-1, IL-6, IL-8, IL-12) by macrophages → This exacerbates vascular inflammation and directly accelerates the formation and progression of AS plaques.

(2). Enhances platelet activity and promotes thrombus formation

LPS activates leukocyte cathepsin G via TLR4-mediated signaling → significantly enhancing platelet aggregation;

LPS upregulates macrophage tissue factor (TF) expression → strongly initiating the coagulation cascade;

In the presence of agonists such as Pam3CSK4, LPS can enhance platelet-induced monocyte production of pro-inflammatory cytokines; ·→ Together, these factors lead to a sharp increase in the risk of hypercoagulability and thrombosis.

(3). Synergistic effects amplify pathological effects

LPS interacts through the two core pathways of "inflammatory activation" and "thrombosis formation," forming a vicious cycle:

The inflammatory environment further promotes platelet activation and coagulation; · Thrombosis-associated cells release additional inflammatory mediators;

→ Together, these factors systematically drive the progression of atherosclerosis and the occurrence of acute clinical events.

LPS exerts dual effects through the "TLR4-inflammatory factor" axis and the "platelet-coagulation" axis, synergistically driving the progression of atherosclerosis from both inflammatory and thrombotic perspectives [1].

4.2.5. Bile Acids

The inhibition of FXR receptors and activation of TGR5-mediated signaling are closely related to lipid metabolism regulation and are considered to be the interaction mechanism of bile acids in chronic diseases induced by a high-fat diet [14].

5. Recent Advances in Related Intervention Studies

Traditional methods such as fecal microbiota transplantation (FMT), antibiotics, and probiotics aim to restore microbial homeostasis as a whole. Current research, however, is more focused on targeting key metabolites and signaling pathways: for example, using selective ADR inhibitors or beta-blockers to reduce the risk of thrombosis caused by the microbial metabolite PAGIn; supplementing butyrate or using FXR receptor modulators (such as specific bile acids) for anti-inflammatory effects and metabolic improvement; and utilizing inhibitors to block the ImP-I1R axis or the Ang-Il signaling pathway downstream of TMAO to suppress immune inflammatory responses, independent of the cholesterol pathway.

Additionally, emerging strategies include aerobic exercise-derived FMT, targeting pathogenic bacterial virulence systems, and multi-pathway interventions such as traditional Chinese medicine formulas (e.g., Zexie Decoction), which repair the intestinal barrier, regulate microbiota composition,

and suppress inflammation, offering new directions for alleviating atherosclerosis. These advancements mark the transition of AS treatment toward precision interventions targeting the microbiota-immune network.

6. Analyzing Research Gaps and Future Research Trends Related to AS

While current research holds promise, there remain numerous unknowns and challenges to be addressed:

First, barriers exist in the application of personalized medicine. Given the high degree of individual variation in gut microbiota, it is essential to identify specific populations (e.g., based on genotype or microbial characteristics) that stand to benefit most from targeted therapies such as IDO modulation or ImP blockade. Second, mechanism research requires further refinement. IDO may exert dual or even contradictory effects across different stages of atherosclerosis and cell types, necessitating a clearer understanding of its regulatory mechanisms to avoid adverse effects from interventions [15]; simultaneously, synergistic effects of combined intervention strategies (e.g., ImP inhibitors + probiotics + Sirt1 activators) should be explored. Third, clinical translation still faces bottlenecks. These include how to achieve precise drug delivery to specific intestinal regions or target cells to reduce systemic exposure, as well as validating the reliability and clinical utility of biomarkers such as ImP and BCAA in large-scale clinical settings [10]. Finally, the interactive mechanisms between diet, microbiota, and the host require systematic elucidation. For example, the Mediterranean diet is associated with lower ImP levels [10]. Future research should elucidate how dietary components precisely regulate microbiota function, inhibit harmful metabolites (such as ImP and TMAO), and promote the production of beneficial metabolites (such as SCFAs and indole), providing a theoretical foundation for nutritional interventions. In summary, future research should continue to expand in terms of mechanism depth, clinical translation, individualized strategies, and holistic dietary interventions, leveraging multi-omics technologies to advance the precision and personalization of atherosclerosis prevention and treatment.

References

[1] Zou, Y., Song, X., Liu, N., Sun, W., & Liu, B. (2022). Intestinal Flora: A Potential New Regulator of Cardiovascular Disease. Aging and disease, 13(3), 753–772. https://doi.org/ 10. 14336/AD.2021.1022.

[2] Luo, Y. T., Cheng, R. Y., Liang, H. J., Wang, J. N., Shen, X., & He, F. (2020). Effects of high-fat diet on glucose and lipid metabolism and gut microbiota structure in mice. Acta Nutrimenta Sinica, *42*(5), 458–464. https://doi.org/10. 13325/ j. cnki.acta.nutr.sin.2020.05.007.

