Seung-Hoon Lee
Min Kyoung Kang *Editors*

Stroke Revisited: Dyslipidemia in Stroke

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Stroke Revisited

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Preface

The *Stroke Revisited* series now presents its final publications. As a principal editor, since Vol. 1: *Diagnosis and Treatment of Ischemic Stroke* published in 2017, I sequentially presented Vol. 2: *Hemorrhagic Stroke*, Vol. 3: *Vascular Cognitive Impairment*, and Vol. 4: *Pathophysiology of Stroke: From Bench to Bedside*. Finally, the contract with Springer Nature to publish six volumes of the *Stroke Revisited* series is now completed together with the current books: Vol. 5: *Dyslipidemia in Stroke* and Vol. 6: *Diabetes in Stroke*. Writing and editing these series in approximately 5 years, I have done my best to create a complete series, not to leave any scratch on the honor of the publisher and me. Looking back over the years, there are some regrets that it would have been a better book series if I had invested a little more energy. However, working concurrently as a clinical professor at Seoul National University Hospital, chair of the Korean Cerebrovascular Research Institute (KCRI), and CEO of a bio-venture company, Cenyx Biotech Inc., I am comforting myself with this level of achievement. Of course, while continuing to monitor the contents of the books, I commit to maintain the latest level of knowledge by revising, reinforcing, or replacing chapters that become knowledge of the past. Vol. 1, 2, and 4 are books I put much effort into as the sole principal editor, whereas for Vols. 3, 5, and 6, I am very grateful for the efforts of the coeditors. In the initial contract, Vols. 5 and 6 were planned to have titles of "small vessel disease" and "large artery atherosclerosis," respectively. Writing Vol. 4, pathophysiology of stroke, I realized that I put a considerable amount of content prepared for Vols. 5 and 6 into Vol. 4. Therefore, I was exceedingly worried about the necessity of proceeding with the original series. Meanwhile, a new era began with the introduction of various new drugs and biologics for the treatment of dyslipidemia and diabetes. Considering the changed circumstances, I thought it would be better to make books that reflect the development of new drugs in these fields. Since the publisher generously agreed with my idea, Vol. 5 and 6 were presented to you with new themes: dyslipidemia and diabetes in stroke.

Stroke Revisited Vol. 5: *Dyslipidemia in Stroke* attempted to deal with dyslipidemia as an important risk factor for stroke, from basics to clinical aspects. Cholesterol is an essential nutrient that is indispensable to human cells; however, the absorption and production of excess cholesterol above the necessary level can produce atherosclerosis in the walls of the vessels, ultimately resulting in stroke and acute coronary diseases. Since the SPARCL trial, which demonstrated that cholesterol lowering by atorvastatin is effective in

preventing subsequent vascular events in patients with stroke, numerous statin drugs have been used for stroke prevention worldwide. Then, for many clinicians majoring in stroke, books to comprehensively provide the basic knowledge of lipid metabolism, the principles of drug use, the mechanisms of action of the drugs, and the clinical impact of the cholesterol level have been awaited for a long time. In addition, proprotein convertase subtilisin/kexin type 9 (PCSK9)-inhibiting monoclonal antibodies have recently been developed, and a new small interfering RNA (siRNA) drug (i.e., inclisiran) has emerged as a new therapeutic drug for dyslipidemia. The need to acquire the latest knowledge on lipid drugs has increased. This book has been completed by inviting relevant knowledge experts from all over the world as authors, from basic to clinical, in line with this need. In terms of stroke and dyslipidemia, I am confident that readers will gain the in-depth knowledge that they have not seen in any other book.

The six-volume *Stroke Revisited* series is now completed. I would like to express my deep gratitude to Springer Nature for providing me with this great opportunity. While producing six books up to this point, the KCRI has provided great support for writing these books, and my colleagues have provided valuable help in various ways. I profoundly appreciate it all. In the future, whenever new information is released regarding the contents of the series, partial or full revisions will be made to offer cutting-edge knowledge as much as possible. When I was studying stroke in my youth, I had hard times because of difficulties finding optimal books in the clinical aspects of stroke. The fact that I have produced some books that will help clinicians worldwide is quite rewarding for the rest of my life.

Seoul, Republic of Korea Seung-Hoon Lee
March 2021

Preface

The field of medicine has always been in constant evolution. Dyslipidemia is a dynamically changing field for new drug development. As a result, clinicians have benefited from learning new findings; however, crushing amounts of results often leave them little time to step back and stay longer with its actual value and relevance to clinical practice.