[3] Liu, H., Zhu, H., Xia, H., Yang, X., Yang, L., Wang, S., et al. (2021). Different effects of high-fat diets rich in different oils on lipids metabolism, oxidative stress and gut microbiota. Food research international (Ottawa, Ont.), 141, 110078. https://doi.org/10.1016/j.foodres.2020.110078.

[4] Wan, Y., Wang, F., Yuan, J., Li, J., Jiang, D., Zhang, J., et al. (2019). Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: a 6-month randomised controlled-feeding trial. Gut, 68(8), 1417–1429. https://doi.org/10.1136/gutjnl-2018-317609.

[5] Jia, X., Xu, W., Zhang, L., Li, X., Wang, R., & Wu, S. (2021). Impact of Gut Microbiota and Microbiota-Related Metabolites on Hyperlipidemia. Frontiers in cellular and infection microbiology, 11, 634780. https://doi.org/10. 3389/ fcimb. 2021.634780.

[6] Qian, Y., Feng, Y. M., Wang, Y. J., Yin, J. Z., & Mi, F. (2022). Research progress on the relationship between gut microbiota and chronic diseases. Chinese Journal of Microecology, *34*(10), 1217–1221. https://doi.org/10. 13381/ j. cnki. cjm. 202210020.

[7] Brandsma, E., Kloosterhuis, N. J., Koster, M., Dekker, D. C., Gijbels, M. J. J., van der Velden, S., et al. (2019). A Proinflammatory Gut Microbiota Increases Systemic Inflammation and Accelerates Atherosclerosis. Circulation research, 124(1), 94–100. https://doi.org/10. 1161/ CIRCRESAHA. 118.313234.

[8] Wen, Y., Sun, Z., Xie, S., Hu, Z., Lan, Q., Sun, Y., et al. (2022). Intestinal Flora Derived Metabolites Affect the Occurrence and Development of Cardiovascular Disease. Journal of multidisciplinary healthcare, 15, 2591–2603. https:// doi. org/10.2147/JMDH.S367591.

[9] Nageswaran, V., Carreras, A., Reinshagen, L., Beck, K. R., Steinfeldt, J., Henricsson, M., et al. (2025). Gut Microbial Metabolite Imidazole Propionate Impairs Endothelial Cell Function and Promotes the Development of Atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology, 45(5), 823–839. https://doi.org/10.1161/ATVBAHA.124.322346.

[10] Mastrangelo, A., Robles-Vera, I., Mañanes, D., Galán, M., Femenía-Muiña, M., Redondo-Urzainqui, A., et al. (2025). Imidazole propionate is a driver and therapeutic target in atherosclerosis. Nature, 645(8079), 254–261. https://doi.org/10. 1038/s41586-025-09263-w.

[11] Niinisalo, P., Oksala, N., Levula, M., Pelto-Huikko, M., Järvinen, O., Salenius, J. P., et al. (2010). Activation of indoleamine 2,3-dioxygenase-induced tryptophan degradation in advanced atherosclerotic plaques: Tampere vascular study. Annals of medicine, 42(1), 55–63. https://doi.org/10.3109/ 07853890903321559.

[12] Nemet, I., Saha, P. P., Gupta, N., Zhu, W., Romano, K. A., Skye, S. M., et al. (2020). A Cardiovascular Disease-Linked Gut Microbial Metabolite Acts via Adrenergic Receptors. Cell, 180(5), 862–877.e22. https://doi.org/10. 1016/j.cell. 2020.02. 016.

[13] Zaric, B. L., Radovanovic, J. N., Gluvic, Z., Stewart, A. J., Essack, M., Motwalli, O., et al. (2020). Atherosclerosis Linked to Aberrant Amino Acid Metabolism and Immunosuppressive Amino Acid Catabolizing Enzymes. Frontiers in immunology, 11, 551758. https://doi.org/10. 3389/ fimmu. 2020.551758.

[14] Chen, J., Xiao, Y., Li, D., Zhang, S., Wu, Y., Zhang, Q., et al. (2023). New insights into the mechanisms of high-fat diet mediated gut microbiota in chronic diseases. iMeta, 2(1), e69. https://doi.org/10.1002/imt2.69.

[15] Chajadine, M., Laurans, L., Radecke, T., Mouttoulingam, N., Al-Rifai, R., Bacquer, E., et al. (2024). Harnessing intestinal tryptophan catabolism to relieve atherosclerosis in mice. Nature communications, 15(1), 6390. https://doi.org/ 10. 1038/ s41467-024-50807-x.