Along with remarkable advances in recent years, particularly in the medical aspect, stroke is no longer considered just a field of neurology. Today, numerous therapies improve the conditions of patients with chronic diseases such as hypertension, diabetes mellitus, and dyslipidemia in stroke care. All risk factors for cardiovascular disease must be treated successfully to achieve favorable outcomes and prevent further stroke. Providing new drugs for the successful treatment of dyslipidemia leads to the success of intra-arterial battle with stroke.

This text is written for this reason. Readers will find that this book is not just about the stroke, but all efforts to reflect the advance in dyslipidemia and real-life challenges in modern society. The editors, authors, and publishers have made every effort to ensure that the knowledge in this book is up-to-date and reliable at the time of publication.

The flow of content is written with the evidence-based practical format to reflect the clinical setting by Professor Seung-Hoon Lee, making it more interesting and easy to read. I do not know if there are words that can genuinely articulate the gratitude I feel for my mentor. His passion, guidance, and support have been the best things to happen in my life. Also, I thank all the authors who have participated in this process.

Through *Stroke Revisited: Dyslipidemia in Stroke*, I hope that physicians in the world will find a timely and effective way to learn about dyslipidemia and its real-life applications in patients with stroke.

Gyeonggi, Republic of Korea — Min Kyoung Kang
March 2021

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Part I

Basic Science: Dyslipidemia and Stroke

Role of Dyslipidemia in Atherosclerosis

1

Akhlaq A. Farooqui

Abstract

Atherosclerosis is a complex inflammatory disease characterized by lipid accumulation within the artery walls. It produces the narrowing of arteries due to the development of intimal plaques. The formation of plaques involves the deposition of small cholesterol crystals in the intima and its underlying smooth muscle. The growth of plaques starts with the proliferation of fibrous tissues and the surrounding smooth muscle producing a bulge inside the arteries. It results in reduction of the blood flow to the heart leading to cardiovascular disease, the leading cause of mortality and morbidity worldwide. Atherosclerosis and cardiovascular disease are not only accompanied by increased levels of cholesterol, cholesterol metabolites, and trimethylamine N-oxide levels in the blood, but also by the involvement of the immune system, which is made up of many cell types, hundreds of bioactive cytokines and chemokines (TNF-α, IL-1β, IL-6, MCP-1), and millions of different antigens. This makes the development of atherosclerosis very challenging. In addition to the development of myocardial infarctions, atherosclerosis is also associated with peripheral artery disease. This pathological condition is also accompanied by different stages of atherogenesis, dyslipidemia, hypertension, oxidative stress, endothelial dysfunction, and inflammation. At the molecular level, these processes involve the generation of reactive oxygen species, reduction in redox status, and increased expression of pro-inflammatory cytokines and chemokines. These mediators can be used as biomarkers for cardiovascular disease, as well as peripheral artery disease.

1.1 Introduction

Cardiovascular disease (CVD) is the biggest killer of the twenty-first century worldwide. It is characterized by the development of atherosclerosis, a multifactorial inflammatory condition that is accompanied by the deposition of plaques, induction of endothelial dysfunction, invasion of the artery wall by leukocytes, and subsequent formation of foam cells, a hallmark of the initial stages of atherosclerosis. The generation of foam cells is associated with an imbalance of cholesterol influx, esterification, and efflux. CD36 and scavenger receptor class A (SR-A) are mainly responsible for the uptake of lipoprotein-derived cholesterol by macrophages. The formation of atherosclerotic plaques starts with the deposition of excessive cholesterol, hydroxycholesterol, and

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lipid oxide products (LOP) in the arterial intimal wall and its underlying smooth muscles, which undergo cellular proliferation and inflammatory reactions [1, 2]. Thus, atherosclerosis can be generally described as an excessive fibrofatty, proliferative, inflammatory response to damage of the artery wall, involving several cell types, such as smooth muscle cells, monocyte-derived macrophages, lymphocytes, and platelets [3]. Then the plaques grow with the proliferation of fibrous tissues and the surrounding smooth muscle and bulge inside the arteries and consequently reducing the blood flow to the heart. The oxidation of low-density lipoprotein (LDL) to oxidized-LDL indicates that the development of atherosclerosis is the first step in the pathogenesis of CVD. Several risk factors have been reported to regulate atherosclerosis and CVD. They include long-term consumption of fatty foods, lack of exercise, hypertension, cigarette smoking, diabetes mellitus, and family history (genetic factors) (Fig. 1.1). Atherosclerosis is also fueled by activation of both innate and adaptive immunity [1, 2]. During the development of atherosclerosis, inflammatory responses are characterized by the recruitment of circulating leukocytes and the production of growth factors that contribute to cell migration and proliferation. Animal model studies have shown that the retention/accumulation of serum low-density lipoprotein (LDL) on intima and sedentary lifestyle are the crucial factors for the initiation and progression of atherosclerosis as well as CVD. The delivery and retention of lipoproteins appear to be dependent on lipoprotein concentration, lipoprotein size, and the integrity of the endothelium. Indeed, modification of retained lipoproteins contributes to the release of phospholipids and phospholipid-derived lipid mediators that can activate endothelium [1, 2]. In addition, recent studies have revealed that atherosclerosis also involves the accumulation and activities of various immune cells. The immune system is a complicated network made up of many cell types, hundreds of bioactive cytokines, and millions of different antigens, making it challenging to readily define mechanisms that con-

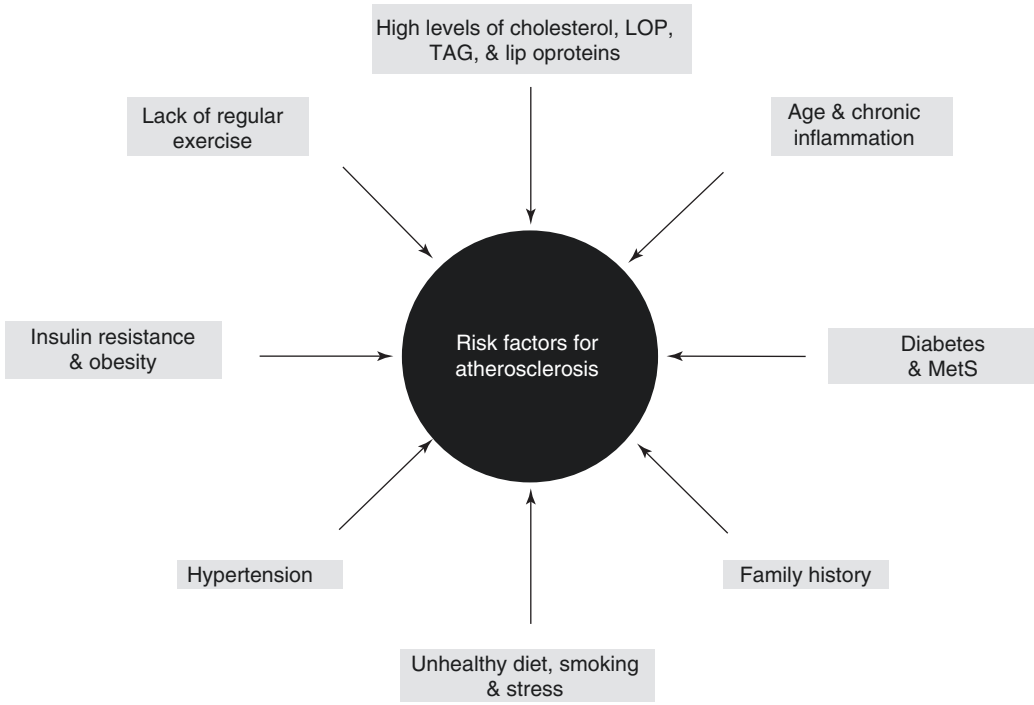

Fig. 1.1 Factors modulating atherosclerosis and cardiovascular diseases. *TAG* Triacylglycerol; *MetS* metabolic syndrome

tribute to atherosclerosis. Finally, the composition of gut microbiota also plays an important role in cholesterol homeostasis. Collective evidence suggests that dyslipidemia, endothelial cells, leukocytes, and intimal smooth muscle cells are the major players in the development of atherosclerosis. The most devastating consequences of atherosclerosis are myocardial infarctions and stroke as well as lower extremities peripheral artery disease (PAD) [1, 2].

CVD and stroke are major health problems in the United States. Approximately 6.5 million Americans suffer from CVD, with 700,000 new cases diagnosed every year. Similarly, about one million people in the United States suffer from stroke each year. On average, in the United States every 40 seconds someone has a stroke and every 4 minutes someone dies from a stroke suggesting that stroke is one of the major causes of death and adult disability in the United States. The likelihood of having CVD and stroke increases with age reaching 10 per 1000 population in individuals older than 65 years of age, and after a continuous decline over the last 5 decades, CVD incidences are increasing again [3, 4]. The most common manifestations of CVD are stable angina pectoris and acute coronary syndromes. Multiple conventional risk factors are known to contribute to the pathogenesis of CVD. "Cholesterol hypothesis" states that high levels of blood cholesterol are a major risk factor for CVD and lowering high levels of cholesterol reduces the risk of CVD. In bio-membranes, the dynamic clustering of cholesterol along with sphingolipids results in the formation of specialized structures called microdomains or rafts. These rafts act as a platform for signal transduction processes. The depletion of cholesterol in bio-membranes induces autophagy, a process by which cells digest their own components. An increase in levels of cholesterol in serum (dyslipidemia) is an abnormality of lipid metabolism. It is characterized by increased circulating levels of serum total cholesterol, LDL cholesterol, triglycerides, and decreased levels of serum HDL cholesterol. High levels of LDL cholesterol and non-HDL cholesterol have been associated with cardiovascular risk, while other cholesterol-related serum markers, such as the small dense LDL cholesterol, lipoprotein(a), and HDL particle measurements, have been proposed as additional significant biomarkers for CVD. Like atherosclerosis, risk factors for CVD include age, sex, genetic predisposition, diet, and regular exercise (lifestyle). These factors not only lead to hypertension and dyslipidemia, but also accelerate aging, and endothelial dysfunction [5].

Protection from atherosclerosis and CVD can be achieved by introducing food restrictions along with appropriate medical treatments according to clinical healthcare guidelines. Both atherosclerosis and CVD are accompanied by inflammatory processes. Inflammation associated with atherosclerosis involves complicated processes, including systemic inflammatory reactions and the accumulation of immune cells, such as monocytes/macrophages, dendritic cells, and lymphocytes. The immune system (innate immunity and adaptive immunity) plays important roles in all stages of atherosclerosis and CVD from initiation through progression, as well as in atherothrombotic complications. Persistent inflammation in atherosclerosis and CVD is also supported by gut microbiota and activated subpopulations of substantial B cells in the vicinity of arterial adventitia. Because atherosclerosis and CVD are global health burden throughout the world especially in developed counties, multidisciplinary therapeutic and preventive approaches should be introduced to achieve protection from these pathological conditions [1, 2, 3].

It is widely accepted that excessive dietary intake of saturated fats and cholesterol (Western diet) and lack of exercise play an important role in the onset and development of atherosclerosis and CVD. It increases levels of apolipoprotein B (apoB) 100–containing lipoproteins and decreases levels of high-density lipoprotein (HDL) in serum [1, 6, 7]. The oxidation of low-density lipoprotein (LDL) to oxidized-LDL is the first step in the pathogenesis of atherosclerosis and cardiovascular diseases. However, non-lipid risk factors can also contribute to the development of CVD. About one-half of the deaths due to this condition occur in individuals with normal cholesterol levels [6]. This is because inflammation is an important

etiological factor for atherosclerosis as well as CVD and current therapeutic options for treating or preventing atherosclerosis and CVD still remain focus on lipid control alone, rather than resolving inflammation [1, 3].

1.1.1 Lipids in the Atherosclerotic Process

Atherosclerosis is a lipoprotein-driven disease that leads not only to plaque formation at specific sites of the arterial tree, but also involves induction of inflammation, necrosis, fibrosis, and calcification. Atherosclerosis can be assessed by monitoring arterial stiffness, which can be monitored using pulse-wave velocity, the cardio-ankle vascular index, the ankle-brachial index, pulse pressure, the augmentation index, flow-mediated dilation, carotid intima-media thickness, and arterial stiffness index-β. Arterial stiffness is generally considered an independent predictor of CVD. The early development of the plaque involves the accumulation of lipids, interactions between damaged endothelial cells, vessel wall smooth muscle cells, circulating inflammatory cytokines, growth factors, and cell adhesion molecules indicating that plaque formation may be a cell-mediated immune phenomenon. Development and progression of atherosclerosis, there is an accumulation of lipid in the plaques, reaching a mean lipid content of 37% in severe plaques. This increase in the lipid content of plaque is mainly due to large increases in cholesterol, over 80% of which are hydroxycholesterols, cholesteryl esters, and cholesterol oxides. This deposition of cholesterol, hydroxycholesterols, cholesteryl esters, and cholesterol oxides in plaque accounts for 20–34% of the total cholesterol content of the plaque. Examples of cholesterol metabolites are 7-ketocholesterol (7-kCh), 26-hydroxycholesterol (26-hCh), 27-hydroxycholesterol (27-hCh), and 5α-cholestane-3β,5,6β-triol (triolCh) suggesting that the main oxidation reactions of cholesterol are peroxidation occurs at carbon C7, C26 and epoxidation of double bond C5-C6 [8, 9]. In addition to cholesterol and its metabolites, human aortic plaques contain free and oxidized fatty acids, phospholipids, triglycerides, and other LOP such as isoprostanes, hydroxy fatty acids, lipid peroxides, and aldehydes [7, 8]. Levels of lipids in normal aortic plaques are low (1–2%). However, human aortic plaques from CVD patients contain high levels of cholesterol and its oxides, free and oxidized fatty acids, triglycerides, and LOP. Among these components, LOP is not only known to impair normal physiological functions, but also stimulate atherosclerotic processes. Unesterified LOP associated with membranes disrupts fluidity and alters signaling pathways associated with oxidative stress, apoptosis, inflammation, and gene expression leading to cellular damage. It has been proposed that the lipoprotein-specific LOP transport not only plays important roles in atherosclerosis-related effects of LDL and HDL but is also produces phospholipid packing defects in cell membranes. Recent studies have indicated that plasma lipoproteins are active carriers of LOP, low-density lipoprotein (LDL) directing transport toward peripheral tissues, and high-density lipoprotein (HDL) being active in the reverse transport [8]. Induction of LOP efflux from macrophages protects against endothelial dysfunction and prevents atherogenesis in mice fed a high-cholesterol diet. Collective evidence suggests that mature atherosclerotic plaques contain a lipid core, which is enriched in cholesterol and its metabolites and a cap composed of fibrillar collagen. It is reported that in sub-endothelial space apoB 100-containing lipoproteins interact with extracellular matrix components, leading to trapping of more lipoproteins with subsequent aggregation and oxidative modification through the involvement of cholesterol, its metabolites, and LOP. These lipids produce cytotoxicity, apoptotic death, and pro-inflammatory effects. They not only participate and damage the endothelium, trigger cell proliferation, modulate vascular remodeling, but also contribute to increased cellular permeability with increased expression of adhesion molecules that bind monocytes and T lymphocytes to create a vicious cocktail of pathophysiological factors. In addition, the expression of chemo-attractants and pro-inflammatory cyto-

kines in arterial intima promote the differentiation of monocytes into macrophages taking up oxidized-LDL uncontrollably to form foam cells and atherosclerotic lesions. Their synthesis has been directly linked with the pathogenesis of atherosclerosis and CVD [9].

1.1.2 Atherosclerotic Plaque Progression and Acute Rupture

Atherosclerosis starts in childhood. After decades of progression, atherosclerosis results in mature plaque formation, which is responsible for the onset of ischemic symptoms. While plaque growth due to smooth muscle cell proliferation, matrix synthesis and lipid accumulation is known to narrow the arterial lumen and ultimately decreasing the blood flow to the heart (coronary heart disease), brain (ischemic stroke), and lower extremities (peripheral vascular disease) [10, 11, 12]. The most common of these manifestations is coronary heart disease, including stable angina pectoris and acute coronary syndromes. After decades of development, the plaque ruptures and develops into a lesion with a large necrotic core with an overlying thin disrupted fibrous cap. The lesion is heavily infiltrated by macrophages and T lymphocytes. Physical interactions between flowing blood and thrombogenic necrotic core result in the development of platelet-rich luminal thrombus, which is superimposed by a proteoglycan-rich matrix. After decades of development, mature plaques may suddenly rupture and cause life-threatening coronary thrombosis presenting as an acute coronary syndrome. At the molecular level, the infiltration of macrophages into plaque not only contribute to the uptake and metabolism of lipoproteins as well as growth factor secretion, but also activate macrophage matrix metalloproteinase (MMPs) activity leading to the exposure of red cell-rich necrotic core materials (lipids, LOP, proteoglycan, and hyaluronan) to smooth muscle cells [13, 14]. Inflammation and immune reactions play a pivotal role in atherogenesis and the destabilization of plaque [13, 15]. Under normal conditions, inflammation produces only temporary incapacitation of heart function, followed by heart tissue restoration and remodeling. However, under pathological conditions, the process becomes chronic and ends with prolonged heart dysfunction. The immune process involves immunocompetent cells: T- and B lymphocytes (the main components of the adaptive immune response). Adhesion of circulating monocytes to activated endothelial cells is associated with the earliest stage of inflammation. The disruption of plaque is facilitated by coronary spasm and calcification of tortious arteries in older individuals [16]. It is known that the disruption of lipid-induced innate immune signaling reduces atherosclerosis in hyperlipidemic murine models. The multifactorial nature of CVD and the complexity of the inflammatory pathways contribute to atherosclerotic plaque development in hyperlipidemic mice model of atherosclerosis. This rat model should be carefully evaluated to compare to the development of plaques in humans. In addition to apoB 100–containing lipoproteins, HDL may also play a dual role in the pathogenesis of atherosclerosis. To this end, chemical modification of HDL by macrophage-derived myeloperoxidase transforms HDL into pro-inflammatory and pro-atherogenic entities indicating that HDL may have a dysfunctional role in atherosclerosis [16].

At the molecular level, plaque rupture not only involves endothelial cell responses to make shear stress, but also induction of inflammation, a process caused by the activation of Toll-like receptors (TLRs) and increased expression of cytokines and chemokines (tumor necrosis factor-beta (TNF-β), interleukin-1β (IL-1β), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1)) through the involvement of pro-inflammatory transcription factor nuclear factor-kappa B (NF-κB). This transcription factor is present in the cytoplasm. Under the influence of oxidative stress, it migrates to the nucleus where it promotes the expression of cytokines (TNF-α, IL-1β, and IL-6), chemokines (MCP-1), and adhesion molecules (intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1)) after interacting with NF-κB response element (NF-κB-RE) (Fig. 1.2).

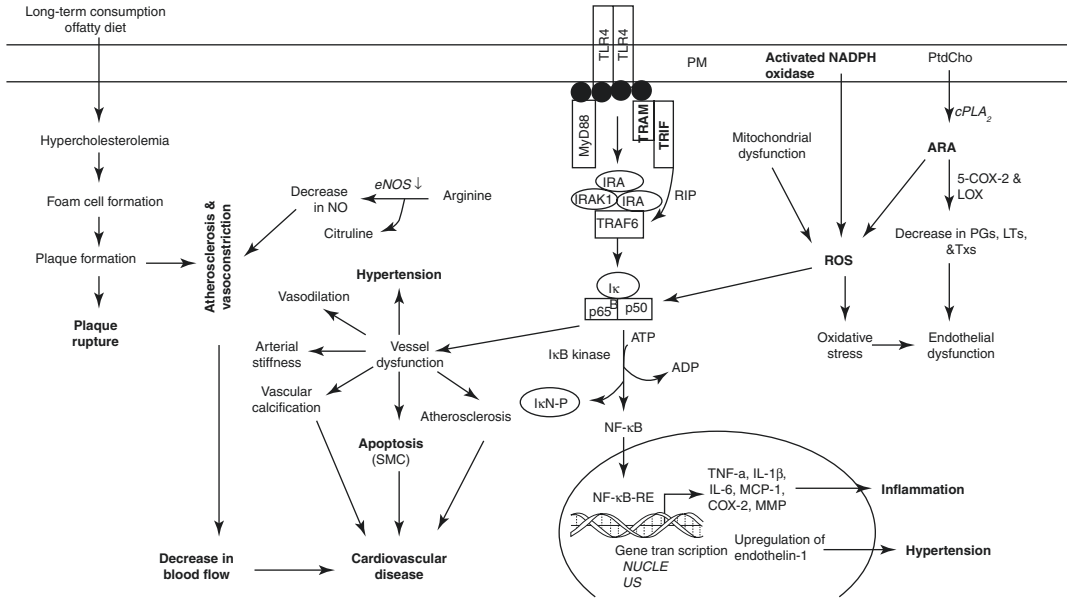

Fig. 1.2 Schematic diagram showing signal transduction mechanisms associated with the pathogenesis of cardiovascular disease. *PM* Plasma membrane; *PtdCho* phosphatidylcholine; *ARA* arachidonic acid; *lyso-PtdCho* lyso-phosphatidylcholine; *PAF* platelet activating factor; *cPLA*$_2$ cytosolic phospholipase A$_2$; *COX* cyclooxygenase; *LOX* lipoxygenase; *ROS* reactive oxygen species; *NF-κB* nuclear factor-kappa B; *NF-κB-RE* nuclear factor-kappa B response element; *TNF-α* tumor necrosis factor-alpha; *IL-1β* interleukin-1beta; *IL-6* interleukin-6; *MCP1* monocyte chemotactic protein-1; *TLR4* Toll-like receptors 4; *MyD88* adaptor protein; *IRAK* IL-1R-associated kinase; *TRAF6* tumor necrosis factor receptor-associated factor adaptor protein 6; *NIK* NF-κB-inducing kinase; *IKK* IκB kinase; *TRIF* TIR-domain-containing adapter-inducing interferon-β; *SMC* vascular smooth muscle cell; *NO* nitric oxide; and *NOS* nitric oxide synthase. Upward arrow indicates increase

TLRs also play important roles in the innate and inflammatory signaling responses to microbial agents. The transcription of cytokines and chemokines is not only involved in the inflammatory process, but also in proliferative responses of cells critical to atherogenesis and ultimately leading to the synthesis and release of antimicrobial peptides and inflammatory cytokines that are associated with adaptive immunity. Moreover, Toll-like receptor 4 (TLR4) expression in macrophages is upregulated by oxidized LDL suggesting a potential mechanism for the synergistic effects of hypercholesterolemia, acceleration of atherosclerosis, and disruption of plaque. Studies on TLR4 and LDL receptors double knockout mice have indicated that a deficiency of TLR4 receptors reduces atherosclerosis without affecting inflammation. Moreover, clinical investigations have revealed that upregulation of TLRs not only contributes to inflammation through the body but also supports the development of atherosclerosis and inflammation leading to clot formation. Similarly, activation of the c-Jun N-terminal kinase pathway leads to the upregulation of stress response genes and is implicated in pathological cardiac events. In normal individuals under physiological conditions generation of nitric oxide (NO) regulates vascular tone, inhibits platelet function, prevents adhesion of leukocytes, and reduces proliferation in the intima. In addition, NO is also involved in the maintenance of metabolic and cardiovascular homeostasis in the heart tissue. Endothelial dysfunction contributes to the pathogenesis of CVD by increasing ROS and decreasing the production of NO (Fig. 1.2). The main enzymes that generate ROS in the vascular wall cells are nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine oxidase, and mitochondrial enzymes, respiratory chain complexes, lipoxygenase, and myeloperoxidase. Major sources for cardiovascular ROS are the activation of NADPH oxidase,

mitochondrial dysfunction, and uncontrolled arachidonic acid cascade. As stated above, an increase in ROS promotes the translocation of NF-κB to the nucleus, where it increases the expression of cytokines, chemokines, and adhesion molecules. These processes are associated with leukocyte adherence, cell permeability, LDL oxidation, platelet activation, and vascular smooth muscle cell proliferation and migration. Elevation in ROS results in oxidation of macromolecules promoting cell apoptosis through the release of cytochrome-c [17].

In the vascular wall, ROS induces proliferation of smooth muscle cells, apoptosis of endothelial cells, and increase the activity of matrix metalloproteinases, therefore providing input to plaque destabilization. A decrease in NO results in vasoconstriction, a process that decreases blood flow to the heart. Endothelial dysfunction is one of the first signs of atherogenesis. It is accompanied by a decrease in NO production. NO is the main regulator of the vascular tone, which limits the synthesis of adhesion molecules and chemokines and prevents platelet aggregation. Endothelial NO is an anti-inflammatory and anti-thrombogenic factor. Endothelial cell death is an important factor in the development of atherosclerosis. During this process, Apoptosis of the endothelial cells is accompanied by the redistribution of phosphatidylserine on the endothelial cell surface and the loss of anticoagulant surface components (thrombomodulin, heparan sulfate, and tissue pathway inhibitor). This increases the procoagulant properties of the endothelium. The involvement of endothelial cell apoptosis in the progression of atherogenesis is supported by the fact that the course of the disease can be controlled by statin therapy [17].

1.1.3 Atherosclerotic Cardiovascular Disease

Atherosclerotic cardiovascular disease is a group of disorders of the heart and blood vessels. It includes CVD, stroke, heart failure, and atrial fibrillation [18]. These diseases are the largest causes of death in the world in the elderly population. Aged CVD patients suffer complex changes that include hypertrophy, altered left ventricular diastolic function, reduced left ventricular systolic reverse capacity, increased arterial rigidity, and impaired endothelial function. The two major initiators of atherosclerotic cardiovascular disease include hyperlipidemia and vascular production of ROS and LOP. During the development of atherosclerosis, the production of ROS is accompanied by rapid loss of anti-inflammatory and anti-atherogenic activities of the endothelium-derived NO resulting in endothelial dysfunction. Production of ROS also results in the activation of the transcription factor NF-κB. This transcription factor in the nucleus induces the expression of vascular pro-inflammatory and pro-thrombotic genes. ROS is also a potent activator of MMPs, which indicate plaque destabilization and rupture leading to a decrease in cardiomyocytes through apoptotic and necrotic cell death. The second initiator of atherosclerotic CVD is the oxidation of LDL. Oxidation of LDL in the vessel wall promotes an inflammatory cascade that activates atherogenic pathway leading to foam cell formation. The accumulation of foam cells leads to fatty streak formation, which is the earliest visible atherosclerotic lesion. In contrast, the cardiac sarco/endoplasmic reticulum Ca^{2+}-ATPase and hepatic apolipoprotein E (apoE) expression can improve cardiovascular function. Ca^{2+}-ATPase regulates the cardiac contractile function by lowering cytoplasmic calcium levels during relaxation, and affecting NO action in vascular cells, while apoE is a critical ligand in the plasma clearance of triglyceride- and cholesterol-rich lipoproteins [18].

Hypertension also plays an important role in the pathogenesis of CVD. Many factors are associated with the pathophysiology of hypertension. Pathogenesis of hypertension is regulated by genetic, environmental, and metabolic factors [19]. Metabolically, renin–angiotensin–aldosterone system, perturbation of G protein-coupled receptor signaling, induction of inflammation, and alteration of T cell function are closely associated with the pathophysiology of hypertension. These processes are linked to increased production of ROS, decrease in NO production, and reduction

in antioxidant capacity in the cardiovascular system [20]. Although ROS production may not be solely associated with the etiology of hypertension, it amplifies blood pressure elevation in the presence of other prohypertensive factors which may contribute to hypertension. As stated above, in the cardiovascular system ROS play an important physiological role in controlling endothelial function, vascular tone, and cardiac function. Among these factors, endothelial dysfunction promotes inflammation, hypertrophy, proliferation, apoptosis, migration, fibrosis, angiogenesis, and rarefaction directly or indirectly. Although convincing data from animal studies support a causative role for oxidative stress in the pathogenesis of hypertension, there is still no solid evidence that oxidative stress causes hypertension in humans. However, biomarkers of excess ROS are increased in patients with hypertension and oxidative damage is important in the molecular mechanisms associated with cardiovascular and renal injury in hypertension. In addition, intake of high salt and consumption of a high-calorie diet may not only increase oxidative stress but may increase the risk of hypertension [21]. Collective evidence suggests that an increase in oxidative stress and inflammatory processes during CVD not only promote a profibrotic environment and impairment in neovascularization capacity due to a reduction of proangiogenic functions but also a decrease in capacity of progenitor cells to functional repair.

Another important factor in the pathogenesis of atherosclerosis and CVD is the involvement of dysbiosis, a process associated with changes in the composition of gut microbiota. Dysbiosis is linked with the pathogenesis of many conditions including atherosclerosis, CVD, hypertension, obesity, and type 2 diabetes [20]. The induction of dysbiosis may produce and release immunogenic endotoxins called lipopolysaccharide (LPS) (Fig. 1.3). It is well known that a large part

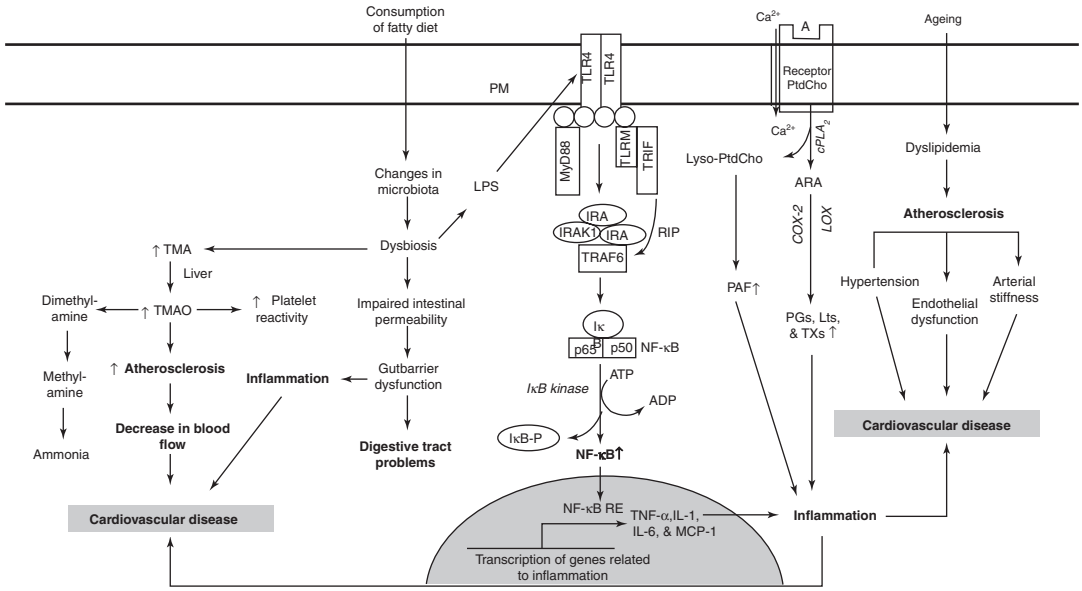

Fig. 1.3 Schematic diagram showing the contribution of microbiota in the pathogenesis of cardiovascular disease. *PM* Plasma membrane; *A* agonist; *R* receptor; *PtdCho* phosphatidylcholine; *ARA* arachidonic acid; *lyso-PtdCho* lyso-phosphatidylcholine; *PAF* platelet activating factor; *cPLA*$_2$ cytosolic phospholipase A$_2$; *COX* cyclooxygenase; *LOX* lipoxygenase; *ROS* reactive oxygen species; *NF-κB* nuclear factor-kappa B; *NF-κB-RE* nuclear factor-kappa B response element; *TNF-α* tumor necrosis factor-alpha; *IL-1β* interleukin-1beta; *IL-6* interleukin-6 ; *MCP1* monocyte chemotactic protein-1; *TLR4* Toll-like receptors 4; *MyD88* adaptor protein; *IRAK* IL-1R-associated kinase; *TRAF6* tumor necrosis factor receptor-associated factor adaptor protein 6; *NIK* NF-κB-inducing kinase; *IKK* IκB kinase; *TRIF* TIR-domain-containing adapter-inducing interferon-β; *LPS* lipopolysaccharide; *TMA* trimethylamine; *TMAO* trimethylamine oxide. Upward arrow indicates increase